This volume contains the extended abstracts presented at the 13th International Chemical and Biological Engineering Conference (CHEMPOR 2018), held in Aveiro - Portugal, from the 2nd to the 4th of October, 2018.

University of Aveiro & Ordem dos Engenheiros

13th International Chemical and Biological Engineering Conference (CHEMPOR 2018)

Book of Extended Abstracts

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Welcome Message

University of Aveiro and Ordem dos Engenheiros proudly welcome all participants to the 13th International Chemical and Biological Engineering Conference - CHEMPOR 2018. Welcome to Aveiro!

During three days, from October 2nd to 4th, more than 370 participants from Industry and Academia gathered at Centro Cultural e de Congressos de Aveiro to discuss recent developments and future directions in different areas of chemical engineering, materials science and engineering, biotechnology and biological engineering. CHEMPOR 2018 aims to be a forum for the debate of societal challenges related to energy, the environment, and the efficient use of natural resources and materials, all of which require innovative solutions complying with sustainability criteria.

In 2017 Portuguese exports increased circa 11 % reaching the highest value of the Gross Domestic Product (in a time horizon of 23 years). Portugal’s Ministry of Economy estimates that capital-intensive industries (such as chemicals and polymers producers) and manufacturing industries (including food, textile, paper, cork, wood panels and metal producers) account for over 50 % of the overall value of the exportations. In this context, CHEMPOR 2018 emerges as a privileged forum to discuss technological and scientific issues that will promote economic growth through innovation.

The Organizing Committee accepted more than 345 communications, of which 83 oral presentations, after peer reviewing. Internationally recognized scientists were invited to deliver 6 Plenary Lectures and 6 Keynote Speeches. On the opening day of CHEMPOR 2018, the Portuguese Minister of Economy, Professor Manuel Caldeira Cabral, made a communication in the presence of dozens of industrial sponsors. Just before the closing session, the Professor Almiro Castro Award (sponsored by PARALAB) was granted, for the second time, and prizes were awarded to the best Oral and Poster Communications presented during the Conference.

The Organizing Committee of CHEMPOR 2018 acknowledges all authors for their contributions, all colleagues involved in the organization of the program, and the generosity of all sponsors.

We wish you a memorable Conference both scientifically and socially.

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[BS] - Biorefinery and Sustainability  
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PLENARY LECTURE SPEAKERS

Professor Gabriele Centi

Gabriele Centi is full professor of Industrial Chemistry at the University of Messina, Italy, and President of the European Research Institute of Catalysis (ERIC). Research interests are in the areas of applied heterogeneous catalysis, sustainable energy and chemical processes, biomass conversion and environment protection.

He was coordinator of the EU Network of Excellence IDECAT, and is actually President of IACS (International Association of Catalysis Societies) and vice-President of the InterUniversity Consortium on the Science and Technology of Materials (INSTM). He was coordinator or PI in over twenty EU projects, besides many other national and industrial projects. He received several awards, and is involved in various editorial activities, between which chairing the editorial board of *ChemSusChem* and be co-editor in chief of *Journal of Energy Chemistry*. He was chairperson of many international conferences (the last one Euрапacat 2017 in Florence) and also chaired the 1st World Congress on Environmental Catalysis in Pisa (Italy) on May 1995. He is author of over 450 scientific publications, 12 books and editor of various special issues. Current h-index is 76 with about 21,250 citations (Google Scholar).

Professor Paul Christakopoulos

Paul Christakopoulos works in the area of Biochemical Process Engineering focusing on the development of biochemical (Green Chemistry) processes for the production and refinement of chemicals, fuels and material from CO$_2$, either captured before it is emitted to the atmosphere (non biomass route) or by recovering it from the atmosphere via photosynthesis in the form of biomass (biomass route).

More specifically his research is focused on cellulose and lignin biorefinery, novel enzymes for modification of plant cell wall material, biocatalysis in non-conventional media, production of prebiotic oligosaccharides, production of biofuels and chemicals, biocatalytic CO$_2$ capture and conversion to chemicals and fuels. Paul has been appointed Chair Professor of Biochemical Process Engineering at Luleå University of Technology, Sweden, from February 2012. He has previously served as a Professor of Industrial Biotechnology at the School of Chemical Engineering, National Technical University of Athens.

He is author of over 230 scientific publications. Current h-index is 50 with about 7600 citations (Google Scholar). Paul had served as an Associate Editor of *World Journal of Microbiology and Biotechnology, Food and Bioproducts Processing, PeerJ, Frontiers in Microbiology, FEMS Microbiology Letters.*
Professor María José Cocero Alonso

María José Cocero Alonso is currently a Professor in the Department of Chemical Engineering and Environmental Technology at the University of Valladolid in Spain. She is director of the research group on High Pressure Process Engineering (http://hpp.uva.es/), and regional editor of The Journal of Supercritical Fluids. Her research is focused in processes and products contributing to the development of bio-economy, as (i) alternative use of natural resources for the production of chemicals and energy by subcritical/supercritical water, (ii) production of health related and high added value formulations from renewable raw materials by supercritical carbon dioxide, and (iii) intensification of processes through implementation of new technologies for upgrading raw materials and waste. She teaches master courses in supercritical fluids separation and reaction processes, and chemical engineering products. María José Cocero Alonso published over 235 articles with ca. 5200 citations and h-index 38 (SCOPUS).

Professor Nien-Hwa Linda Wang

Professor N-H Linda Wang is the Maxine Spencer Nichols Professor of Chemical Engineering, Purdue University. She joined Purdue faculty in 1980 as the first female professor in Chemical Engineering. She received her BS in Chemical Engineering from National Taiwan University in 1971, her MS in Chemical Engineering from the University of Wyoming in 1973, and her PhD in Chemical Engineering from the University of Minnesota in 1978. She is a Fellow of the American Institute of Medical and Biological Engineering and a Fellow of the American Institute of Chemical Engineers (AIChE). She was a Director of the Separations Division, American Institute of Chemical Engineers (2001-2004) and a Director of the International Adsorption Society (2001-2007). She was the Chair of Separations Division of the AICHE in 2013. She is well respected as a leader in separations. She consulted for NSF, NIH, several national labs and many chemical, food, and pharmaceutical companies.

She is internationally known for her research contributions in adsorption, ion exchange, multicomponent chromatography, and simulated moving bed technologies. She has two patents, four patent applications, more than 120 technical publications, and more than 200 invited lectures and presentations at national and international meetings.

She is a leading expert in adsorptive separation methods. She made major contributions and published extensively in multicomponent chromatography and simulated moving bed (SMB) technologies for separating complex mixtures. She invented the seminal Standing Wave Design method for SMB processes. She developed the VERSE simulation software package, which is a powerful tool for understanding quantitatively adsorption and reaction phenomena in such processes. Recently she developed the first graphical solutions, which can be used to design affinity chromatography processes without any simulations.

In addition to her significant contributions to the fundamentals of chromatography, she developed the first tandem SMB process for insulin purification, and the first five-zone SMB for the recovery of six...
sugars from biomass hydrolysates. Recently she also
developed a new capture chromatography process
for the production of an important medical isotope.
She developed the first separation process to recover
polycarbonates and flame retardants from electronic
wastes. She also developed the first ligand assisted
sequential chromatography method based on inorganic adsor-
bents for the production of high-purity rare earth ele-
ments. These new methods are expected to have a
major impact on the environment and on the produc-
tion of important chemicals and biochemicals.

Professor Nikos Hadjichristidis

Nikos Hadjichristidis is a Distinguished Profes-
sor of Chemical Sciences at King Abdullah Univer-
sity of Science and Technology, Saudi Arabia, and
Emeritus Professor at the University of Athens, Gre-
ee.
The research of Professor Hadjichristidis focuses
mainly on the synthesis of novel homopolymers and
copolymers with well-defined complex macromole-
cular architectures, by using anionic polymerization
(AP) high vacuum techniques as well as, combina-
tion of AP with other polymerizations (C1 and
C3, ROP, ROMP, ATRP, etc.). These polymers are
ideal models for checking the theory (e.g. de Gen-
nes tube theory and hierarchical motion theory in
branched polymers), understanding/improving the
performance of industrial polymers (e.g. PE and
PMMA), and are potential candidates for high-tech
applications (e.g. nanolithography, high temperature
membranes and drug delivery). Also his research
interest includes the synthesis of hybrids of poly-
mers with polypeptides, CNTs, silica and Au nano-
particles by using surface initiating polymerizations
(anionic and C1) in order to overcome their inherent
insolubility and make these materials more useful
in nanotechnology applications. He has published
more than 500 scientific papers in referred scientif-
ic journals (citations until May 31th 2018: 19990,
h-index: 69, Web of Science; citations: 20160, h-
index: 69, Scopus; citations: 27300, h-index: 78,
Google Scholar), 25 patents, is the editor of three
books and author of one book on “Block Copolym-
ers” (Wiley 2003). Professor Hadjichristidis has
received several awards including the Macro Group
United Kingdom Medal for Outstanding Achieve-
ments (2016), the ACS National Award for Polymer
Chemistry (2015), the ACS Rubber Division Chem-
istry of Thermoplastic Elastomers Award (2011),
the ACS Polymeric Materials Science and Enginee-
ring (PMSE) Division Cooperative Research Award
(2010), and the International Award of the Society of
Polymer Science, Japan (SPSJ, 2007).

Professor Rajamani Krishna

Rajamani Krishna received his PhD degree in
Chemical Engineering in 1975 from the University
of Manchester in England, and soon thereafter joined
the Royal Dutch Shell Laboratories, Amsterdam
in The Netherlands. At Shell, he was involved in a
wide range of research and developmental activities
in separations and reaction processes. Since 1990,
he is Professor at the Van ‘Hoff Institute for Mole-
cular Sciences, University of Amsterdam.
Professor Krishna’s research interests range from molecular modelling, bubble and particle dynamics to reactor scale up to process synthesis. The main goal of this research concerns the development of unifying concepts in multicomponent diffusion and multiphase hydrodynamics, both in separations and reaction engineering. He has pioneered the development of the Maxwell-Stefan diffusion formulation and its application to fluid phases, porous solids, and complex three-phase (vapour-liquid-liquid) systems. The Krishna Group has investigated the hydrodynamics of various types of multiphase reactors, using the Computational Fluid Dynamics technique. Improved design procedures have been developed for bubble columns, slurry and air-lift reactors, gas-solid fluidized beds, catalytically structured reactors, distillation trays, and catalyst containing trays. Professor Krishna has also contributed to the understanding of the influence of low-frequency sound waves on gas and liquid flow in bubble columns. The current focus of Krishna’s research is on development of novel energy-efficient separation technologies using ordered crystalline nanoporous adsorbents.

Thanks to his wide experience, based both on his academic and industrial careers, Professor Krishna is recognised worldwide as a leading scientific expert. He has received several awards, which include the prestigious CONRAD Prize of the Dutch Institute of Engineers, and the Akzo Nobel Science Award. He is the recipient of the prestigious 2013 ENI award in the category New Frontiers of Hydrocarbons (Downstream) for his research theme Improving Process Technologies with Molecular Insights.

Krishna is included in the 2017 list of the most Highly Cited Researchers (category: Chemistry); see https://clarivate.com/hcr/2017-researchers-list/. He has published more than 500 peer-reviewed journal articles, and two text books, one of which has been translated into Chinese. He holds several patents. A complete list of his research contributions can be found on Google Scholar; see http://scholar.google.nl/citations?user=cKqtQ0MAAAAJ&hl=en. His publications have been cited more than 36 000 times, with an h-index of 101 (Google scholar).

KEYNOTE SPEAKERS

Adélio Mendes

Professor Adélio Mendes (born 1964) received his PhD degree from the University of Porto in 1993, and is currently Full Professor at the Department of Chemical Engineering of the Faculty of Engineering - University of Porto. Coordinates a large research team with research interests mainly in dye sensitized solar cells and perovskite solar cells, photoelectrochemical cells including water splitting and solar redox flow batteries, photocatalysis, redox flow batteries, electrochemical membrane reactors (PEMFC, H-SOFC, chemical synthesis), methanol steam reforming, membrane and adsorbent-based gas separations and carbon molecular sieve membranes synthesis and characterization.

Professor Mendes authored/co-authored ca. 330 articles in peer-review international journals, possesses nearly 5970 citations and h-index 38. He filled 23 families of patents and is the author of a textbook. He received an Advanced Research Grant from the ERC on dye-sensitized solar cells for building integrated of ca. 2 M€ and since 2013 he is partner in 2 more EU projects leading one of them. He received the Air Products Faculty Excellence 2011 Award (USA).
for developments in gas separation, Solvay & Hovione Innovation Challenge 2011 prize, the Prize of Coimbra University of 2016, and the prize of Technology Innovation - 2017 by the University of Porto. Presently, he is the Coordinator of CEner-FEUP, the Competence Center for Energy of the Faculty of Engineering at the University of Porto.

**João Rocha**

João Rocha is Full Professor of Inorganic and Materials Chemistry at the Department of Chemistry, University of Aveiro, and Director of CICECO - Aveiro Institute of Materials. He is member of the European Academy of Sciences (EURASC) and of the Lisbon Academy of Sciences, and Fellow of Royal Society of Chemistry and of the Chem-PubSoc Europe. He has received the Madinabeitia-Lourenço award from the Real Sociedad Española de Química, the prize Ferreira da Silva (Portuguese Chemical Society), and the prize for Scientific Excellence from the Portuguese Science Foundation. He was member of the National Science and Technology Council (advising the Prime Minister). He headed the Commission on Inorganic and Mineral Structures of the International Union of Crystallography and is consultant for the Commission on NMR Crystallography and Related Methods. Professor João Rocha published ca. 490 SCI papers and 24 book chapters with ca. 16,000 citations (h-index 60) and 4 patents; gave over 200 talks at conferences; and supervised 40 post-docs and 25 PhDs. His present main research interests encompass microporous transition metal and lanthanide silicates, photoluminescent lanthanide-bearing materials, and Metal Organic Frameworks for sensing applications, including nanothermometry; nanosystems for multimodal (magnetic resonance, optical and thermometry) imaging and small molecules drug delivery; solid-state NMR and X-ray diffraction.

**José António Teixeira**

José António Teixeira is currently full Professor at Biological Engineering Department, University of Minho (since 2000). He has a degree in Chemical Engineering from University of Porto (1980) and a PhD in Chemical Engineering also from University of Porto (1988). He has been involved in different management activities, being Head of the Department of Biological Engineering, Univ. Minho, 2000-2012, and Head of Biological Engineering Research Centre, 2012-2015.

Prof. José Teixeira has a wide research expertise in Industrial Biotechnology (bioprocess development for the transformation of lignocellulosic materials into 2nd generation bioethanol and chemicals; valorization of agro-industrial residues; bioreactor development and continuous processing) and Food Biotechnology (non-conventional food processing; edible films for packaging; food nanotechnology, process development for production of prebiotics). He was responsible/co-responsible for the supervision of 31 PhD theses and 20 Post-docs and has been
the coordinator of 32 scientific research projects, 7 of which international. José Teixeira was awarded the “Stimulus to Excellence”, 2006, from FCT, the “Seeds of Science” in “Engineering and Technology”, 2011, from “Ciência Hoje” and the “Scientific Merit Award”, Universidade do Minho, 2015. He is the co-editor of the books “Reactores Biológicos-Fundamentos e Aplicações” (in Portuguese), Engineering Aspects of Milk and Dairy Products and Engineering Aspects of Food Biotechnology, and the author/co-author of over 535 peer reviewed papers with more than 14260 citations and h-index 58.

Maria da Ascensão Reis

Maria Reis is a Full Professor in Environmental Biotechnology at FCT-NOVA. Main research interests have been in the area of Environmental/Industrial Bioengineering, with special focus on the development of sustainable bioprocesses for the removal of pollutants from water and wastewater streams and for the exploitation of industrial wastes for the production of biopolymers and bulk chemicals.

Within this research area, published more than 220 papers in scientific journals with peer review, possesses around 7070 citations and h-index 44. She is the co-author of 18 Book Chapters. Presented more than 200 oral presentations in International conferences. Is co-author of 4 National patents and 6 International patents. Coordinated 20 national and international projects (team leader), out of which 12 European Projects and 6 were co-funded by industrial companies, and participated as team member in 22 research projects. Co-supervised 20 PhD students and is currently co-supervisor of 11 PhD students. Her scientific work has been distinguished with several awards and she was the winner of the Solvay & Hovione Ideas Challenge SHIC’08 Prize- Solvay prize in 2008. Nominated and Elected to the Portuguese Academy of Engineering in 2009 and Elected as IWA Fellow, September 2010. She is Editor of the Water Research (Elsevier).

Ramesh L. Gardas

Ramesh Gardas was born and brought up in Surat, Gujarat, India. He completed B.Sc. (Chemistry) with First Class from P.T. Sarvajanik College of Science, Surat in 1998 and M.Sc. (Physical Chemistry) with First Class from Department of Chemistry, South Gujarat University, Surat in 2000. He has been awarded the Ph.D. degree in Chemistry in 2004 by the Veer Narmad South Gujarat University, India, for his work on thermodynamic studies of non-electrolyte binary and ternary liquid mixtures under the supervision of Prof. S.L. Oswal. Then, he joined the research group of Prof. Abel Ferreira and Prof. Isabel Fonseca at the University of Coimbra, Portugal, for his first post-doc research on PVT data of pure and binary liquid mixtures. Later, in 2006, he joined University of Aveiro, Portugal, to work with Prof. João Coutinho and Prof. Isabel Marrucho. His second post-doc focused on water solubility, octanol-water partition coefficients, and thermophy-
sical properties prediction of ionic liquids. Then, in May 2008, he moved to Queen’s University Belfast, Belfast, UK, and joined the QUILL Research Team to work with Prof. Chris Hardacre and Prof. David Rooney for his third post-doc on confidential project sponsored by Petronas, Malaysia. Research experience from Aveiro and QUILL improved his knowledge of ionic liquids and more precisely their physico-chemical properties and applications.

He joined the Indian Institute of Technology Madras, India, in August 2010, as an Assistant Professor in Chemistry and promoted to Associate Professor in July 2015. He has more than 18-years of research and 8-years teaching experience. He is a co-author of 4 patents, 2 book chapters and 143 research papers with ca. 3900 citations with h-index 30 (SCOPUS).

Rosa Quinta-Ferreira

Rosa Maria de Oliveira Quinta Ferreira, name for scientific publications Rosa M. Quinta-Ferreira, was born in 1952, graduated in Chemical Engineering at the University of Coimbra, Portugal, in 1975, received the DEA - Diplome d’Études Approfondies - from the Université Pierre et Marie Curie, Paris, France, in 1981 and the PhD degree in Chemical Engineering from the University of Porto, Portugal, in 1988 and had the Aggregation degree in 2015. Full Professor in the Department of Chemical Engineering at the University of Coimbra since 2018 and Coordinator of the Group on Environment, Reaction, Separation and Thermodynamics (GERST) of the Chemical Processes Engineering and Forest Products Research Centre (CIEPQPF) of the University of Coimbra. Published 143 papers in specialized scientific journals with ca. 1800 citations, 15 chapters of books and 210 communications to scientific meetings. Supervised 11 PhD thesis, 34 Master theses and co-supervised 3 besides the supervision of 91 final scientific dissertations for graduation in chemical engineering in the pre-Bologna period (equivalent to the actual master thesis). Principal investigator of 9 financed projects by the Research National Foundation (FCT) and researcher in 6 other national projects. As coordinator of the UC team, integrated a transnational bilateral cooperation project, was member of the team of an EU FP6 project and an EU FP7 project and is member of a POC-TEP Portugal-Spain project. The general research specialization domain is Reaction Engineering, including computational modeling, through phenomenological models and advanced Computational Fluid Dynamics (CFD) frameworks, as well as experimental work dedicated to the environmental preservation from polluted liquid, gas and solid wastes. In particular, Advanced Oxidation Processes (AOPs) for liquid effluents treatment have been developed under the common denominator of heterogeneous catalysis, including wet oxidation, ozonation, Fenton’s, photo-assisted systems and electrochemical processes. Spin-off companies were created from her research group with the support of the research carried out in her laboratorial facilities. Advanced Biotechnology Processes embrace novel solutions for environment safeguarding. For VOCs decontamination, autothermal systems are investigated, whereas fly ashes recycling and composting methodologies are targeted as solid wastes valorization. Chemical, Environment and Biotechnology Reaction Engineering are joint domains to search sound environmental technologies for the planet remediation. Health concerns about the effect of biological and chemical contaminants on public welfare are reinforced within multidisciplinary pharmaceutical, biophysics and medical sciences areas.
Simulated moving beds: Fundamental principles, enabling technologies, and applications

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Chromatography methods are highly selective separation methods, which are used for manufacturing many chemicals and biochemicals. Simulated moving beds (SMBs) are advanced continuous chromatography methods, which are more efficient and can have one or two orders of magnitude lower manufacturing costs than conventional batch chromatography. SMB methods, however, are not widely taught in conventional science or engineering curricula. SMB processes designed empirically usually have low yield, productivity, and solvent efficiency. In this talk, the fundamental principles of SMB and two key tools, a rate model simulation tool (VERSE) and the Standing Wave Design and Optimization tool (SWD), will be briefly introduced. The tools can be used to help understand, analyze, simulate, design, optimize, and develop various types of SMBs. Three examples will be used to show the applications of the tools: (1) a tandem SMB for the purification of insulin from a ternary mixture, (2) a five-zone SMB for the recovery of six sugars from biomass hydrolysates, and (3) a three-zone non-isocratic SMB for protein capture or purification.

Acknowledgements
The materials of this talk are based on the research results of many past and present members of my research group: VERSE: R. Whitley; Standing Wave Design and Optimization: Z. Ma, B. Hritzko, Y. Xie, S. Mun, G. Weeden, N. Sopriatna, and D. Harvey.

References
Novel hybrid organosolv: Steam explosion-based integrated biorefinery of the lignocellulosic biomass (an evolution from pretreatment to fractionation processes)

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A novel biomass pretreatment was established in the current work, which combines the fractionation efficiency of the traditional organosolv cooking with the size reduction and improvement of the hydrolysability that steam explosion offers. This hybrid method was applied on both hardwood (birch) and softwood (spruce) biomass where various process parameters were optimized toward achieving high fractionation efficiency. The pretreated solids presented superior hydrolysability, allowing their use to various biotechnological applications such as ethanol fermentation, biogas, microbial oil and cellulosoligosaccharides production. High purity lignin was also isolated permitting its use in advanced applications such as nanoparticles formation and as lubricant additive. The newly established hybrid method can play a significant role towards the establishment of a biomass biorefinery, allowing the use of all biomass components for the production of fuels, chemicals and materials.

Introduction
The current state of biorefinery development is focused almost entirely on the production of fuel ethanol. However, a glucose/ethanol-centric approach misses the crucial example set by the petrochemical industry. The ability to fractionate a raw material, rather than simply pretreat it, enables the parallel production of low value, high volume fuels and high value, low volume chemicals. The main role of pretreatment is the reduction of the natural recalcitrance observed in lignocellulosic biomass and enhancement of its enzymatic hydrolysis yield to glucose. A further prerequisite for future whole biomass utilization strategies is the efficient fractionation of the biomass components into clean process streams. It is often seen that not all available pretreatment methods can be equally effective in both these goals. Steam explosion for example excels as a pretreatment method but has limiting potential for fractionation; on the other hand, organosolv is an excellent delignification method without the benefits of steam explosion in biomass deconstruction.

Experimental
A hybrid method (OSH) has been developed. This method combines the fractionation of a conventional organosolv pretreatment, with the size reduction of an explosive discharge of the cooking mixture at the end of the pretreatment. The explosion step aims to increase the enzymatic saccharification of the pretreated. The method has been studied for representative softwood and hardwood derived biomass, i.e. spruce and birch, and the effect of process parameters (cooking time, ethanol content and addition of sulfuric acid) was studied. The pretreated solids were subjected to enzymatic hydrolysis trials and they were further utilized in microbial conversion processes (ethanol fermentation, anaerobic digestion and microbial lipids from microalgae for biodiesel and nutraceutical applications) and enzymatic conversion processes for COS production. The isolated lignin was used for the formation of nanoparticles and as lubricant additive.

Results and Discussion
OSH resulted in efficient delignification of spruce and birch biomass resulting in pretreated solids with a cellulose content in the range of 72 -78% w/w. The pretreated solids presented high saccharification yields, which in the case of birch reached 68% at low enzyme load (6 FPU/g) and complete saccharification at high enzyme load (22.5 FPU/g) [1]. Spruce biomass was more recalcitrant with the saccharification reaching up to 61% [2]. High gravity saccharification and fermentation have shown the highest ethanol titre reported thus far for spruce (ca. 62 g/L) and birch (ca. 80 g/L) [1]. Apart from ethanol production, and in an attempt to demonstrate the versatility of our process, we used the cellulose-rich pulp for two other processes, namely biomethane production through anaerobic digestion and microbial lipids production by microalgae. For the anaerobic digestion, the pulp was used directly without the enzymatic saccharification. Yields up to 327 mL CH₄/gVS were obtained using OSH treated birch residues and up to 177 mL CH₄/gVS for the spruce residues. Based on these results it can be concluded that organosolv pretreated forest material are ideal substrates for biogas production applications like the co-digestion of forest residues with manure. Moreover, the pretreated solids from spruce and birch biomass (after enzymatic saccharification) were also used for the heterotrophic growth of the green microalgae Aueneochlorella protothecoides. The biomass of the algae obtained was 8.56 g/l and 8.37 g/l when growing on birch and spruce respectively. Under these conditions, the lipid content was 66% and 63.1% w/w respectively with the corresponding lipid concentration to be 5.7 g/L (for birch) and 5.3 g/L (for spruce) [3]. To the best of our knowledge, this was the first time that was reported the growth of microalgae on wood hydrolysates in the literature, with the lipid concentration obtained during our work to be among the highest reported when algae are cultivated in hydrolysates for plant biomass. The lipid profile obtained had similar composition to vegetable oils and was suitable for biodiesel production. Apart from the production of microbial lipids suitable for biodiesel, we also examined the production of lipids of nutritional value (such as omega-3 and omega-6 fatty acids). To this direction, we studied the marine microalgae Phaeodactylum tricornutum for ω-3 PUFAs under autotrophic and mixotrophic cultivation conditions. Autotrophic growth took place in photobioreactor with yeast.
extract (3.35 g/L) as nitrogen source under 18/6 h light/dark regimen. For the mixotrophic cultivation, glucose (20 g/L) was added to the medium. Biomass productivity, cell dry weight and total lipid concentration were higher in the mixotrophic mode, reaching 19.69 mg/L-h, 5.2 g/L, and 1.8 g/L, respectively. After that, the commercial glucose was replaced with glucose obtained from OSH treated birch and spruce hydrolysates. Increased amount of TAG and decreased amount of pigments were obtained in mixotrophic condition after TLC separation of extracted lipids. The FAME profile of this algae under mixotrophic condition was rich in eicosapentaenoic acid (EPA).

The pretreated solids were also evaluated for the production of other dietary compounds (cellobiooligosacharides – COS) in the form of prebiotics. This was done by fine-tuned enzymatic hydrolysis of the pretreated spruce and birch solids with a custom-made cocktail composed of cellobiohydrolases and endoglucanases. During the hydrolysis of the substrates with the use of the optimal enzyme cocktail, cellulose conversion to cellobiose reached as high as 35% [4]. Apart from cellobiose, the COS mixture contained small amounts of cellotriose, cellotetraose (traces) and oxidized oligosaccharides with a degree of polymerization (DP) of 3-6, which are also considered prebiotic candidates. The sustainable production of COS from non-edible novel sources, such as pretreated wood pulps, can lead to a bio-based economy and achieve a significant impact on the prebiotic market share.

Acknowledgements
The work was funded by the following projects: SolveFuels (funded by Swedish Energy Agency), ForceUpValue (funded by Vinnova, BioInnovation) and LIGNOFLOT (funded by Vinnova’s Strategic Innovation Programme for the Swedish mining and metal producing industry). The authors would also like to thank the Kempe Foundations and Bio4Energy, a strategic research environment appointed by the Swedish government, for supporting this work. Sveaskog (Sweden) is greatly acknowledged for providing the forest materials, Novozymes A/S (Denmark), for providing the Celllic® CTec2 enzyme solution and Lesaffre Advanced Fermentations (France) for providing the Ethanol Red® that were used during this work.

References

The lignin stream obtained by precipitation from the liquid after the organosolv pretreatment was of high lignin purity (>90%) and already in the form of fine spherical particles [5]. By using a simple process involving homogenizing of the lignin in an ethanol/water solvent, lignin nanoparticles were also formed (sizes <50nm are observed). These particles had a zeta-potential lower than -30 mV in the majority of the cases, indicating the formation of stable colloidal in water, thus permitting the preparation of aqueous lignin solutions. Finally, we also evaluated the application of lignin as an additive in lubricants. It was demonstrated that the addition of lignin in EG-based lubricants contributes to an outstanding lubricating performance. More specifically, the wear volume of the disc of the lignin-EG mixture was only 8.9% of that lubricated with pure EG [6].

Conclusion
OSH is proven to be a superior method for the treatment of forest biomass as it combines an efficient fractionation and results in pretreated solids with high hydrolysability. The pretreated solids were efficiently applied in various bioprocesses and the isolated lignin was of high purity and suitable for advanced applications. This is an important step forward towards establishing the biomass biorefinery of the future.
The most commonly used adsorption separation strategies involve distinguishing molecules on the basis of (a) size and mobility, (b) van der Waals interactions and polarizability, (c) electrostatic interactions, and (d) π-electron transfers. The use of ordered crystalline microporous materials, such as zeolites (crystalline aluminosilicates), metal-organic frameworks (MOFs), and zeolitic imidazolate frameworks (ZIFs), with channel dimensions in the 3 Å – 20 Å range, opens up new separation strategies that are not realizable with amorphous adsorbents. Particularly noteworthy are possibilities of separations on the basis of subtle differences in molecular configurations, and efficiencies with which molecules pack or stack within the microporous channels. Packing effects are of particular importance in separations of mixtures that operate under conditions of pore saturation. Broadly speaking, the component that is preferentially adsorbed is the one that packs most efficiently; this preference may be rationalized by \( S = k_B \ln(W) \). My talk will highlight a wide variety of industrially important separations that exploit entropy effects. The examples considered include separations of mixtures of (a) hexane isomers, (b) xylene isomers, (c) linear alkanes, and (d) linear alcohols.

### Separating Xylene Isomers

The separation of p-xylene from mixtures containing o, m, p-xylene, along with ethylbenzene is currently carried out in Simulated Moving Bed (SMB) adsorption devices that operate in the liquid phase. In current industrial practice, the adsorbent used is BaX zeolite, that selectively adsorbs p-xylene. The hierarchy of adsorption strengths in BaX is dictated by molecular packing, or entropy, effects that prevail under pore saturation [1, 4]. For realizing improvements in the SMB adsorber, there is considerable scope for development of MOFs that have both higher uptake capacity and selectivity to p-xylene as compared to BaX zeolite. Improved MOF adsorbents will result in lower recirculation flows of eluent, and solid adsorbent in the SMB unit and this will result in significant economic advantages. Due to the differences in the molecular dimensions of the xylene isomers, the efficiencies with which the xylene isomers stack within the channels of different dimensions are different. We can deliberately choose a material with a specified channel dimension in order to allow the optimum stacking of one or other of the xylene isomers. Stacking xylenes within 1D channels of MOFs is analogous to stacking books within bookshelves [4, 5]. In MIL-47 and MIL-53 with 1D rhombohedric channels of 8.5 Å, o-xylene stacks best and is the component that is preferentially adsorbed. Optimum stacking of p-xylene molecules is possible in MAF-X8 with square 10 Å channels [1, 5, 6], and within the 1D zig-zag shaped channels of Co-CUK-1, allowing optimal vertical stacking of p-xylene [2, 3]; see Figure 1.

### Separating Hexane Isomers

The separation of linear, mono-branched, and di-branched isomers of hexane is industrially important in the context of production of high-octane gasoline [6]. With the 1D triangular shaped channels of Fes(BDP), the linear isomers align optimally along the gutters (vertices), and are preferentially adsorbed [7, 8]. Within the 5.5 Å intersecting channels of MFI zeolite, the branched isomers can only locate at the channel intersections (see Figure 2), whereas the linear hexane molecule can locate anywhere along the straight and zig-zag channels. Configurational entropy considerations in mixture adsorption in MFI lead to near exclusion of the branched isomers at pore saturation conditions [9, 10].
Separating linear alcohols with SAPO-34

Remy et al. [11] report experimental data on transient breakthroughs of ethanol/1-propanol mixtures in a fixed bed adsorber packed with SAPO-34, that has the same structural topology as CHA zeolite, consisting of 316 Å³ sized cages separated by 3.8 Å × 4.2 Å sized windows. The experiments carried out in the liquid phase are quite remarkable because the component that is eluted first from the adsorber is the alcohol with the longer chain length; see Figure 3.

The reversal of selectivity in favor of the shorter molecule can be explained on the basis of the entropic contribution \( S = k_B \ln(W) \) to the free energy of mixture adsorption: \( G = H - TS \). Each cage of SAPO-34 can accommodate a maximum of 4 ethanol molecules, or 2 molecules of 1-propanol. At low pore occupancies, the adsorption selectivity is driven by the enthalpic contribution, i.e. dictated by the binding strengths that favors 1-propanol. With increasing pore occupancies, the maximum entropy is realized when the cages are predominantly occupied with ethanol, because this enables increasing number of possible “arrangements” \( W = \text{Wahrscheinlichkeit} \) and a lattice model (Figure 4) allows the calculation of \( S \) [4]. Configurational-Bias Monte Carlo simulations [12] demonstrate that at total pore saturation, the pores will be exclusively filled with ethanol.

The same separation principle applies to the selective adsorption of ethanol from mixtures with 1-hexanol. Indeed, Remy et al. [11] conducted breakthrough experiments with ethanol/1-hexanol liquid feed mixtures to find that the component that is eluted first is the longer chain alcohol.

### Potency of entropy separations

The reversal of selectivity in favor of the shorter molecule in a homologous series has widespread practical implications for separation of linear alkanes, and linear alkenes [4, 13]. Of particular interest are MOFs that allow commensurate adsorption of one of the constituents, in order to enhance the packing efficiencies [4]. Maes et al.[14] and Remy et al.[15] have demonstrated that MIL-47 (V) and MIL-53 (Al) have the potential for separation of mixtures of styrene and ethylbenzene. Styrene is a flat molecule; by contrast, ethylbenzene is not a flat molecule; the ethyl branch is not in the same plane as the benzene ring [16]. Being flat, styrene molecules stack more efficiently within the 1D rhombohedral channels of MIL-47 (V) and MIL-53 (Al). MOFs can be synthesized to yield 1D Tolbrerone-shaped channels [7]; unique separation possibilities emerge; for example, 1,3,5 trichlorobenzene (TCB) can be selectively separated from its isomers, 1,2,3 and 1,2,4 TCB due to optimum face-to-face stacking within the triangular channels [17]. Entropy-based adsorption separations have vast potential in industry.

### References

Overcoming the challenges of the sustainable biorefinery: Supercritical water ultrafast processes

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The concept of biorefinery was proposed more than 30 years ago but so far it has not been fully implemented, mainly because of the cost and complexity of processing biomass to generate useful chemicals and energy. Efficient biomass fractionation is currently one of the major challenges of biorefineries, due to the calcitrance of the lignocellulose matrix and the high crystallinity of cellulose, which make typical feedstock difficult to separate into its components. Conventional processes require long reaction times and the use of strong reagents, leading to degradation of the non-cellulosic fractions as well as large volumes of effluent, which require expensive treatment to reduce their environmental load.

Another challenge is the production of aromatics; much fundamental research has been devoted to investigate lignin transformation to aromatics, but little of this effort has been transformed into products close to market [1]. The market for bio-based polymers is well established, but the aromatic components in many bio-based polymers are obtained from non-renewable sources [2].

In the recent years sub and supercritical water (SCW) have been proposed as promising media to process biomass, based on their physical properties around the critical point (Tc = 374, 22 MPa), which are very different from those of ambient liquid water. The key points of this process are: (1) Around the critical point, the dielectric constant is decreased by increasing temperature, enhancing the solubility of small organic compounds. (2) Above the critical point, the ionic product (Kw) decreases drastically (from \(10^{-14}\) to \(10^{-24}\)), promoting free-radical reaction mechanisms instead of ionic reaction mechanisms. (3) Operating at supercritical conditions substantially reduces or eliminates interphase mass transfer resistances, allowing faster reaction rates [3, 4].

It is possible to take advantage of SCW properties and avoid biomass degradation by controlling the residence time: Arail et al. demonstrated that this was possible working at residence times below 1 second [5].

Our group has developed the ultrafast sudden expansion micro reactor concept. It involves heating the biomass suspension by injecting supercritical water in a mixing tee junction, and the fast cooling by the Joule Thomson effect in the depressurization step. The heating and cooling steps take place with residence times below 1 millisecond: in our facility it is possible to operate with residence times from 4 milliseconds [6]. The concept of this reactor allows developing supercritical water ultrafast processes.

A continuous pilot plant with a suspension biomass concentration of 15%, inlet flow of 10 kg/h, reactor temperature up to 450°C, pressure up to 25 MPa, and reaction time from 0.06 to 1 second is used for the fractionation of lignocellulosic biomass. The main equipment is high pressure pumps for increasing the pressure of the biomass suspension and heating the water, together with heaters to produce the SCW, the reactor, the depressurization valve, the flash for effluent concentration and the heat exchangers for energy recovery.

Fractionation of local agricultural sub products such as cereal bran or beet pulp has been studied. The main products are C5 and C6 sugars and a solid of aromatics of high molecular weight with similar properties to lignin [7].

Reactions with lignin in SCW mainly result in a phenolic char as the dominant product in the majority of literature studies, as well as a minor yield of aromatic hydrocarbons. Char is formed through crosslinking of phenolic units and the formation of recalcitrant C-C aromatic-to-aromatic bonds. These condensation reactions take place too fast, and it is not possible to control them in conventional reactors. Whilst poor lignin depolymerization selectivity is common to both sub- and SCW, there are some significant differences between the reaction media due to the significant reduction of the ionic product of water that favors the radical reactions that are prevalent under SCW conditions. The SCW reaction media allow a selective lignin depolymerization and the reduction of the char by controlling the residence time.

Kraft lignin has been depolymerized by SCW ultrafast reactions, and we have demonstrated that the char production could be minimized by the control of the residence time. It is possible to transform more than the 60% of the lignin in an aromatic oil with a key monomeric composition of approximately 20%.

There is a huge potential in SCW ultrafast reactions for biomass processing. If the SCW hydrolysis process is combined with a
gas turbine or a motor, the heat integration between both processes could minimize energy consumption [8]. Furthermore, the use of water as solvent and reaction media and the important reduction in the reactor size by the achieved processes intensification allow sustainable biorefinery processes to be implemented in the rural areas where the biomass is produced [9].

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References
Beyond fossil fuels for a transformative energy and chemistry

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In the last few years, a new theme is becoming of increasing relevance, driven in part from sustainable (mitigate climate change) and geopolitical (security and diversification of resources) motivations, but now largely from economic ones: make chemistry and energy without fossil fuels. It is thus necessary to reconsider the possibilities to make base chemicals from alternative sources, waste materials being clearly on obvious choice also in consideration of a circular economy, and to look also at the role of chemical energy storage in this renewable energy panorama.¹,² The lecture will first analyze the scenario opened from this transition to a new (sustainable and low carbon) chemistry and energy, in relation to the change in their nexus and related influence on economics. It will then discuss some options for a short, medium and longer term change. For short term, the use of waste as alternative C-source is discussed,³ with an analysis of the waste to chemical possibilities and an analysis of the role of chemical energy storage to enable the transition to a renewable-energy-driven economy.⁴ For medium- and longer-term, the role of electrocatalytic and photo-electrocatalytic technologies, the latter in relation to the development of artificial-leaf type devices, is discussed.

References

The importance of model polymers in polymer science and industry

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Access to model polymers (high degree of structural, compositional and molecular weight homogeneity) is necessary in order to elucidate structure-property relationships, which are the key in improving polymer performance and designing new materials. The synthesis of model polymers with different structures (a few are given in Figure 1) is demanding, time consuming and often leads to a small quantity of products [1-3]. Nevertheless, this is a small price to pay given the tremendous potential of model macromolecules for selecting the appropriate structures needed for specific applications. Among others these model polymers serve in: Better testing existing theoretical concepts; Developing new theoretical concepts to explain experimental results on novel structures; Understanding/Improving the properties of industrial polymers.

Figure 1. Model polymers with different macromolecular architectures. Different colors represent chemically different chains [PS: polystyrene, PDMS: poly(dimethyl siloxane), PI: polyisoprene, P2VP: poly(2-vinylpyridine), PBd: polybutadiene, PE: polyethylene, PCL: polycaprolactone].

References
The importance of being porous: Silicates and functional Metal Organic Frameworks

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Some of the work carried out in Aveiro on nanoporous transition-metal and lanthanide (Ln) silicates, and on Ln-bearing coordination polymers (or metal organic frameworks, MOFs) will be reviewed.

The main focus of this talk will be on the design of (nano)materials for light emission sensing temperature [1-4] and health-related applications, such as drug delivery [5] and treating bone tissue disorders. Another outstanding example of the latter is the use of zirconium silicates as pharmaceuticals for treating hyperkalemia (excess K⁺ in serum), providing an intriguing case study of a real translation from the bench to the bedside. A final example of health-related and sustainability applications is provided by the anti-mosquito activity of a titanium-based metal–organic framework supported on textile fibres [7].

While nanoporous (zeolite-like) silicates are highly robust (thermal and chemical) systems, allowing applications in relatively harsh conditions, it is very challenging to synthesise the desired architectures and modify them post-synthesis. In contrast, MOFs operate in milder conditions and often lack robustness but they are much more amenable to ‘rational synthesis’ and post-synthetic modification. Thus, together, metal silicates and MOFs provide a wonderful playground for chemists and a toll box for engineering applications.

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Advanced reaction processes, wastewater treatment and reuse

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Water supplies from natural resources are reaching dramatic low levels imposing high stress on the water quality available for human activities. Polluted streams returning to the environment after usage in domestic and industrial systems are leading to contaminants accumulation in lakes and rivers. Lack of fresh water is therefore pushing scientists, governments and society to become aware of the need to properly treat wastewaters targeting their reuse. Novel treatments urge then to be found to fulfill environment legislations aiming at water recycling. Chemical Reaction Engineering has a fundamental role in this domain through the proposal of advanced reaction processes able to produce strong oxidant species such as the hydroxyl radicals. Heterogeneous catalysis is also crucial for improving the efficacy of such technologies ensuring adequate catalysts separation. The high diversity of effluents claims a large spectrum of reactor types aiming to tailor the best solutions to specific treatments.

The water cycle seems to make water lasting infinitely in our planet. Water in rivers, lakes and oceans goes to the atmosphere and comes back through rain falls. However, the usage along this cycle include taking off water for domestic and industrial purposes, involving manufacturing of new products, textile mills, pharmaceutical enterprises, agricultural and animal farming, among many others. This necessarily leads to the discharge on nature of water streams that are more polluted than the initial ones taken from hydric resources. This is one of the major problems our world is facing and it will certainly increase in the future due to the spread of industrialization. The countries economic growth and the search for better life quality do not include adequate care in the delivery of effluents to earth. Normally wastewater treatment plants encompass end systems that are not profitable for municipalities and enterprises. Thus, treatments tend to be the less costly even though they are often not enough to clean properly the water that was taken from nature. Undoubtedly, such pollution accumulation in natural sources, with time, leads to the reduction of the quality of the available water to be captured again for the operative continuity of domestic and production systems. Environmental legislation is crucial to oblige stakeholders to ensure treatment procedures to avoid highly polluted water discharges. For this, more restrictive thresholds have to be imposed demanding efficient processes to reduce pollution in earth water. Reaction processes are then required targeting the pollutant compounds transformation in final harmless molecules. Wastewater treatments are nowadays mainly performed by low costly biological systems using bacteria consortium that metabolize those pollutants. However, two main drawbacks can be pointed out: the production of high amounts of final contaminated sludge and the inability of microorganisms to degrade various toxic chemicals. Alternative and complementary systems are therefore demanded aiming to reach efficient degradation. Advanced Oxidation Processes (AOPs) are, in this regard, as a chemical engineering area, focused on delivering innovative treatments. These are needed to fulfill legal compliance levels for the good sake of human health. The increasing large numbers of water borne diseases calls the attention of scientists, governments and society to the relevance of more powerful treatments to eliminate injurious contaminants in wastewater discharges. Strong oxidative species are then required aiming at effective removal of refractory pollutants, including the so-called emerging contaminants. Processes able to lead to in situ production of hydroxyl radicals, which are powerful oxidants in liquid environment, are then strongly recommended. At ambient operating conditions, various advanced reaction processes, such as ozonation [1, 2], Fenton’s [3, 4], electrochemical [5, 6] and photocatalytic [7] systems have been addressed in our research group. Due to the huge diversity of effluents, this environmental preservation area deals with different reactor types where heterogeneous catalysis has an important contribution. Also more severe operating conditions were studied under catalytic wet oxidation [8, 9]. Chemical engineers with strong reaction and process skills are then fully entitled to act in this noble domain to test and install reaction systems dedicated to treat specific effluents, targeting tailored solutions. These advanced processes can be employed either before less costly biological treatments for microorganisms toxicity reduction or after activated sludge plants for refining water quality aiming its reuse. Indeed, the dramatic scarcity of clean water is compelling the reuse of polluted waters simultaneously requiring highly efficient treatments and secure disinfection methodologies to lead to specifications safely targeted for water recycle. Reclaimed water imbeds integration of various technologies associating reaction and separation areas to tackle different aspects of the wastewater recovery. The main goal is to embrace altogether waste waters as sources of raw materials, energy and clean water. Valorization of such polluted water courses is fully in line with future goals of converting harmful wastes, nowadays polluting the environment, into additional financial profits. This involves using advanced technologies simultaneously tackling more stringent legal thresholds for planet safeguarding. Environment is thus a noble area where chemical engineers have large expertise to help earth and ecosystems preservation.
References
Harvesting and storage of solar energy: a promising new world for off the grid residences?

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The photovoltaic (PV) electricity is expected to reach the lowest price of fossil fuel electricity by 2020 and below that reference price after 2020. Obviously that the actual price of the PV electricity depends on the production location and in Portugal the PV electricity is already cheaper than the fossil fuel electricity. This remarkable achievement is fueling the development and use of technologies for harvesting energy from renewable sources using namely photovoltaic panels and wind mills. Compared to the fossil fuel electricity, PV electricity is not dispatchable making necessary the combined use of storage systems. A worldwide tremendous effort is now being developed for producing stationary battery systems suitable for handling electricity from renewable sources, which display intermittence. Redox flow batteries are taking the lead for storing energy easily and efficiently transformed in electricity.

In the 16th century Thomas More described an idealized and sustainable place in his book Utopia. Today’s an ideal city should comply with the Near Zero Energy Building directive (nick name for the Energy Performance of Buildings Directive (2010/31/EU)) and going beyond. PV electricity is already the cheapest if produced in countries with high solar irradiance; the world’s cheapest price for electricity of 2017 was for a PV farm in Saudi Arabia – 1.46 €/kWh [1].

Besides silicon based PV-cells, the recently emerged perovskite solar cells (PSC) promise to fight for a relevant place among the PV technologies – Figure 1. PSC are a thin film technology, where the absorber layer has a thickness of 350 nm to 450 nm, potentially cheaper than c-Si or p-Si PV panels and easier to produce; this makes PSC suitable for local manufacturing, in opposition to the centralized production of silicon-based panels. PV electricity is, however, generated only daytime and then just partially dispatchable. To make it fully dispatchable it is necessary to store it and batteries are a technology of choice. Among the storage technologies, redox flow batteries (RBs) emerged as promising, offering very low storage costs [2] and reliable and robust operation. RBs are energy storage devices that easily and efficiently transform chemical energy into electricity, displaying very high thermodynamic cycle efficiencies that can reach 99%. Vanadium RBs display an energy density of ca. 50 Wh/L but the use of non-aqueous solvents for dissolving the redox pairs promises to bring this energy density to values that ideally can reach 1 kWh/L. In a flow battery, stored energy and power are independent parameters; energy is stored in the redox pairs dissolved in an aqueous solution of a supporting electrolyte – an easily stored and transported electrochemical fuel; and power is related to the size of the electrochemical cells and stack. The energy storage cost is expected to reach 120 €/kWh by 2030 [3], which corresponds to an electricity storage cost of ca. 1.5 €/kWh/cycle, assuming a life expectancy of 10 000 cycles. With the grid electricity high prices, owners dream about energy autonomous houses, more environmentally sustainable and displaying lower energy costs. Few companies in Portugal are already working hard to address this dream and expect to delivery very soon solutions that promise conquer hurdles of clients.

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Oscillatory Flow Reactors – A platform for process intensification

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Process intensification demands the development of innovative and more sustainable process design alternatives in order to tackle the challenges related with mixing efficiency. OFR – oscillatory flow reactor – have been proposed as an alternative design to overcome these limitations. OFR, basically a column provided with periodic sharp constrictions, operated under oscillatory flow, have shown to improve significantly processes where mixing is relevant. The application of OFR to several processes will be presented and perspectives for its development discussed.

Introduction

Process intensification (PI) has been receiving increased attention and importance because of its potential to obtain innovative and more sustainable process design alternatives. Several definitions have been proposed being one of the most “complete” the targeted improvement of a process at the unit operations scale, the task scale, and/or the phenomena scale [1].

It is well known that mixing efficiency is the key factor for the success of several processes and improper mixing can result in non-reproducible processing and lowered product quality. Stirred tank reactor (STR) is commonly used at the industry, however, problems associated with bad mixing, scale-up, product quality, and process reproducibility, are typically reported. In order to overcome these limitations, reactors with alternative designs have been proposed, including oscillatory flow reactors (OFR) [2,3] as shown in Figure 1 where a modular “LEGO” type OFR is presented.

OFR is basically a column provided with periodic sharp constrictions, called baffles, operating under oscillatory flow mixing (OFM). The liquid or multiphase fluid is typically oscillated in the axial direction by means of diaphragms, bellows or pistons, at one or both ends of the tube, developing an efficient mixing mechanism where fluid moves from the walls to the center of the tube with intensity controlled by the oscillation frequency (f) and amplitude (x0). The formation and dissipation of eddies, in these reactors, has proved to result in a significant enhancement in processes such as heat transfer, mass transfer, particle mixing and separation.

OFR have been applied to several systems like bioprocess, gas-liquid absorption, liquid-liquid extraction, precipitation, and crystallization, at different scales, and the advantages of its use demonstrated. OFR have also been proven to be very efficient in systems where a good solid dispersion must be achieved.

Results and conclusions

The most important characteristics related to mixing and mass transfer of OFR will be described and the effect of the oscillation frequency and amplitude will be highlighted. For the particular case of oxygen transfer, it will be clearly shown that OFR does allow for a several-fold increase in the transfer rate and the relative effect of the oscillation frequency and amplitude on the transfer mechanisms will be discussed. A particular emphasis will be given to the effect of the operating conditions on solids suspension and the way how solids concentration acts on mass transfer in these type of reactors.

Several case studies describing the use of OFR will be presented going from cases where oxygen mass transfer is the rate-limiting to cases where mixing intensity defines the rate and yield of the process.

Finally, a discussion will be made on recent developments and future perspectives for the application of OFR

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The wide use of biodegradable biopolymers has been limited by the cost. The use of wastes or industrial by-products as substrate is the most promising strategy to reduce the cost of microbial biopolymers and make them more competitive. An overview of strategies for the production of biopolymers from wastes at lab to pilot scale will be presented, with the main focus on the production of polyhydroxyalkanoates (PHA) and polysaccharides using pure and mixed cultures.

Given its availability, large diversity, lack of valuable alternative utilizations, and low cost, the use of wastes or industrial by-products as substrate is the most promising strategy to reduce the cost of biopolymers produced by microbial processes. An overview of production of biopolymers using less energy intensive processes, such as low oxygen consumption, and wastes as feedstocks will be presented. The main focus will be on the production of polyhydroxyalkanoates (PHA) and polysaccharides using pure and mixed cultures from lab to pilot scale. The PHA process optimization at pilot scale will be presented. The characterization of the produced biopolymers as well as the applications will be described.

Acknowledgements
Utilizing ionic liquids as additives for enhancing the extraction, absorption and dissolution processes

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Solvents are major contributors and high on the list of environmental damage chemicals, mainly because of their large usage and high volatility. The widespread use of volatile organic compounds (VOCs) in many industrial chemical processes is an issue of great environmental concern. It is an extremely important task to search of potentially green and environment friendly alternatives for VOCs. At least a partial solution to this problem may offer by a novel class of molten salts referred to as ionic liquids (having melting point, generally, below boiling point of water), as they possess unique combination of particular properties, unlike molecular liquids, namely negligible vapour pressure (~ $10^{-11}$ to $10^{-10}$ bar at room temperature), wide thermal window (~ -50 °C to +250 °C), wide electrochemical window (~ ±3 Volt vs. NHE), non-flammability, high ionic conductivity and a highly solvating capacity for organic, inorganic and organometallic compounds. This unique combination of particular properties leads them to be exploited as “green solvents” and giving them increasing attention in academic and industrial research.

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References
Oral Session

BIOLOGICAL ENGINEERING AND BIOTECHNOLOGY
Introduction

In the last century, the added sugar products consumption has increased and also the number of metabolic diseases [1]. Since these events seem to be related, the necessity of finding healthier options to added sugar, such as low-calorie sweeteners, became a priority [2]. Sugar alcohols, natural sweeteners, are used as sugar substitutes [1]. Erythritol, a four-carbon polyol, is a biological sweetener widely found in nature [1,3]. This sweetener has around 70 % of sweetness of sucrose [1,3]. Erythritol is a small molecule, with low calorific value and it is easily absorbed by the human body, so it does not cause the common intestinal problems related with the use of other sweeteners [1]. Erythritol can be used as food sweetener by people with diabetes and obesity because this sweetener is not metabolized by the human body and is excreted unchanged in the urine without changing blood glucose and insulin levels. It is a not toxic sweetener and noncariogenic, since it cannot be fermented by the bacteria that cause dental caries [1]. Erythritol is industrially produced by fermentation of glucose, sucrose or glucose from chemically and enzymatically hydrolyzed wheat and corn starches by yeast-like fungi such as Torula sp. and Monilinia pollinis [3,4]. Erythritol can also be produced by some osmophilic yeasts and some bacteria [3-5].

Yarrowia lipolytica has the ability to grow in several carbon sources, including agro-industrial wastes, and produced a variety of value-added products such as organic acids, microbial lipids, lipases, biosurfactants and polyols like mannitol and erythritol [6]. Yarrowia divalgata was recently described. This species can be found in animal related and marine sources [7]. This species can use several carbon sources like sugars, alcohols, organic acid and n-hexane. The production of erythritol, mannitol, citric acid and accumulate lipids was described [7,8].

The main goal of this work was to compare the production of erythritol by strains from two species of Yarrowia genus, Y. lipolytica and Y. divalgata. Using selected strain, the bioprocess was scaled-up from flasks to lab-scale bioreactor at different operating modes (batch and step-wised fed-batch).

Material and Methods

In this work, the production of erythritol was studied for 5 strains of two species of Yarrowia. The three strains from Y. lipolytica were Y. lipolytica W29 (ATCC 204600), Y. lipolytica Ch 1/5 and Y. lipolytica Ch 3/4, the last two were isolated from cheese, and Y. divalgata M 445/4 and Y. divalgata 5257/2, were isolated from ground raw meat. Batch cultures to select the best strain were carried out in Erlenmeyer flasks with 200 mL of production medium. After pre-growth in YPG medium (in % (w/v): glycerol 2, yeast extract 1 and peptone 2), 1 g L⁻¹ of cells were added to the production medium, that was composed by (g L⁻¹): crude glycerol 100; yeast extract 1; NH₄Cl 3; NaCl 25, KH₂PO₄ 0.2; MgSO₄·7H₂O 1; dissolved in phosphate buffer 0.72 M, pH 3. The experiments were performed at 27 °C and 200 rpm. Experiments in bioreactor were performed in a Biengineering fermenter (RALF PLUS SOLO, Biengineering, Switzerland) of 1.7 L Batch cultures were carried out at 27 °C, 3vvm of aeration rate, 900 rpm of stirring rate and pH 3, using the same inoculum preparation as in flasks. In the step-wise fed-batch, after 72 h, 100 g L⁻¹ of glycerol was added. For all experiences, culture samples were collected for analysis of cell concentration (optical density measured at 600 nm and then converted to dry cell mass per liter), glycerol consumption and erythritol production. Glycerol and erythritol concentrations were determined by HPLC using an ion-exchange column (Aminex HPX-87 H) attached to a RI detector. The column, at 60 °C, was eluted with H₂SO₄ 5 mM at 0.7 mL min⁻¹.

Results and discussion

All strains tested were able to produce erythritol (Figure 1). Only the results between W29 and M 445/4 were statistically different. However, the higher concentration was obtained by W29 strain (25 g L⁻¹). The strain with lower concentration of erythritol was Y. divalgata M 445/4 that produced only 9.8 g L⁻¹.

Rakicka et al. [8] studied the production of erythritol by several species from Yarrowia clade, including a strain of Y. lipolytica and one of Y. divalgata. In that studied Y. divalgata presented a better result than Y. lipolytica, although the production medium composition was very different. The optimum culture
conditions to produce erythritol by *Y. divulgata* can be different, and are not described. No statistically differences were observed between *Y. lipolytica* strains, the strain W29 was selected to further studies in bioreactor since it presented the highest erythritol concentration.

During the batch mode, the erythritol production was increasing without showing a decrease or a slowing down until the glycerol is completely consumed (Figure 2). This profile suggests that if the carbon source had not been exhausted, the cells would continue to produce erythritol. Thus a step-wise fed-batch was planned (Figure 3). In this experiment, after 72 h of batch culture more glycerol was added to the bioreactor. As expected the production of erythritol continued and no cellular growth as observed. In this experiment was possible to double the final concentration of erythritol (64 g L⁻¹). The productivity was the same though all experiment. After adding glycerol to the bioreactor, the yield (0.56 g g⁻¹) in erythritol increased 1.65-fold comparing to batch phase. With this step-wise fed-batch strategy the yield achieved was closed to the theoretical value (0.66 g g⁻¹) [9].

**Acknowledgments**

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Comparing the operation of aerobic granular sludge bioreactors under different hydrodynamic regimes during the treatment of textile wastewater containing silver nanoparticles

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Comparing the operation of aerobic granular sludge bioreactors under different hydrodynamic regimens during the treatment of textile wastewater containing silver nanoparticles

Two aerobic granular sludge (AGS) sequencing batch reactors (SBRs) were used in the treatment of a textile wastewater (TWW) containing an azo dye and silver nanoparticles and monitored for granule development and maintenance, N-acetyl-homoserine lactone (AHL) content as a quorum sensing (QS) indicator and effluent detoxification. Each SBR was run using a different feeding strategy and hydraulic shear pattern to assess the impact of these operational conditions. The use of lower hydraulic shear was shown to benefit granule formation during the initial stages of the operation but became less relevant in time. AHL QS appeared to be an important feature of granulation, as well as the applied shear. However, biomass washout episodes in later stages led to the removal of AHL-producing bacteria and markedly reduced AHL. Overall, the two SBRs displayed similar performances in terms of TWW toxicity changes during treatment, model yeast growth being inhibited by over 60% after dye reduction.

Introduction

Textile wastewaters (TWWs) contain high organic loads, recalcitrant dyes (mainly azo dyes), and diverse chemicals. As one of the biggest contributions for water pollution worldwide, they are the subject of intense research striving to develop remediation strategies [1]. Anaerobic-aerobic sequencing batch reactors (SBRs) are one of the most investigated alternatives for the bacterial decolorization of azo dye-laden TWWs. The process comprises two stages: the anaerobic cleavage of azo bonds with the formation of colourless and often toxic aromatic amines, and the aerobic degradation of these metabolites [2]. Yet, many cases of null or incomplete aerobic arylamine removal have been reported, the consequent possible toxic process effluent raising concerns over the effects of prolonged exposure for the process biomass [2]. In addition to dye-related toxicity, the increasing occurrence of nanoparticles (NPs) in TWWs has been noted, with further possible negative impacts during and after treatment. Silver NPs (Ag NPs), a finishing textile additive, has been shown to penetrate and/or change the permeability of microbial cell membranes and to unleash a range of unspecific toxic effects through the release of Ag⁺ [3]. Given these concerns, aerobic granular sludge (AGS) SBRs, lauded for their ability to endure higher toxic loads than conventional activated sludge (CAS), emerged as a promising solution for TWW treatment [1]. However, the evolution of wastewater toxicity during treatment has rarely been assessed, leaving a knowledge gap in the possibility of safe environmental discharge [1]. Also, the impacts of NPs on the properties and stability of AGS systems are still largely unknown [4]. Many doubts persist on the basis of AGS technology, including the influence of bacterial quorum sensing (QS) on the development and maintenance of aerobic granules (AGs) and how it can be affected by specific operational parameters [5]. In an effort to answer some of these questions, this paper reports on the use of microplate-based assays employing a Saccharomyces cerevisiae toxicity model and an Agrobacterium tumefaciens biosensor to describe the evolution of wastewater toxicity and QS signal molecule concentrations (N-acetyl-homoserines, AHLs) during the long-term operation of two anaerobic-aerobic AGS SBRs, fed with an azo dye- and Ag NP-laden TWW and run under different hydrodynamic regimens.

Materials and Methods

Two 1.5-L SBRs seeded with CAS from a wastewater treatment plant in Chelas (Lisbon, Portugal) were operated for 192 days. The SBRs were run in 6-h cycles with five stages – fill, reaction (stirred anaerobic stage and aerated stage), settling, drain and idle – a volumetric exchange ratio of 50% and a 12-h hydraulic retention time. The synthetic TWW included a starch-based sizing agent (Emsize E1, 1 g O₃ L⁻¹, as chemical oxygen demand), nutrients, pH buffering phosphates and the azo dye Acid Red 14 (40 mg L⁻¹) [6] and was fed under static and anaerobic conditions as described in the Graphical Abstract; Ag NPs were supplied separately at 10 mg L⁻¹. Settling periods were reduced from 60 to 5 min over the first 28 days to promote granulation and maintained at 5 min thereafter. AG contents were assessed through sieving [6], analysis of total and volatile suspended solids (TSS and VSS) and of sludge volume indexes after 5 and 30 min of settling (SVI₅ and SVI₃₀) [6]. To assess the impact of QS mechanisms on AG formation and maintenance, AHL contents were evaluated weekly in sonicated and clarified AG extracts using a microplate colorimetric biosensor assay involving the AHL-mediated synthesis of β-galactosidase in reporter strain A. tumefaciens NTL4(pZLR4) and the hydrolytic activities measurement with chromogenic substrate X-gal [6]. Cell-density normalized activity levels were determined in unclarified biosensor cultures using dual-wavelength optical density (OD) readings [7] and expressed as nmol-equivalents of the reference AHL N-(3-oxo-dodecanoyl)-L-homoserine lactone (30C12-HSLeq). Wastewater toxicity was assessed against a dye-free feed solution (WWcontrol) during several cycles, using the synthetic TWW prior to treatment (WWaerob) and samples collected at the end of the anaerobic reaction (WWanaerob) and from the final effluent (WWanaerob). All samples were sterilized by filtration (0.2 µm) and evaluated for potential toxicity using a yeast-based growth inhibition microplate susceptibility assay [1]. A standardized population of S. cerevisiae BY4741 Δcwp1Δcwp2 grown to mid-exponential phase was diluted to an OD₆₀₀nm=0.01 using fixed sample and concentrated YPD growth medium volumes. The mixtures were then distributed in a microplate and incubated for 22 h (30 °C and 1200 rpm) before new OD measurements were collected. Potential toxicity levels were expressed as the yeast growth inhibition ratio.
ODx/ODx_WControl, where ODx and ODx_WControl represent the OD640nm of the samples and the control, respectively.

**Results and Discussion.** AG formation in SBR1 and SBR2 was induced by decreasing settling times, high hydrodynamic shear forces (mechanical stirring and aeration), and a feast-famine regimen. Granulation was achieved after 50 days in both SBRs, SVI3 and SVI30 reaching minimum values (< 75 and 50 mL g⁻¹ TSS, respectively; Fig. 1A). The SBRs were then able to operate at maximum sludge retention capacity for 3-4 weeks, before experiencing severe washout episodes (days 63-85; Fig. 1A) that brought TSS levels down. Subsequently, periods of settleability improvement (e.g., days 85-119) and deterioration (e.g., days 119-148) alternated until the last day of the experimental run, reflecting on TSS and causing operational instability (Fig. 1A).

This behaviour could be related to Ag NP toxicity, which has previously been shown to impact cyclically on sludge properties through accumulation in the biomass [3]. Overall, two distinct operational periods could be discerned: one in which SBR1 performed better in terms of SVI, TSS and AG contents (until day 79) and one in which SBR2 came to be the superior system (mostly after day 148) (Fig. 1). The lower stirring speed in SBR1 was likely the main factor determining sludge properties at first, allowing better AG development and settleability improvement [8]. In the long run, however, other factors, such as higher AG stability from better substrate penetration during filling in the plug-flow-fed SBR2 [6], likely became dominant.

QS mechanisms. AHL synthesis was evident during granulation, with 3OC12-HSLeq values rising steeply during the first 21 days of operation and again after day 28 (Fig. 1B). In the remaining operation time, however, signal molecules contents in AGS extracts decreased, dropping markedly during periods of heavy sludge washout or slow biomass accumulation (e.g., days 21-28, 44-77, 126-148; Fig. 1), hinting at large changes in the structure of both microbial communities – possibly involving the removal of AHL-producing bacteria. While apparently suggesting a lesser role for bacterial QS in long-term AGS maintenance, the results may also reflect the proliferation of microorganisms with a quorum quenching activity, which promote the breakdown of signal molecules. Both phenomena have been described for AGS systems [9,10], further supporting this hypothesis. In agreement with the results on sludge properties, SBR1 displayed higher AHL contents during granulation; distinctions between the SBRs became less evident in the long run, showing no clear relation with AGS settleability, TSS or AG content (Fig. 1B).

![Figure 1. TSS (A), SVI (A), 3OC12-HSLeq (B) and granular mass fractions (B) in SBR1 (pink) and SBR2 (blue) along the operation. SVI50 followed the same general trends as SVI. Error bars for 3OC12-HSLeq are standard errors of the mean (n=6).](image-url)

**References**


**Acknowledgements**

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Assessing the influence of the adsorption time on the build-up of natural-origin polymeric multilayered systems: how fast can we go?

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The Layer-by-Layer (LbL) approach is a straightforward technology to fabricate highly ordered multilayered architectures with applications in the biomedical field. However, physicochemical, biological and mechanical properties of the multilayered systems are greatly influenced by both intrinsic and extrinsic parameters, being pivotal to understand their influence at molecular level. This work investigates the effect of chitosan (CHT) and alginate (ALG) biopolymer adsorption and rinsing times on the physicochemical, biological and mechanical properties of CHT/ALG free-standing membranes. Different combinations of biopolymer deposition and rinsing times were studied regarding film thickness, mass deposited, swelling ability, mechanical and biological properties. This work demonstrates that membranes with similar properties can be quickly developed by extensively shortening the adsorption time needed for building blocks interaction, turning LbL technology more prone for commercial use.

Over the last decades, the significant advances in biotechnology aided the design of highly versatile platforms with wide range of applications, including the biomedical field. These tailored made platforms combine a plethora of materials with distinct physical, chemical and biological properties and a set of technologies that are suitable for the target application. The technologies herein mentioned, are grouped into two categories, namely top-down and bottom-up approaches, the latter relying on the spontaneous self-assembly of small molecular components into order molecular assemblies. Among the latter, the Layer-by-Layer (LbL) assembly technology enables the fabrication of functional 2D or 3D structures by resorting to a plethora of surfaces and materials displaying complementary interactions.

The LbL approach is a simple, inexpensive, flexible and highly versatile technology that enables the modification of surfaces, as well as the fabrication of highly ordered polymeric multilayered architectures by engineering and unlimited number and variety of building blocks. Those multilayered systems include robust free-standing multilayered membranes that can be produced with precisely layered structures, thickness, composition (e.g. proteins, polysaccharides), properties (e.g. magnetic), and functions over virtually any type of substrate regardless its surface chemistry [1, 2]. It relies on the sequential adsorption of complementary multivalent molecules on a surface, occurring either via electrostatic as well as non-electrostatic interactions [3, 4]. Not only its straightforwardness is an advantageous characteristic, it also requires simple laboratory equipment. On top of it, it allows the combination of a myriad of building blocks ranging from proteins and polysaccharides to living cells [5-10]. An unprecedented number and variety of combinations of building blocks can be assembled into diverse sizes and shapes, therefore adding new functionalities and capabilities to the multilayered films. The architecture and overall properties of the developed systems are highly influenced by the charge density of the adsorbed materials, as well as by the ionic strength of the liquid medium. However, many other parameters influence the development of such multilayered systems. For instance, several external parameters, such as the temperature, materials’ adsorption time, rinsing time or even the number of deposited layers also play a key role during the assembly process.

This work aims to investigate the influence of the biopolymer deposition and washing times on the physicochemical, mechanical and biological properties of free-standing multilayered membranes, foreseeing its application in the field of tissue engineering and regenerative medicine. The behaviour of the multilayered thin films at the nanometer scale level was assessed using the quartz crystal microbalance with dissipation (QCM-D). The morphological properties, swelling behaviour and robustness were assessed by scanning electron microscopy (SEM), water uptake and tensile loading respectively. The in vitro biological performance of the developed membranes was studied by assessing the metabolic activity and proliferation of human adipose-derived stem cells.

The build-up of the multilayered assemblies comprising oppositely charged marine-origin polysaccharides, positively charged chitosan (CHT) and negatively charged alginate (ALG), at a concentration of 0.5 mg mL⁻¹, onto the gold-coated quartz crystal sensors was monitored in situ by QCM-D in a liquid environment (0.1 M acetate buffer at pH 5.5, 0.15 M NaCl). The multilayered film formation was monitored in real-time and the combinations and assembly conditions tested are listed in Table 1.

Table 1 - Set of conditions used to assess the influence of chitosan and alginate adsorption and washing times on the development of multilayered thin films by QCM-D.

<table>
<thead>
<tr>
<th>Adsorption time (min)</th>
<th>Washing time (min)</th>
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<tr>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>10</td>
<td>1</td>
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</table>

Then, the fabrication of robust free-standing multilayered membranes comprising the aforementioned biopolymers was performed on low surface energy substrates using an automatic dipping robot. The membranes were developed by keeping the
same biopolymer deposition rinsing times (1 min-1 min, 2 min-2 min, 5 min-5 min, respectively). However, two different number of bilayers (100 and 200 bilayers) and materials concentration (1 mg.mL$^{-1}$ and 2 mg.mL$^{-1}$) were tested for each condition to assess their influence on the physicochemical, mechanical and biological properties.

As observed by QCM-D, a higher shift in frequency (more negative values) was substantially more pronounced for the long adsorption time. This led to more material being deposited and consequently an increase in film thickness. Despite the fact that longer washing times (from 1 min to 10 min) slightly decreased the thickness, the film behaviour switches from and viscoelastic (higher variation in dissipation energy) to a rigid-like (lower variation in dissipation energy). This shift suggests that a longer exposure is likely to aid the molecular reorganization both of chitosan and alginate and the film is likely to become more stable. The thickness of the films increased while increasing the adsorption time and decreased with longer washing times. However, if we keep the adsorption time unchangeable and increase the rinsing time, the difference in terms of film thickness between shorter (1 min) and longer washing periods (10 min) is negligible. This suggests a strong electrostatic interaction between oppositely charged CHT and ALG biopolymers.

This work demonstrates that membranes with similar properties can be more quickly developed by extensively shortening the time needed for the deposition of the building blocks, as well as the rinsing times thus turning the LbL technology more prone for commercial use.

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References

Recovery of value-added antibodies from serum samples

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In this work, ionic-liquid-based aqueous biphasic (liquid-liquid) systems were applied in the downstream processing of antibody-based biopharmaceuticals, namely immunoglobulin G antibodies, from human serum samples. The nature of the phase-forming components and phases’ compositions were investigated in order to selectively extract antibodies to the top phase, allowing to recover antibodies with a high purity level and integrity.

Recovered human antibodies are used in the treatment of several diseases, e.g. autoimmune thrombocytopenic purpura, Kawasaki disease, polymyositis/dermatomyositis, Guillain-Barré syndrome, among others [1]. Despite their relevant therapeutic potential, the cost of recovering these biopharmaceuticals at high doses, with high quality and purity, is still extremely expensive due to the absence of a cost-effective downstream platform. The main challenge in the purification of immunoglobulin G (IgG) from human serum corresponds to the elimination of most abundant protein - human serum albumin (HSA). In the last years, the purification of human IgG has been essentially carried out by precipitation and/or chromatographic approaches [2,3]. However, these processes lead to low recovery yield/low resolution, require long processing times, and are difficult to be transposed to a large-scale, resulting in the inability to meet the high demand for plasma-derived therapeutic molecules. Herein we propose the use of polymer-polymer aqueous biphasic systems (ABS), using ionic liquids (ILs) as adjuvants, to purify and recover IgG human antibodies from human serum samples. The nature of the phase-forming components and their concentration was investigated aiming at selectively extracting IgG for one of the coexisting phases. Several ILs were studied. The use of appropriate ILs allows an increase of 16 fold on the IgG purity when compared to its purity in the original sample, and of 10 fold when compared with the respective ABS in which no ILs were used. Under the optimized conditions, an IgG extraction yield of 93% with a purity level of 93% was obtained in a single-step, while keeping the stability/integrity of the protein.

Acknowledgements

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References

Aerobic granular sludge process treats real fish canning wastewater

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Introduction

Aerobic granular sludge (AGS) is a promising technology for treating wastewater and presents several advantages compared to conventional activated sludge systems. The AGS process allows a higher biomass retention which decreases the need for energy and space. Besides, the aerobic granules are composed of extracellular polymeric substances (EPS) which protect bacteria towards stress conditions. Therefore, aerobic granules are stable structures with a higher tolerance to toxic substrates and higher resistance to variable wastewater composition [1]. The fish canning wastewater is characterized by a variable composition in organic matter, mainly containing nitrogen and phosphorous as nutrients. This type of wastewater often contains salt, which can be very high and affect the biological processes. This variability occurs along the year but also throughout the day, depending on the processing at the plant [2].

The main objective of this work is to use an AGS-SBR for treating wastewater from a fish canning plant. The performance of the AGS process was evaluated in terms of chemical oxygen demand (COD), phosphorous and nitrogen removal.

Methods

A lab-scale AGS-SBR, with a working volume of 2.5 L, a total height of 110 cm and an internal diameter of 6.5 cm, was operated in four successive treatments of 6-h-cycles per day. The reactor was inoculated with granular sludge from a municipal AGS-SBR. Operation followed procedures previously described [3]. The reactor was fed with wastewater from a fish canning plant, collected after screening and coagulation/flotation processes performed at the plant. The wastewater was collected at different time points for sequential treatment.

The performance of the AGS-SBR was assessed during 142 days for COD, ammonia, nitrate, nitrite and phosphorous removal. Chloride in the feed (wastewater) and in the outlet was also assessed. Samples were filtered using syringe nylon membrane filters (0.45 µm pore-size) in order to remove suspended solids. COD was analyzed in accordance with standard methods [4]. Ammonia, nitrate, nitrite, phosphorous and chloride concentrations were determined with photometric test kits (Spectroquant®, Merck Millipore), according to the manufacturer’s instructions.

Results and discussion

The AGS-SBR performance was evaluated during 142 days of operation which were divided into five phases (I, II, III, IV and V). The organic loading rate (OLR) of the wastewater fed to the reactor was variable, as described in Table 1. Throughout phases I, II and III, most of the COD removal was performed during the anaerobic feeding period, reaching less than 125 mg O₂ L⁻¹ at the outlet. A constant and higher OLR was applied during phase IV, temporally affecting COD removal. During this phase, part of the COD was still present in the reactor bulk liquid after the anaerobic feeding. On phase V, the OLR decreased and COD values closer to 125 mg O₂ L⁻¹ were obtained at the outlet.

Phosphate accumulation and removal were stable during phases I, II and III, reaching values below 10 mg L⁻¹ at the outlet. However, a lower phosphate removal was observed during phase IV. In the presence of a higher amount of carbon, PAOs (Polyphosphate Accumulating Organisms) can be outcompeted by other microorganisms, such as GAOs (Glycogen Accumulating Organisms), affecting phosphate removal. During phase V, phosphate concentration was very low in the wastewater and after the anaerobic feeding. Concerning nitrogen removal, during phases I, II and III most of the ammonium was converted to nitrate, without nitrite accumulation. On phase IV, the ammonium concentration was higher in the feed. The conversion to nitrate decreased, while nitrite accumulated in the reactor. The presence of extra COD after the anaerobic feeding could have led to the overgrowth of heterotrophic microorganisms, which compete with the nitrifiers for oxygen. This possibly affected the activity of nitrite oxidizing bacteria (NOB). During phase V, most of the ammonium was converted to nitrite and nitrate.

Chloride concentration in the feed and outlet of the AGS-SBR was measured along the operation. During phase IV, chloride concentration values in the outlet were between 3.7 and 4.6 g L⁻¹. While, throughout phases I, II, III and V, chloride concentration in the outlet was more variable and between 0.5 and 3.2 g L⁻¹.

Conclusion

The AGS-SBR presented a good performance while treating a variable composition of real fish canning wastewater. During periods when a higher organic loading rate was applied, the excess of COD present in the bulk liquid during the aeration
phase possibly affected phosphate removal and the nitrification process. The higher concentration of salt, associated to the higher OLR values, could also have disturbed the different biological processes. Nevertheless, the process performance was resumed, as observed by the COD removal. The AGS-SBR reactor operation is still ongoing to investigate the key parameters that affect the process and to promote the recovery of the initial performance on phosphate and nitrogen removal. Further, EPS from the AGS will be a source for recovery of biopolymers with application in different fields.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Phase I</th>
<th>Phase II</th>
<th>Phase III</th>
<th>Phase IV</th>
<th>Phase V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Period (days)</td>
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<td>36 – 57</td>
<td>58 – 89</td>
<td>90 – 115</td>
<td>116 – 142</td>
</tr>
<tr>
<td>OLR (g COD L⁻¹ day⁻¹)</td>
<td>0.8 – 2.3</td>
<td>0.2 – 0.9</td>
<td>0.5 – 0.8</td>
<td>2.1 – 2.4</td>
<td>0.4 – 1.2</td>
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C.L. Amorim acknowledges the research grant from Fundação para a Ciência e Tecnologia (FCT), Portugal (SFRH/BPD/96481/2013) and Fundo Social Europeu (Programa Operacional Potencial Humano (POPH), Quadro de Referência Estratégico Nacional (QREN)). This work was financed by FCT through the project MultiBiorefinery-POCI-01-0145-FEDER-016403. We also thank the scientific collaboration of CBQF under the FCT project UID/Multi/50016/2013.

References
Water2Return: towards the total recovery of nutrients from the sewage water in a slaughterhouse

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Nowadays, water scarcity and resource recovery are global concerns. Therefore, there is rising demand for alternative and sustainable solutions to use water in the best possible way and to recover resources from going to waste. These solutions become a must when dealing with industrial sectors like slaughtering, a water-intensive industry that generates wastewater containing a high proportion of organic matter. This wastewater can be a valuable source of nutrients if properly recovered.

Water2Return project adopts a Circular Economy approach where nutrients present in wastewaters from the meat industry can be recycled and injected back into the agricultural system as new raw materials. Water2Return proposes a full-scale demonstration process for integrated nutrients recovery using biochemical and physical technologies. The project also proposes innovative business models for the resulting products that will open new market opportunities in the environmental and agricultural sectors.

Figure 1. Water2Return flow-sheet.

Water2Return is not only about recovering soluble nitrogen and phosphorous from slaughterhouse wastewater as nitrate and phosphate concentrates (SRM1) for the manufacture of fertilizers. Water2Return will also use an innovative fermentation process for sludge valorization to make biostimulant products (SRM2) that have low development costs but high added value for plant nutrition and agriculture. In addition, algal biomass (SMR3) will be produced to capture and recycle CO₂: that is released from the biogas unit, which will be used to make an algal-based product.

An environmental, economic, social and risk assessment of the technology performance and the products obtained will be also performed.

The Water2Return consortium is made up of 15 multidisciplinary actors: 9 SMEs, 2 EU associations and 4 research institutions. Water2Return brings together the experience from stakeholders covering the whole value chain in eight EU countries (Spain, Portugal, Slovenia, Romania, Italy, Belgium, Germany and United Kingdom): experts in wastewater treatment and nutrient recovery, sludge valorisation, energy production and IT; fertiliser manufacturers that will invest in nutrient recycling and end-users of the resulting nutrients recovery system (organization representing

Around 750,000 m³ of wastewater is produced per year in pig, cattle and poultry slaughterhouses (estimations made by the project consortium), which together constitute 98% of the EU slaughtering sector. This wastewater represents a huge source of nutrients. However, the current approach for treating it is not focused on nutrients recovery and recycling, but rather, nutrients removal.

The EU Action plan for the Circular Economy, published by the European Commission in December 2015, that promote the transition to an economy model where the value of products, materials and resources is maintained in the economy for as long as possible, and the generation of waste is minimised, contributing to a sustainable, low carbon, resource efficient and competitive economy [1]. Under a Circular Economy model, materials that can be recycled are injected back into the economy as new raw materials, thus increasing the security of supply. These “Secondary raw materials” (SRM) can be traded and used like primary raw materials from traditional extractive sources, and are therefore treated as a resource rather than a waste [2]. Recycled nutrients are an important category of SRM and are widely present in organic wastewaters [3] (e.g. wastewater produced in the food industry). These nutrients can be returned to soils as new manufactured fertilizers, to meet nutrient demands for crops. The benefits of safely “recycling”, otherwise value less industry by-products, are multiple: promote synergies among key industrial sectors (e.g. agriculture, food processing and water treatment), maximize land use efficiency, decrease the environmental impacts of food production and increase the competitiveness of recycling companies by obtaining revenues from byproducts with a potential value in the market as fertilizers.

Water2Return proposes a viable, cross-sectoral and integrated full-scale demonstration process by using a novel combination of biochemical and physical technologies and processes in cascade, all while aiming for a positive balance in terms of energy footprint –biological aeration systems, membrane technologies, anaerobic processes for bio-methane production and algal technologies, all combined in a zero-waste emission system, with an integrated monitoring control tool that improves the nutrient flow data quality –.
the meat industry at EU level) and the APs (landowners’ association representing EU farmers), as well as an agriculture cooperative that will test the three APs in its own land.

Acknowledgements
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References
Yarrowia lipolytica as a potential producer of 2-phenylethanol from L-phenylalanine biotransformation

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2-Phenylethanol (2-PE) is an aromatic alcohol with a delicate fragrance of rose petals. The non-conventional yeast Yarrowia lipolytica is extensively explored for flavor compounds production, but the production of 2-PE has been very poorly described. This study investigated the potential of different Y. lipolytica strains (W29, CBS2075, CH 1/5 and CH 3/4) for 2-PE production. It was confirmed that all strains were able to produce 2-PE by L-phenylalanine (L-Phe) biocconversion, but were inhibited by 2-PE concentrations above 2 g L⁻¹. The strain Y. lipolytica CH 1/5 was selected for further studies since it produced the highest 2-PE titer (2.2 g L⁻¹). Afterwards, the effect of L-Phe concentration and carbon source (glucose and crude glycerol) on 2-PE production was studied, and it was observed that increasing L-Phe concentration decreases the aroma production, and that the highest titer was obtained with glycerol. This study demonstrates the promising production of 2-PE using Y. lipolytica as biotechnological platform for flavors production.

Introduction

2-Phenylethanol (2-PE) is an aromatic alcohol with a fresh rose scent, and is the second most widely used flavor after vanillin [1]. It is commonly used in the cosmetics and perfumes industries [1], with a world market volume of nearly 10 000 t, mainly produced by chemical synthesis. Nevertheless, the use of the chemically synthesised flavor compounds is restricted to some applications and natural flavor compounds are preferred by consumers [2]. However, its natural production includes the extraction from plants and this process involves several steps of down-stream operations, which makes the market price of natural 2-PE more expensive ($1000/kg) than the chemically synthesised ($5/kg) [3]. Hence, great interest has been arising for the biotechnological production of 2-PE. Several microorganisms have been described as possessing the ability to synthesise 2-PE [1]. 2-PE can be synthesized in yeast through shikimate and Ehrlich pathways (Graphical abstract). Either way, the main bottleneck for yeast fermentation of 2-PE is its toxicity [1] due to the fact that concentrations between 2 and 3 g L⁻¹ inhibit the cellular growth [1] and the results obtained so far do not seem viable for industrial scale-up. Among several microorganisms able to produce 2-PE, the yeast Y. lipolytica appears to be promising due to its interesting characteristics, such as the Crabtree negative trait and absence of ethanol production, however this process has been fairly described.

Material and Methods

The strains used in this work were Y. lipolytica W29 (ATCC 20460), CBS2075, CH 1/5 and CH 3/4 (isolated from cheese). Y. lipolytica strains were cultivated for 16-17 hours in of YPD medium (glucose 20 g L⁻¹, peptone 20 g L⁻¹, yeast extract 10 g L⁻¹) at 200 rpm and 27 °C, and further used to inoculate the biocconversion experiments with an initial OD₆₀₀ of 0.5. Bioconversion of L-Phe to 2-PE was carried out in cultivation medium containing per liter of deionized water: glucose or glycerol 40 g, KH₂PO₄ 15 g, MgSO₄·7H₂O 0.5 g, YNB without amino acids 0.02 g, thiamine 3 mg, pH 6.5, supplemented with L-Phe 4 g or 6 g incubated at 27 °C and 200 rpm. Glucose and glycerol were quantified by high-performance liquid chromatography. The 2-PE and L-Phe quantification was obtained using a SHIMADZU UHPLC system equipped with a diode array detector (SPD-M20A) at a fixed wavelength of 215 nm. LC separation was carried out with a YMC ODS-Aq (250 mm × 4.6 mm) reverse phase column at 25 °C. For elution, water (solvent A) and acetonitrile (solvent B) were applied as the mobile phases at a flow rate of 1 mL min⁻¹. A gradient was used, where the amount of solvent A was increased stepwise: 0 min – 100% A, 10 min – 100% A, 16.7 min - 70% A, 26.7 min – 70% A, 33.3 min – 100% A; 41.7 min – 100% A.

Results and Discussion

The main challenge for microbial production of 2-PE is its cytotoxicity [1]. To study the tolerance of different Y. lipolytica strains toward 2-PE, cells were cultivated in solid medium containing various concentrations of 2-PE. As shown in Fig. 1 the growth of all strains was completely repressed at a 2-PE concentration of 2 g L⁻¹. Comparing the performance of the four Y. lipolytica strains herein studied it was possible to observe that W29 and CBS2075 strains were more tolerant to 2-PE.

Figure 1. Inhibitory effect of 2-PE for the Y. lipolytica W29, CH 1/5, CH 3/4 and CBS2075 strains. Cells were incubated in YPD medium for 16 h and diluted to an OD₆₀₀ of 0.6. Cells (3 μL) at a dilution of 10⁰, 10⁻¹, 10⁻², 10⁻³ and 10⁻⁴ were spotted on solid media (glucose 40 g L⁻¹, agar 20 g L⁻¹ and (NH₄)₂SO₄ 2 g L⁻¹) containing different concentrations of 2-PE (0 (control), 1, 2 and 3, g L⁻¹). Cells were incubated at 30 °C during 48h.
Since the 2-PE production by Y. lipolytica was poorly explored, it was studied the ability of the four Y. lipolytica strains to produce this aroma through L-Phe biotransformation (Table 1).

Table 1. Maximum concentration, productivity and yield of 2-PE for the Y. lipolytica strains with 4 g L\(^{-1}\) of L-Phe. (Data are presented as average and standard deviation of two independent experiments).

<table>
<thead>
<tr>
<th>Strains</th>
<th>2-PE (g L(^{-1}))</th>
<th>Yield (g g(^{-1}))</th>
<th>Productivity (mg L(^{-1}) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>W29</td>
<td>1.01 ± 0.07</td>
<td>0.24 ± 0.01</td>
<td>5.8 ± 0.4</td>
</tr>
<tr>
<td>CH 1/5</td>
<td>2.17 ± 0.27</td>
<td>0.7 ± 0.1</td>
<td>11.4 ± 0.3</td>
</tr>
<tr>
<td>CH 3/4</td>
<td>1.71 ± 0.14</td>
<td>0.8 ± 0.2</td>
<td>8.9 ± 0.7</td>
</tr>
<tr>
<td>CBS 2075</td>
<td>0.80 ± 0.02</td>
<td>0.19 ± 0.01</td>
<td>4.8 ± 0.1</td>
</tr>
</tbody>
</table>

Comparing the performance of the four strains tested, it was possible to observe that all strains were able to produce 2-PE, although with different strain-dependent production efficiencies, being the CH 1/5 strain the one with the highest production titer (Table 1), 2.17 ± 0.27 g L\(^{-1}\). Taking into consideration the 2-PE titters reported by Huang et al. [4] (0.5 g L\(^{-1}\) of 2-PE from 1 g L\(^{-1}\) of L-Phe with P. fermentans L-5) the obtained results elucidate the potential of the tested Y. lipolytica strains for this metabolite production. Therefore, given that the bioconversion yields of 2-PE with respect to L-Phe, Y. lipolytica CH 1/5 and CH 3/4 were the best producers, since the obtained yield was 68% and 75% higher than the values obtained with the strains W29 and CBS2075, respectively. Taking the above into account and considering that the highest 2-PE concentration and productivity were obtained with CH 1/5 strain, this strain was selected as the best candidate for the following experiments. Previous reports [5] have shown that the L-Phe concentration in the media influences 2-PE production. Since that, in the previous experiments L-Phe was completely consumed and new experiments were carried out to analyze the behavior of the Y. lipolytica CH 1/5 strain in the presence of 6 g L\(^{-1}\) of L-Phe. Alternatively it was also studied the ability of this yeast to produce 2-PE in the presence of crude glycerol, a by-product of the biodiesel industry, that can be naturally consumed by Y. lipolytica [6] (Table 2).

Table 2. Maximum concentration, productivity and yield of 2-PE for the Y. lipolytica CH 1/5 with 6 g L\(^{-1}\) of L-Phe using glucose and glycerol as carbon sources. (Data are presented as average and standard deviation of two independent experiments).

<table>
<thead>
<tr>
<th>Carbon source</th>
<th>2-PE (g L(^{-1}))</th>
<th>Yield (g g(^{-1}))</th>
<th>Productivity (mg L(^{-1}) h(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>0.57 ± 0.0005</td>
<td>0.13 ± 0.03</td>
<td>2.6 ± 0.1</td>
</tr>
<tr>
<td>Glycerol</td>
<td>1.2 ± 0.1</td>
<td>0.33 ± 0.01</td>
<td>5.0 ± 0.5</td>
</tr>
</tbody>
</table>

Regarding the 2-PE concentrations obtained under these conditions, it was possible to observe that, for glucose, increasing the L-Phe concentration decreases the titer of 2-PE (0.57 vs 2.17 g L\(^{-1}\), for the experiments with 4 and 6 g L\(^{-1}\) the L-Phe, respectively). However, the 2-PE titer, yield and productivity attained with glycerol are 53 % higher than the ones obtained with glucose, under the same conditions. Despite the preference of Y. lipolytica to use glucose as carbon source for cell growth, glycerol is a better substrate for 2-PE production. The same behavior was also reported by Huang et al. [4] with P. fermentans L-5. The authors also reported a higher 2-PE yield with glycerol (0.56 mol mol\(^{-1}\)) when compared with glucose (0.31 mol mol\(^{-1}\)). The low titer of 2-PE obtained with 6 g L\(^{-1}\) of L-Phe in the presence of glucose can be probably attributed to the production of other metabolites in the Ehrlich pathway, such as phenylacetate (Graphical abstract). It seems that some enzymes in this pathway can be inhibited in the presence of glycerol. Comparison of the obtained final titters of 2-PE with the literature data indicates that Y. lipolytica shows the potential to efficiently produce this metabolite. For example P. fermentans L-5 produced 0.5 g L\(^{-1}\) 2-PE from 1 g L\(^{-1}\) L-Phe [4], strains of K. marxianus produced up to 0.9 g L\(^{-1}\) of 2-PE [7] in a non-optimized process. In the scope of the above, the process of 2-PE production with Y. lipolytica CH 1/5 appears promising, however it requires further research and optimization.

Acknowledgements

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References

A microfluidic platform for optimization of downstream process of biopharmaceuticals

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Miniaturization platforms as high-throughput screening tools to speed up process optimization are described.

The number of biotechnology-based pharmaceuticals in the late-stage pipeline has been increasing more than ever in particular mAbs represent a quarter of all biopharmaceuticals in clinical trials. As a result, there is an enhanced demand for more efficient and cost-effective processes. Here, the potential of miniaturization as a high-throughput screening tool to speed up process optimization is explored.

The ATPS-microfluidic developed setup allowed the rapid screening of a wide range of concentrations inside the microchannel by varying the flow rates of the solutions while using sub-mL volumes for each ATPS-forming system.

A Chip chromatography microfluidic developed platform allowed the effective screen of multiple adsorption and elution conditions within a few minutes for early stage multimodal chromatography optimization. This technique can be used with any target molecule or resin assuming a previous labeling procedure with a photostable and pH insensitive fluorophore.

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Sustainable production of plastic building blocks by enzymatic biocatalysis

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Galactose oxidase (GAO) and aryl alcohol oxidase (AAO) have been shown to be promising biocatalysts for the oxidation of primary and secondary alcohols. However, the use of free enzymes on an industrial scale presents low stability and the possibility of reusing the biocatalyst depends on the viability of separation of the free enzyme in ultrafiltration membrane systems. Different enzyme immobilisation strategies have been proposed, with the aim of improving activity and protein stability. This study presents different immobilisation techniques for GAO and AAO on supports based on ionic interactions and covalent bonds: Agarose-glyoxyl, Relizyme, Lifetech ECR8204/F, Lifetech ECR8215/F, magnetized agarose-glyoxyl, magnetized fumed silica nanoparticles, silica-coated and polyethilenimine-coated magnetic nanoparticles. Benchmarking among the different supports was conducted taking into account the optimization of several factors such as enzyme retention capacity, loss of enzyme activity during immobilization, ease of the support activation process and cost. Moreover, envisaging the use of immobilized GAO and AAO as a first stage in enzyme cascade synthesis, the biotransformation of 5-HMF was performed and compared with free enzyme.

Introduction

For many years, fossil fuels have been the main source for plastic building blocks. However, due to the growing environmental concerns, there is an enormous interest to gradually move from traditional fossil fuel derived feedstocks towards a more sustainable and renewable biomass. In this context, 5-hydroxymethylfurfural (HMF) has emerged as one of the 14 top biomass platform molecules for the sustainable future [1,2]. In fact, recently new catalytic routes have been developed to transform HMF into building blocks for plastics such as dimethylfuran (DFF) or 5-formyl-2-furancarboxylic acid (FFCA).

New unexploited enzymes as Galactose oxidase (GAO) and Aryl alcohol oxidase (AAO) reported to oxidize HMF and produce DFF and FFCA using oxygen as electron acceptor (Figure 1). Nevertheless, to scale-up the process, the reusability of the biocatalyst is a crucial point and therefore, an immobilization strategy should be selected. This study aims to produce a robust biocatalyst for the transformation of HMF to DFF and FFCA with GAO and AAO, respectively. For this purpose, enzymes were immobilized on different magnetic and non-magnetic supports and taking into account different factors a benchmarking was assessed.

Materials and methods

Supports and enzymes. Agarose-glyoxyl (Ag, with a size of 100 µm), Relizyme (with a size of 200-500 µm), Lifetech ECR8204/F, Lifetech ECR8215/F, magnetized agarose-glyoxyl (mAg), magnetized fumed silica nanoparticles (fsMNP, with a size of 7 nm), silica-coated magnetic nanoparticles (sMNP, with a size of 20 nm) and polyethilenimine-coated magnetic nanoparticles (PEImNP, with a size of 10 nm) were used as immobilization support materials.

Immobilization of GAO and AAO on agarose and epoxy based supports. The supports were incubated with GAO or AAO enzyme (1.5 U mg⁻¹ support) at 4ºC for 24 h at pH 10. The non-covalently bound enzyme was washed in five centrifugation/re-suspension steps. The immobilization yield was calculated as the ratio of the laccase activity theoretically immobilized and the final enzyme loading.

Immobilization of GAO and AAO on magnetic nanoparticles. Prior to immobilization fsMNP and sMNP were aminofunctionalized under the reaction of APTES (0.8 mmol g⁻¹ support) for 24 h at room temperature. The support was incubated with GAO or AAO enzyme (1.88 U mg⁻¹ support) at 4ºC for 2 h at pH 7. Thereafter, 8 mmol glutaraldehyde g⁻¹ support was added and the reaction was completed at 4ºC for at least 12 h. Once formed, the nanoparticles were washed. Immobilization efficiency was calculated as previously mentioned.

Material and scaffold (MS) conjugates (0.8 mmol g⁻¹) were dispersed in a final volume of 1 mL containing 200 µg mL⁻¹ of DNA in 100 mM of PBS. A control was run in parallel containing only DNA in PBS. The mixtures were incubated for 1 h at 37°C.
After incubation, the testing solutions were subject to electrophoresis in 0.75% (w/v) agarose gels, prepared with 40 mM Tris buffer (pH 7.6) containing 20 mM acetic acid and 1 mM EDTA. Gels were run in the above buffer, at 2.5 A, and the DNA bands were visualized.

**Results**

Immobilization yields were poor for supports based on agarose glyoxyl and epoxide groups (Table 1). However, the immobilization on magnetic nanoparticles attained satisfactory immobilization yield (IY) values higher than 40%. The best immobilization support for both supports result on the sMNP with enzyme loadings (EL) of 1.19±0.02 and 1.08±0.03 U mg⁻¹ sMNP for GAO and AAO, respectively.

**Figure 2.** HMF (2 mM) biotransformation by AAO-sMNPs. Therefore, sMNP where selected for further experiments of biotransformation of HMF. HMF biotransformation by GAO and AAO was demonstrated and the nanoparticles could be easily recovered by applying the magnetic field in less than 1 min. Complete biotransformation of HMF on FFCA was observed by AAO in less than four hours (Figure 2), whereas GAO biotransform up to 48% of HMF into DFF after 18 h (Figure 3). However, an enhance on biotransformation rates was observed in comparison with free enzyme. Finally, it was no found DNA damage when applying the nanobiocatalyst based on sMNP.

**Figure 3.** HMF (2 mM) biotransformation by GAO-sMNPs.

**Conclusions**

The study compared different supports for the immobilization of GAO and AAO by covalent bonding, as well as their use as nanobiocatalyst for HMF biotransformation. In summary, the most efficient biotransformation and the best performance of the activity was achieved for those immobilization strategies that considered the use of magnetic nanoparticles as an immobilization support. One of the main results of this study is that the nanobiocatalyst is magnetically recoverable and can be reused in repeated HMF biotransformation cycles. The easy recovery of the nanobiocatalyst from the reaction media is a significant advantage from an operational perspective. Further research should focus on the development of an enzymatic magnetic reactor to demonstrate the scalability of the process.

<table>
<thead>
<tr>
<th>Supports</th>
<th>GAO</th>
<th>AAO</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>IY (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>1.53±0.04</td>
<td>0.07±0.02</td>
</tr>
<tr>
<td>mAg</td>
<td>5.40±1.40</td>
<td>-</td>
</tr>
<tr>
<td>Epoxy</td>
<td>1.11±0.01</td>
<td>0.05±0.01</td>
</tr>
<tr>
<td>Relzyyme</td>
<td>25.9±2.4</td>
<td>2.17±0.33</td>
</tr>
<tr>
<td>ECR8204/F</td>
<td>30.9±10.8</td>
<td>2.86±1.00</td>
</tr>
<tr>
<td>ECR8215/F</td>
<td>42.3±1.99</td>
<td>1.19±0.02</td>
</tr>
<tr>
<td><strong>EL (U mg⁻¹)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>0.07±0.02</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>mAg</td>
<td>0.12±0.03</td>
<td>-</td>
</tr>
<tr>
<td>Epoxy</td>
<td>0.05±0.01</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>Relzyyme</td>
<td>2.17±0.33</td>
<td>0.03±0.01</td>
</tr>
<tr>
<td>ECR8204/F</td>
<td>2.86±1.00</td>
<td>0.04±0.01</td>
</tr>
<tr>
<td>ECR8215/F</td>
<td>1.19±0.02</td>
<td>46.05±7.71</td>
</tr>
<tr>
<td><strong>NF (%)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ag</td>
<td>0.07±0.02</td>
<td>0.11±0.01</td>
</tr>
<tr>
<td>mAg</td>
<td>0.12±0.03</td>
<td>-</td>
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<tr>
<td>ECR8215/F</td>
<td>1.19±0.02</td>
<td>46.05±7.71</td>
</tr>
</tbody>
</table>

| **EL (U mg⁻¹)**|     |     |
| Ag             | 0.11±0.01 | <0.01 |
| mAg            | -       | -     |
| Epoxy          | 0.03±0.01 | <0.01 |
| Relzyyme       | 0.03±0.01 | <0.01 |
| ECR8204/F      | 0.04±0.01 | <0.01 |
| ECR8215/F      | 1.08±0.03 | <0.01 |

3IY: Immobilization yield
4EL: Enzyme loading

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**References**

Designing humanized hydrogels toward tissue engineering and disease modeling

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Currently, 3D in vitro cell cultures that lead to more predictive models for research are emerging not only as a new tool in early drug discovery, but also as potential therapeutics to treat disease. For example, basement membrane extracted from the Engelbreth-Holm-Swarm (EHS) mouse sarcoma or collagen matrices have shown to drive cellular self-organization and complex morphogenetic processes to result in sophisticated 3D cell culture platforms. However, drug screening models using this type of materials go against current animal-free approaches for cell culture and restrict clinical compliance. Despite the design flexibility of synthetic alternatives, they are typically expensive and their synthesis can be time consuming. To overcome these drawbacks, we developed a next-generation of cost effective, easy do handle human based materials for 3D cell culture and microtissue development (patented). This innovative product consists in a bioactive platelet-rich plasma derivative precursor (PLMA) that can be cured upon light exposure to form soft materials with tuneable mechanical properties.

We have designed PLMA platforms that provide the necessary biological, biochemical and structural support for 3D cell culture. This platform is the first to offer complete human based material for 3D cell culture and an easy-to-use solution for clinical purposes. PLMA hydrogels are formed by UV crosslinking of methacrylated plasma and platelet derived proteins and growth factors. The PLMA materials are fully compatible with tissue engineering protocols and suitable for human in vivo use. This is an easy-to-use and cost effective platform as the raw material to produce the gels is easily available, compliant with regulatory requirements, and processed using a simple and cost-effective protocol. The synthesized gels have proven to support distinct human derived cell cultures. Since day 1 the encapsulated cells could perform important biological processes such as growth and migration. [1]

Complex 3D models of osteosarcoma are currently being studied using PLMA hydrogels. Preliminary results reveal an increased invasion rate of MG63-osteosarcoma cells through PLMA gels when compared with Matrigel. Studies are ongoing to look at the biological function of encapsulated mesenchymal stem cells and the synergistic interaction between the cells of the tumour microenvironment. The validation of the 3D in vitro disease model is a crucial step in assessing the technical feasibility of our platform. We will use a library of standard-of-care FDA and EMA approved drugs used to treat or reduce the symptoms of osteosarcoma.

These innovative products are envisioned to have a major societal impact since it addresses the development of humanized cell culture platforms that can be employed to accelerate drug discovery and/or increase our understanding of the mechanisms behind tissue morphogenesis and specific diseases, reduce drug development costs and increased interest of pharmaceutical and industrial/medical biotechnology.

Acknowledgements

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References

Valorization of coffee wastes through acidogenic fermentation

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Spent coffee grounds (SCG) are a waste of low value with potential to be valorized into polyhydroxyalkanoates (PHAs). PHAs are bio-based polymers, posing as sustainable alternative to conventional plastics, and can be produced by mixed microbial cultures (MMCs) using wastes or by-products, leading to a significant reduction in the production costs. In this work, the conditions for producing short-chain organic acids (SCOAs), the preferred substrate for PHAs production, from SCGs were evaluated. Different sources of inoculum were studied, and an aerobic MMC showed the best performance. Acidogenic fermentation of SCG using the aerobic inoculum was tested in two reactor configurations, CSTR and FBBR. FBBR showed better results, with a production of 1.53 gSCOAs/L.

Pretreatments of SCG were tested, and acid hydrolysis led to the highest increase on SCOAs production. This work showed the potential of SCG valorization via acidogenic fermentation into SCOAs that can be used for PHA production.

### Introduction

Spent coffee grounds (SCG) are an industrial waste of low value, but with great potential to be valorized [1]. SCG high content on carbohydrates, fatty acids and phenolics has the potential to be used as substrate for the production of several added-value products, like polyhydroxyalkanoates (PHAs) [2]. PHAs are bio-based polymers, biodegradable and biocompatible representing a more sustainable alternative to conventional plastics. They can be produced by mixed microbial cultures (MMCs) using wastes or by-products, leading to a significant reduction in the production cost of these polymers [3].

The main goal of this work was the production of short chain organic acids (SCOAs), the preferred substrates for PHAs production by MMCs, using SCGs as carbon source. Acidogenic fermentations of SCGs was studied by testing different sources of inoculum and different reactor configurations. A continuous stirred-tank reactor (CSTR) and a fluidized bed biofilm reactor (FBBR), were conducted to determine the optimal conditions for the process.

### Methods

Two sources of inoculum were used in this study, aerobic and anaerobic activated sludge collected from the wastewater treatment plant of Aveiro Sul, SIMRia. The anaerobic sludge was submitted to a pretreatment, in the oven at 93 °C for 30 min. SCGs were obtained from a coffee shop at the Department of Chemistry of University of Aveiro, Portugal. SCGs were then dried to a constant weight in an oven at 95 °C for 24 h and used as carbon source diluted in a mineral solution with pH corrected to 6.5.

The studies of the inoculum and pretreatments of the SCGs were conducted in encapsulated flasks with 100 mL of working volume, maintained at 35°C with constant stirring and purged with nitrogen to ensure anaerobic conditions. The reactors were operated with the aerobic MMC under the operational conditions described in Table 1.

### Results

The MMCs that resulted from the different inoculum studied (aerobic, anaerobic, and anaerobic with pretreatment) were able to produce SCOAs. In the assay incubated with anaerobic sludge only acetic acid was produced, 0.41 g/L on day 21. The aerobic inoculum with heat treatment led to a lower concentration of acid, with a maximum concentration of acetic acid of 0.35 g/L on day 18. The aerobic inoculum led to the best overall performance, with the production of acetic, propionic, butyric and valeric acids, with a maximum concentration of 1.68 gSCOAs/L after 15 days. For this reason, the aerobic sludge was used to inoculate the bioreactors.

### Table 1. Operational conditions of the CSTR and FBBR

<table>
<thead>
<tr>
<th>Reactor</th>
<th>OLR (gCOD/L.d)</th>
<th>HRT (days)</th>
<th>Volume (L)</th>
<th>Temp. (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSTR</td>
<td>4.4</td>
<td>3</td>
<td>2</td>
<td>31</td>
</tr>
<tr>
<td>FBBR</td>
<td>4.4</td>
<td>4.8</td>
<td>3.2</td>
<td>31</td>
</tr>
</tbody>
</table>

The SCOAs production in the CSTR produced occurred only during the first 12 days, with highest concentration, 0.52 gSCOAs/L, on the first day of operation after the batch period. A decrease of the biomass concentration was observed during the operational time, indicating that the sludge retention time (SRT) and the hydraulic retention time (HRT) imposed to the reactor were too low, and the biomass was washed out. The FBBR configuration showed better results, with a maximum of 1.53 gL of SCOAs in the beginning of operation and production of the same profile of SCOAs as in the CSTR. A decrease of biomass concentration, and consequent decrease of organic acids production, was also observed. However, under these conditions, the culture was able to maintain a low but constant concentration of acetic acid of 0.07 g/L for 32 days. The low performance of the two reactor configuration could result from the low HRT imposed but also to the difficulty of the MMC to hydrolyze the more complex components of SCGs. In order to increase the acidification yield a study of possible pretreatments of the SCG was conducted. Acid hydrolysis, ultrasonication, microwave and autoclave pretreatment were conducted, and the resultant hydrolysates were incubated with the aerobic MMC. Acid hydrolysis was the pretreatment that led to the best results, with a production of 2.11 gSCOAs/L on the 20th day. This result was slightly higher than the concentration obtained with non-treated SCGs that had the second-best result, 1.96 gSCOAs/L. These results...
may indicate that some of the pretreatments could lead to sugar degradation or to inhibitors production. The profile of SCOAs obtained was similar in all tests, being produced acetic, propionic, butyric and valeric acids.

Conclusions
This work showed the potential of SCGs valorization via acidogenic fermentation into SCOAs, that can be used as carbon source for the production of PHAs. The aerobic activated sludge proved to be the best source of inoculum, producing a diverse SCOAs profile and with the highest concentration. FBBR configuration had better results than CSTR probably due to higher sludge and hydraulic retention times, but other parameters need to be studied and optimize to achieve higher acidification yields. The pretreatment study showed that acid hydrolysis can be used to improve the production of SCOAs.

Acknowledgements
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References
Polyhydroxyalkanoates (PHAs) are biodegradable plastics with microbial origin, stored by bacterial cells as granules. PHAs are considered as future substitutes of traditional plastics. However, their application is still limited by their high production costs. In the last years, the possibility of reducing such costs by developing a low-cost PHAs production by mixing microbial cultures using agro-industrials wastes as substrate has been largely explored. However, only few studies were dedicated to the extraction and purification process proved to strongly affect the production costs.

In this work, an alternative PHAs extraction procedure was developed, based on biomass pretreatment with non-ionic surfactants followed by the use of dimethyl carbonate (DMC) as a more sustainable extracting agent. The developed procedure allowed recovering up to 57% of the polymer content that represents a good result given the heterogeneity of the starting mixed microbial culture.

In order to evaluate the effect of the concentration of the three surfactants, the three best values of S/L ratios were tested at a fixed surfactant concentration. The results obtained were in general similar or slightly lower with respect to the highest concentration tested.

In any alternative adopted (concentration and S/L ratio), Surfactant 1 gave the lowest results in terms of polymer recovery yield with respect to the other two surfactants. For this reason, Surfactant 1 was discarded while Surfactant 2 and Surfactant 3 were adopted as the most appropriate digestive agents to non-PHA cells.

The best results were obtained at Concentration 1 and at the lowest S/L ratio, allowing reaching a final polymer recovery yield of 57% and 55%, respectively for Surfactant 2 and Surfactant 3. To underline the similarity between the extracted polymers with the selected conditions and the commercial polymer (P(3HB)), an analysis by FT-IR spectra was performed.

In order to evaluate the effect of reducing the DMC amount obtaining similar recovery yields, a last test was performed. The obtained results showed a clear reduction in the amount of extracted polymer. Probably these evidences can be explained by the formation of a hydrocolloid gel that made impossible to separate the DMC phase rich in PHA from the pretreated biomass.

By adopting the best conditions in both steps of the extraction procedure developed, it was possible to increase the polymer recovery yield by 50% with respect to that obtained performing the extraction method developed by Samori et al. [4] on the PHA-rich MMC selected on fermented agro-industrial wastes.
Acknowledgements
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. J. Pereira thanks FCT for her PhD grant (SFRH/BD/130003/2017). The authors thank FCT for the doctoral grant SFRH/BD/122220/2016 of M. Martins and for the IF contract IF/00402/2015 of S.P.M. Ventura and for the project PAC – Programa de Atividades Conjuntas ref: SAICTPAC/0040/2015.
This work was partially financed by Regione Lombardia, Italian Government and European Community-Programma Operativo Regionale 2014–2020, Obiettivo “Investimenti in Favore della Crescita e dell’Occupazione Asse Prioritario 1 – Rafforzare la Ricerca, lo Sviluppo e l’Innovazione. Azione 1.1.b.1.3—Sostegno alle attività collaborative di R&S per lo sviluppo di nuove tecnologie sostenibili, di nuovi prodotti e Servizi. Project ID 141082: Renewable RAw materials valorization for INnovative BiOplastic production from urban Waste (RAINBOW).

References
Design of a periodic counter-current chromatography process for efficient oncolytic virus purification

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Over the past two decades, biopharmaceutical industry observed a shift in the allocation of manufacturing limiting steps, from upstream to downstream processing. The use of continuous chromatography for biologics presents a way to address the productivity improvement challenges required for debottlenecking downstream operations. Although the adoption of these type of processes is becoming more common in the biopharmaceutical industry, namely for mAbs purification, there is still a long path to fully adopt these technologies as a standard practice in downstream processing. We report on the design of a periodic counter-current chromatography process applied to the intermediate purification of an oncolytic adenovirus.

The developed ion-exchange chromatographic purification process was carried using a four-column (4C) setup - 4C-PCC (GE Healthcare, Uppsala, Sweden). A mechanistic mathematical model was formulated and used to support the design and analysis of the cyclic process.

Two different anion-exchange resins were compared in terms of dynamic binding capacity. Moreover, the impact of column cycling on column capacity was assessed and the steps taken to minimize it will be discussed, highlighting the optimization of the cleaning-in-place step procedure and the need to include organic solvents to promote the stripping of tighter-adsorbing impurities.

The use of 4C-PCC enabled the achievement of a stable cyclic steady state operation with obtained virus recoveries (> 60%) and impurity reductions (> 80% DNA, and > 70% total protein) in line with batch purification. Additionally, due to the wealth of data generated through the cycling operations, such as historic column breakthrough and elution peak profiles a deeper insight on product quality is gained. The robustness of the existing dynamic control strategy and its ability to overcome perturbations originated in precedent stages was demonstrated through the use of feeds with different impurity profiles and titers, showing that it is possible to generate elution pools with consistent quality.

Periodic counter current chromatography offers a transversal solution to match the scale of the manufacturing plant to any of the throughputs, easing technology transfer and lowering investment costs with the potential productivity increase, and the benefits of process automation and control.

Acknowledgements

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References

**Introduction**

Nanotechnology is one of the most promising approaches assisting antimicrobial therapy design as it can provide novel vehicle systems to deliver antimicrobial agents. Nanoparticles exhibit exceptional properties for drug delivery via different routes with excellent pharmacokinetic profiles minimizing systemic side effects [1]. Generally, nanoparticles are entities that are usually less than 100 nm in diameter, in some cases between 100 and 500 nm, which are represented in the form of biodegradable or non-biodegradable drug carriers [1]. In clinical context, liposomes have been intensively investigated for drug delivery. Liposomes are spherical vesicles composed by bilayer membranes of amphiphilic lipids with an internal aqueous space and its relevance arises from their high biocompatibility, ability to incorporate both hydrophobic and hydrophilic drugs, safe drug delivery profiles and ease of preparation. However, liposomes still present limitations, such as storing stability, drug leakage and lack of size control and a limited loading capacity of lipophilic drugs. Hence, the use of liposomes for drug delivery is limited [2].

Nanosized dispersions of liquid crystalline phases had attracted our attention because of their interesting approach of encapsulation and release of drugs [3,4]. This liquid crystal structures are composed by amphiphilic molecules that when mixed with right solvent have the ability to spontaneously self-assemble leading to the formation of well-defined thermodynamically stable complexes, such as the cubosomes [4]. The colloidal dispersion of bicontinuous cubic liquid crystalline structures in water using suitable surfactant can result in cubosomes exhibiting 100 to 300 nm [5]. Cubosomes are attractive drug delivery systems because they can incorporate a great variety of chemical compounds in their domains and exhibit larger surface area, lower viscosity and high heat stability [6,7]. Although these unique physicochemical characteristics and the potential for controlled release through functionalization, the production of cubosomes has some challenges that need to be overcome, such as the presence of liposomes vesicles in the cubosomes dispersions and high energy input [4,6].

Therefore, the main goal of this study was to strengthen the assumption that cubosomes will be the new trend in drug delivery system improving their preparation and drug encapsulation method. We aimed to develop and optimize the production of controlled-size cubosomes to further load them with ciprofloxacin (CIP), a fluoroquinolone antibiotic that is administrated to treat infections caused by gram-negative bacteria. Through an optimized controlled-size and an ease of preparation, cubosomes applicability as drug delivery systems would be greatly enlarged.

**Methods**

In this work, a bottom-up approach was used to form the cubosomes through the dispersion of a mixture comprising the liquid crystal forming lipid, the polymer and a hydrotrope in excess of water with minimal energy input [6]. Based on phase diagram of the phytantriol (PT) – ethanol (ET) – water system described by Y. Chen et al. [8], different mixtures of phytantriol/ethanol (lipid/hydrotrope) were prepared as described in Table 1. The preparation of cubosomes also required a stabilizer in order to provide colloidal stability, prevent re-coalescence to bulk cubic phase and create stable dispersions [6,9]. In this study, we used the block copolymer Pluronic F127 (or Poloxamer 407) dissolved in water. The initial solutions of phytantriol/ethanol described in Table 1 were used to form cubosomes by solvent shifting. Briefly, phytantriol/ethanol solutions were added at once (solvent shifting) to different F127 solutions resulting in distinct PT: F127 ratios (Table 2). The resulting dispersions were stirred for 2 min and kept at room temperature. To verify whether ethanol had influence on the structure and size of formed cubosomes, dispersions were led at room temperature overnight to promote ethanol evaporation. All the final dispersions prepared were labeled as, for instance, A1, the letter to identify the initial solution (Table 1) and the number to discern the final PT: F127 ratio (Table 2).

Table 1. Composition of the initial solutions used to form cubosomes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Composition (phytantriol (w/w))</th>
<th>Composition (ethanol (w/w))</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.01</td>
<td>0.99</td>
</tr>
<tr>
<td>B</td>
<td>0.05</td>
<td>0.95</td>
</tr>
<tr>
<td>C</td>
<td>0.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

The final dispersions were characterized in terms of particle size (hydrodynamic diameter) and polydispersity index (PDI) using a dynamic light scattering instrument (DLS) at angles of...
90° and 173°. Measurements were performed at 25°C, during 4 runs at 90 s each run. The results were analyzed using MATLAB software according to the functions described by Hassan et al. [10] with some optimizations.

Table 2. Different final phytantriol/F127 ratio in the formed cubosomes.

<table>
<thead>
<tr>
<th>Sample series</th>
<th>PT: F127 ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>0.5</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>10</td>
</tr>
</tbody>
</table>

To encapsulate CIP into cubosomes, the same method described above was used. CIP was dissolved in the mixture of phytantriol and ethanol and adding 1 M of NaOH until the mixture was completely dissolved and clean. At the end, the final dispersions were centrifuged at 2000 rpm for 5 min to remove the non-encapsulated CIP. The supernatants containing the cubosomes loaded with CIP were collected for DLS analysis.

Results & Discussion

Cubosomes were prepared using a bottom-up approach because it needs less energy input and produces cubosomes more efficiently, with smaller size, and long-term stability. Moreover, this approach is easily scale-up to commercial and industrial batches [6]. Even though monolein is the most common lipid used to prepare lipid nanoparticles, we used phytantriol due to its superior chemical stability, absence of the ester group and availability of commercial phytantriol with some optimizations.

Before DLS measurements, the dispersions were first screened visually in order to discard the dispersions containing visible phase separation. For instance, dispersions from series 5 (A5 to C5) exhibited visible aggregates indicating that the resultant cubosomes were too large and not suitable for drug delivery proposes. Therefore, only transparent and clean dispersions were analyzed by DLS.

DLS data demonstrated that our innovative experimental setup was able to produce cubosomes with diverse diameters in a controlled-size way. Using the dispersions A3, B2 and C1, we produced cubosomes with reduced size (hydrodynamic diameter), ranging from 150 to 270 nm, whereas using the dispersions B3 and C2 larger cubosomes (>300 nm) were obtained.

Table 3. Hydrodynamic diameter and polydispersity index of the cubosomes dispersions.

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Hydrodynamic diameter (nm)</th>
<th>Polydispersity index (PDI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A3</td>
<td>152</td>
<td>0.19</td>
</tr>
<tr>
<td>A3*</td>
<td>148</td>
<td>0.17</td>
</tr>
<tr>
<td>B2</td>
<td>209</td>
<td>0.16</td>
</tr>
<tr>
<td>B2 + CIP</td>
<td>265</td>
<td>0.28</td>
</tr>
<tr>
<td>B3</td>
<td>327</td>
<td>0.30</td>
</tr>
<tr>
<td>B3 + CIP</td>
<td>344</td>
<td>0.52</td>
</tr>
<tr>
<td>C1</td>
<td>264</td>
<td>0.19</td>
</tr>
<tr>
<td>C2</td>
<td>354</td>
<td>0.25</td>
</tr>
</tbody>
</table>

*Ethanol evaporation overnight.

In terms of the polydispersity, all the dispersions showed to be monodisperse as PDI scores varied from 0.08 to 0.7 [12]. This result suggested that dispersions contained a unique and homogeneous population of cubosomes, indicating that controlled-size cubosomes were attained. Moreover, these data reinforced the role of F127 in cubosomes stabilization without compromising the particle size.

DLS results also showed that ethanol seemed not interfere in the structure of the cubosomes. The hydrodynamic diameter of cubosomes derived from A3 dispersion with and without ethanol evaporation was similar, as well as the PDI indicating both dispersion as monodisperse.

CIP encapsulation into cubosomes demonstrated to be an easy procedure with satisfactory encapsulation efficacies. More importantly, cubosomes loaded with ciprofloxacin seemed not exhibit structural alterations. DLS data demonstrated that cubosomes derived from B2 and B3 dispersions with and without CIP had identical hydrodynamic diameter, approx. 286 nm, and controlled-sized of cubosomes were still maintained as PDI score was below 0.7.

In conclusion, our data revealed that controlled-size cubosomes with different range of diameters can be produced using a simple experimental workflow with minimal energy input. Ciprofloxacin encapsulation into cubosomes was easily performed and no structural alterations were detected. These findings will greatly impact on the applicability of cubosomes as drug delivery systems.

References
Genotoxicity analysis of different magnetite-based nanoparticles applied in chemical catalysis processes

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Eco-toxicity is an important consideration in understanding the potential environmental impacts of the release of nanomaterials into the environment. In this study, the potential genotoxicity of nanosized magnetic iron-based oxide particles used in the treatment of contaminated effluents by chemical catalysis processes such as Fenton reaction or photocatalysis was investigated using a simple method for the quantitative evaluation of DNA degradation. The selected nanoparticles were magnetite (Fe3O4), silica, citrate, poly (acrylic acid) and polyethylenimine coated magnetite (Fe3O4@SiO2, Fe3O4@Citratete, Fe3O4@PAA, Fe3O4@PEI) and two different magnetite nanocomposites with TiO2 and ZnO. The obtained results highlight the need for caution during the use and disposal of these manufactured nanomaterials, as DNA oxidation effects may be observed at very low concentrations.

1. DNA solution
2. DNA-Nanoparticles incubation
3. Electrophoresis
4. DNA degradation (%)

Introduction
In the last decade, research has increased with the application of nanomaterials to the treatment of industrial wastewater using different advanced oxidation processes (AOPs) such as heterogeneous oxidation of Fenton or heterogeneous photocatalysis [1,2]. In addition, the use of iron oxide-based magnetic nanoparticles as solid catalysts presents great advantages as unique magnetic properties that allow easy recovery and reuse of the nanocatalyst when applying a magnetic field. However, these heterogeneous processes must ensure the complete retention of nanoparticles to ensure their use in long-term processes and also to avoid possible adverse effects on the environment.

Nanomaterials with a specific DNA solution to estimate the pro-oxidant effects of these nanoparticles.

Methods
Deoxyribonucleic acid (DNA) from calf thymus (Sigma, Germany) at 0.025 mg mL−1 sterile water solution was prepared and stored at 4 °C up to seven days. This DNA solution was incubated in the presence of different concentrations of the selected magnetic nanoparticles (1-0.0125 g L−1). After 1 h at 37°C, an electrophoresis assay was run. A positive control (without nanoparticles) was carried out for each case.

Samples of DNA-nanoparticles solution (10 μL) were mixed with loading buffer (1:4; 25 mg bromophenol blue, 10 mL Tris EDTA 1x buffer and 20 mL of glycerol; pH 8) and the electrophoresis test was performed in a 0.75% (w v−1) agarose gel (Ntech, Portugal) prepared with Tris-Acetate EDTA buffer (TAE; Grisp, Portugal) and 0.03 μL mL−1 of GreenSafe Premium (Ntech, Portugal). The test was run for 1.25 h at 150 mV in PowerPac Universal (BioRad, USA). The resulting DNA bands were viewed using a molecular imager GelDOC XR+ (BioRad, USA) and analysed with Image Lab™ Software v5.1.

The results were expressed as the percentage of inhibition of DNA, calculated as follows,

\[\text{Intensity}_{\text{sample}} \times 100 \text{Intensity}_{\text{DNA control}}\]

Where \(\text{Intensity}_{\text{sample}}\) is the intensity of the band area of each sample and \(\text{Intensity}_{\text{DNA solution}}\) is the manually measured intensity of DNA solution control band. To ensure the reproducibility of the assay, duplicate of sample-DNA solution was performed for each nanoparticle sample. Also, duplicate of electrophoresis band sample was carried out, obtaining four replicates for each evaluated sample.

Results
The main characteristics of the selected nanoparticles: magnetite (Fe3O4), silica, citrate, poly (acrylic acid) and polyethylenimine coated magnetite (Fe3O4@SiO2, Fe3O4@Citratete, Fe3O4@PAA, Fe3O4@PEI) and two different magnetite nanocomposites with TiO2 and ZnO are shown in Table 1.

The results of the DNA degradation after the incubation in presence of Fe3O4 nanoparticles (1-0.0125 g L−1) are displayed in Figure 1. The obtained results indicated that low concentrations (0.05-0.0125 g L−1) of magnetite nanoparticles present a negative effect on DNA stability.

![Figure 1. DNA degradation effect of Fe3O4 nanoparticles with concentration between 1 and 0.0125 g L−1.](image)

In the case of magnetite coated with different stabilizers, a similar effect was observed. The higher degradation percentages were obtained in the case of citrate coating with 7.2% of DNA degradation for a nanoparticle concentration of 0.0125 g L−1.
In regard to the magnetic nanophotocatalysts (Fe₃O₄/TiO₂ and Fe₃O₄/ZnO), DNA damage alterations were observed not only at low concentrations (0.05-0.0125 g L⁻¹), but also at intermediate concentrations (0.6-0.1 g L⁻¹), reaching DNA degradation values of 17.8% for 0.2 g L⁻¹ of Fe₃O₄/TiO₂. Therefore, a nanoparticle concentration effect on the increase of the DNA degradation was detected.

**Conclusion**

This work contributes to the understanding of the potential effects of the iron-based nanoparticles disposal may have on the environment, through the genotoxicity study of different nanoparticles in a wide range of concentrations. Therefore, this study underscores the importance of further research on the mechanisms and factors that increase toxicity to enhance nanomaterials use and disposal awareness.

**Table 1. Characteristics of nanoparticles under study.**

<table>
<thead>
<tr>
<th>Nanoparticles</th>
<th>Characteristics</th>
<th>Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe₃O₄</td>
<td>Magnetite magnetic nanoparticles</td>
<td>9.4 ± 1.4</td>
</tr>
<tr>
<td>Fe₃O₄@SiO₂</td>
<td>Silica coated magnetite nanoparticles</td>
<td>21.5 ± 1.3</td>
</tr>
<tr>
<td>Fe₃O₄@PAA</td>
<td>Magnetite stabilized with Poly (acrylic acid)</td>
<td>10.1 ± 2.4</td>
</tr>
<tr>
<td>Fe₃O₄@Citrate</td>
<td>Citrate coated magnetite nanoparticles</td>
<td>10.9 ± 3.9</td>
</tr>
<tr>
<td>Fe₃O₄@PEI</td>
<td>Polyethyleneimine (25 kDa) coated magnetite nanoparticles</td>
<td>8.5 ± 2.3</td>
</tr>
<tr>
<td>Fe₃O₄@TiO₂</td>
<td>Titanium dioxide magnetic nanocomposite</td>
<td>9-10</td>
</tr>
<tr>
<td>Fe₃O₄@ZnO</td>
<td>Zinc oxide magnetic nanocomposite</td>
<td>15-30</td>
</tr>
</tbody>
</table>

**Acknowledgements**

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**References**

Oral Session

BIOREFINERY AND SUSTAINABILITY
Production of ethanol from *Pinus pinaster* stump wood extracted pulp and lignin recovery

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*Pinus pinaster* stump wood was processed by soda (SA) and alkaline organosolv (AOS) pulppings using different operating conditions. Black liquors of soda and organosolv cooking were acidified in order to precipitate dissolved lignins. Both pulps and precipitated lignins were characterized in terms of hemicelluloses, cellulose and lignin contents. The purities of precipitated lignins were similar in AOS cookings (around 95% vs. 90%)

### Materials and methods

PPSW was milled into 1 mm particles, and fractioned by SA and AOS cookings, as schematically described in graphical abstract. All cookings were performed at 170 °C in a 1 L agitated autoclave reactor using 50 g (dry weight) of PPSW. In SA cookings, the PPSW was processed for 120 min using a wood/solvent ratio (W/S) of 1/10 and 50 wt.% of NaOH (based in biomass dry weight) as catalyst. All AOS cookings were performed using a W/S of 1/8. Initially, the PPSW was cooked for 120 min, in a 35 wt.% ethanol/water solution and 40 wt.% NaOH (AOS1). In the following cooking, the amount of NaOH charge was firstly reduced to 30 wt.% (AOS2), and then the reaction time was reduced to 90 min (AOS3). No matter the cooking process used, the whole final liquid-solid mixtures were processed in the same way. After each experiment, the liquid-solid mixture was vacuum filtered to separate the solid fraction (pulp) from the liquid (liquor). The so obtained pulp was washed with distilled water. The liquor was then dropwise acidified to pH = 4.5 using a H2SO4 solution (10 M), and the obtained precipitate was separated by vacuum filtration in order to collect the formed solid (mainly lignin), and rinsed with distilled water in order to remove soluble salts.

### Results and discussion

Pulp yield (Table 1) in AOS cookings showed an increasing tendency when the cooking severity was reduced (AOS1 > AOS2 > AOS3), and was always higher than the yield of SA pulping. On the other hand, lignin yield (Table 1) were higher in SA cookings. In AOS pulppings the increase of lignin yield was only perceptible from AOS2 to AOS3 cookings. Cellulose content of the AOS pulps were similar, but lower than that of SA pulp. The opposite was observed for hemicelluloses content.

Comparably lignin content (SA=5.2% vs. AOS1=5.7%), cellulose content was higher in SA pulp (SA=85.0% vs. AOS1=77.8%) mainly due to lower hemicelluloses content (SA=9.1% vs. AOS1=11.1%). Therefore, the SA process allowed to obtain a purer pulp, but with lower yield.

By comparing the AOS cookings with each other within the tested range, the results indicate that the amount of catalyst (40 wt.% vs. 30 wt.%) had higher influence in lignin removal than the cooking time (120 min vs. 90 min). The purities of precipitated lignins were similar in AOS cookings (around 95% vs. 90%).
no matter the severity of the experimental conditions, and were higher than in SA pulping. No cellulose was found in AOS precipitated lignins, and its content in SA lignin was also very low (0.3%). Moreover, the hemicelluloses contents of the precipitated lignins were also very low in AOS1 (1.7%) and absent when the pulping severity was reduced (AOS2 and AOS3). Furthermore, the hemicelluloses contents of SA lignins were also low (2.5%). These results indicate that the operating conditions used in the pulping experiments were effective in the separation of these two wood components. The pulps obtained from the pine stump treatments were efficiently converted to second generation ethanol, as presented in Table 2. For the same pulp consistency (10%), different initial carbohydrates concentrations (98-104 g/L monosaccharides) were available for enzymatic saccharification and ethanol production, thus leading to different ethanol concentrations (all higher than 40 g/L). Maximum ethanol concentrations were produced between 24 and 48 h. Carbohydrate-to-ethanol conversion yields above 82% were obtained, in contrast with the results presented by [1], using different pretreated pitch pine pulps (organosolv with catalyst), but the same pulp consistency (maximum ethanol yield of 70%). These results confirm the significant role of the selected catalyst and its concentration in organosolv-based treatments. The differences in ethanol yields obtained in the SSF was attributed to the different lignin content in pulp samples (Table 1). SA and AOS1 pulps, with similar lignin content, were converted to ethanol with comparable yields. A decreasing ethanol yield (from 88.1 to 82.8%) was observed between AOS pulps with lignin content increase (from 5.7 to 8.1%).

Table 2. SSF efficiency.

<table>
<thead>
<tr>
<th>Pulp</th>
<th>[EtOH]_{max} (g/L)</th>
<th>E_{EtOH} (g/(L h))</th>
<th>Y_{EtOH} (g/g)</th>
<th>Y_{EtOH} (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SA</td>
<td>47.0</td>
<td>1.00</td>
<td>0.454</td>
<td>89.1</td>
</tr>
<tr>
<td>AOS1</td>
<td>43.9</td>
<td>1.66</td>
<td>0.449</td>
<td>88.1</td>
</tr>
<tr>
<td>AOS2</td>
<td>42.2</td>
<td>1.58</td>
<td>0.419</td>
<td>82.2</td>
</tr>
<tr>
<td>AOS3</td>
<td>41.9</td>
<td>1.56</td>
<td>0.422</td>
<td>82.8</td>
</tr>
</tbody>
</table>

(a) Percentage of theoretical yield (0.51 g/g)

Conclusions

The cellulose content of the AOS pulps were similar, but lower than the SA pulp, whereas the hemicelluloses content was higher for AOS pulps. Globally, the SA cooking allowed to obtain purer pulps, but with lower yield than in AOS pulping. For similar pulp lignin contents, the cellulose content in SA pulp was higher, mainly due to hemicelluloses removal. Lignin yield was higher from SA liquor than from AOS liquor, while AOS cookings allowed to obtain precipitated lignin having higher purities. Second generation ethanol was produced from SA and AOS pulps by SSF with high yields (above 82%) and productivity. A decrease in ethanol yield with lignin content increase in the pulp sample was observed.

Table 1 – Pulps and lignins characterization (P. = pulp, L. = Lignin).

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Fraction</th>
<th>P. Yield (a)</th>
<th>L. Yield (a)</th>
<th>Composition (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>T. Lignin</td>
<td>Cellulose</td>
<td>Hemicelluloses</td>
</tr>
<tr>
<td>None</td>
<td>Wood</td>
<td>-</td>
<td>-</td>
<td>29.2</td>
</tr>
<tr>
<td>SA</td>
<td>Pulp</td>
<td>38.1</td>
<td>-</td>
<td>5.1</td>
</tr>
<tr>
<td></td>
<td>Lignin</td>
<td>-</td>
<td>-</td>
<td>88.4</td>
</tr>
<tr>
<td>AOS1</td>
<td>Pulp</td>
<td>42.1</td>
<td>-</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>Lignin</td>
<td>-</td>
<td>-</td>
<td>94.9</td>
</tr>
<tr>
<td>AOS2</td>
<td>Pulp</td>
<td>46.8</td>
<td>-</td>
<td>7.2</td>
</tr>
<tr>
<td></td>
<td>Lignin</td>
<td>-</td>
<td>-</td>
<td>95.8</td>
</tr>
<tr>
<td>AOS3</td>
<td>Pulp</td>
<td>48.2</td>
<td>-</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>Lignin</td>
<td>-</td>
<td>-</td>
<td>95.6</td>
</tr>
</tbody>
</table>

(a) Calculated from the total lignin content of wood.

Acknowledgements

This work was funded by the Portuguese Foundation for Science and Technology (FCT) under the scope of the MultiBiorefinery project (POCI-01-0145-FEDER-016403).

References

Toward the improved sustainability of bioplastics: process modelling and life cycle assessment of an FDCA production route

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Leading research trends nowadays are geared towards the production of alternatives that compete with fossil-based materials. Issues like the increase of plastic waste or the finite nature of oil make it imperative to study sustainable production routes. Process modelling with Aspen Plus® has allowed to assess the production of 2,5-furandicarboxilic acid (FDCA) from lignocellulosic 5-hydroxymethyl furfural (HMF). FDCA is the precursor of bioplastics such as polyethylene furanoate, which has a great potential to be a direct competitor of polyethylene terephthalate. This study has been focused on the modelling of FDCA synthesis in a catalytic reactor with two different downstream options: A) crystallization, B) distillation. When dealing with renewable feedstocks, one of the most relevant challenges is to guarantee their sustainability. Thus, life cycle assessment has allowed to pinpoint the processing areas with most environmental burden: the production of HMF and the energy consumption.

Introduction

The need to tackle plastic waste, the finite nature of oil or the global warming issue make it imperative to study more sustainable production routes. The European union in the Horizon 2020 initiative has urged to achieve some environmental improvement objectives to drastically reduce the amount of resources used for production, the emissions to the environment which promote climate change and other closely related issues [1,2].

This can be addressed through strategies based on the research on sustainable alternatives such as the exploitation of lignocellulosic wastes. Lignocellulosic matter, to the contrary of crops, does not compete with food and feed markets with the aim of producing biofuels, bioenergy and biomaterials. Woody biomass does not spark the issue of land use change and it provides a useful alternative to the management of, for instance, wood and crop waste from sawmills and cropping activities, enhancing circular economy measures [3]. Today’s green economy initiatives, although still in their infancy, will probably be the foundation of the consumption trends of future generations.

Objectives

This study aims to provide an overview of one of the most relevant biochemicals with high potentiality to substitute fossil-based products: FDCA [4]. Due to its novelty, production routes from lignocellulosic HMF are not considered to be completely optimized. Process modelling and life cycle assessment (LCA) are tools that allow to detect technical and environmental hotspots of processes in early developmental stages. Their application in this study has aimed to provide useful insight on the next steps towards the realistic implementation approaches of industrial FDCA production.

Methods

Process modelling through the commercial software Aspen Plus® has allowed to provide data for the LCA methodology implementation [5]. LCA is a reliable tool to determine environmental hotspots of a process or a product, which goes in line with the early determination of feasible optimization routes. LCA methodology parting from the inventory tables retrieved from the simulation (Table 1 and Table 2) considers all inputs and outputs as well as emissions from cradle to gate perspective of the modelled FDCA production process. The process flow diagram, system and subsystem boundaries for the study can be seen in the graphical abstract. The functional unit for the study was the production of 1 kg/h of FDCA through the two alternative scenarios: A) in which the downstream process was based on a crystallization unit and B) in which a distillation train was implemented.

Table 1. Inventory for the production of 1 kg/h of FDCA from HMF through the processing steps of scenario A (purification through crystallization).

<table>
<thead>
<tr>
<th><strong>SS1. Catalytic reaction</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inputs</strong></td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>HMF</td>
</tr>
<tr>
<td>PtZrO₂ catalyst</td>
</tr>
<tr>
<td>P-1 electricity</td>
</tr>
<tr>
<td>R-1 heating</td>
</tr>
<tr>
<td>C-1 electricity</td>
</tr>
<tr>
<td>C-1 cooling</td>
</tr>
<tr>
<td>E-1 heating</td>
</tr>
<tr>
<td><strong>Outputs</strong></td>
</tr>
<tr>
<td>E-2 cooling</td>
</tr>
<tr>
<td>P-2 electricity</td>
</tr>
<tr>
<td><strong>Emissions to air</strong></td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>Nitrogen</td>
</tr>
<tr>
<td>Oxygen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SS2. Flash separation</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inputs</strong></td>
</tr>
<tr>
<td>E-2 cooling</td>
</tr>
<tr>
<td>P-2 electricity</td>
</tr>
<tr>
<td><strong>Outputs</strong></td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>Nitrogen</td>
</tr>
<tr>
<td>Oxygen</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>SS3. Crystallization</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inputs</strong></td>
</tr>
<tr>
<td>CR-1 cooling</td>
</tr>
<tr>
<td>FDCA</td>
</tr>
<tr>
<td><strong>Outputs</strong></td>
</tr>
<tr>
<td>Wastewater</td>
</tr>
</tbody>
</table>
were: climate change (CC), ozone depletion (OD), terrestrial acidification (TA), freshwater eutrophication (FE), marine eutrophication (ME), human toxicity (HT), photochemical oxidant formation (POF), freshwater ecotoxicity (FET), marine ecotoxicity (MET) and fossil depletion (FD).

Table 2. Inventory for the production of 1 kg/h of FDCA from HMF through the processing steps of scenario B (purification through distillation).

<table>
<thead>
<tr>
<th>SS1. Catalytic reaction</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inputs</strong></td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>HMF</td>
</tr>
<tr>
<td>PtZrOx catalyst</td>
</tr>
<tr>
<td>P-1 electricity</td>
</tr>
<tr>
<td>R-1 cooling</td>
</tr>
<tr>
<td>C-1 electricity</td>
</tr>
<tr>
<td>C-1 cooling</td>
</tr>
<tr>
<td>E-1 heating</td>
</tr>
<tr>
<td><strong>Emissions to air</strong></td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>Nitrogen</td>
</tr>
<tr>
<td>Oxygen</td>
</tr>
<tr>
<td>Wastewater</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SS2. Flash separation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inputs</strong></td>
</tr>
<tr>
<td>F-1 heating</td>
</tr>
<tr>
<td>F-2 cooling</td>
</tr>
<tr>
<td><strong>Outputs</strong></td>
</tr>
<tr>
<td>Emissions to air</td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>Nitrogen</td>
</tr>
<tr>
<td>Oxygen</td>
</tr>
<tr>
<td>Wastewater</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SS3. Distillation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inputs</strong></td>
</tr>
<tr>
<td>D-1 heating</td>
</tr>
<tr>
<td>D-1 cooling</td>
</tr>
<tr>
<td>P-2 electricity</td>
</tr>
<tr>
<td><strong>Outputs</strong></td>
</tr>
<tr>
<td>FDCA</td>
</tr>
<tr>
<td>Wastewater</td>
</tr>
<tr>
<td><strong>Emissions to air</strong></td>
</tr>
<tr>
<td>Water</td>
</tr>
<tr>
<td>Acetic acid</td>
</tr>
<tr>
<td>Nitrogen</td>
</tr>
<tr>
<td>Oxygen</td>
</tr>
</tbody>
</table>

**Results**

Simulation results provide a series of optimized material and energy streams according to the simplified process diagram shown in the graphical abstract. The implementation of inventories in the SimaPro software provides the environmental profiles shown in figures 1 and 2 for downstream options A and B respectively. The comparative graph of overall environmental results is displayed in figure 3. Results show that the downstream option for FDCA recovery through purification is less environmentally burdening due to the greater recycling of HMF. HMF was found to be one of the more relevant hotspots of the process due to the considered route of production, which included the use of non-environmentally friendly solvents.

![Figure 1. Process relative contributions (in %) per subsystem to obtain 1 kg/h of FDCA (Scenario A: Crystallization).](image1)

![Figure 2. Process relative contributions (in %) per subsystem to obtain 1 kg/h of FDCA (Scenario B: Distillation).](image2)

![Figure 3. Comparative environmental profiles (in %) for Scenarios A and B.](image3)

**Conclusions**

The study of production routes in the scope of the biorefinery concept has been proven to have the potential to provide useful solutions for a sustainable future. Further process optimization should be considered to minimize energy requirements of the studied system. The use of HMF as feedstock has been found to be a great bottleneck of the process environmentally. Further research should focus on finding alternative routes to produce HMF with higher yields and lower environmental burdens.

**Acknowledgements**

This research was supported by EnzOx2 BBI JU-Project. The authors belong to the Galician Competitive Research Group GRC ED431C 2017/29 and to the CRETUS Strategic Partnership (AGRUP2015/02). All these programs are co-funded by FEDER (EU).

**References**

The aim of this work was to evaluate the starch accumulation in the microalga *Chlorella sorokiniana* and process its whole biomass in a biorefinery approach. Nitrogen starvation triggered starch accumulation in *C. sorokiniana*, resulting in a maximum starch content of 26% (w/w). The starch-rich biomass was harvested by sedimentation and the fresh cells were subjected to a treatment with ethanol in order to extract pigments prior to the hydrolysis step. After the extraction, the ethanol impregnated cells were more easily dried than the fresh cells, reducing the energy requirements of the drying step. Extracted dried cells were then milled for cell wall disruption, resulting in a biomass highly susceptible to hydrolysis with amylases, with glucose yields higher than 90% within 4 hours of processing. The resulting hydrolyzed biomass is rich in proteins and lipids and could be used for human or animal nutrition.

**Introduction**

Microalgae research has been growing in number and importance in the last decades. Besides high added value products, such as carotenoids, polyunsaturated fatty acids and proteins, microalgae are being studied for chemicals and biofuels production, such as biodiesel [1] and bioethanol [2]. Therefore, microalgae can be part of the solution for two current issues: finding new renewable feedstocks for substituting petroleum and providing quality food for a growing population.

Cultivation time and conditions are essential for dictating microalgal composition, which can be tuned to contain high amounts of starch as a carbon storage molecule [3]. The intracellular starch can be hydrolyzed by amylases to give a glucose syrup for use in several fermentation processes, yielding building blocks such as ethanol, 1,3-propanediol and succinic acid that can be turned into several other chemicals [4]. After starch hydrolysis, the remaining fraction is rich in proteins and nutraceutical lipids such as PUFAs, which could be used for human nutrition [5]. Moreover, pigments, such as chlorophylls and carotenoids, can be extracted and used in the food and cosmetics industry as antioxidants with several health benefits, improving the revenue of the microalgae-base biorefinery [6].

Major bottlenecks for microalgal cultivation and processing are the harvesting and drying steps, which are usually expensive and energy intensive [7]. Therefore, the aim of this work was to evaluate the starch accumulation in the green microalga *Chlorella sorokiniana* and process its whole biomass in a biorefinery context using green technologies and addressing the costliest steps in order to achieve a more economically attractive process.

**Materials and Methods**

*Chlorella sorokiniana* was grown in Bold's Basal Medium in an orbital shaker at 225 rpm, 30 °C and 100 μmol.m⁻².s⁻¹ for 20 days for starch accumulation. For the hydrolysis assays, an enzyme preparation rich in amylases was produced with the fungus *Aspergillus awamori*. Hydrolysis assays were conducted initially at 50 °C, pH 4.8 and 1% (m/m) solids loading in Eppendorf® tubes containing 1.25 mL of the hydrolysis mixture (enzymes, buffer and microalgal cells); microalgal cells were used freeze-dried with or without a previous milling treatment. Temperature and pH values were studied to maximize amylase activity and the new condition of 60 °C and pH 4.0 was then used in hydrolysis experiments with solids loading varying from 5% to 30% (m/m) in flasks containing 12.5 mL of hydrolysis mixture. Glucose released from the hydrolysis experiments was analyzed using a biochemical analyzer (YSI 2700). Sedimentation experiments were carried out with cells with low and high starch contents for comparison of the sedimentation velocity. Pigment extraction experiments were performed with freeze-dried and milled cells before or after the hydrolysis step and with fresh cells with methanol or ethanol as extraction solvents.

**Results and discussion**

*C. sorokiniana* was initially cultivated for different time periods in order to achieve high starch accumulation, reaching a 26% (w/w) dry weight content of intracellular starch and a dry weight of 0.7 g/L after nitrogen starvation. This starch-rich biomass had greater cell size than cells with low starch content, which allowed its harvesting by sedimentation, a low-energy process.

High pigment loss was observed in the hydrolyzed microalgal cells, with a reduction of 50% in the chlorophyll content and 80% in the carotenoids content when extracted with methanol. Therefore, cells were subjected to the pigment extraction process prior to the hydrolysis step. The extraction was performed in fresh whole cells with ethanol and the results were similar to the ones achieved with dry milled cells when methanol was used as solvent, making it possible to eliminate the cell lysis step and to change the solvent to a green one. Pigment extraction yielded, on a dry biomass basis, 2.5% (w/w) of chlorophyll a, 1.0% (w/w) of chlorophyll b and 0.5% (w/w) of carotenoids, as shown in Figure 1. After the extraction, the ethanol impregnated cells were more easily dried than the fresh cells, reducing energy requirements of the drying step.

Extracted dried cells were then subjected to enzymatic hydrolysis with an enzyme pool rich in amylases. The cell wall proved to be an obstacle for the enzymatic hydrolysis of the intracellular starch, making it necessary to disrupt this structure. After cell wall disruption, the starch was easily hydrolyzed with the sole use of amylases, with yields of 75% after 4 hours of hydrolysis. It was possible to increase the final...
glucose yield achieved by the same amount of enzyme, after 4 hours of hydrolysis, from 75% to 94% just by the adjustment of the temperature and pH of the enzymatic hydrolysis process. The increase in solids loading in the hydrolysis process is important, since this will lead to lower water consumption, smaller reactors and a more concentrated product stream. However, increasing solids loading may lead to loss in hydrolysis yields due to problems in homogenization and mass transfer [8]. The hydrolysis of dried and milled Chlorella sorokiniana cells were evaluated with solids loading from 5 to 30 % (m/m) and the results are shown in Figure 2.

![Figure 1. Pigment extraction from Chlorella sorokiniana.](image)

The hydrolysis yield decreased 24% when the solids loading increased from 5 to 25%. Nevertheless, the glucose concentration was increased by 350%, indicating the potential of high solids loading processing. A further increase in the solids loading, to 30%, resulted in a thick mixture with poor flowing properties, leading to a further reduction of the glucose yield to 63%. However, in a scaled-up system with better mixing properties, this result could be further improved, with the possibility of reaching glucose concentrations higher than 90 g/L.

**Acknowledgements**
The authors thank CNPQ for supporting this research.

**References**
Harvesting of carotenogenic phase *Dunaliella salina* by membrane filtration at lab and pilot scale

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Introduction

*Dunaliella salina* is industrially produced due to its high content in carotenoids induced by low nitrogen and high salinity conditions (carotenogenic phase “orange” *D. salina* cells). These cells, with no cell wall, are even more sensitive to shear stress than the non-carotenogenic phase “green” *D. salina* cells. The harvesting of microalgae involves high-energy costs. The harvesting process may represent up to 20-30% of the microalgae cultivation costs [1]. The most common method of harvesting is centrifugation [2,3]. However, this technique requires high capital investment and energy costs. Membrane technology have been reported as being economically adequate for microalgae harvesting and suitable to process dilute media [4–6]. An integrated process of microalgae harvesting by membrane filtration followed by centrifugation may lead to a reduction of investment cost and energy demand [7,8].

Objectives

This work aims at pre-concentrating carotenogenic *D. salina* using membranes prior to a final concentration by centrifugation, in order to reduce energy and capital costs in comparison with harvesting solely using centrifugation. It is also aimed to maximise permeate volumetric fluxes, while maintaining cell integrity, at maximised concentration factors.

Methods

The harvesting of carotenogenic *Dunaliella salina* at lab and pilot scales was studied, using a cross-flow configuration and controlled permeate flux conditions (using the membrane set-up represented in Figure 1). Optimisation of the pre-concentration process by ultrafiltration was performed by evaluating the impact of: cross-flow velocity, permeate flux, relaxation and washing cycles as well as membrane pore size (similar with the optimisation performed in Monte et al. 2018) [8]. Additionally, controlled pressure conditions were performed similar with conditions tested in Monte et al. 2018 [8]. Furthermore, a Spiral Plate centrifuge (Evodos 50, Evodos, The Netherlands) with a hydraulic capacity of 3 m³/h, was used for an economic evaluation of the process comparing harvesting in two-steps (ultrafiltration followed by centrifugation) and in one-step (only centrifugation).

Results

The effect of several operating parameters on the pre-concentration of *Dunaliella salina* was evaluated by monitoring the permeate flux, transmembrane pressure and the cell integrity. The harvesting of *Dunaliella salina* at controlled pressure conditions was not successful. However, when using controlled permeate flux conditions the results were promising. The harvesting of *Dunaliella salina* was optimised at lab scale under controlled permeate flux conditions at 25 L/(m².h) and feed cross-flow velocity of 0.35 m/s. These operating conditions assured a cell integrity loss lower than 15% for a concentration factor of 4. At pilot scale, the controlled permeate flux was set at 21 L/(m².h) and the cross-flow velocity at 0.30 m/s for a concentration factor of 10. An economic evaluation was performed for the harvesting of carotenogenic *Dunaliella salina* at pilot scale, comparing the performance by one-step approach (only centrifuges) or by a two-step approach (integrating membrane processing and centrifuges). The total cost of ownership for the one-step approach is 2.27 €/m³, while for the two-step approach...
(integrated process with membranes and centrifuges) is 0.67 €/m$^3$. In this case, a reduction of energy consumption of 76% and a reduction of the operational and capital costs of 70% were observed (when comparing harvesting by a two-step approach with harvesting in one-step).

**Conclusion**

At pilot scale, carotenogenic *Dunaliella salina* was successfully harvested using an ultrafiltration membrane (with a molecular weight cut-off of 100 kDa) operated under controlled permeate flux followed by centrifugation. The economic evaluation performed shows clearly that harvesting of carotenogenic *Dunaliella salina* using a two-step approach is significantly more economic than harvesting in a single step by centrifugation, with a reduction of operational and capital costs of 70% and a reduction of energy consumption of 76%.

**Acknowledgements**

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**References**

Polyols: efficient solvents to extract phenolic compounds from walnut leaves

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In this work, a set of polyols was studied to extract phenolic acids and flavonoids from walnut leaves (Juglans regia), a rich source of bioactive phenolic compounds. The solvent screening included glycerol and a series of diols (1,2-ethanediol, 1,2-propanediol, 1,3-propanediol, 1,3-butanediol, 1,2-pentanediol, 1,5-pentanediol, 1,2-hexanediol, 1,6-hexanediol). The extraction yield was quantified and the bioactivity of the extracts was evaluated by measuring the antioxidant, cytotoxic and anti-inflammatory activities. The best combined results were obtained with 1,2-propanediol and 1,3-propanediol. The extraction yields were similar to the results obtained with ethanol. Moreover, those extracts do not present cytotoxicity for non-tumor cells (PLP2) and reveal significantly higher antioxidant activity and cytotoxicity for a tumor cell line (HeLa) when compared to the aqueous extract. The results obtained provide relevant information regarding the use of alternative solvents that could potentially be used as formulation media in the pharmaceutical or cosmetics areas.

Introduction

Plants are an important source of natural and bioactive phytochemicals namely phenolic compounds. A significant number of studies has studied the bioactivity of different plant extracts, evaluating their antioxidant, anti-inflammatory, antiviral, analgesic, anticarcinogenic and/or antimicrobial activities, suggesting the inclusion of phenolic compounds in different preparations, namely, in the pharmaceutical, cosmetics and food areas [1]. Due to their abundance in phenolic acids, naphthoquinones, tannins, flavonoids, etc., walnut leaves stand out as an important source to be studied. Traditional medicine has already applied this matrix for both enteric and cutaneous preparations [2,3]. The use of safe and sustainable solvents with better performance than conventional volatile organic solvents is desirable. Currently, glycolic extracts (usually, 1,2-propanediol or glycerol mixed with water) are example of liquid extracts that may find direct application [2].

In this work, a set of polyols were studied as promising green solvents to recover phenolic compounds from Juglans regia L. (walnut) leaves. Some of them are already allowed to be included in food, pharmaceutical and cosmetic applications due to their biocompatibility. The antioxidant activity of the extracts was evaluated as well as the cytotoxic (in tumor and non-tumor cells) and anti-inflammatory potential of each extract and pure solvent.

Methods

The selected solvents include 1,2-ethanediol, 1,2- and 1,3-propanediol, 1,3-butanediol, 1,2- and 1,5-pentanediol, 1,2-hexanediol and glycerol and were used with a certain amount of water (20 % wt). The conventional water and ethanol solvents were also studied for comparison purposes [3]. All the extractions were carried out under the following conditions: 50 °C (extraction temperature), 2 h (extraction time), 600 rpm (stirring rate), and solid-liquid ratio of 30 mg/mL.

The extraction yield of each solvent was evaluated according to the HPLC-DAD results at 280 nm (phenolic acids) and 370 nm (quercetin derivatives) for the four most abundant phenolic compounds: 3-O-caffeoylquinic acid, p-coumaric acid, quercetin 3-O-glucoside and quercetin O-pentoside.

The antioxidant activity of the extracts was evaluated through the Oxygen Radical Absorbance Capacity (ORAC) assay. The biocompatibility of the solvents and extracts was assessed by anti-inflammatory (inhibition of LPS-induced NO production by Murine macrophages) and cytotoxic (inhibition of cell growth in a human cervical carcinoma cell line- HeLa and in non-tumor porcine liver primary cells- PLP2) assays.

Results

Figure 1 presents the extraction yields (mg/g dry plant) obtained for all the studied solvents.

![Extraction yield of the main phenolic compounds present in the extracts of walnut leaves.](image-url)
As can be seen in Figure 1, the highest extraction yield was obtained using 1,2-ethanediol (27.6 ± 0.2 mg/g dry plant). This solvent has higher extraction capacity compared to water (12.8 ± 0.2 mg/g dry plant) and ethanol (25.1 ± 0.1 mg/g dry plant). However, the 1,2-ethanediol extract has shown to be toxic for the tested cell lines. The cytotoxicity of all the extracts of walnut leaves, evaluated in tumor (HeLa) and non-tumor (PLP2) cells, are shown in Figure 2 (A and B).

The second best set of solvents are the selected propanediols with an extraction performance very close to that of ethanol: 24.6 ± 0.4 mg/g dry plant for 1,2-propanediol and 24.8 ± 0.2 mg/g dry plant for 1,3-propanediol. Both solvents and extracts show no cytotoxicity in non-tumor cells (GI<sub>50</sub> > 400 µg/mL) and similar cytotoxicity in HeLa tumor cell line, comparable to the ethanolic extract. 1,3-Butanediol achieves a lower extraction yield but, even so, close to the propanediols. On the other hand, the heavier diols (pentanediols and hexanediol) as solvents already present cytotoxicity in PLP2 even when very diluted, limiting its range of application.

Furthermore, none of the extracts exhibits anti-inflammatory activity, neither the solvent interferes in the study regarding the NO formation inhibition (IC<sub>50</sub> > 400 µg/mL or 4% of solvent). Finally, the antioxidant activity results obtained by the ORAC method are also shown in Figure 2 (C). As can be seen, the extract obtained by using 1,3-propanediol aqueous solution exhibits the highest antioxidant activity (66 ± 9 nM F/mL extract), being the aqueous extract the least active one (3.4 ± 0.4 nM F/mL extract).

The combination of the bioactivity results and the extraction yields suggests the use of the extracts of 1,2-propanediol and 1,3-propanediol as a potential source of bioactive compounds.

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**References**

Supercritical CO₂ extraction of *Eucalyptus globulus* bark: Techno-economic optimization of the industrial process

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This work aimed the design and simulation of an industrial scale process of supercritical fluid extraction (SFE) of *E. globulus* bark, as well as the techno-economic optimization for different process responses: Total Yield, Cost of Manufacturing (COM), Productivity and Process Energy. An extensive matrix of SFE datapoints was prepared, including new simulated extraction curves produced using Broken plus Intact Cells modeling. Then, an experimental optimization was applied to five operating conditions: pressure (120 – 200 bar), temperature (40 – 60 °C), cosolvent (ethanol) content (0 – 5 wt.%), solvent (CO₂) flow rate (6 – 12 g min⁻¹) and extraction time (1, 3 and 5 h). The optimal results scored 1.15 wt.% for Total Yield, 28.1 € kg⁻¹ of extraction for COM, 364 ton⁻¹ of extraction year⁻¹ for Productivity, and 0.436 GJ kg⁻¹ of extraction for Process Energy. The present study provides strong techno-economic arguments towards the investment in SFE technology for the valorization of *E. globulus* bark.

*Eucalyptus globulus* is an important tree species for the Portuguese, Spanish, Brazilian and Chinese economies, being abundantly employed in the production of paper. The underlying activity generates considerable amounts of byproducts that end up being burned for energetic purposes only. Under the scope of biorefinery, an extended research line has been developed in the last years at the University of Aveiro, devoted to the supercritical fluid extraction (SFE) of high added-value molecules known as triterpenic acids (TTAs) present in the bark of *E. globulus*. So far, the research pathway has covered studies of chemical identification [1], extracts characterization [2], preliminary SFE of TTAs [3], equilibrium and kinetics of the process [4], experimental optimization [5], phenomenological modeling and selection of scale-up criteria [6], and scale-up assays [7]. As the most recent stage of this research line, the present work aimed to design and simulate the industrial scale process of SFE of *E. globulus* bark, as well as to perform the techno-economic optimization for different process responses.

At first, an extensive matrix of SFE datapoints was prepared either by compiling existing data from the literature [5–7] or by generating new extraction curves using Broken plus Intact Cells (BIC) modeling [8], with the final goal of completing a Design of Experiments (DoE) plan of 48 runs. The latter comprised a study of 5 different factors: pressure (120 – 200 bar), temperature (40 – 60 °C), cosolvent (ethanol) content (0 – 5 wt.%), solvent (CO₂) flow rate (6 – 12 g min⁻¹) and extraction time (1, 3 and 5 h). In turn, these operating conditions were optimized by applying the Response Surface Methodology (RSM) to the following responses: Total Yield (wt.%), Productivity (ton⁻¹ of extraction year⁻¹), Cost of Manufacturing (COM, € kg⁻¹ of extraction) [9], and Process Energy (GJ kg⁻¹ of extraction). For this, the industrial process was designed and scaled according to the amount of bark produced from a local pulp and paper mill, and was fully simulated using Aspen Plus® software. A novel aspect of the process simulation work was related to the use of ethanol as cosolvent, opening the way to track cost implications in terms of recovery and reuse in the SFE process to minimize the material losses and increase the sustainability of the process.

The modeling of existing SFE curves was successfully accomplished with an average deviation (AAD) of 7.01 %, opening the way to the subsequent simulation of 7 new SFE curves at different CO₂ flow rates aided by empirical correlations involving Sherwood (Sh), Reynolds (Re) and Schmidt (Sc), like $Sh = a Re^β Sc^γ$.

The optimum values for each response are shown in Figure 1. The respective conditions were similar in terms of pressure, 200 bar (with the exception of Process Energy, 120 bar), temperature, 40 °C, and cosolvent content, 5 wt.%. As for the other two factors, solvent flow rate varied between 6 g min⁻¹ for optimal Process Energy and 12 g min⁻¹ for both Productivity and Total Yield. Lastly, the optimum extraction time scored 5.0 h for Total Yield, 3.0 h for COM, 2.6 h for Productivity and 1.0 h for Process Energy.

![Figure 1. Optimum values of the responses under study: Total Yield (wt.%), COM (€ kg⁻¹ of extraction), Process Energy (GJ kg⁻¹ of extraction) and Productivity (ton⁻¹ of extraction year⁻¹) (clockwise from top left).](image)

Concerning the main effects affecting the responses, the Total Yield presented four main effects (extraction time, pressure, cosolvent content and solvent flow rate) affecting the response positively. Similarly, Productivity denoted cosolvent content, pressure and solvent flow rate as the main
positive effects, with the time effect standing out as a complex trade-off between the extraction rate (under different BIC model extraction periods) and the total number of reload cycles (non-productive) accumulated during a year of operation. In general, these two responses reflected the following trends: (i) the increase of CO$_2$ solvent power under higher pressures; (ii) the higher extract solubility when employing ethanol as cosolvent; and (iii) the decrease of external mass transfer limitations with higher solvent flow rates.

The COM response revealed pressure to be the main positive effect while cosolvent content, although significant, had much lower impact. Finally, Process Energy response showed the same positive effects as COM, but with solvent flow rate standing as the second most important one and with a negative effect. Throughout all responses, the increase of temperature presented a constant and important negative effect, related to the decrease in the CO$_2$ solvent power and the higher energy necessary. Globally, one can conclude that, given the different factors that can be changed as operating conditions, there is great and versatile margin for improving the performance of this SFE process. As a result, the presented study provides strong techno-economic arguments towards the investment in SFE technology for the valorization of $E. globulus$ bark, specially, if integrating the proposed process in existing industrial facilities that already handle this vegetal part.

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References
Aqueous solutions of surface-active agents on the recovery of violacein from Yarrowia lipolytica cells

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The focus of this work is to optimize the extraction and purification of a purple-blue pigment - violacein, with appealing biological activities. With this aim, a large array of tensioactive agents was tested in solid-liquid extraction processes. Furthermore, some operational conditions, such as the solid-liquid ratio, the effect of consecutive extractions, the time of extraction and the concentration of the solvent were evaluated. Once the optimized extract was attained, the application of purification systems was pursued. Aqueous biphasic systems (ABS) composed of surfactant and cholinium-based ionic liquids (ILs) were characterized and applied in the purification of violacein from the remaining cellular contaminants, namely proteins. Thus, the development of an integrated process for the recovery of this biomolecule was achieved with success and in a sustainable way.

Introduction
Violacein is a natural purple-blue hydrophobic pigment[1] that, in addition to fulfill one of the current priorities linked to sustainability, it is also associated to very interesting biological activities such as antitumoral, anti-parasitic, antifungal, antiviral, antiprotozoal, antioxidant, antiulcerogenic and immunomodulatory.[2] Violacein is produced intracellularly. Thus, it is of utmost importance the integration of a cell wall disruption step for the extraction, i.e. to release the pigment from the cells. Currently, there are several mechanical methods available to achieve intracellular biomolecules. However, pros and cons (such as, the energy consumption, compatibility, high costs, non-selective character and effects on the biological activity of the target compounds) can be argued over most of them. Non-mechanical techniques are more selective and gentler. Conventional solid-liquid extractions use volatile solvents that can contribute to increase their environmental footprint and are often associated with the poor selectivity of the extraction process.[3] Surfactants are amphiphilic molecules, that above their critical micellar concentration (CMC), aggregate in particular structures called micelles. Aqueous solutions of surfactants take advantage over other more commonly solvents used in that they require lower concentrations leading to cheaper and more sustainable processes.[3],[4] The spontaneous insertion of cations in the lipid bilayer causes the swelling of membranes and, when at concentrations above the CMC, leads to an easier cell membrane breakdown.[5] ABS are recognized as a suited technology for the recovery and purification of biomolecules.[6] ILs’ features combined with the potential benefits of ABS set out high performance extractions besides presenting other advantages like their quick phase separation, and low viscosity, favoring the mass transfer.[7] Thus, ABS create an opportunity for the recovery of violacein.

Objectives
The main objective of this work was to optimize the extraction and purification of violacein, produced by genetically modified Yarrowia lipolytica cells. Bearing this in mind, alternative solvents were proposed aiming at the use of more sustainable and less toxic approaches in both solid-liquid and liquid-liquid extractions. The effect of different classes of solvents, including surfactants, tensioactive and non-tensioactive ILs, on the yield of extraction of violacein was studied. Additionally, the effect of the ILs’ chemical structure in the cell membrane disruption and therefore, the violacein removal were also assessed. Some operational conditions such as the solid-liquid ratio, the effect of consecutive extractions, the time of extraction and the concentration of the solvent were accessed. After the optimization, the focus was directed to the purification of the biomolecule. ABS were prepared aiming to separate violacein from the proteins of the raw extract. The phase diagrams for ABS composed of surfactant - Tween 20 and five different cholinium-based ILs were characterized. These systems were then applied on the purification of violacein.

Methods
A large array of 21 different tensioactive and non-tensioactive agents, including common surfactants and ILs with different properties and chemical features, was tested. The screening was performed under fixed conditions of surfactant concentration, time of extraction, and solid-liquid ratio (SLR). The biomass was placed in contact with aqueous solutions of these solvents. After extraction, the samples were centrifuged to efficiently separate the cell debris from the aqueous solutions rich in violacein. The aqueous supernatant containing violacein was collected, and the absorption read. The yield of extraction was calculated according to equation 1:

\[
\text{Yield of Extraction} = \frac{\text{weight of pure violacein (mg)}}{\text{weight of raw cells (mg)}}
\]  

in which the direct correlation between the concentration of violacein (mg.mL⁻¹) and the absorbance (nm) was considered. The most performant tensioactive compounds were selected to evaluate the effect of the SLR, extraction time and surfactant concentration, as well as, successive solvent extractions. The ternary or quaternary phase diagrams were determined using the cloud point titration method at 278 K and atmospheric pressure. [8]

A fixed biphasic mixture point was selected to conduct studies on the violacein partition behavior. The raw extract rich in violacein was added to the ternary mixture. Each system was subjected to centrifugation to reach the complete phase separation and total partition between both phases. The phases were then separated, weighed, and the violacein content in each phase was evaluated. The partition coefficient of violacein and the main contaminant, total proteins (TP), Kviolacein and KTP, were determined according to equations 2 and 3, respectively:

\[
K_{\text{violacein}} = \frac{\text{Yield of violacein}}{\text{Yield of total proteins}}
\]

\[
K_{\text{TP}} = \frac{\text{Yield of total proteins}}{\text{Yield of total proteins}}
\]
where [Violacein] and [TP], represent the concentration of violacein and total proteins, respectively, either in the surfactant-rich and the IL-rich phases. The selectivity was also calculated according to equation 4:

$$\text{Selectivity} = \frac{K_{\text{Violacein}}}{K_{\text{TP}}}$$

(Eq. 4)

**Results and Discussion**

A screening of different aqueous solutions of tensioactive and non-tensioactive compounds was carried to find the solution that better extract violacein. It was found that only the aqueous solutions of tensioactive compounds were able to extract violacein, being the solution of surfactant A the most efficient. Besides, the operational conditions of extraction such as solid-liquid ratio, consecutive extractions, time of extraction, and solvent concentration in water were optimized. The violacein purification was accessed considering the proteins as the main contaminant. For all tested systems about 100% of the violacein content was concentrated in the surfactant A-rich phase, while a partially separation of the proteins among the two phases was observed. In the end, the integrated downstream process able to extract and purify violacein was defined, considering all the conditions optimized in both steps, extraction (i) and purification (ii).

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**References**

From a global perspective, world population growth, as well as the society modernization, have been raising concerns about environmental protection and establishing new concepts of sustainability, green chemistry and circular economy. Wastes are being increasingly recovered, transformed and used in new technologies.

The Kraft black liquor, a waste from the pulping process is normally burned to produce energy and recover chemicals. But the lignin remaining in the Kraft black liquor can be retrieved from the process and be used as additive and/or raw material for other processes [1]. Among several alternative uses, Kraft black liquor may be a phenolic precursor for phenolic resin production. For that, the liquor has to go through a polymerization reaction with formaldehyde and a catalyst [2]. There are different possibilities of use for those resins: as catalyst support, carbon precursor, electrodes, supercapacitors and adsorbents [3–5].

The adsorption process has gained increasing attention in environmental chemistry. That is due to the need of technologies for emerging micropollutants removal, such as drugs and pesticides. Since these pollutants are not biodegradable and are resistant to biological treatment, they are not completely eliminated with conventional wastewater treatment [6].

Even though drugs are nowadays found in trace-level concentrations in aquatic systems, they may cause chronic effects and ecotoxicological impacts on ecosystems [6]. Among all the drugs, amoxicillin is a widely used antibiotic [7].

In line with current research efforts in the field of new adsorbent materials, this work aims to show the potential of the renewable phenolic resin as an adsorbent for amoxicillin removal from wastewater. For comparison purposes, the adsorption process was also carried out using the adsorbent for Babassu coconut activated carbon.

In order to produce the phenolic resin, a blend of Kraft black liquor and tannin was first hydroxymethylated with formaldehyde at 70 °C and, then, crosslinked and cured at 90 °C. The resin was also subjected to a heat treatment by means of a ramp at 100 °C for 30 minutes followed by another at 170 °C for 2 hours, both at a rate of 5 °C min⁻¹. The produced resin, as well as the activated carbon, had their characteristics investigated, as shown in Table 1. Their textural analyses were conducted in a Quantachrome NOVA-1200 and their structural analyses (Figure 2) in a Bruker VERTEX 70V equipment. Finally, the pH point of zero charge were performed according to Regalbuto & Robles (2004) methodology [8].

Adsorption experiments were conducted in batch. For that, 10 mg of the adsorbent were mixed with 20 mL of 150 mg.L⁻¹ amoxicillin solution. The adsorbate-adsorbent solution was constantly agitated in an orbital shaker at 150 rpm and 25 °C. The amoxicillin concentration was measured in an UV HACH DR5000 spectrophotometer, at a wavelength of 227 nm [7] and the removal rates are illustrated on Figure 1.

Table 1: Adsorbents and adsorbate characteristics.

<table>
<thead>
<tr>
<th>PHENOLIC RESIN</th>
<th>ACTIVATED CARBON</th>
</tr>
</thead>
<tbody>
<tr>
<td>BET (m².g⁻¹)</td>
<td>5.759</td>
</tr>
<tr>
<td>VMICRO (cm³.g⁻¹)</td>
<td>0.001</td>
</tr>
<tr>
<td>VMESO (cm³.g⁻¹)</td>
<td>0.010</td>
</tr>
<tr>
<td>VTOTAL (cm³.g⁻¹)</td>
<td>0.011</td>
</tr>
<tr>
<td>ZAVERAGE (Å)</td>
<td>45.85</td>
</tr>
<tr>
<td>pH ZERO CHARGE</td>
<td>8.6</td>
</tr>
</tbody>
</table>

AMOXICILLIN

<table>
<thead>
<tr>
<th>Dimensions (Å)</th>
<th>15.622 x 18.785 x 6.645</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution pH</td>
<td>6.77</td>
</tr>
<tr>
<td>pKa</td>
<td>3.23 and 7.22</td>
</tr>
</tbody>
</table>

Textural characterization reveals the phenolic resin lower specific surface area and the predominance of mesopores. On the other hand, micropores prevail on activated carbon, assigning a specific surface area 120 times higher. The adsorption kinetic reveals a faster adsorption of amoxicillin onto phenolic resin, which occurs at higher rates and, as a result, the equilibrium is achieved sooner, at 480 min. Then,
the removal rate is higher for the phenolic resin (60%) than for the activated carbon (48%). However, the amoxicillin removal onto activated carbon increases continuously, achieving an efficiency of 77% at 1440 min. According to Figure 1, that time was not enough for achieving the equilibrium on activated carbon. The higher adsorption capacity may be due to the adsorbent higher specific surface area, characteristic of microporous material (Table 1). Moreover, amoxicillin diffusion along narrower pores contributes to the slower adsorption rate, once a resistance may persist.

Even though the phenolic resin has lower specific surface area and wider pores, its surface functional groups influence the adsorption process and the pH of zero charge [9]. The phenolic resin pH of zero charge (Table 1) is 8.6, which indicates the presence of functional groups with pKa higher than 7. This was also confirmed in the FTIR spectrum in Figure 2. FTIR analysis reveals the phenolic resin aromatic nature (1496 and 1593 cm⁻¹) and the diversified surface functional groups, which facilitates the adsorbent-adsorbate interaction and, as a result, the adsorption process. The absence of surface groups in the activated carbon is notorious in Figure 2, and Table 1 exposes the activated carbon neutral surface charge.

Figure 2: Adsorbents FTIR spectra.

All in all, the phenolic resin prepared through polycondensation reaction of a blend of Kraft black liquor and tannin was used as an adsorbent for amoxicillin removal. The material reveals a great potential for adsorption process, once it has diversified surface groups. That way, the external mass transfer involving the adsorbent surface occurs rapidly, favoring the adsorption rate in the early phase. As the material has low specific surface area and is predominantly mesoporous, the internal diffusion does not control the adsorption process and the equilibrium is fastly achieved, as the pores are around 3 times bigger than the amoxicillin dimensions. As a result, with the new resin the process will demand less time to be performed, with an adsorption capacity of 210.81 mg.g⁻¹ and an amoxicillin removal of 60%.

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References
FCC feedstocks/bio-oils co-processing: Towards understanding of phenolic compounds impact on Ni- and V-USY zeolites

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An option to produce second generation bio-fuels could consist of co-feeding hydrotreated bio-oils with traditional Fluid Catalytic Cracking (FCC) feedstocks. A key aspect of this co-processing is the presence of phenolic compounds, which have a detrimental impact on the acidic zeolites in FCC catalysts. However, metal contamination by Ni and V species in the feedstock also takes place during the process, which might influence this deactivating effect. Thus, catalytic tests were performed with a model hydrocarbon (n-heptane) and a model bio-oil derived molecule (guaiacol) at 450°C, on Ni- and V-USY zeolites. The deactivating effect of guaiacol was observed to be partially attenuated with V and fully suppressed by Ni at 2% incorporation, owing to the Lewis acidity increase upon metal impregnation, especially for Ni. As a result, guaiacol molecules will preferentially adsorb on the Lewis acid sites, protecting the Brønsted acidity responsible for the cracking reactions.

Introduction

Concerns about fossil fuels are encouraging the search for new sources of liquid fuels. From the renewable energy sources currently available, biomass is the only renewable source of carbon that can be directly converted into liquid transportation fuels. One option for the upgrading of biofuel could be the co-feeding of bio-oils (produced by pyrolysis of lignocellulosic biomass) with conventional feedstock in the FCC process of existing petroleum refineries [1-4]. A huge number of research works have been carried out, using complex mixtures of bio-oils or model compounds, and the majority agree that coke formation increases when co-processing bio-oil with petroleum feedstocks. However, this increase in coke formation has been shown to be lower than what would be expected if conversion of bio-oil was carried out in the absence of a hydrocarbon co-feed, due to the occurrence of synergetic reactions involving hydrogen from the hydrocarbon feed [4]. As the amount of heavy feedstock that is used in refining operations has been increasing worldwide and these feedstocks usually present a high amount of metals that are in part responsible for the FCC catalyst deactivation, the objective of this work is to understand the influence of nickel and vanadium on the deactivating effect of oxygenated compounds and catalytic properties of the FCC catalyst. A model compound study was conducted using guaiacol, a phenolic compound representative of hydrotreated bio-oil and n-heptane as representative of conventional hydrocarbon feedstock. The deactivating effect of guaiacol on the USHY zeolite was studied with and without metal impregnation.

Methods

The parent zeolite, ultra-stable Y zeolite, CBV500 provide by Zeolyst in the ammonium form, was impregnated with 0.5%, 1% and 2% nickel and vanadium to resemble the industrial composition of the ECAT mixture. Nickel and vanadium were introduced in the zeolite using dry (incipient wetness) impregnation and the samples were calcined under air at 500°C. Catalyst samples with and without impregnated metal, were characterized before and after catalytic tests. The zeolites acidity was measured by pyridine adsorption followed by IR spectroscopy on a Nicolet Nexus spectrometer. For structural analysis X-Ray Diffraction (XRD) powder patterns were obtained in a Bruker AXS AdvanceD8 diffractometer. Nitrogen adsorption measurements were carried out at -196°C on a Micrometrics ASAP 2000 apparatus. UV-vis spectroscopy using a Varian Cary 5000 UV/Vis/NIR spectrophotometer was performed on fresh samples impregnated with nickel and vanadium. The carbon content retained within the zeolites was analysed by total combustion with a mixture flow of helium and oxygen in a Fisons Instruments EA 1108 CHNS-O. The catalytic tests were carried out in a pyrex fixed-bed reactor at 450°C, under atmospheric pressure. The reactor feed was constituted by 10 mol. % of n-heptane (Merck, 99%) and 90 mol. % of N₂. In the poisoning tests, 1.2 wt. % of guaiacol (Sigma 99%) was injected with the reactant. The reactor effluent samples were taken for different time-on-stream (TOS) values: 1.5, 5, 10, 15, 30 and 60 min, using a 10-position valve. The tests were performed for a contact time \( (\tau = 1/\text{WHSV}) \) of 4 min, corresponding to about 200 mg of catalyst. The reaction products were on-line analysed by a SHIMADZU GC-14B gas chromatograph. The methodology and operating conditions are those previously used by I. Graça et al. [5,6].

Results

The characterization of fresh samples showed that crystallinity did not change upon nickel or vanadium impregnation, but a small decrease in the mesoporous volume and external surface area was observed for impregnated samples. A significant increase in Lewis acid sites concentration (54%) was observed for Ni samples compared to USHY zeolite, while in the case of V impregnation only a slight increase (12.6%) occurred (Table 1). The Brønsted acid sites concentration slightly decreased (about 8%) for nickel samples and remained constant in the case of vanadium samples.

<table>
<thead>
<tr>
<th>Acidity (μmol/g)</th>
<th>USHY</th>
<th>0.5% Ni</th>
<th>0.5% V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brønsted</td>
<td>816</td>
<td>744</td>
<td>796</td>
</tr>
<tr>
<td>Lewis</td>
<td>442</td>
<td>682</td>
<td>498</td>
</tr>
</tbody>
</table>

In the transformation of pure n-heptane over nickel and vanadium impregnated samples, a clear decrease in n-heptane conversion was observed for both metal-impregnated catalysts (Figure 1). The decrease was also more pronounced at increasing
metal contents, and for nickel samples (from 0.5% to 2% Ni) the reduction was more prominent. Higher carbon contents were found for deactivated nickel catalysts, which is consistent with the decrease in activity. These differences observed with Ni samples are due to the effect on the dehydrogenation reactions leading to an increase in coke and, therefore, a decrease in activity. During the cracking reaction, only nickel impregnated catalysts showed a relatively high decrease of Lewis acid sites, likely due to an interaction of nickel hydroxyls with coke molecules. From nitrogen adsorption, it was concluded that coke is mostly located in the micropores of the zeolite for all studied catalysts. Concerning selectivity, it was observed that aromatics selectivity increases slightly when USHY is impregnated with Ni or V, and an increasing importance of the occurrence of light products C1-C2 was observed in the case of Ni catalysts. Regarding the effect of guaiacol poisoning, for both nickel and vanadium samples, it was observed a decrease in n-heptane conversion when guaiacol is added to the reaction mixture, as compared to pure n-heptane. From the evolutions of n-heptane conversion before and after guaiacol addition shown in Figure 1, it can be noticed that the additional deactivation induced by the phenolic molecule is lower with increasing metal content. This effect is more pronounced for Ni than for V catalysts and can be related to the higher amount of Lewis acid sites in the nickel samples (Table 1). Additional Lewis acid sites can indeed protect the Brønsted acid sites from the poisoning of guaiacol. A slight decrease in aromatics selectivity was also observed in the case of guaiacol poisoning for both metals, likely due to a decreasing importance of hydrogen transfer and dehydrogenation reactions when guaiacol bonds to metal particles. Compared to pure n-heptane cracking, no very significant changes can be detected in the detailed product distribution when introducing guaiacol. An increase in iso-paraffin / n-paraffin ratio was seen for nickel impregnated catalyst when introducing guaiacol to the reaction mixture. This is consistent with the increase in coke deposition and Lewis acid sites concentration upon nickel impregnation, which results in more potential places for guaiacol to replace coke molecules.

![Figure 1. N-heptane conversion at 1.5 and 60 minutes time-on-stream (TOS) with and without guaiacol as a function of metal content impregnated on USHY zeolite (Ni on left and V on right).](image)

**Conclusions**

Guaiacol was less poisonous for nickel and vanadium zeolite (USHY > V-USHY > Ni-USHY), due to an increase of Lewis acid sites concentration upon metal impregnation. Guaiacol adsorbs preferentially onto Lewis acid sites, which result in relatively more Brønsted acid sites available for cracking reactions. Both nickel and vanadium induce an increase in hydrogen transfer and dehydrogenation reactions, which led to some changes in cracking products selectivity during pure n-heptane cracking. During guaiacol poisoning, no additional changes in product distribution were observed.

**Acknowledgements**

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**References**

Oral Session

REACTION AND SEPARATION PROCESS
Separation of nadolol racemates by high pH reversed-phase simulated moving bed chromatography

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Nadolol is a pharmaceutical drug marketed as a mixture of four stereoisomers, used to treat cardiovascular diseases. This drug is a mixture of two pairs of racemates, therefore, its complete separation represents a challenging task. Recently, our research group reported the pseudo-binary separation of nadolol by SMB chromatography using both coated Chiralpak AD and Chiralpak IA immobilized chiral stationary phases. In this work, we present an alternative strategy, implementing a first achiral separation step, by using C18 columns to perform the separation of the two pairs of nadolol racemates under reversed-phase mode. Extensive experimental and simulation results will be presented including solvent screening, measurement of equilibrium and kinetic data, and both fixed-bed and SMB preparative separations. Extensive experimental and simulation results will be presented, including solvent screening, measurement of equilibrium adsorption isotherms, breakthrough measurements, and SMB (FlexSMB-LSRE unit) experimental preparative separation using C18 columns.

Introduction

The main objective of this work is to improve the recent findings on the pseudo-binary separation of nadolol stereoisomers and to achieve the real multicomponent separation of all its four components. This introduces much more deep and new challenges involving optimization of the packing to be used, the solvent composition, and the strategy for defining the different separation steps and its sequences. Different separation strategies can be designed and optimized, enlarging the packing materials possibilities, from fully chiral (Chiralpak) to achiral (C18) – chiral (Chiralpak) separation combinations and, so, the used of both normal and reversed phase chromatography. For each step, the optimization of the solvent composition will be carried out, through the use of pure alcohol, alcohol-hydrocarbon and alcohol-water mixtures, all with a basic modifier (diethylamine), taking into account the strong basic nature of the nadolol stereoisomers. The separation technique to be used will also be tested, including fixed-bed, SMB and JO chromatography. The different alternatives will be evaluated in terms of the real capacity to achieve complete separation of all the four nadolol stereoisomers and in terms of system productivity and solvent consumption.

Considering the previous tasks, both modeling-simulation and experimental tools will be fully used, namely in what concerns the knowledge of the equilibrium adsorption isotherms, kinetic data (axial dispersion and resistance to mass transfer), and the prediction of fixed-bed, SMB and JO operation. This chemical engineering approach will allow the deep knowledge of all the separation processes and its optimization at preparative scale.

At the end is expected the clear definition of the best separation strategy for the complete separation of nadolol stereoisomers and the experimental availability of all the four pure stereoisomers for new pharmaceutical and therapeutical tests.

Materials and methods

The mixture of the four nadolol stereoisomers of nadolol and 1,2,3-tri-tert-butylbenzene, used as the non-retained compound, were both obtained from Sigma-Aldrich (Schnelldorf, Germany). The basic modifier diethylamine (DEA), methanol (M), acetonitrile (ACN), all HPLC grade solvents, were obtained from Fluka (Buchs, Switzerland). All reagents and solvents were used without further purification. Different types of chromatographic columns were used. One chiral column, named Chiralpak IA, and one achiral column, named Waters XBridge C18 column, both with analytical dimensions (4.6mm ID x 250mm L) and particle size diameter of 5 μm. Seven achiral Waters XBridge columns with the preparative dimensions (19mm ID x 100mm L) and a particle size diameter of 10 μm, named as “SMB columns”.

The preliminary screening of the solvent composition and the analytical pulses of nadolol were performed using a Knauer analytical HPLC system equipped with one Smartline 1050 pump with a 10 mL pump head, a Smartline UV detector 2520 set at a 270 nm wavelength, and a manual Rheodyne injection valve with a 20 μL loop. A constant flow-rate of 1 mL/min was used with all the analytical columns.

The loading experiments, the measurements of the equilibrium adsorption isotherms and the breakthroughs experiments were carried out using a preparative Knauer HPLC system equipped with a Smartline UV detector 2520 set at 270 nm wavelength, two Smartline 1050 pumps with 50 mL pump heads, a manual injection valve and two different loops (100 and 1000 μL). A constant flow-rate of 5 mL/min was used.

The pseudo-binary SMB separation of nadolol stereoisomers were performed on a laboratory-scale SMB unit. This unit was completely built on the LSRE group, Faculty of Engineering, University of Porto, and is designated as “FlexSMB-LSRE”. The SMB unit was operated using a [1-2-2-1] columns configuration.

Results

An extensive set of experimental and simulation results will be presented. Results will include the identification of the stereoisomers present in both nadolol racemates by means of using UV and polarimeter detectors in series.
Figure 1. Comparison between experimental and model results on the adsorption equilibrium isotherms for nadolol stereoisomers using 30\% ethanol/70\% water and 40\% ethanol/60\% water solvent compositions. Left figure, the multicomponent adsorption equilibrium isotherms and its fitting to a linear + Langmuir competitive model (solid line, 30E70W) and Langmuir model (dashed line, 40E60W); right figure, the experimental selectivity (points) and its fitting using the selected isotherm models (lines), between the racemate A and the racemate B, as a function of their feed concentrations.

Then a complete methodology, developed during the last years by our group and explained to scale-up the separation process from analytical to preparative chromatography [1-3].

Figure 2. SMB complete separation regions for the pseudo-binary separation of racemate B (stereoisomers 2 and 3) from racemate A (stereoisomers 1 and 4) using XBridge C18 stationary phase using 30\% ethanol/70\% water (left plot) and 40\% ethanol/60\% water (right plot) solvent compositions and for total feed concentrations of 2 g/L and 10 g/L.

This large set of results will include the screening of solvent composition using an achiral adsorbent and measurements of the column efficiency; the experimental determination of the equilibrium adsorption isotherms and the fitting to an appropriate equilibrium adsorption model (See Fig. 1); then, the model validation by means of breakthroughs measurements with the estimation of the Peclet and mass transfer resistance coefficient; the prediction of the separation regions (See Fig. 2) and the main performance parameters, such as the productivity and solvent consumption (See Fig. 3); finally, the final results of the experimental separation of the nadolol racemates by simulated moving bed chromatography.

Figure 3. Prediction of SMB: (left) productivity and (right) solvent consumption for the two solvent compositions, as a function of the feed concentration (mixture of the four stereoisomers). Solid line for the 40E60W and dashed line for the 30E70W composition.

Conclusions
The results recently obtained by our research team for this topic, clearly supports the capacity to enhance the knowledge on the chromatographic separation of chiral pharmaceuticals using preparative and SMB chromatography. In this communication we will introduce original and innovative challenges through the real separation of multicomponent (quaternary) chiral mixtures which will represent an important step forward for the pharmaceutical industry.

Acknowledgements
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References
Separation of hexane isomers in metal organic framework ZIF-8

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The performance of porous metal organic framework ZIF-8 in the separation of all five hexane isomers (nHEX, 2MP, 3MP, 23DMB, 22DMB), is evaluated through a series of multicomponent breakthrough adsorption experiments, at the temperatures of 373, 423 and 473 K and up to partial pressures of 0.5 bar. The reported data shows for all experiments the following sorption hierarchy: nHEX >> 2MP > 3MP >> 23DMB > 22DMB. It is also demonstrated that the sorption of nHEX is equilibrium based in contrast with the sorption of branched isomers which is kinetic controlled. The experimental data is also simulated through a mathematical model developed in MATLAB code, being the results in qualitatively agreement. This paper shows that it is possible to separate the hexane isomers in ZIF-8 by classes linear/mono-branched/di-branched if proper experimental conditions are set-up, being the result of importance for the octane upgrading of gasoline.

Figure 1. Breakthrough curves for an equimolar mixture of hexane isomers in ZIF-8 at 373 K and total hydrocarbon pressure of 0.50 bar.

Figure 2. Breakthrough curves for an equimolar mixture of hexane isomers in ZIF-8 at 423 K and total hydrocarbon pressure of 0.50 bar.

The goal of this work is to contribute to a better understanding of the adsorption of hexane isomers on ZIF-8 through fixed bed adsorption experiments. For the first time, experimental breakthrough curves with an equimol quinary mixture of all 5 isomers (nHEX, 3MP, 2MP, 23DMB and 22DMB) are measured. The influence of temperature and total hydrocarbon pressure are analyzed to evaluate the sorption dynamics behavior. Moreover, the data obtained are modeled by the Langmuir-Freundlich isotherm model and finally, all calculated data are used to development of a mathematical model, in MATLAB code, to predict the experimental breakthrough curves and validate the thermodynamic isotherm model.

Figure 3. Breakthrough curves for an equimolar mixture of hexane isomers in ZIF-8 at 473 K and total hydrocarbon pressure of 0.50 bar.

The adsorption behavior of all five hexane isomers nHEX, 2MP, 3MP, 23DMB and 22DMB in ZIF-8 was investigated by performing a set of multicomponent breakthrough curves. The experiments were performed at temperatures 373, 423 and 473 K, total hydrocarbon pressure of 0.10, 0.25 and 0.50 bar and using helium to set-up a total pressure in the column of 1 bar.

A mathematical model was used to simulate the adsorption in a fixed bed column by using a system of partial differential equations (PDE’s), constituted by the mass and energy conservation laws, a general isotherm to describe the adsorption equilibrium and a control mechanism for the kinetic mass transfer from the bulk gas phase to the adsorbent. The adsorption data was described by the Langmuir-Freundlich isotherm [6]:

\[ q_l = q_{\text{max}i} \frac{b_i p_i^{n_i}}{1 + \sum_{k=1}^{5} b_k p_k^{n_k}} \]
where \( q_{\text{max}} \) is the maximum saturation loading, \( b_i \) is the adsorption equilibrium affinity constant, \( p \) is the hydrocarbon partial pressure and \( n_i \) is the heterogeneity index. When \( n \) is 1 the material has a homogeneous surface and the model reduces to Langmuir isotherm applicable for ideal surfaces. The index \( t \) refers to component and \( j \) is the number of components present in the mixture.

Figures 1, 2 and 3 show the breakthrough curves for 373, 423 and 473 K, respectively, at a total hydrocarbon pressure of 0.50 bars, plotted in terms of normalized molar flow rate \( F/F_0 \) and as a function of time (min) where the points are the experimental data and the continuous lines correspond to the numerical simulations. Generally, the results shown in these Figures indicate that the sorption hierarchy of hexane isomers in ZIF-8 is always \( n_{\text{HEX}} \gg 2\text{MP} > 3\text{MP} > 2\text{DDB} > 23\text{DMB} \) (normal hierarchy), just like the order of the normal boiling point of the compounds. However, the shape of the breakthrough curve of \( n_{\text{HEX}} \) is always different from the branched ones, which means that its sorption is essentially equilibrium based. In contrast, the branched isomers start their elution practically at the contact time of the gas in the column due to strong diffusional limitations. But, the approach to feed concentration of the branched isomers is different since the di-branched isomers 22DDB and 23DDB rapidly saturate at the early stage of the experiments, in contrast with the mono-branched isomers 3MP and 2MP that due to less diffusion limitations produce a less sharp approach to inlet concentration. These observations show that ZIF-8 separates the hexane isomers by classes linear>mono-branched>di-branched, which is an important result for petrochemical applications viewing the octane improvement of gasoline.

The Graphical Abstract shows the adsorption isotherms of a mixture of hexane isomers in ZIF-8. Points are experimental data and the continuous lines represent the Langmuir–Freundlich model predictions for a mixture loading with the temperature at 373 K (○), 423 K (●) and 473 K (△). The experimental data was satisfactorily represented, and the isotherms are pronounced as type 1 in IUPAC classification. Next, the experimentally determined isotherm fits (Graphical Abstract) were inserted in the mathematical model developed in MATLAB code. Figures 1, 2 and 3 show that the numerical model developed is capable to describe the profiles of the breakthrough curves of hexane isomers very well. To prove that the separation between \( n_{\text{HEX}} \) and branched isomers, and mono-branched 2MP/3MP from di-branched 22DDB/23DDB in flow system is kinetically driven, we decided to perform numerical simulations assuming thermodynamic equilibrium. Figure 1, 2 and 3 b) shows the simulations performed at the three studied temperatures and at total hydrocarbon pressure of 0.5 bar. In this case, the breakthroughs from equilibriums simulations show a completely different approach for the branched isomers in comparison with experimental data. Their shapes become more sharped and a pronounced roll-up for the branched isomers can be seen, reaching more than 1.5 of the feed concentrations for 22DDB and 23DDB at 373 K in Figure 1 a). One should also note that the overshoot of mono-branched isomers 2MP and 3MP is much higher that obtained from experimentally breakthrough curves. These overshoots are an evidence of the interactions occurring in the material in equilibrium separations, disregarding the strong diffusional limitations.

Concluding, this is the first experimental work dealing with the sorption of all five hexane isomers \( n_{\text{HEX}} / 2\text{MP} / 3\text{MP} / 22\text{DDB} / 23\text{DMB} \) on ZIF-8. A series of multicomponent (equimolar) breakthrough curves were measured analyzing the effect of temperature and partial pressure on the hexane isomers sorption. All experiments showed a normal sorption hierarchy \( n_{\text{HEX}} \gg 2\text{MP} > 3\text{MP} > 23\text{DMB} > 22\text{DDB} \), being the di-branched isomers 22DDB and 23DDB completely sieved from the others. In addicting, the parameters obtained by the Langmuir-Freundlich model fitting were used to develop a mathematical model in MATLAB code for better understanding the sorption of all five hexane isomers in a fixed bed with ZIF-8. Firstly, the numerical simulation proves that the \( n_{\text{HEX}} \) sorption is essentially equilibrium based, since the experimental breakthrough curves of branched isomers were qualitatively reproduced by accounting for diffusional influences. Secondly, the contact time between the gas and the solid in the bed has extremely important, once very low contact times doesn’t allow the penetration of mono-branched isomers 2MP and 3MP in the structure of ZIF-8. Thirdly, in a global view, the results of numerical simulations clearly show that the agreement between the experimental data and the Langmuir-Freundlich model is reasonably good considering the wide range of studied conditions. Fourthly, this work has proved the great importance of using mathematical modelling to describe and understand experimental results and for futures designs of adsorptive processes.

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References
Ethylene/ethane separation by gas-phase SMB in binderless Zeolite 13X monoliths

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In this work binderless zeolite 13X monoliths were tested as a new promising material for high purity ethylene production from olefin/paraffin mixtures by gas-SMB technology. In order to characterize the adsorbent and its potential for the target separation, the equilibrium isotherms of ethane, ethylene and propane were measured gravimetrically, in a temperature range from 323 K to 423 K, and pressure up to 6 bar. Dynamic studies were performed at 373 K and 1.5 bar in the gas-SMB bench scale unit. For the experiment with 0.48/0.52 ethane/ethylene feed composition, the adsorbent under evaluation was obtained an ethylene purity of 97.7%, with recovery and productivity of 98.0 % and 16.6 kg\textsubscript{C2H4} h\textsuperscript{-1} m\textsubscript{bed}\textsuperscript{-1}, respectively. This work experimentally demonstrated that the gas-phase SMB can be an efficient technology for olefin/paraffin separations and that it is possible to produce high purity grade ethylene with a high recovery using binderless zeolite 13X monoliths as adsorbent and propane as desorbent.

Introduction
Petrochemicals play an important role in the chemical and petroleum industries due to their utilization as a base product for the fabrication of a wide range of materials. These substances are starters/intermediates to create a diversity of products, which are used in several industries worldwide, from pharmaceuticals and cosmetics, to all kind of plastics and textiles [1]. The main basic petrochemicals are light olefins and aromatics. However, ethylene’s production exceeds that of any other organic compound, because of its application in polyethylene manufacture [2]. Ethylene is also used in the production of other polymers such as polyethylene terephthalate, polyvinyl chloride and polystyrene, as well as other chemicals [3].

The most extensively used process to produce ethylene is by thermal cracking of the hydrocarbons in the presence of steam, but since the required polymer grade purity is above 99.9%, for ethane/ethylene separation, an effective distillation column is required due to the proximity of their boiling points. This step is frequently performed at cryogenic temperatures, in the C2-splitter distillation towers containing between 100 - 130 plates and operating at a very high reflux ratio. These extreme operating conditions make this the most cost-intensive separation process in the petrochemical industry. Several technologies have been studied and among the alternatives, adsorption-based separations, such as gas phase simulated moving bed (gas-SMB), appear to be one of the most promising energy-effective option [4-7].

Our research group explored olefin/paraffin separation by the SMB technology, first by computer simulation [6, 7], resulting in a patent on this technology to separate propylene from propane. Thereafter, a study on the separation of propylene from propane on zeolite 13X in the gas-phase SMB bench unit, home built, has shown experimentally that it is possible to produce polymer-grade olefin with a high recovery, at a bench scale [8]. More recently, polymer-grade ethylene production from ethaneethylene mixtures by four-zones gas-phase SMB with recycle was studied [9]. In that work, enhanced capacity binderless zeolite 13X beads were used as adsorbent and propane as desorbent. A high level of purity and similar recovery were obtained. In this study, enhanced binderless 13X zeolite monoliths were used as adsorbent and propane as desorbent. This work makes evident the efficiency of the gas-phase SMB for olefin/paraffin separations.

Methods
Fixed-bed breakthrough experiments and the SMB cycles were carried out on the gas-phase SMB unit described in detail in a previous work of our group [8]. Briefly, this bench scale unit contains eight columns interconnected in series, each with an internal cross-sectional area of 9 cm\textsuperscript{2} and a length of 10.5 cm. The columns are inside of an air-forced convective oven with an operation range between 303 and 523 K. The gas temperature history in the wall of the monolith was monitored by one type K thermocouple located at the middle of the axial direction of the column. The pressure of the system is controlled using a back pressure regulator from 1 to 5 bar. The system’s pressure history is assessed by four pressure transducers with a 0-7 bar range located at the top of every two columns. The simulated countercurrent contact between the mobile fluid phase and the stationary solid phase is accomplished by the periodic switching of inlet and outlet ports in the same direction of the fluid flow. The feed allows a maximum of three components, measured and controlled separately by three mass flow controllers (MFCs). The desorbent gas is also measured and controlled by a MFC. Similar to the input streams, the flow rates of extract and raffinate are measured and controlled by two MFCs. The unit is remotely controlled by a Labview interface and a relay controller software from OMROM. The sampling system of this setup allows the collection of samples on a multiloop valve from a sampling point placed at the exit of the column 6. The posterior sample analysis is performed by a gas chromatograph, equipped with a flame ionization detector.

Results
The adsorbents studied were binderless zeolite 13X monoliths provided by Chemiewerk Bad Köstritz GmbH. To test the potential of the monoliths as adsorbent and propane as
desorbent, pseudo-ternary runs were performed by feeding two monoliths in series with a 50/50 % mixture of olefin/paraffin in pre-saturated columns with propane. The total feed flow rate was 200 ml min\(^{-1}\) while the desorbent flow rate was 500 ml min\(^{-1}\). The experiment was carried out at 373 K and 1.5 bar. Based on the information collected in the dynamic study, several SMB cycles were proposed for production of polymer-grade ethylene from 0.48/0.52 feed mixtures. All the columns packed with the monoliths were kept at the operating temperature of 373 K during the experiments. The operating conditions are summarized in Table 1. The switching time in both experiments was 250 s.

<table>
<thead>
<tr>
<th>Config.</th>
<th>Feed flow rate, SLPM</th>
<th>Desorbent flow rate, SLPM</th>
<th>Extract flow rate, SLPM</th>
<th>Raffinate flow rate, SLPM</th>
</tr>
</thead>
<tbody>
<tr>
<td>4-2-2</td>
<td>0.093 C₂H₆, 0.102 C₃H₈</td>
<td>1.505</td>
<td>1.117</td>
<td>0.641</td>
</tr>
<tr>
<td>3-2-2-1</td>
<td>0.093 C₂H₆, 0.102 C₃H₈</td>
<td>1.505</td>
<td>1.106</td>
<td>0.460</td>
</tr>
</tbody>
</table>

By the end of the 5th cycle the cyclic steady state was achieved. Figure 1 shows the composition of the three components for the eight SMB monoliths. It can be seen that the ethylene molar fraction decreases in section III and that only ethane moves forward with the fluid to the raffinate port. In section IV, the molar fraction of ethane also decreases and a pure propane stream is obtained at the end of the section. Ethane’s molar fraction decreases in section II, and that only ethane moves with the solid, is obtained in the extract port, at the end of section I. In section I the ethylene’s concentration decreases and the adsorbent is regenerated.

### Acknowledgements
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### References

In summary, all the experiments indicate that the binderless 13X monolith is a potential adsorbent for the separation of ethane/ethylene with propane as desorbent. Indeed, using structured adsorbents, such as monoliths, presents two main advantages, the lower pressure drop and easier up-scaling.

**Conclusion**
The process performance was evaluated based on purity, recovery, productivity and desorbent consumption. For the experiment with 0.48/0.52 ethane/ethylene feed composition, ethylene was obtained with 97.7% purity, with recovery and productivity of 98% and 16.6 kg\(\text{C}_2\text{H}_4\) h\(^{-1}\) m\(^3\) bed\(^{-1}\), respectively. In the same experiment, ethane was obtained with a purity of 97.5%, with a recovery of 97% and a productivity of 14.5 kg\(\text{C}_2\text{H}_6\) h\(^{-1}\) m\(^3\) bed\(^{-1}\).

This work experimentally demonstrated that the combination of gas-phase SMB with structured adsorbents can be an efficient technology for olefin/paraffin separations and that it is possible to produce polymer grade ethylene with a high recovery using binderless 13X zeolite monolith as adsorbent and propane as desorbent.

![Figure 1. - Internal profile at step half time as function of column number.](image-url)
An alternative method for the separation of C2/C3 hydrocarbons mixtures by pressure swing adsorption using the MOF MIL-100(Fe)

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Introduction
The separation of olefin/paraffin is achieved conventionally by cryogenic distillation, being one of the most challenging and energy consuming separations at industrial scale [1]. The high energy requirements and the increasing demand have been the driving forces to develop new technologies which must be cheaper, yet environmentally friendly. Among the competing alternatives towards this end, cyclic adsorption processes such as vacuum pressure swing adsorption (VPSA) [2] and simulated moving bed (SMB) [3] technologies appear to be the most promising and energy-efficient options.

In the last years, several studies were carried out in order to alternatively, separate gas mixtures by SMB technology and more recently this technology was applied to the olefin/paraffin separation in gas phase [4]. Besides the choice of a suitable adsorbent, to perform the SMB it is also necessary to deal with the choice of a desorbent, which should be easily separated from both components of the binary mixture. The efficiency of the downstream separations, necessary to recover the desorbent, is intrinsically related to the desorbent choice.

For the case study, ethylene/ethane separation by SMB, with propane as desorbent, propane must be recovered from the extract and raffinate streams by two mandatory downstream separations: ethylene/propane and ethane/propane [3, 5]. In the present work two VPSA cycle schemes with five steps were proposed to separate 0.30/0.70 ethane/propane and ethylene/propane using an iron MOF (metal-organic framework) MIL-100(Fe) as adsorbent. The composition of both mixtures were chosen based on the raffinate and extract expected compositions [3, 5]. The MIL-100(Fe) sample was synthesized and provided by KRICT (Korea Research Institute of Chemical Technology).

Results
The study started with the characterization of the adsorbent sample by measuring the adsorption capacity of pure gases over MIL-100(Fe). The measurements were performed by a gravimetric method on a magnetic suspension microbalance from Rubotherm® (Germany) operated in a closed mode described in detailed elsewhere [6]. For each isotherm point obtained, the equilibrium was considered when the values of pressure, temperature and mass persisted constant for at least 30 min. Desorption branch equilibrium points were executed to check the reversibility of the adsorption. The adsorption isotherms of ethane, ethylene and propane on MIL-100(Fe) granulates were determined at 323, 373 and 419 K in the pressure range of 0–700 kPa. Figure 1 represents the comparison among adsorption isotherms of ethane, ethylene, and propane on MIL-100(Fe) at the temperature of 323 K (temperature chosen to operate the PSA).

Further, the adsorption kinetics was studied by single and multicomponent fixed bed experiments. Single and multicomponent breakthrough curves were conducted at 0.5 SLPM, 323 K and 150 kPa feed conditions on the VPSA single-column unit described in detail elsewhere [7]. The single-column is made of stainless steel with 0.021 m internal diameter and 0.79 m length. Prior to the breakthrough experiments, the column was packed with 111.7 g of hydrated MIL-100(Fe) granulates and placed inside of an air-forced convection oven and after activation the adsorbent had lost 25% of its initial mass.

![Image of adsorption isotherms](image-url)

Figure 1. Adsorption isotherms of ethane, ethylene, and propane on MIL-100(Fe) granulates at 323 K. Filled and open symbols represent adsorption and desorption branches, respectively. The solid lines correspond to the Langmuir isotherm model.

The main objective of this work is the separation of 0.30/0.70 ethane/propane and 0.30/0.70 ethylene/propane, representative mixtures of the outlet streams of the ethane/ethylene separation by gas-phase SMB, using propane as desorbent. Since adsorption equilibrium data are essential for the process design, the adsorption isotherms of ethane, ethylene and propane were measured at 323, 373 and 419 K by a gravimetric method. Fixed-bed experiments were conducted at 0.5 SLPM, 323 K and 150 kPa feed conditions on a lab-scale VPSA unit to obtain fundamental information for the process modelling. Two five-steps VPSA cycles were designed using gPROMs software and performed experimentally. The performance parameters demonstrated the high viability of the proposed processes, since ethane and ethylene were obtained at high purities (99.9%), with a recovery of 76.2% and 63.9%, respectively.

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A mathematical model describing the dynamic behavior of multicomponent adsorption in a fixed bed was implemented in gProms® environment (Process Systems Enterprise, London, UK) and numerically solved. Figure 2 shows the comparison between model and experimental results for the displacement of the propane by ethylene. The experimental information collected and validated by modeling showed that MIL-100(Fe) granulates has the potential to be used for ethane/propane and ethylene/propane separation by VPSA.

The VPSA cycle schemes proposed were experimentally performed in the same VPSA single-column unit. Both cycle schemes start with the pressurization of the bed with a pure C2 stream counter-currently at a pressure (P_H) of 150 kPa. In the adsorption step, performed at P_H a C2 enriched stream is produced. The rinse step was implemented after the adsorption step, to increase the purity of the C2:Hs in both cycles. During this step a C2 enriched stream is recovered at the end of the column, while the C2:Hs stream is continuously being fed to the adsorber. At the end of the rinse step, the adsorber is mainly filled with the C2:Hs and a blowdown step is applied counter-currently. The components that retained in the solid are desorbed by lowering the gas-phase partial pressure inside the column to 10 kPa (P_H). During this step, a C2:Hs enriched stream is produced. The last step implemented was a purge, with a stream of pure paraffin operating in counter-current, to regenerate the adsorbent.

Acknowledgements

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References

Simulated moving bed reactor for the production of p-xylene: isomerization, disproportionation and transalkylation reactions of xylene isomers, ethylbenzene, and toluene in liquid phase

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An experimental and parametric behavior study of xylene isomerization and the considered main side reactions that may occur in a Simulated Moving Bed Reactor (SMBR) for the production of p-xylene, was conducted in liquid phase, over beta zeolite. Different reaction schemes were investigated; the linear reaction scheme, taking into account the thermodynamic equilibrium, proved to be the best representation for the isomerization of xylene. By conducting mixture experiments of the C8 isomers with and without toluene, ethylbenzene disproportionation and ethylbenzene-toluene transalkylation were identified as the main side reactions in the studied conditions. Both reactions were confirmed to be second order following the deethylation-ethylolation mechanism; similar values obtained from pure ethylbenzene and ethylbenzene-toluene experiments indicated the absence of competition between the two reactions. Kinetic parameters and activation energies were obtained for all reactions studied.

A Simulated Moving Bed Reactor (SMBR), based on the conventional Simulated Moving Bed (SMB) but comprising a homogeneous mixture of adsorbent and catalyst, can overcome the equilibrium constraints based on the concept of process intensification. By allowing xylene isomerization and adsorption in a single unit in liquid phase, it is possible to reduce the consumption of raw materials and has huge potential to increase the p-xylene yield [1]. In this work, xylene isomerization and main side reactions like disproportionation and transalkylation between C8 aromatics and toluene that may occur in the SMBR were investigated, as part of the development of a SMBR unit for the production of p-xylene in liquid phase.

An experimental and parametric behavior study of xylene isomerization, as well as disproportionation and transalkylation between C8 aromatics and toluene were conducted through fixed bed experiments to gain a deeper insight into the field. Toluene was included in the experiments since it has been used as promising desorbing in the separation of xylenes [2]. Monofunctional acid beta zeolites with SiO2/Al2O3 ratio (SAR) of 35 (BEA35) and 38 (BEA38) were used as catalysts for the xylene isomerization experiments. The following techniques were employed to characterize the solids: NH3-Temperature Programmed Desorption and FTIR spectroscopy were adopted to investigate the acid sites of the catalysts; X-Ray diffraction (XRD) patterns to study the structure and crystallinity; Scanning Electron Microscopy (SEM) and Energy-Dispersive X-Ray Spectroscopy (EDS) for morphologic characterization; mercury porosimetry analyses to determine the density and pore size distribution; the specific surface area, micropore size and the pore volume were determined with nitrogen adsorption at 77 K and carbon dioxide adsorption at 273 K. The fixed bed experiments were carried out under the following conditions: 513, 493, 473, and 453 K at 2.1 MPa in liquid phase. The existence of external and intraparticle mass-transfer limitations was carefully examined. With the Carberry number, Ca, lower than 0.05, and the effectiveness factor η, near unity, both external and internal mass-transfer resistance can be neglected. All reactions were verified to be in the kinetic-controlled regime. Firstly, xylene isomerization is the main reaction in the SMBR, as presented in Figure 1; kinetic constants were estimated with four different models. For the triangular reaction scheme, xylenes convert through mutual interconversion, allowing the 1,3-methyl shift mechanism, whereas for the linear reaction scheme, α-xylene can only convert to p-xylene via m-xylene as intermediate through the 1,2-methyl shift mechanism. Two of the models included the xylene isomerization thermodynamic equilibrium from the literature [3]. The linear reaction scheme, including the thermodynamic equilibrium from the literature, presented a better fit to the experimental values. Higher conversion of p-xylene was observed when compared with the conversion of the other two isomers. This may be attributed to its smaller molecular size. BEA35 presented better performance due to its higher amount of Brønsted acid sites. Activation energies of xylene isomerization as well as the interconversion kinetics over the catalysts were estimated and they are presented in Table 1. The center-type Arrhenius equation was used where the centered temperature was 493 K for BEA38 and 473 K for BEA35. The activation energies present similar values over the catalysts, it can be concluded that the isomerization on both catalysts follows the same mechanism.

Secondly, two experiments were carried out to identify the main side reactions during xylene isomerization in liquid phase, that is C8 aromatics mixture experiments with and without toluene. This second study was conducted only on BEA35 since it was the catalyst offering better performance on xylene isomerization. By analyzing the products distribution of the two experiments, ethylbenzene disproportionation and ethylbenzene-toluene transalkylation were identified as the main side reactions that may occur in a SMBR in liquid phase, as presented in Figure 1. Both reactions were verified to be second-order. Similar kinetic constants obtained respectively from pure ethylbenzene and ethylbenzene-toluene experiments indicated the absence of competition for the adsorption sites between the two reactions under the studied conditions. Additionally, ethylbenzene disproportionation and ethylbenzene-toluene transalkylation, undergo through the deethylation-ethylolation mechanism; it was
verified that the bulky diphenylethane-based mechanism was suppress by the narrow channels of the zeolite. The activation energies are presented in Table 1. Moreover, by conducting ethylbenzene-toluene experiments with different ratios, it was concluded that the existence of a parallel reaction, as well as the feed concentration, show no effect on the p-diethylbenzene selectivity. The p-diethylbenzene selectivity is reduced as temperature increases at low conversions; in other words, at higher temperatures, diethylbenzene isomerization rate is increased at a higher extent than actual production of p-diethylbenzene. It can also be deduced that, the lowest flow rate led to highest conversion and lower selectivity towards p-diethylbenzene.

Finally, simulated results of the experiments showed excellent agreement with all the experimental data. Reliable parameters for further investigation of a SMBR unit for the production of p-xylene in liquid phase were obtained.

Table 1. Kinetic parameters of xylene isomerization, ethylbenzene disproportionation, and ethylbenzene-toluene transalkylation over beta zeolites with SiO2/Al2O3 ratios of 35 and 38 [4,5]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>BEA35</th>
<th>BEA38</th>
</tr>
</thead>
<tbody>
<tr>
<td>T_0 (K)</td>
<td>473</td>
<td>493</td>
</tr>
<tr>
<td>E_{a,1} (kJ/mol)</td>
<td>91 ± 7</td>
<td>91 ± 9</td>
</tr>
<tr>
<td>E_{a,3} (kJ/mol)</td>
<td>84 ± 6</td>
<td>90 ± 8</td>
</tr>
<tr>
<td>k_01 × 10^9 (m^3/kg_s cat s)</td>
<td>8.7 ± 0.5</td>
<td>3.5 ± 0.3</td>
</tr>
<tr>
<td>k_03 × 10^9 (m^3/kg_s cat s)</td>
<td>4.4 ± 0.2</td>
<td>2.35 ± 0.12</td>
</tr>
<tr>
<td>E_{a,5} (kJ/mol)</td>
<td>66 ± 3</td>
<td></td>
</tr>
<tr>
<td>k_05 × 10^12 (m^3/kg_s cat s mol)</td>
<td>7.2 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>E_{a,6} (kJ/mol)</td>
<td>57 ± 8</td>
<td></td>
</tr>
<tr>
<td>k_06 × 10^12 (m^3/kg_s cat s mol)</td>
<td>7.3 ± 0.5</td>
<td></td>
</tr>
</tbody>
</table>

Parameters of reverse reactions (E_{a,2}, E_{a,4}, k_{02}, k_{04}) were calculated with the thermodynamic equilibrium equations proposed by Gonçalves and Rodrigues [3].

Acknowledgements
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References
Removal and recovery of technology-critical elements from aqueous solutions using Fe₃O₄/graphite nanoplatelets

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Magnetic graphite-like nanoplatelets (Fe₃O₄/GNPs) were successfully prepared by the electrostatic assembly of graphite nanoplatelets and magnetite nanoparticles. Their potential as sorbent for the removal of technology-critical elements was investigated. The Fe₃O₄/GNPs exhibited good sorption properties towards trivalent lanthanides (La, Eu and Tb). The sorption process is sensitive to solution pH and to the amount of sorbent used. Removal efficiencies up to 80% were achieved using alkaline solutions (pH 8) and 50 mg/L of sorbent. In ternary model solutions, Fe₃O₄/GNPs showed preferential removal for Eu and Tb. The kinetic is well fitted by the pseudo second-order kinetic model, and the equilibrium evidenced a rare but interesting behaviour well depicted by the isotherm measured. At low equilibrium concentration, the sorption system is approximately irreversibly but becomes essentially vertical as the concentration increases. The composite can be reused without loss of efficiency.

Introduction

There has been great concern about future supplies and increasingly cost of trace elements that are essential for key technologies such as renewable energy, electronics, energy efficiency and aerospace industry [1]. These elements are very often called technology-critical elements (TCE) due to their increasing use and demand for high-technology advances, and at the same time due to their vulnerability to politically or economically driven fluctuations in supply[1–3].

Graphite-like nanoplatelets (GNPs) have attracted great attention in the past decade, as a viable and inexpensive materials for many engineering applications, given the excellent in-plane mechanical, structural, thermal, and electrical properties of graphite[4]. Our own interest in the preparation of magnetic nanomaterials as new adsorbents for water treatment applications led us to explore the preparation of magnetic GNPs composites. In principle, these hybrid structures combine the sorption properties of GNPs, with the magnetic properties of magnetite (Fe₃O₄), allowing the implementation of magnetic separation technologies in water remediation units using sorption processes.

Objectives

In line with the high demand of TCE for high-tech applications, we investigate the application of a magnetic composite to capture selected TCE (La, Eu and Tb), from urinary and ternary solutions, by a simple and environmental friendly procedure, with potential for future application in the recovery of TCE from industrial or other water systems.

Methods

The GNPs are obtained by ultrasonic treatment (5h) of graphite (5 g) in 100 mL of dimethylformamide. The Fe₃O₄ NPs were prepared by oxidative hydrolysis of FeSO₄ in alkaline media [5]. The Fe₃O₄/GNP was prepared by electrostatic assembly. Briefly, 100 mg of GNPs and 100 mg of Fe₃O₄ NPs, suspended in 100 mL of 0.1 mol/L HNO₃, were sonicated (Sonics Vibra Cell Sonicator VC70, 130 W, 20 kHz), during 1 h.

The capability of Fe₃O₄/GNPs to remove TCE from water was assessed by exposing different amounts of composite to urinary or ternary solutions of La(III), Eu(III) and Tb(III) with an initial element concentration of 100 µg/L. Batch experiments were conducted at a maximum period of 24 h at 22 °C, with mechanical stirring (250 rpm). The pH effect on the capability of the composite to remove TCE from water was studied in the range 2 to 10, exposing 5 mg of Fe₃O₄/GNPs to 100 mL of ternary solutions of La(III), Eu(III) and Tb(III). Equilibrium of TCE removal process was studied by exposing different amounts (2, 4, 8, 12, 16, 20, 30, 40 and 60 mg/L) of composite to unary and ternary solutions of La(III), Eu(III) and Tb(III) (solutions pH 7.5±0.2) during 24 h. Quantification of TCE in water samples was performed by inductively coupled plasma optical emission spectroscopy (Horiba Jobin Yvon Activa M).

Results

Figure 1 displays the removal efficiency of Fe₃O₄/GNPs toward the selected TCE as function of solution pH. After a 24 h of exposition to a ternary solution of La(III), Eu(III) and Tb(III), equal amounts of Fe₃O₄/GNPs removed from 0 to 94.3±1.1% of La(III), 3.9±0.8 to 94.3±9.0% of Eu(III) and 0 to 94.4±1.0% of Tb(III), by increasing the solution pH from 2 to 10. The results obtained with this experiment allow us to conclude that the removal of La(III), Eu(III) and Tb(III) from water by the Fe₃O₄/GNPs depends strongly on pH. A pronounced increase on the removal efficiency occurs for a pH about 6. This behaviour can be explained based on the sorbent surface chemistry and aqueous phase chemistry. The isoelectric point (IEP) of the Fe₃O₄/GNPs was found to be 5.2, hence a positive charge exists on the surface of the composite for pH values lower than the IEP, which gradually decreases and attains a negative value for pH values higher than 5.2. When the surface of Fe₃O₄/GNPs is positively charged, the predominant lanthanide species are the hydrated trivalent ionic[6] (La³⁺, Eu³⁺ and Tb³⁺). In these acidic conditions, the sorption of the lanthanide cations is not favourable and the removal of TCE by the composite is very low (less than 5%). At pH above 5.2, the number of negatively charged sites at Fe₃O₄/GNPs surface increases, favouring the sorption of cationic species due to electrostatic attraction. As a result, the predominant species[6] of the selected TCE for the pH range 5-9 (La³⁺/La(OH)²⁺, Eu³⁺/Eu(OH)²⁺, Eu⁴⁺ and Tb³⁺/Tb(OH)²⁺/TbO²⁻/TbO₃⁻) are sorbed onto the composite.
Figure 1. Effect of pH on TCE removal from ternary (La, Eu and Tb) solutions. Initial concentration of each TCE 100 μg/L and mass to volume ratio (m/V) of 50 mg/L.

For all sorbent-sorbate(s) systems the kinetic profiles are characterized by an abrupt increase of the solid loading of each TCE in the nanomaterial, followed by a less pronounced increase, reaching a horizontal branch (plateau). Kinetic models were used to fit the experimental data. The fitting curves based on pseudo-first order, pseudo-second order and Elovich provided good adjustments to the experimental data, for all the studied sorbent-sorbate(s) systems, with most of the absolute average relative deviation below 5% and the coefficient of determination ($R^2$) above 0.90.

Regarding the equilibrium data, all isotherms exhibit a very unfavourable trend, with an almost square convex shape to the concentration axis ($C_{eq}$). In terms of the theoretical classification of BDDT (Brunauer, Deming, Deming, Teller), their shape falls within type III, and according to the Giles classification [7], the isotherms follow S-type curve pattern. The most noteworthy feature of these systems is that sorption becomes easier as concentration rises. As more solute is sorbed, the easier it is for additional amounts to become fixed, which implies a side-by-side association between sorbed species, helping to hold them to the surface (the so called “co-operative adsorption”)[7]. According to Giles[7], the S curve usually appears when the solute molecule is monofunctional, has moderate intermolecular attraction, causing it to pack vertically in regular array in the sorbed layer, and meets strong competition, for substrate sites, from molecules of the solvent or of another sorbed species.

The recovery of TCE was investigated by means of composite regeneration, by its treatment with 0.1 mol/L HNO$_3$ for a 24 h period of exposition to a ternary solution of La(III), Eu(III) and Tb(III). The TCE removal efficiency of the composite after each regeneration cycle was also evaluated. During subsequent cycles, the recovery efficiency of the three TCE ranged from 82 to 92% for La(III), 88 to 104% for Eu(III) and 82 to 97% for Tb(III), suggesting that 0.1 mol/L HNO$_3$ is an effective eluent for the recovery of these elements from the Fe$_3$O$_4$/GNPs. More, the capability of the composite to remove the selected TCE from water was rarely affected since the values of the removal efficiency remained nearly constant during all cycles, confirming also the stability of the sorbent in diluted nitric acid solution. All these results allow us to state that Fe$_3$O$_4$/GNPs is a recyclable sorbent material and can be used in several cycles.

**Conclusions**

A Fe$_3$O$_4$/GNPs composite was successfully prepared by electrostatic approach, and exhibited very attractive sorption properties toward trivalent lanthanides (La, Eu and Tb), from diluted unary and ternary model solutions. The sorption mechanism is based on electrostatic interactions making this removal process only applicable for pH values higher than 5.2, the isoelectric point of the composite. Removal efficiencies up to 80% can be achieved at pH 7.5 with only 50 mg/L of composite. Moreover, the recoveries and reutilization rates, at consecutive cycles, highlight the recyclability of the Fe$_3$O$_4$/GNPs composite without loss of efficiency, and the combination of these two steps constitutes thus a promising procedure to recover TCE from the diluted aqueous streams, pointing to the potential success of this methodology towards one of the greatest difficulties encountered in the recovery and reuse of these critical elements.

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In this work, the morphological structure of a fabricated alumina monolith was reconstructed using 3D X-ray tomography data. Subsequently, OpenFOAM, an open-source CFD tool, was used to simulate the essential parameters for monoliths’ performance characterisation and optimization, i.e. velocity and pressure fields, fluid streamlines, shear stress and residence time distribution (RTD). Firstly, the flow patterns of a single-phase incompressible laminar flow of a Newtonian fluid (water) were simulated in a computational domain originated from 3D image reconstruction of the monolithic porous column under study. Subsequently, using the previously obtained velocity profiles, a pulse injection of the tracer (acetone) was simulated and its concentration at the outlet was followed to obtain the RTD curves. Additionally, the monolith’s tortuosity was directly assessed by measuring lengths of the streamlines. To test the reproducibility of the simulations, they were performed in two different subvolumes, A and B, with average edge length of 107 and 142 µm, respectively (Fig. 1).

It was observed (for the case of the monolith studied) that fluid transport was dominated by flow heterogeneities and advection, while the shear stress at pore mouths was significantly higher than in other regions, which is especially important in protein separations. The computed tortuosity of the monolith (~1.1) was found to be in the same range of the results obtained by known experimental, analytical and numerical equations. Besides, the proposed modelling approach was successfully validated by an experimentally obtained residence time distribution (Fig. 2).

Porous monolithic alumina columns find a wide variety of applications, including in chromatography, due to increased surface area and good accessibility to the ligands and reduced diffusional hindrances. The monolith morphology plays a key role in determining its hydrodynamic and mass transfer properties.

In this work, the morphological structure of a monolith was reconstructed using 3D X-ray tomography data. Subsequently, OpenFOAM CFD package was used to simulate the essential parameters for monoliths’ performance characterization such as velocity and pressure fields, streamlines, shear stress and residence time distribution (RTD). Moreover, the tortuosity was directly assessed by measuring lengths of the streamlines which, in laminar regime and steady state, represent the fluid pathways. It was observed (for the case of the monolith studied) that fluid transport was dominated by flow heterogeneities and advection, while the shear stress at pore mouths was significantly higher than in other regions, which is especially important in protein separations. The proposed modelling approach was successfully validated by an experimentally obtained RTD. The herein presented CFD modelling approach can be used to optimise and virtually test the performance of chromatographic materials before being printed/manufactured, thus leading to production of target materials.

Figure 1. Two subvolumes of the 3D virtual reconstruction of the monolith used for CFD simulations.

Figure 2. Comparison of computational fluid dynamics (CFD) and experimental dimensionless residence time distribution (RTD) curves.

The experimental RTD data is slightly wider and lower than the computed RTD curves. Such a curve shape suggests that the experimental fluid dispersion is slightly larger than the dispersion in the computed domain [2]. This result is not unexpected, since the skeleton is considered as non-porous in the computed domain, as pores smaller than 1 µm in diameter were not captured by the X-ray tomography. Therefore, the predicted dispersion is slightly lower than the experimentally observed one. A higher image resolution, than that provided by the tomography apparatuses used in the present study, would allow for capturing a more detailed monolith’s morphology, and...
will lead to an even better approximation between the modelling results and the physical reality.

The modelling approach presented in this work obviates the need for model-dependent fitting parameters, thus is an important step towards monoliths (or other porous structures) optimisation. Additionally, it is expected that in a very close future, chromatographic materials will be produced by additive manufacturing (3D-printing). The .stl (stereolithography) files used to give information about geometry, shape and dimensions of 3D printed object are the same type of files used in this work to create computational mesh and perform CFD studies. Consequently, the herein presented CFD modelling approach can be used to optimise and virtually test the performance of chromatographic materials before being even printed/manufactured, thus leading to production of materials “right at the first time” and according to the “design-for-purpose” approach.

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References
Mixing of dissimilar fluids in confined impinging jets – Mayonnaise equation

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Two working liquids were defined with the following properties:

\( \mu_1 = 0.04 \text{ Pa} \cdot \text{s}, \rho_1 = 1000 \text{ kg/m}^3 \)

CIJs can efficiently mix fluids with different viscosities at non-unitary flow rate ratios. In this communication it is presented the experimental and numerical characterization of the flow in CIJs for fluids with a viscosity ratio of 1:2. Experimental characterization is made with Planar Laser Induced Fluorescence, and numerical study is based on 3D CFD simulations of CIJs. Experimental and numerical results show that the balance of kinetic energy rate of the opposed jets plays the key role on the jets impingement point positioning, although viscosity also has an impact on this. Mayonnaise equation, previously introduced, makes a good prediction of the best CIJS design to effectively mix dissimilar fluids.

Introduction

An emulsion is a mixture of two or more immiscible liquids. One liquid (the dispersed phase) form microscopic droplets dispersed in the other phase (continuous phase). Mayonnaise is an example of an oil-water emulsion (o/w) where oil is the dispersed phase while water is the continuous phase [1]. The traditional emulsification process is a batch process where one phase is slowly added to the other under intensive agitation. Nevertheless, in this procedure mechanical energy is not solely injected in the dispersion of one phase. Currently, continuous industrial processes are already available and consist on feeding both phases into a mixer following subsequent continuous homogenization [2].

Confined Impinging Jets (CIJs) is a continuous process that is applied in this work to an emulsification process. CIJs are highly efficient mixing devices composed by a semi-confined mixing chamber, two opposed injectors near the closed top of the chamber and an outlet in the open end from where the fluid leaves the reactor [3]. The fluids enter into the mixing chamber through two injectors as high-speed opposed jets that decelerate in the short distance from the injector to the chamber axis causing strong energy dissipation, which promotes very effective mixing. Two main flow regimes have been identified in CIJs, one at low Reynolds number, where the flow is steady and the fluids injected from opposed jets are kept almost segregated, and a chaotic or dynamic flow field where a street of vortices is formed upon the impingement of the opposed jets. Chaotic flow regime is onset with strong dynamics of interfacial area generation, which injects the energy on the work of phase dispersing.

The operation at chaotic flow regime is a necessary condition for CIJs efficient operation and the jets Reynolds number is the key parameter for this [4-6]. The other parameter which plays a critical role on chaotic flow onset is the balancing of the jets, this was clearly demonstrated by Fonte et al. [3] who later proposed the elastic analogue model [7],

\[ \xi = \left( \frac{Re_1 d_1}{10 D} + 1 \right) \left( \frac{Re_1 d_1}{Re_2 d_2} - \frac{Re_1 d_1}{Re_2 d_2} \right) \left( \frac{Re_1 d_1}{10 D} + 1 \right) \]

where \( Re_1 \) and \( Re_2 \) are the Reynolds numbers of injector 1 and 2, respectively, \( d_1 \) and \( d_2 \) are the injector diameter, \( D \) is the distance between the nozzles and \( \phi_K \) is the ratio of kinetic energy, \( \phi_K = \frac{\rho_1 d_1 v_{inj1}^2}{\rho_{inj} d_1 v_{inj1}^2} \). This model describes the mechanics of the equilibrium of two opposed jets enabling to design CIJs for the mixing of fluids at non-unitary flow rate ratios by changing the diameter of the nozzles so that \( \xi = 0 \). Furthermore, this model predicts the influence of fluid viscosities on the jets equilibrium. The viscosity influence is taken into account in the Reynolds number term.

In many emulsification processes, the two phases, which typically have different viscosities, are mixed for a specific stoichiometric ratio of mass fractions. For example, in mayonnaise production, the oil (dispersed phase) is added to water (continuous phase) in order to produce an emulsion with 70-80% (mass concentration) of oil. A continuous process to produce mayonnaise using a CIJ mixing chamber could be designed using the elastic analogue model. Therefore, from now, this model is called mayonnaise equation.

This equation was already validated for several flow rate ratios but only in a very limited range of values for mixing of dissimilar fluids, i.e., with different viscosities [8].

Objective

This work studies the mixing of dissimilar fluids, namely with different viscosities, in CIJs using Planar Laser Induced Fluorescence (PLIF) experimental data and Computational Fluid Dynamics (CFD) results, for conditions typical of those used in industrial applications. The objective is the mayonnaise equation validation for mixing of fluids with different viscosities.

Methods

The CIJs used in this work have typical dimensions of those used in industrial RIM machines. CIJ has a height of \( H = 50 \text{ mm} \) and a diameter of \( D = 10 \text{ mm} \) while the injectors have a diameter of \( d = 1.5 \text{ mm} \) placed at \( h = 5 \text{ mm} \) from the closed top of the mixing chamber.

The flow in CIJ mixing chamber was studied using the Finite Volume software package ANSYS/Fluent. The boundary conditions set were no slip conditions at the CIJs walls, an uniform pressure at the outlet and a parabolic velocity profile at each inlet, \( v(r) = 2v_{inj} \left( 1 - \frac{r^2}{r^2} \right) \), where \( r \) is the distance from the injector axis, \( v_{inj} \) is the average velocity at the inlet and \( d \) is the diameter of the injector.

Two working liquids were defined with the following properties:

\[ \mu_1 = 0.04 \text{ Pa} \cdot \text{s}, \rho_1 = 1000 \text{ kg/m}^3 \]

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\[ \mu_2 = 0.02 \text{ Pa} \cdot \text{s}, \rho_2 = 1000 \text{ kg/m}^3. \]

Experiments were run in an acrylic CIJ mixer having the same dimensions of the CFD model. PLIF experimental technique was used to predict the impingement point of the two streams in CIJs. Both fluids are aqueous solution of glycerol. Fluid 1 is an aqueous solution with 68% glycerol (mass concentration) \( (\mu_1 = 0.02 \text{ Pa} \cdot \text{s}) \) and fluid 2 is a solution with 80% glycerol \( (\mu_2 = 0.04 \text{ Pa} \cdot \text{s}) \).

The fluid 1 was injected from the left CIJ injector while the fluid 2 was injected from the right side. In all experiments, the left hand side Reynolds number was fixed at \( Re_1 = 100 \).

Results

In CFD simulations, the right hand side jet flow rate was varied from a flow rate from \( \phi_1 = 0.1 \) to \( \phi_1 = 10 \).

Figure 1 show the plot of the normalised displacement of the jets impingement point from the mixing chamber axis, \( \xi \), as a function of the jets kinetic energy ratio, \( \phi_K \). This plot shows the impingement point is extremely sensible to \( \phi_K \) values. The results show that the impingement point at \( \phi_K = 1 \) is no longer coincident with the mixing chamber axis, \( \xi = 0 \). Therefore, the balancing condition is not at \( \phi_K = 1 \), which is changed by the fact that \( Re_1 \neq Re_2 \), causing a slight offset in the equilibrium condition. This shows that kinetic energy flux is the dominant parameter regarding the equilibrium of the opposed jets \([7]\), although viscosity also plays a role. As observed from Figure 1, CFD results are coincident with mayonnaise equation. PLIF experiments were made in order to validate these results. Figure 2 show that the impingement point, calculated from elastic analogue model, fits the results obtained from PLIF images. The bars in this figure represent the standard deviation of \( \xi \) measured from 10 PLIF images for each \( \phi_K \). The value of \( \xi \) is obtained from the plot of the image colour value plotted in a line defined from the injectors’ axis, the contacting of the two jets is marked by a steep gradient in this plot. These results provide an experimental validation of mayonnaise equation. The validation of the ability of this equation to describe the opposed jets impingement point enables to design CIJs with directly opposed jets impinging at mixing chamber axis for fluids with different viscosities. This equation is of particular relevance for emulsification processes, such as, for mayonnaise production.

Conclusion

CIJs enables the mixing of two fluids with different viscosities under a chaotic flow regime. The necessary condition is the balancing of the jets, making them to impinge at the CIJs chamber axis. Mayonnaise equation predicts the tendency and actual position of the jets impingement point. The validation of this equation was made from PLIF data and CFD results showing that the mayonnaise equation is a design tool for CIJ mixers for dissimilar fluids, for instance, for mayonnaise production.

Acknowledgements

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References

Removal of the mixture of pharmaceuticals sulfamethoxazole and diclofenac from water streams by a polyamide nanofiltration membrane

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Surface and groundwater are the main resources for sustainable drinking water production throughout the world. However, large amounts of this water are being contaminated by refractory organic substances derived from industrial, domestic and farming activities [1,2]. Pharmaceutical active compounds (PhACs) are a highly relevant issue in this context since their use and diversity is increasing every year. Recently, these types of contaminants were considered as emerging contaminants (EC), which means that they are still unregulated or under regularization process and in the near future regulations regarding their maximum concentration in water must be established [3,4]. Domestic and pharmaceutical industry effluents and hospital and animal wastes as well are the major source of pharmaceutical products, containing a wide variety of these compounds [4].

The conventional biological treatment processes based on activated sludge are the cheapest available technology to remove and degrade organic contaminants from water streams. But, in the case of pharmaceuticals, these substances are not completely removed because they are toxic and bio-refractory, which have contributed to their continuous release into the environment.

In the past few years, pressure-driven separation membrane technologies like Nanofiltration (NF) or Reverse Osmosis (RO) have been the focus of many studies, as an alternative to conventional treatment processes since they are energetically efficient and environment-friendly.

Thus, the main goal of the present paper is to study the effect of several operative variables (pH, drop pressure and ratio between contaminants’ concentration) on the removal of a mixture of sulphamethoxazole (SMX) and diclofenac (DCF) from aqueous solutions by nanofiltration (NF) with a Trisep TS80 membrane. Moreover, it aims to evaluate the impact of the water matrix on contaminants rejection and on the inhibitory effect caused by the toxicity of the raw effluent and the treated samples.

This work aims to investigate and optimize the performance of the Trisep TS80 nanofiltration (NF) membrane for the removal of a mixture of two most detected pharmaceutical compounds in municipal wastewaters worldwide, Sulfamethoxazole (SMX) and Diclofenac (DCF). Several NF tests were carried out to study the rejections of these contaminants spiked both in demineralized water, filtrated natural water and secondary effluent. pH was the variable that most affected the contaminants rejection and membrane permeability. In the case of synthetic effluent, an applied pressure of 10 bar and pH 7 were determined as the best operating conditions, which allowed achieving almost total COD retention and a global contaminants rejection of 96.3%. The application of different water matrices (river water and secondary municipal effluent) had no significant impact on process efficiency. Vibrio fischeri luminescence inhibition tests revealed that treatment by nanofiltration reduced acute toxicity of all studied effluents.

The main drawback of the membrane filtration systems is the membrane’s fouling which occurs due to the deposition of the pharmaceuticals and/or organic matter on its surface. This phenomenon leads to a decline in the permeate flux, considerably changes the membrane surface characteristics such hydrophobicity, morphology and surface charge, affecting this way the transport of the contaminants and, hence, the process separation performance. Thus, this work also aims to analyze the membrane’s fouling and compare two types of membrane cleaning (with demineralized water and with acid cleaning with HCl) on the membrane fouling index (FI).

Sulfamethoxazole and diclofenac sodium salt of high purity grade (>98%) were obtained from Alfa Aesar and stored at 5°C. Solutions of both PhACs were prepared using demineralized water, filtrated water from Mondego river and municipal secondary effluent coming from a biological treatment process (trickling filter beds), Coimbra, Portugal.

Nanofiltration experiments were carried out in a cross-flow operation mode using flat-sheet laboratory-scale membrane filtration unit (SEPA CF II, Osmonics). The feed stream was pumped to the membrane module by a diaphragm pump. The drop pressure (∆P) was regulated using a concentrate flow control valve and measured with a precision manometer (SPAN) and the concentrate flow rate was monitored by a flowmeter. The membrane filtration system was operated in recycle mode, since both the permeate and the concentrate returned to the feed reservoir.

The target compounds (SMX and DCF) were detected and quantified by HPLC. Chemical oxygen demand (COD) of the raw effluents and treated samples was determined by potassium dichromate closed reflux method described by ISO 6060. Bioluminescence tests were used to determine the toxicity of the raw wastewaters and treated samples in order to assess their impact in ecosystems. Experiments were run in duplicate to ensure the reproducibility of the results. LUMISStox tests (Dr. Lange, Germany) were performed according to the standard...
method DIN/EN/ISO 11348-2. Atomic Force Microscopy (AFM) was performed in order to compare surface topography of the virgin and used membrane. Total Dissolved Solids (TDS) in the natural water matrices were determined according to standard methods. The amount of inorganic ions Ca$^{2+}$ and Mg$^{2+}$ dissolved in river water and secondary effluent was measured by atomic absorption spectroscopy (Perkin-Elmer 3300).

The speciation of SMX and DCF mixtures in aqueous solutions and their fractionation by nanofiltration is dependent of the solution pH, which also determines the charge density of the membrane surface with negative zeta potential throughout the pH range tested. In other words, the feed pH interferes on the rejections mechanisms. Thus, a pH scanning was done and the individual and global rejection were calculated with the results of COD (Fig. 1a)) and HPLC (Fig. 1b)) analysis.

Feed water pH was the operating variable that most affected contaminants rejection and membrane permeability, since it influences the membrane and solutes properties and, hence, the dominant rejection mechanisms. Regarding to the mixture, at pH below 4 and above 7, high retentions were reached by size exclusion and electrostatic interactions, respectively. However, at intermediate pH (4-6) a decrease on chemical oxygen demand (COD) and global contaminants rejection was observed, reaching the minimal values of 37% and 46% at pH 6, possibly due to relevant electrostatic interactions between the two compounds, which make them highly hydrophilic and with a high dipole moment.

An applied pressure of 10 bar and pH 7 were determined as the best operating conditions. At these conditions, for the synthetic effluent, an almost total COD (Fig. 1a) retention and a global contaminants rejection of 96.3% were achieved (Fig. 1b). For the same conditions, the use of natural water matrixes had no significant impact on the process efficiency, with a COD retention of 90.9% and 96.1% for secondary effluent and river water, respectively. Regarding to HPLC, a global retention of 98.5% and 96% were achieved for the secondary effluent and river water. Moreover, nanofiltration was able to reduce the acute toxicity of both matrices towards Vibrio fischeri; however, the light inhibition percentage for the treated samples was higher for the river water.

AFM analysis revealed the increase of zed quota and roughness of the membrane. Moreover, a higher flux decline was obtained for the synthetic effluent which indicates the deposition of the PhACs on the membrane surface. A similar maximal Fouling Index was attained for both water and acid cleaning, 49.8% and 47.4%, respectively.

Acknowledgements
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References
Assessment of the influence of clearance and agitation on the nucleation rate and particle size distribution in anti-solvent crystallization process of a drug substance

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The impact of reactor hydrodynamics on the nucleation rate and Particle Size Distribution (PSD) of a drug substance produced in an anti-solvent crystallization process was evaluated. For this purpose, a Design of Experiment (DoE) approach was employed, where the suspension height:clearance ratio and power per volume were varied along the crystallization. Subsequently, in order to better understand the interaction among the variables, correlations were found by Multiple Linear Regression (MLR). It was found that both nucleation rate and the particle size distribution can be influenced by the suspension height:clearance (H/C) ratio and power per volume (PV). The nucleation rate seems to increase when the ratio decreases and increases when the power per volume increases. Larger particles seem to be produced when a higher ratio and lower power per volume are employed.

Introduction

Crystallization is a vastly used solid-liquid separation process in the pharmaceutical industry due to its capability to purify while obtaining a product with the desired physical properties [1]. Anti-solvent crystallization is a method extensively employed in the pharmaceutical field, due to a lower energy consumption in comparison with cooling or evaporative techniques [2]. However, crystal formation is related to reactor hydrodynamics, since the latter affects the incorporation of the anti-solvent into the solution and as consequence, the distribution of supersaturation (driving-force), which in turn, affects the nucleation and growth kinetics and thus, it can compromise the particle size distribution of the product [2], [3]. In order to reduce batch-to-batch variability at industrial scale, the impact of the hydrodynamics on the metastable zone width (MSZW), which is the zone where growth occurs and thus, influence the final product properties, must be understood.

Regarding studies that report the impact of clearance on the crystallization process, Kačmíř et al. [4], for instance, studied the influence of the impeller clearance in a dual pitch blade turbine (PBT) impeller system on the crystal growth kinetics of Borax during a cooling crystallization and concluded that the clearance influences the crystal size over process time [4]. Shimizu et al. [5], studied the effect of the clearance of a turbine impeller on the crystal size distribution of aluminium potassium sulphate in a batch crystallizer by cooling with seed crystallization and observed that when increasing the clearance, the median diameter of the crystal increased [5]. Additionally, Jin Liu et al. [6], assessed the influence of agitation rate, impeller type and diameter and clearance, and the presence of baffles on the primary nucleation on cooling crystallization of butyl paraben in ethanol and m-hydroxybenzoic acid in 1-propanol [6]. They found that the induction time seems to decrease with increasing agitation rate and in presence of baffles, the induction time is shorter when using a Rushton turbine than when using a marine propeller and a disk impeller. Regarding the influence of impeller clearance on the average induction time of butyl paraben in ethanol at different agitation rates, with a disk impeller, no clear difference was observed [6].

Nevertheless, most of the studies that report the influence of the clearance on the induction time only address cooling crystallizations and thus, the impact of clearance on the nucleation rate and particle size distribution during an anti-solvent crystallization of a drug substance is still unknown.

The aim of this study is to investigate the impact of hydrodynamics on the nucleation rate and PSD parameters (Dv10, Dv50 and Dv90) of drug substance obtained through an anti-solvent crystallization method. For this purpose, a DoE approach was employed, varying the height of suspension:clearance ratio and power per volume. The experimental data was further used to investigate possible correlations among the variables by multiple linear regression (MLR).

Methods

Anti-solvent crystallizations of a drug substance were performed using a 500 mL glass reactor and a retreat curve impeller (RCI), where the clearance was varied along with the power per volume. The suspension height was maintained constant in all experiments. The temperature was controlled with a Huber cryostat bath and the nucleation rate was detected with FBRM (Focused Beam Reflectance Measurement) Lasentec probe. The resulting crystals were filtered under vacuum. The wet crystals were dried under vacuum at a temperature not more than 60 °C. The PSD of the dry crystals was measured using Malvern Mastersizer 2000 particle size analyzer.

Statistical models were obtained correlating the suspension height:clearance ratio and power per volume with the nucleation rate and PSD parameters by MLR. The models were optimized by using quadratic, logarithmic and squared-root terms. The quality of the models was evaluated in terms of adjusted coefficient of determination (R²).

Results

The models obtained as well as the respective R² and adjusted R² are summarized in Table 1. From the data gathered in Table 1, it is possible to observe relevant correlations among the H/C ratio, power per volume, nucleation rate and PSD parameters, since R² is higher than 0.67 [7]. The regression coefficients are depicted in the Graphical Abstract. These indicate that both the nucleation rate and the PSD parameters can be influenced by the hydrodynamics inside the reactor. The nucleation rate seems to be higher when the H/C ratio decreases and when the power per volume increases. This can be explained by the fact that higher
agitation rates promote collision among crystals, baffles and impeller, which therefore increases secondary nucleation and increases the formation of crystals. The PSD parameters also seem to be affected by the H/C ratio and the power per volume. Larger particles seem to be produced when a higher H/C ratio is employed, whereas, on the other hand, higher particle size is obtained when the power per volume decreases.

Table 1. R² and adjusted R² of the selected (optimized) models. NR – Nucleation Rate; a, b – regression coefficients.

<table>
<thead>
<tr>
<th>Model</th>
<th>R²</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>NR = a.(H/C)² + b.PV</td>
<td>0.68</td>
<td>0.52</td>
</tr>
<tr>
<td>Dv10 = a.(H/C)² + b.PV</td>
<td>0.72</td>
<td>0.58</td>
</tr>
<tr>
<td>Dv50 = a.(H/C)² + b.PV</td>
<td>0.81</td>
<td>0.71</td>
</tr>
<tr>
<td>Dv90 = a.PV²</td>
<td>0.83</td>
<td>0.80</td>
</tr>
</tbody>
</table>

Conclusions
• Both nucleation rate at induction time and the PSD parameters can be influenced by the suspension height:clearance ratio (H/C) and power per volume.
• The nucleation rate seems to increase when the H/C ratio decreases and increases when the power per volume increases.
• Larger particle size seems to be produced when a higher H/C ratio and lower power per volume are employed.

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References
Glycerol steam reforming for hydrogen production: traditional versus membrane reactor

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A phenomenological model was developed for predicting the performance of a traditional reactor (TR) versus a membrane reactor (MR) for hydrogen production via glycerol steam reforming (GSR). A parametric analysis was carried out for different ranges of conditions, namely: temperatures of 773-973 K for the TR and 673-773 K for the MR (due to restrictions of existing Pd-based H2 permselective membranes), water-to-glycerol feed ratios (WGFRs) of 4-12 and total pressures of 100-500 kPa; the membrane thickness influence in the MR was also assessed. For the same operating conditions (773 K, WGFR of 4 and 500 kPa), the MR showed an enhancement in the H2 yield over 220 % when compared with the TR. Finally, when the maximum amount of H2 production was analyzed for both reactor configurations, an increase of 6 % was observed in the MR while allowing reducing the operating temperature by 250 K.

Introduction
Developing alternatives of renewable and eco-friendly energy sources has begun to be part of our society goals since global warming is a problem that arises essentially from the use of fossil fuels. Biodiesel has received widespread attention being one of the auspicious alternative energy sources, once it reduces a lot of CO2 emissions (over 52 % [1]) when compared to fossil fuels, apart from being a renewable energy source. However, biodiesel is not competitive in terms of price yet; one way of lowering the biodiesel production cost would be to use its main product, glycerol, to produce H2 (or syngas) [2]. The global reaction of glycerol conversion into hydrogen through steam reforming is defined by Eq. \( \text{Error! Reference source not found.} \):

\[
\text{C}_3\text{H}_3\text{O}_3 \text{(g)} + 3\text{H}_2\text{O} \text{(g)} \rightarrow 7\text{H}_2 \text{(g)} + 3\text{CO}_2 \text{(g)} \quad (1)
\]

This reaction is considered to be a combination of glycerol decomposition (Eq. (1)) and water-gas shift (WGS, Eq. (2)) [3]:

\[
\text{C}_3\text{H}_3\text{O}_3 \text{(g)} \rightarrow 3\text{CO} \text{(g)} + 4\text{H}_2 \text{(g)} \quad (1)
\]

\[
\text{CO} \text{(g)} + \text{H}_2\text{O} \text{(g)} \rightarrow \text{CO}_2 \text{(g)} + \text{H}_2 \text{(g)} \quad (2)
\]

However, the formation of secondary products must be considered as well [3], namely as described by Eq. \( \text{Error! Reference source not found.} \) (CO methanation):

\[
\text{CO} \text{(g)} + 3\text{H}_2 \text{(g)} \rightarrow \text{CH}_4 \text{(g)} + \text{H}_2\text{O} \text{(g)} \quad (3)
\]

Innovative reactor configurations such as membrane reactors (MRs) have been studied in the perspective of process intensification and/or aiming improving performance of traditional reactors (TRs). In the case of a MR, the selective removal of H2 from the reaction zone (by using an H2-permselective membrane) during GSR shifts the thermodynamic equilibrium towards the products side, producing a higher H2 yield than in a TR [2]; furthermore, it is expectable that it can also inhibit other undesirable reactions [3].

Although some phenomenological models have been developed on membrane reactors, there are few modeling studies on glycerol steam reforming performance in a membrane reactor.

This work aims to simulate the behavior of each species involved on GSR (glycerol decomposition, CO methanation and WGS), both in TR and MR, and assess the range of operating conditions where the latter can be beneficial, and quantify such improvement. Moreover, the species simulated profiles with and without hydrogen removal are compared. Parameters like hydrogen recovery and membrane thickness are also evaluated for the MR.

Phenomenological model
The model proposed in this work considers steady-state conditions, non-isothermal operation, pressure drop along the bed, non-constant velocity, constant porosity of the bed, ideal gas behavior, the use of a membrane selective only towards hydrogen (e.g. dense Pd-based membrane) and absence of internal (intra-particle) and external (film) mass-transfer resistances. To simulate such system, it was necessary to use 7 differential equations for the partial mass balances (one for each species – Eq. (4)), one for the total mass balance (Eq. (5)) (because the gas velocity along the reactor is not constant), one for the energy balance (to obtain the temperature variation (Eq. (6)) and, finally, the differential equation to define the total pressure change along the reactor length – Ergun equation (Eq. (7))). Furthermore, a dense Pd-Ag membrane selective only towards H2 was considered to be used in the MR and the Sievert’s law was applied to describe the H2 permeation flux. Danckwerts boundary conditions were used to solve the system of ODEs. The differential equations were solved using the bvp4c function (MATLAB software (R2016b version), with a finite difference code which implements the three-stage Lobatto IIIa formula.

\[
\begin{align*}
\frac{d}{dx} (u_i) &= \frac{W_{\text{out}}}{V_{\text{eq}}} \left[ (v_i R_1 + v_2 R_2 + v_3 R_3) - 2n R_i f_i^i \right] \Delta \frac{A_r}{A_v} = 0 \quad (8) \\
\frac{dW_{\text{out}}}{dx} &= \frac{W_{\text{in}}}{V_{\text{eq}}} \sum_{i=1}^{3} v_i R_i f_i^i \frac{2n R_i f_i^i \Delta A_r}{A_v} = 0 \quad (9) \\
\frac{dT}{dx} &= - \frac{W_{\text{out}}}{V_{\text{eq}}} \sum_{i=1}^{3} \left( (-\Delta H_i) (v_1 R_1 + v_2 R_2 + v_3 R_3) \right) \\
&\quad - 2 \frac{h}{h_{\text{ref}}} (T - T^*) = 0 \quad (10) \\
\frac{dP}{dx} &= \frac{150}{\rho_{\text{g}}} \frac{(1 - \varepsilon_b)^2 \mu{R}}{u_b^2} \frac{1.75 \times 10^{-6}}{u_c^2} \left( 1 - \frac{\rho_{\text{g}}}{\rho_{\text{p}}} \right) u_i^2 = 0 \quad (11)
\end{align*}
\]
Results and Discussion
In order to verify if the results obtained are reliable, validation of the model has been done by comparing predicted results with others reported in the literature. Table 1 shows that there is a good agreement, particularly in what concerns to H₂ formation. However, the slight discrepancy with respect to the other species might be due to the fact that not all the reactions involved in the GSR process were considered. Furthermore, the kinetics used in this work do not consider the formation of liquid and solid products, which are present in the GSR reaction and whose formation depends on the experimental conditions and catalytic system employed. Nevertheless, the results displayed evidence that the model predictions are reliable.

The parametric analysis has shown that higher temperatures and pressures, and lower WGFRs, increase the glycerol conversion ($X_{\text{glycerol}} = \frac{r_{\text{glycerol}}}{r_{\text{glycerol}} + r_{\text{H₂}}}$) for both types of reactors. Furthermore, for improving the H₂ recovery in the MR ($\eta_{\text{H₂}} = \frac{r_{\text{H₂}}}{r_{\text{H₂}} + r_{\text{H₂,ret}}}$), lower WGFRs and higher temperatures and pressures should be used, combined with a thinner membrane. However, a commitment between the membrane thickness and the H₂ purity in the permeate side must be taken into account since the purity of hydrogen decreases along with the decrease of the membrane thickness.

Comparing both reactors performance at 773 K, WGFR of 4 and 500 kPa (Figure 1), it can be seen that the MR provides an enhancement of 224 % in terms of H₂ yield ($\eta = \frac{r_{\text{H₂}}}{r_{\text{H₂}} + r_{\text{H₂,ret}}}$). However, and even more importantly, a significant decrease in the CH₃ yield of 46 % is attained, being the main undesirable by-product (since each mole of produced methane consumes 3 mol of hydrogen – Eq. (3)).

Table 1. Comparison between the model predictions and experimental works from other authors.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Operating conditions</th>
<th>Products selectivities (%)</th>
<th>Products yield (p/p_{glycerol feed})</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni/Al₂O₃</td>
<td>T = 923 K P = 100 kPa</td>
<td>H₂ = 59 H₂ = 61</td>
<td>-</td>
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<tr>
<td>(10 wt.%)</td>
<td>WGFR = 12 WHSV = 10 h⁻¹</td>
<td>CO₂ = 22 CO₂ = 27</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH₄ = 6 CH₄ = 6</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO = 13 CO = 6</td>
<td>-</td>
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<tr>
<td></td>
<td>Ref. [4] This work</td>
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<td>-</td>
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<tr>
<td>Ni/Al₂O₃</td>
<td>T = 973 K P = 100 kPa</td>
<td>H₂ = 61 H₂ = 61</td>
<td>-</td>
</tr>
<tr>
<td>(4 wt.%)</td>
<td>WGFR = 6 WHSV = 10 h⁻¹</td>
<td>CO₂ = 22 CO₂ = 21</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH₄ = 3 CH₄ = 4</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO = 14 CO = 14</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ref. [5] This work</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ni/Al₂O₃</td>
<td>T = 823 K P = 100 kPa</td>
<td>H₂ = 3.3 H₂ = 3.3</td>
<td>-</td>
</tr>
<tr>
<td>(15 wt.%)</td>
<td>STCR = 2.6 GHSV = 5.0×10⁶ (mL·g-cat⁻¹·h⁻¹)</td>
<td>CO₂ = 1.60</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CH₄ = 0.23</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CO = 0.28</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>CO = 0.50</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Ref. [6] This work</td>
<td>-</td>
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</table>

Acknowledgements
M.S. Macedo acknowledges LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, and the project NORTE-01-0145-FEDER-000005 - LEPABE-2-ECO-INNOVATION, supported by the North Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF), for her research grant. M. A. Soria is grateful to the FCT for his postdoctoral grant (SFRH/BPD/88444/2012), with financing from the European Social Fund (ESF) and the Human Potential Operational Program (POPH).

References
Monitoring the transesterification reaction by continuous off-line density measurements

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The continuously monitoring of transesterification reaction in industrial production is of high relevance in order to avoid operational problems, eliminate batch sampling and analysis delay and guarantee the quality of the final product through a process control system. Also it is essential for kinetic studies. In this work, the transesterification of cottonseed oil at 50 °C and 70 °C, with methanol/oil molar ratios of 4.5, 6.0 and 7.5 were carried out and the oil conversion as a function of reaction time was evaluated by 1H NMR. The density of the reaction mixture, at these experimental conditions, was measured as a function of the reaction time by an off-line methodology. From the density-time and FAME conversion-time profiles the operative relationships between conversion, density, alcohol to oil ratio and temperature were established. A very good correlation was obtained.

Introduction

Biodiesel is a biofuel used in compression ignition engines. The success of introducing biodiesel into the market is mainly due to the efficient standardization as consequence of the wide variety of oils and fats that can be used in the production. The continuously monitoring of transesterification reaction in industrial production is of high relevance in order to avoid operational problems, eliminate batch sampling and analysis delay and guarantee the quality of the final product through a process control system. An appreciable number of analytical procedures have been developed to assess the quality of biodiesel in terms of the specified standards. However, due to the rapid conversion of methanolysis reaction, most of the analytical techniques such as GC, HPLC, 1H NMR and different methods based on IR, are not suitable for on-line monitoring of the process, since they are impractical, time-consuming and requiring instrumentation not available in all standard laboratories. For this purpose, analytical methods based on easily measurable physical properties are preferred. Properties, such as the refractive index, viscosity, density and speed of sound vary significantly for the main components of biodiesel (TG, Me, FAME and Gly). Therefore, the changes during the oil conversion to biodiesel are suitable to continuous monitoring of the reaction, as evidenced by the growing number of studies in this field. Previous studies include the use of viscosity [1], refractive index [2, 3], acoustic techniques [2], pH [4] and impedance measurements [5] used for real-time monitoring. Also, a methodology using calibration curves for density, refractive index and viscosity of biodiesel was used to determine the composition in TAGs and FAMEs [6]. Recently, an off-line monitoring of transesterification reaction was made through density variations measured by using an electronic analytical balance [7]. The main goal of this work is to study a simple, cheap and easy monitoring methodology based on the measurements of the density variations of the reaction medium during the course of the transesterification reaction, with emphasis on real-time applications. 1H-NMR spectroscopy was used as reference method.

Method

The methanolysis of cottonseed oil, at 50 °C and 70 °C, with molar alcohol/oil ratios of 4.5, 6.0 and 7.5 were carried out and the oil conversion as a function of reaction time was evaluated by 1H NMR. For each reaction condition a minimum of three replicates were performed. The density of the reaction mixture was measured by an off-line methodology, using an Anton Parr DMA 60 vibrating tube densimeter with a DMA measurement cell 512P. The set up for the transesterification described in a previous work [8] was used with the only difference that tubes were adapted to circulate de the mixture through the 512 P cell, so that the density was measured continuously and in real time under the same conditions at which the reaction occurs. From the reaction mixture, ten samples were collected over the course of the reaction and dissolved in 600µl of deuterated chloroform. The 1H NMR spectra were recorded on a Bruker Avance III, operating at 400 MHz at 298.2 K.

Results and discussion

The evolution of measured density during the methanolysis of cottonseed oil, at 70 °C and alcohol/oil molar ratio r=6 is shown in Figure 1.

![Figure 1](image_url)

Figure 1. Evolution of density with reaction time, at 70°C and r = 6.0. Stages: (1+2) oil+MeOH mixture, dispersion of oil and slow rate; (3) minimum density; (4) fast reaction; (5) chemical equilibrium.

As methanol is not soluble in the oil the first minutes, corresponding to stages (1+2), are spent to the dispersion/diffusion of catalyst and methanol in the oil. Due to mixing process, the density of oil falls abruptly to a minimum density value, ρm, which corresponds to the reaction time tm at
stage (3). This almost vertical profile of density was also described by Tubino et al. [7] in the monitoring using mass variation techniques, which can be considered as derived from the density monitoring. After sequence (1+2+3), a fast reaction stage is observed (4). According to kinetic data of several oils, this region is characterized by a strong decrease in the concentration of triglycerides and a marked increase in the concentration of FAMEs and glycerol. It is interesting to see that the shape of density profile in this region is quite similar to the one observed from kinetic studies, corresponding to the increasing concentration of FAMEs and also is comparable to that found in this study, for oil conversion curves. After 10 to 15 minutes the chemical equilibrium is reached and density increases slowly with time (stage 5), corresponding to the slowdown of the formation of FAMEs and glycerol over time. Stages 4 and 5 are related with production of FAMEs, therefore it is important to find some analytical relations, between the density and the oil conversion, of practical interest in biodiesel production.

The density measured as a function of time, for several methanol/oil ratios (r) and for T=323 K and T=353 K, were used to establish a linear correlation, which allows us to find out the densities of the reaction mixture at the same times at which the FAMEs conversion was determined by 1HNMR. From the analysis of experimental data (ρ; %C), for each combination of T and r, the following relationship between the density and the CSO conversion was found:

\[ \rho = A_0 + A_1 \cdot T + A_2 \cdot r + A_3 \cdot (%C) + A_4 \cdot r \cdot T + A_5 \cdot r \cdot (%C) + A_6 \cdot (%C)^2 \]  \hspace{1cm} (1)

where A1 are parameters obtained from the fitting of (% C, density) data, resulting in correlation coefficient \( r^2 = 0.990 \) and standard deviation \( \sigma = 0.9 \text{ kg m}^{-3} \), a value which is close to the combined uncertainty on density measurement. From Eq. (1), the oil conversion %C can be calculated, from the process variables (T and r) and the measurement of density, as follows:

\[ \%C = \frac{-C_2 \pm (C_2^2-4C_1)^{1/2}}{2} \]  \hspace{1cm} (2)

where

\[ C_1 = \frac{(A_1+A_2 \cdot r) \cdot A_6}{A_4} \]  \hspace{1cm} (3)

\[ C_2 = \frac{(A_0+A_1 \cdot T+A_2 \cdot r+A_3 \cdot T \cdot r)-\rho}{A_6} \]  \hspace{1cm} (4)

and the signs + and – apply for the time conditions (t<t\text{min}) and (t>t\text{min}), respectively.

The monitoring of methanolysis reactions through off-line density measurements and RMN (both expressed as %C) are displayed in Fig. 2, for 323.15. In this figure, the oil conversion values calculated from Eq. (2), were compared with the values obtained by RMN analysis. The relative deviation between the two set of values are usually in the range ± 5 % (84 % of all data for both temperatures).

![Figure 2. Conversion (%C) of CSO as a function of time, at 323.15 K. Colours blue, red and black are relative to r = 4.5, 6.0 and 7.5. Lines represent %C calculated from Eq. (2).](image)

**Conclusion**

In this work, a simple and easy methodology was developed to monitor the reaction of methanolysis under different conditions, through continuous measurements of density of the reaction medium. The transesterification of cottonseed oil at 50 °C and 70 °C, with methanol/oil molar ratios of 4.5, 6.0 and 7.5 were carried out and the monitoring of the reaction was made by following the changes in density of the mixture. The oil conversion as a function of reaction time was evaluated by \(^1\)H NMR. From the density-time and FAMEs conversion-time profiles, relationships between conversion, density, alcohol/oil molar ratio and temperature were established. The obtained AARD=3.6% and standard deviations of 3.8%, for T=323.15 K and AARD=3.0% and σ=3.5%, for 353.15 K allow concluding that the new developed correlation can describe the oil conversion evolution during the reaction time with good accuracy.

**References**


Multifunctional reactors for biogas upgrading through CO₂ methanation: thermodynamic considerations

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Prior to injection into the natural gas grid or use as vehicle fuel, biogas must undergo an upgrading process for CH₄ purification whereby, globally, the CO₂ is commonly removed by pressure swing adsorption and thereafter emitted to the atmosphere. This work studies an alternative, considering the conversion of CO₂ present in the raw biogas into more CH₄ using renewable-based H₂. The feasibility of such process was assessed thermodynamically, focusing on the Sabatier reaction taking place in the presence or absence of water separation in relevant temperature, pressure and biogas composition ranges. The results showed that the CH₄ yield increases, but only up to an optimum water removal fraction; above this value, coke formation is favored. Still, considering water removal represents a minor impact on CH₄ production, but can be particularly useful to minimize the production of CO₂ allowing to fulfill with the biogas quality specifications for injection into the natural gas grid.

Introduction

The consumption of fossil fuel reserves, climate change, fuel prices, as well as political issues have accelerated the development and dissemination of technologies to exploit renewable sources for securing the energy demand in a sustainable way. Among them, biogas is an interesting option to replace natural gas [1]. However, for injection of biogas into the natural gas grids or to use it as a vehicle fuel, a biogas upgrading stage is required where CO₂ is separated and, generally, vented to the atmosphere. Instead of the conventional biogas upgrading separation processes, an alternative would be to reuse that CO₂ present in the raw biogas for producing more CH₄ (Eq. (R1), in Table 1), using renewable-based H₂ (the so-called Power-to-Gas concept, as explained in the Graphical Abstract). Since the CO₂ methanation is a reversible reaction, this work compares, from the thermodynamic point of view, the performance of multifunctional reactors (membrane or sorption-enhanced reactors) wherein water is selectively removed (through a water selective membrane or sorbent, respectively) to that obtained with a traditional reactor, i.e., without water removal. Moreover, the effect of water removal on the biogas specifications to be injected into natural gas grids as well as the influence of temperature, pressure and biogas compositions on CH₄ yield and selectivity are also assessed.

Methods

Simulations were performed using the Gibbs reactor model (RGibbs) available in the Aspen Plus V8.8 software applying the nonstoichiometric method based on direct minimization of the Gibbs free energy. Table 1 lists other reactions that can take place, besides the CO₂ methanation.

To simulate the in situ water removal in the reactor, a modular approach was used considering Z sub-separators and (Z+1) sub-methanators, as described elsewhere [2,3]. The value of the targeted global H₂O removal fraction (R) is expressed as follows:

\[ R = \frac{F_{H_2O,removed}}{F_{H_2O,produced} + F_{H_2O,feed}} \]  

Hence, the developed modular approach allows to simulate a traditional reactor (TR, \( R = 0 \)), a membrane reactor (MR, 0<\( R < 0.99 \)) or a sorption-enhanced reactor (SER, \( R > 0.99 \)), the later operating before water breaks through the reactor.

The analysis was performed at temperatures between 200 °C and 450 °C, while the total pressure was varied between 1 atm and 31 atm. Based on realistic biogas compositions, the H₂O and O₂ fractions in the raw biogas were kept constant (1.08 % and 0.43 %, respectively) while the CH₄ and CO₂ contents were changed in the simulations, considering the boundaries of 50-75 % and 25-45 %, respectively [4]; this way, the following methane to carbon dioxide ratios were analyzed: i) CH₄/CO₂=1.11 (51/46), ii) CH₄/CO₂=1.92 (64/33) and iii) CH₄/CO₂=2.96 (72/25). H₂ was added to the process according to the stoichiometric H₂/CO₂ ratio of 4.

Results and Discussion

For the traditional reactor case (not shown for brevity reasons), CO₂ conversion was never complete in the whole range of temperature and pressure studied, regardless of the CH₄/CO₂ ratio considered. As foreseen by the exothermic nature and stoichiometry of CO₂ methanation reaction (Eq. (R1)), the CO₂ conversion and CH₄ yield show the same dependence on the operating conditions, increasing with pressure and decreasing with temperature. Concerning the CO and coke formation, both are favored at high temperatures and low pressure. CO production occurs through reaction R2 and reverse of R3, while coke mostly from reaction R5.

Fig. 1 displays the CO₂ conversion as well as CH₄ and coke yields variation with the water removal fraction (R) and the CH₄/CO₂ ratio at 325 °C and 1 atm. There is a unique amount of water removal which maximizes the CH₄ yield depending, simultaneously, on the temperature, pressure and CH₄/CO₂ ratio. This value, which henceforth will be referred as the optimum water removal fraction (R*), is depicted in Fig. 1b, by way of example at 325 °C and 1 atm for each of the three cases of CH₄/CO₂ ratios considered. Above the R*, the CH₄ yield starts decreasing. Higher values of R* are obtained when lower CH₄/CO₂ ratios are employed.

The decrease of CH₄ yield shown in Fig. 1b is related to the displacement of the equilibrium of reactions (R6) and (R7) leading to coke formation (Fig. 1c). Due to the exothermic nature of these reactions and the removal of water, coke can thus be formed in the whole range of temperatures considered,
whereas in the traditional reactor case it was confined to temperatures above 300 °C. Coke formation through reactions (R6) and (R7) is further supported by the values obtained for CO$_2$ conversion (Fig. 1a); the conversion increases with $R$ and decreases with the temperature, as a result of the occurrence of exothermic reactions (R1), (R6) and (R7), all of them having water as product. Complete CO$_2$ conversion was observed for the whole range of temperature considered, regardless of the CH$_4$/CO$_2$ ratio and pressure, when $R$ was 0.99. The obtained results show that removing water up to $R^*$ still represents a minor impact on CH$_4$ yield (e.g. shown in Fig. 1b). However, the water removal approach can be useful to decrease the amount of carbon oxides in the reactor outlet stream, as shown in Fig. 2, where both CO and CO$_2$ specifications (grey area) for methane injection into natural gas grids are always guaranteed by carrying out the operation with a multifunctional reactor (MR or even a SER).

![Figure 1](image1.png)

**Figure 1.** Conversion of CO$_2$ (a), yield of CH$_4$ (b) and coke (c) at 325 °C and 1 atm as function of $R$ and CH$_4$/CO$_2$ ratio.

The maximum $R^*$ obtained in the present work was 0.6 (for CH$_4$/CO$_2$ =1.11 in the 200 °C to 325 °C temperature range at a pressure of 5 and 10 atm) and thus a SER for biogas upgrading is not recommended, from the thermodynamic point of view, since the $R^*$ value was found to be always lower than 0.99. The reverse water gas shift reaction (R1) is exothermic ($\Delta H_f^{298 K}$ = -165 kJ mol$^{-1}$), whereas the water gas shift reaction (R2) is endothermic ($\Delta H_f^{298 K}$ = 41 kJ mol$^{-1}$), as shown in Table 1, where the exothermic reactions (R3-R7) are also considered.

![Figure 2](image2.png)

**Figure 2.** CO and CO$_2$ content (graphs in the first and second row, respectively) in the SNG produced (dry basis) for the TR and the MR case (graphs in the first and second column, respectively).

**Conclusion**

A thermodynamic analysis of biogas upgrading based on CO$_2$ conversion by the Sabatier reaction featuring water removal showed that the optimum water removal fraction, i.e. which maximizes the methane production, depends on the CH$_4$/CO$_2$ ratio in the biogas stream as well as on the operating temperature and pressure. Although removing water through an H$_2$O-permselective membrane represents a minor impact on CH$_4$ yield, this approach shows its usefulness to decrease the CO$_2$ content in the outlet stream and thus fulfill the biogas quality specifications to be injected into natural gas grids.

<table>
<thead>
<tr>
<th>Reaction</th>
<th>$\Delta H_f^{298 K}$ (kJ mol$^{-1}$)</th>
<th>Equation number</th>
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</thead>
<tbody>
<tr>
<td>Sabatier reaction</td>
<td>CO$_2$ + 4H$_2$ $\leftrightarrow$ CH$_4$ + 2H$_2$O</td>
<td>-165</td>
</tr>
<tr>
<td>Reverse water gas shift</td>
<td>H$_2$ + CO$_2$ $\leftrightarrow$ CO + H$_2$O</td>
<td>41</td>
</tr>
<tr>
<td>CO methanation</td>
<td>CO + 3H$_2$ $\leftrightarrow$ CH$_4$ + H$_2$O</td>
<td>-206</td>
</tr>
<tr>
<td>Carbon formation</td>
<td>2CO $\leftrightarrow$ C + CO$_2$</td>
<td>-172</td>
</tr>
<tr>
<td></td>
<td>CH$_4$ $\leftrightarrow$ 2H$_2$ + C</td>
<td>74</td>
</tr>
<tr>
<td></td>
<td>CO + H$_2$ $\leftrightarrow$ H$_2$O + C</td>
<td>-131</td>
</tr>
<tr>
<td></td>
<td>CO$_2$ + 2H$_2$ $\leftrightarrow$ 2H$_2$O + C</td>
<td>-90</td>
</tr>
</tbody>
</table>

**Acknowledgements**

A. Catarina Faria acknowledges LEPABE – Laboratory for Process Engineering, Environment, Biotechnology and Energy, and the project POCI-01-0145-FEDER-006939 funded by the European Regional Development Fund (ERDF), through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds, through FCT - Fundação para a Ciência e a Tecnologia, for her research grant. The authors acknowledge financial support by: project “LEPABE-2-ECO-INNOVATION” - NORTE-01-0145-FEDER-000005, funded by the Norte Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through ERDF; Project POCI-01-0145-FEDER-030277 funded by FEDER funds through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES.

**References**

Effect of TiO\textsubscript{2} plate supported nanotubes on the solar photocatalytic oxidation and ozonation of parabens mixtures

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TiO\textsubscript{2} nanotubes supported on titanium foils has been tested for photocatalytic degradation. This kind of approach can be a very suitable option for emerging contaminants degradation considering the possibility of reuse and recover of the catalyst comparing to the case where catalytic powder is used. In this study, besides photocatalytic degradation with sun and UVA radiation also ozonation and photocatalytic ozonation were tested on the degradation of a mixture of three parabens. Ozone coupled with the plates prepared at 20 and 40V improves the single ozonation allowing a reduction on the amount of ozone required for total parabens removal. The UVA radiation with the TiO\textsubscript{2} nanotubes does not promote significant degradation. On the other hand, sunlight radiation for the same plates enhanced the parabens degradation comparing with photolysis. Also catalytic ozonation with the same plates aided by sunlight radiation was tested for the abatement of the parabens mixture.

The endocrine disruptors compounds have been referred as a problem for human health. Among them, parabens are mentioned as potential toxic compounds present in the municipal wastewaters. Parabens are used as preservative and antimicrobial in a lot of pharmaceutical and personal care products. Normally, parabens are applied as a blend to enhance the spectrum of action. These compounds can be founded at micro and nano scale in the municipal wastewaters and drinking waters [1]. Current municipal wastewaters treatment technologies are not able to satisfactorily promote the degradation of these compounds.

Advanced oxidation processes appear as suitable alternatives to promote the degradation of these emerging compounds. Several studies can be found at the literature involving a wide range of AOP methodologies. Ozonation seems to be a good option for the removal of parabens [2]. Otherwise, heterogeneous catalysis with titanium dioxide seems to be a good option since allows the use of sunlight radiation for production of reactive oxidative species. Titanium dioxide is the most used semiconductor in the photocatalytic degradation due to its chemical stability and the low cost. Recently TiO\textsubscript{2} nanotubes have been used for photocatalytic degradation instead of powders due to the specific surface area and adsorption increase [3]. On the other hand, looking for the development of continuous catalytic reactors for wastewater treatment, nanotubes supported in plates seem to be a better option than powder TiO\textsubscript{2}.

One of the major drawbacks of the TiO\textsubscript{2} nanotubes is the high bandgap (3 – 3.2 eV) needing UVA radiation to promote the generation of electron-hole pairs. However, the heterogeneous photocatalysis can be enhanced if couple with ozonation. Ozone is a powerful oxidant that can promote the degradation of parabens [2]. Meanwhile, if the radiation source was good enough to produce the electron-holes pairs, they can reduce ozone to produce more oxidative species. Moreover, the sunlight radiation, even though the low amount of UVA in solar spectrum, can be used. This is an important advantage since no operating costs related with light will be present. Moreover, since Portugal is located in the Mediterranean area there is a long-time availability of Sun during the year. Despite of this, the sunlight radiation presents also UVB radiation that can be helpful to activate the TiO\textsubscript{2} nanotubes and also enhance the degradation of emerging compounds.

To the best of our knowledge, this kind of catalysts were never been tested coupled with ozone. One of the goals was to verify their efficiency on the depletion of a mixture of methyl (MP), ethyl (EP), propylparaben (PP) when coupled with ozone under the presence or not of UVA radiation and sunlight. The TiO\textsubscript{2} nanotubes were prepared by one-step anodization method using three different voltages 20, 30 and 40V. The effect of these three different voltages of preparation was also verified for catalytic and photocatalytic ozonation.

TiO\textsubscript{2} nanotubes have grown from titanium foils (5 x 2 cm). The anodization experiments were carried out in two-electrode electrochemical set-up [3]. After the preparation the TiO\textsubscript{2} samples were washed with deionized water and drying at 80 °C for 24 h. Followed by calcination at 450 °C during 1 h. During the experiments seven plates of (5 x 2 cm) were used. A simulated solution with 1 mg/L of each one paraben was prepared using ultrapure water. The experiments with ozone were carried out in a 2 L glass reactor under controlled temperature (25±1 °C) for 60 min. The ozone concentration entering and leaving the reactor was measured by gas ozone analyzers, with gas flow rate of 0.2 L/min, and the results were expressed as a function of transferred ozone dose (TOD) and time in the case of UVA and sunlight radiation. For sunlight experiments a tubular photoreactor was used equipped with a CPC collector with 0.5 L of volume. In all cases, the spectrum of light entering the reactor was recorded using an Ocean Optics USB4000 fiber optic spectrometer. The parabens concentration along reaction time was determined by HPLC.
Preliminary results reveal that ozone is effective on the mixture of parabens degradation, since in 10 min of contact is able to remove all the parabens (TOD = 3.8 mg/L). Otherwise the UVA radiation alone does not promote any degradation in 60 min. Sunlight photolysis after 60 min promoted about 55% of MP and EP degradation and 67% for PP. This reveals that the UVB and Visible light present on the composition of sunlight radiation have a positive effect on this mixture degradation. Therefore, the effect of visible light with the catalysts for parabens mixture degradation was tested using a visible light lamp (600W of power). However, no degradation was verified after one hour which means that probably UVB radiation may be the explanation of the higher efficiency of the sunlight experiments.

UVA radiation coupled with TiO$_2$ nanotubes for different voltages was analyzed for the parabens mixture degradation. The results were very similar for different voltages tested. The best one was 40V which led to up to 12% of removal for MP and 11% of removal for EP and PP. These results reveal a very low photocatalytic activity of nanotubes under UVA radiation. Therefore, bearing in mind the industrial application and the results achieved with the photolysis described above, the sunlight was tested using the different plates prepared under three voltages. After 60 min the best result was achieved for 40V which removed 74% of MP, 76% of EP and 81% of PP. The worst result was achieved with the plates of 20V where the removal was only 65% for MP and EP and 74% for PP.

Ozone was capable of removing all parabens mixture. To decrease the amount of ozone needed for total degradation, it was coupled with TiO$_2$ nanotubes (catalytic ozonation) and with UVA radiation (photocatalytic ozonation). As it can be seen in Figure 1 the presence of UVA radiation with catalyst and ozone does not enhance the parabens mixture degradation. TOD for totally removal was even higher than single ozonation. An improvement was verified in the catalytic ozonation using the 20 V and 40 V nanotubes which allowed a TOD reduction of about 2 mg/L comparing with single ozonation.

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References
Acidic aqueous biphasic systems: a new paradigm for the ‘one-pot’ extraction of critical metals

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Herein, acidic aqueous biphasic system (AcABS) based on ionic liquids (ILs) is proposed as a new extraction-separation platform for the integrated hydrometallurgical treatment of critical metals. AcABS, in which the inorganic salt component of traditional aqueous biphasic system (ABS) is replaced by the acid from the leaching solution, is shown to selectively separate cobalt from nickel, a separation relevant to the recycling of NiMH batteries. Selective electrodeposition of cobalt directly from IL rich phase was optimised based on the partition of the different system components, namely Cl– and H2O as well as water content. Selective deposition of cobalt in the presence of co-extracted manganese impurities was achieved in various AcABS derived systems resulting in highly tailored cobalt deposits. These results highlight the potential of AcABS derived systems as a ‘one-pot’ approach for metal treatment from leaching to electrodeposition.

Introduction

A sustainable, effective and affordable metal extraction process is an issue of critical importance. To alleviate metal criticality and reduce the environmental impact of mining operations, the development of a simple, robust and flexible platform is of particular relevance for the recycling of metals from secondary ‘ores’. The extraction, separation and purification of critical metals from complex matrices such as minerals or electronic wastes is technologically challenging, often requiring a series of demanding processing steps.[1,2] A traditional flowsheet for the hydrometallurgical treatment of metal feedstocks follows a series of concentration (leaching), purification (solvent extraction) and refining stages (electrodeposition or precipitation).[3] Process intensification, i.e. the ability to conduct multiple hydrometallurgical operations within a single processing unit, is of great interest industrially as this can reduce the economic and environmental impact associated with metal consumption.

For over a decade, ionic liquids (ILs) have been considered as promising solvent alternatives to volatile organic compounds (VOC). In the field of metal ions extraction, ILs are currently used at a laboratory scale as extracting media for various metals with excellent results. Nevertheless, industrial application of such ILs is limited by (i) the range of available hydrophobic ILs, most being based on fluorinated anions such as bis(trifluoromethanesulfonyl)imide ([NTf2]–), or on ILs incorporating long alkyl chains, (ii) the viscosity of ILs and (iii) production costs significantly higher than those of classical extracting systems currently in operation. IL-based acidic aqueous biphasic systems (AcABS) represent a promising alternative to solvent extraction process for the recovery of critical metals in which the substitution of the inorganic salt by an acid allows for the ‘one pot’ approach to the leaching and separation of metals. The work presented in this paper expands the scope of previously reported ionic liquid-based AcABS processes for the sustainable recycling of metals.[4-6]

Results and Discussion

In the newly reported AcABS composed of the IL tributyltetradecyl phosphonium chloride ([P44414]Cl), the acid present in the leaching solution was used to salt-out the IL. This is markedly different from conventional aqueous biphasic systems (ABS) in which the molar entropy of hydration of an inorganic salt dictates the formation of a reversible biphasic system composed of a salt-rich phase and organic-rich phase.[7] The extraction and separation of Co(II) from Ni(II) and Mn(II) using [P44414]Cl in hydrochloric acid media is investigated using a mixed ABS-AcABS approach, with the results presented in Figure 1.

Figure 1. Co(II), Mn(II) and Ni(II) extraction efficiencies (EE%) in ABS-AcABS system with varying NaCl to HCl concentrations. System compositions are provided in Table S2 of the ESI.

Variations in the HCl concentration and the chloride source (either NaCl or HCl), selective separation of Co(II) from Ni(II) was achieved whilst controlling the partition of the various system constituents. By conducting the extraction process in aqueous media, issues associated with conventional ILs mediated solvent extraction were circumvented whilst retaining the inherent advantages of ILs. Performing the extraction in aqueous media reduces the overall environmental impact associated with IL application as (i) lower quantities are required compared to solvent extraction and (ii) hydrophilic ILs are significantly less toxic and more biodegradable than their hydrophobic counterparts.

The distribution of HCl was found to have a profound influence on the subsequent electrodeposition of Co(II). To address this, a mixed ABS-AcABS extraction system was proposed which combines the advantages of the AcABS and ABS systems whilst...
diminishing the acid concentration required to induce phase separation as well as its distribution to the IL-rich phase. This enables the use of more environmentally friendly operating conditions and facilitates the metal deposition from the IL-rich phase. Selective deposition of Co(II) is optimized as a function of various parameters including water content, HCl concentration and NaCl concentration. By altering the water content of the [P44414]Cl-rich phase after extraction and separation of Co(II) from Ni(II), high quality dendritic deposits of pure metallic cobalt were obtained in presence of Mn(II) impurities, Figure 2. Furthermore, ABS-AcABS systems can be tailored to obtain metal deposits with varied properties. The results presented in this work demonstrate the applicability of AcABS for a ‘one-pot’ approach for the sequential leaching, solvent extraction and electrodeposition of metals. Moreover, this work provides an understanding of the fundamental mechanisms governing the extraction and electrodeposition in AcABS required to extend these findings to the recovery of other critical metals.

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References
The development of an adsorbent material by 3D printing technology with high CO2 adsorption capacity and electrical conductivity is presented for application in Electric Swing Adsorption process. The adsorbent is composed by 13X zeolite, activated carbon and binder. The prepared ink was characterized in order to determine the rheological properties of the mixture. The monolith was printed with dimensions of mm of mm. Textural characterization was performed by diverse techniques, including CO2 adsorption isotherm at 273 K measurement, being expected that the 3D printed monolith would have high CO2 adsorption capacity. Heating of material was tested by Joule effect through an electric current passage in the monolith. An increase of temperature of about 80 K during 180 s was verified with a power consumption of 3.25 W, demonstrating that the printed material is suitable for ESA processes applied for CO2 capture.

**Material and Methods**

**Monolith printing.** Three different powders were used to prepare the ink: 13X zeolite (Chembwerk, Kosttrz); Maxsorb MBCS-30 activated carbon (Kansai Coke and Chemicals Co Ltd); and Carboxymethylcellulose (VWR). A mixture with 21.1 g of 13X zeolite, 9.1 g of activated carbon and 1.5 g of CMC of dried powders was prepared in a mixing chamber of a Multi Lab Extruder (Caleva Process Solutions Ltd, England) during 60 minutes at 60 rpm. After that, 42 mL of deionized water was added drop by drop while mixing continuously at 70-80 rpm. At this point, the mixture presented a homogeneous and wet aspect and the ink production was completed. The mixture was immediately inserted in a syringe and placed in a Disco3ry Extruder machine (Structur3D Printing, Canada) to be printed. In order to create the desired monolith design, SolidWorks 2017® software was used. After, Ultimaker Cura 3.1.0 software was used to set the printing properties. For monolith printing, a conical polypropylene tip of 0.84 mm of diameter was used. The final printed monolith was dried at room temperature during 48 hours.

**Characterization.** Textural characterization of the 3D printed monolith was done by different techniques, such as, nitrogen adsorption at 77 K, carbon dioxide adsorption at 273 K, mercury intrusion and SEM/EDS analyses. Furthermore, the electrical properties of the monolith were analysed by connecting two crocodile tweezers to the printed structure to conduct the electric current. A power supply unit (Keithley, 2440, Newark, USA), that can deliver constant voltage or constant current intensity within 0 to 42 V and 0 to 5 A, respectively, was used. A type K thermocouple was placed in contact with the centre of the printed piece, in order to measure the temperature history.

**Results**

Rheological measurements of the ink demonstrated that at low values of shear rate, between 0.001 and 0.01 s⁻¹, viscosity remains constant and equal to 15701 Pa.s. After 0.01 s⁻¹, the results demonstrated an exponential decrease in viscosity as shear rate increases. The experimental data were fitted by the Cross model. These tests verified that the ink has a shear-thinning behavior and high near-zero viscosity, promoting the deposition process in printing step and the extrusion with pressure, respectively. For a successful printing step, the important parameters defined were layer height (0.6 mm), wall thickness (0.84 mm), infill density (100%) and print speed (1.5 mm/s). The final printed monolith is shown in the graphical abstract.

**Introduction and Objectives**

3D printing technology, in particular, Direct Ink Writing method allows to print structures with solid free-form fabrication from an ink with high viscosity. The printing occurs with a pressure delivery of an ink through one or multiple capillaries or syringes [1]. The layers, printed individually, can be treated by UV irradiation or dried in ambient air. This method offers great flexibility in the raw materials and a large range in the printed structure’s dimensions and resolutions [2, 3]. The main requisite for a successful structure construction with this technique is the development of an ink with suitable rheology [2, 4]. The ink should flow through the nozzle at high shear stress and then be able to suddenly set at low stress (i.e., once out of the nozzle) to achieve shape retention. Recently, additive manufacturing gained worldwide attention in the development of adsorbents for gas separation processes applications. Moreover, this method can be used as an alternative or a complement to the extrusion process, which is one of the most used processes for adsorbents production, namely in monolithic shaped adsorbents development.

In a previous work, we reported the development of a monolith made with 13X zeolite and activated carbon (AC) by extrusion for Electric Swing Adsorption (ESA) applications which demonstrated a poor electrical conductivity [5]. In this context, the need to develop a material, now by 3D printing process, in order to obtain high electrical conductivity and high CO2 adsorption capacity appears. There are only a few studies about the preparation of adsorbent materials by 3D printing processes especially for CO2 capture applications. Recently, a study of zeolite/electrical conductive carbon monolith suitable for ESA process is described by Masala et al. (2017) [6]. The monolith was developed with 78 wt. % of H-ZSM-5 and 22 wt. % of phenolic resin. A piece of adsorbent with 6x6x6 mm of dimension was tested and CO2 adsorption capacity of a zeolite (H-ZSM-5) in powder form and in the shaped monolith were compared. The authors reported a resistance value measured by a multimeter at a 20 cm distance of 29 Ω. This work reports the development of monolith by 3D printing process with 30x30x43 mm. The adsorbent was produced with an ink composed by 13X zeolite, AC, carboxymethylcellulose (CMC) and water. Rheological measurements of the ink were carried out. Textural characterization of the printed material and heating tests through Joule effect were done in order to demonstrate the feasibility of using this monolithic material in ESA process applications.
Textural characterization demonstrated a N\textsubscript{2} isotherm at 77 K with large adsorbed amount at low pressures, usual in materials with large micropore volume, and with an increase of N\textsubscript{2} adsorbed after P/P\textsubscript{0}=0.05, typical behavior of activated carbon, due to the presence of pores with larger sizes. CO\textsubscript{2} adsorption isotherm at 273 K of the 3D printed material showed a CO\textsubscript{2} adsorption capacity of 3.49 mol/kg, at 0.15 bar. Comparing with the previous material presented with the same composition, an increase of 1.37 mol/kg (about 40\%) was obtained, demonstrating an improvement of the CO\textsubscript{2} adsorption capacity at low pressure.

The ability to use monolith for Electric Swing Adsorption applications was assessed by applying an electric current in order to observe the Joule effect on the adsorbent. Figure 1 shows the results of three tests developed.

![Figure 1. Heating measurements of the 3D printed sample (Exp 1 and 2) and of an activated carbon monolith (Exp 3). Green line at 60 min represents the moment when the power source was turned on.

Experiments 1 and 2 were carried out with the 3D printed piece with \(10\times10\times4\) mm of dimensions, at constant voltages of 16 and 25 V, respectively. Experiment 3 was done in a piece of an activated carbon monolith from Mast Carbon\textsuperscript{®} with 10 mm of height, at constant voltage of 10 V. All experiments were conducted with the power source turned off from 0 to 60 seconds, during which the thermocouple reading represents the initial temperature of the experiment. At 60 s, the power was turned on until the maximum temperature was reached. From that moment, the Power Supply Unit (PSU) was turned off and the temperature decreased.

In experiment 1, the PSU was on during 484 s, until a temperature of 333 K was reached. At this point, the experiment of heating the power source was turned off. To reduce the heating time, experiment 2 was carried out at a higher voltage (25 V), and as can be seen in Figure 1, a higher maximum temperature was reached in a shorter time, about 377 K in 180 s. In the case of the activated carbon, experiment 3 showed that a more efficient heating step was obtained, reaching a temperature of 384 K in 88 s only.

Based on these facts, the produced 3D printed monolith is a good adsorbent for ESA process applications. Though it presents high resistance, the electric current passage demonstrated that the Joule effect was verified in the material with high efficiency, and a low energy consumption was measured to increase the temperature about 80 K.

**Conclusion**

3D printing technology was used in order to create an adsorbent for CO\textsubscript{2} capture, composed by 70\% of 13 X zeolite and 30\% of activated carbon. Rheological measurements demonstrated that a zero-shear viscosity of about 15701 Pa.s is needed to start the flow in the 3D printing process. The honeycomb monolith was printed using an Ultimaker 3D printer connected to Disco3ry Extruder. Characterization of the material demonstrated the high-expected CO\textsubscript{2} capacity of this adsorbent. Considering the aim of this study, that is, the development of a viable adsorbent for Electric Swing Adsorption process, some experiments were done to heat the material by Joule effect. 3D printed monolith demonstrated that, when submitted to a voltage of 25 V, a current between 0.05 and 0.13 A was needed to heat the material until 377 K, representing a maximum power consumption of about 3.25 W in 180 seconds. Therefore, it was demonstrated that this 3D printed monolith material can be used in ESA process for CO\textsubscript{2} capture.

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**References**

Modelling studies of supercritical fluid extraction of oils from grape and chia seeds

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This work reports the modelling of the kinetics of supercritical extraction of oils from grape seed samples (obtained from a Portuguese industry), and from two sets of chia seeds - high oil content seeds (HOCs), and underutilized low oil content seeds (LOCS). A rigorous thermodynamic framework was applied to calculate the solubility in the supercritical solvent of the grape and chia seeds oils. The oils, for the purposes of modeling, were represented by triolein. Second order polynomial functions are then fitted to the solubility data and used in the kinetics model of Sovova and Stateva [1] to simulate the extraction curves in gPROMS ModelBuilder [2]. The qualitative and quantitative agreement between the experimental and simulated extraction curves in terms of yields was good (2% <AARD < 9%) taking into consideration the very complex nature of the systems examined.

Introduction
Nowadays, increasing attention is drawn to the effective use of waste biomass and vegetal material as renewable resources of high added value compounds with applications in food, cosmetics, pharmaceutical industries, biodiesel production, etc. For example, seed biomass from *Vitis vinifera* L. contains typically (8–15) % (w/w) of oil which is rich in long chain polyunsaturated fatty acids (PUFAs) and antioxidants [3,4]. Yet, in many countries the seeds, which represent about (20-25) % of the biomass generated by the wine industry, are considered a disposable material.

Chia (*Salvia hispanica* L.) is also attracting great attention due to its health beneficial properties, especially the seeds, which contain large amounts of oil rich in PUFAs, mainly the omega-3 α-linolenic acid (ALA). Extraction with scCO₂ (SCE) prevents or minimizes the degradation of bioactive compounds and allows obtaining solvent-free products and is currently establishing itself as the viable and eco-friendly alternative to the use of organic solvents. Yet, kinetic data are not abundant, and, particularly for chia seeds oil extraction they are scarce and superficial.

In view of the above, the aim of our work is to model the kinetics of the SCE of oils from industrial grape seeds, obtained directly from a Portuguese industry [5], and from two sets of chia seeds - high oil content (HOCs), and underutilized low oil content (LOCS) seeds, by applying an efficient solution method to a recently introduced kinetics model [1].

Materials and Methods
The SCE of the grape seeds biomass was performed at *T* = (313 and 333) K and *p* = (20, 30 and 40) MPa and flow rates of (1.8, 2.3 and 2.8) g min⁻¹ of CO₂. Figure 1 shows the cumulative extraction curves plotted to assess the effect of pressure, temperature and flow rate in the process of SCE, as well as the simulated profiles.

The SCEs from LOCS were carried out at two pressures (25 and 45 MPa), two temperatures (313 and 333 K), 40 g min⁻¹ of CO₂ flow rate and 240 min extraction time. The extractions of HOCs were performed at 45 MPa, 313 K and several CO₂ flow rates (27, 40 and 54) g min⁻¹, which represent a CO₂-to-seed ratio of 50, 70 and 100, respectively. Figure 2 shows the experimental kinetic curves and the simulated profiles for the SCE of chia oil from LOCS.

**Figure 1.** Experimental (symbols) and simulated extraction curves (lines) at a scCO₂ flow rate of 1.8 g min⁻¹ at different pressures and temperatures for grape seeds.

**Figure 2.** Experimental (symbols) and simulated extraction curves (lines) at a scCO₂ flow rate of 40 g min⁻¹ at different pressures and temperatures for LOCS.

The grape and chia seed oils are very complex mixtures of mainly triacylglycerols (TAGs) with minor amounts of other...
compounds. For the purpose of modelling, they have to be represented by model TAGs. Thus, following the results of the composition analyses, the grape seed oil is represented by triolein [5].

The most suitable model representative of chia seed oil is trilinolenin. However, taking into consideration two very important issues, namely: i) lack of any experimental information on the VLE of trilinolenin+scCO₂; ii) total lack of data (both experimental and estimated) on the thermophysical properties of trilinolenin, we chose triolein as the TAG to represent the chia seed oil.

To calculate the solubility of the TAGs in the scCO₂, the VLE of the two component (triolein+CO₂) system should be modelled, which requires a reliable thermodynamic model. In our study we apply the predictive Soave-Redlich-Kwong (PSRK) cubic EoS [6], with the triolein properties estimated by us.

In order to calculate the yield and composition of the oil extracts at the experimental temperatures, pressures and scCO₂ flow rates for the grape and chia seeds, the novel model of Sovova and Stateva [1] was applied. Although the model was developed for multicomponent extraction, it remains valid for a single component system.

This model considers homogeneous concentration in the extractor at both solid and fluid phases and assumes that the extracts are located on the surface of the solid particles. This assumption allows neglecting internal diffusion, which is compatible with finely ground substrates where the diffusion path in the particles is short and the extract is easily accessible, resulting in negligible internal mass transfer resistance.

The model was deployed, validated and executed using gPROMS ModelBuilder [2], an equation-oriented modelling and optimisation platform for steady-state and dynamic systems. gPROMS offers an integrated framework where all the tasks can be performed within the same environment, with embedded solvers that can usually complete the simulations without further user input if a complete and well formulated model, with the adequate initial conditions, is provided.

**Results and Conclusions**

Using gPROMS Modelbuilder parameter estimation, the partition coefficient \(K\) was estimated for all the cases. The maximum oil content corresponding to monolayer adsorption \(u_i\) was also estimated for the chia seeds case. gPROMS uses a maximum likelihood parameter estimation problem and attempts to determine values for the uncertain physical and variance model parameters that maximize the probability that the mathematical model will predict the measurement values obtained from the experiments.

In order to compare the fitting accuracy obtained with other works, a standard deviation measure, the absolute average relative deviation, AARD, was also calculated after parameter estimation using Eq. 1, where \(N\) is the total number of experimental points, and \(e_j^{\text{exp}}\) and \(e_j^{\text{est}}\) the \(i\)-th experimental and estimated point, respectively.

\[
\text{AARD} = \frac{100}{N} \sum_{j=1}^{N} \left| \frac{e_j^{\text{exp}} - e_j^{\text{est}}}{e_j^{\text{exp}}} \right|
\]

(1)

The AARD values obtained ranged from (2 to 9) % for the grape seeds and LOCS, and from (2 to 7) % for HOCS, which may be considered as a good agreement taking into consideration the very complex nature of the systems under study.

The new modelling approach applied incorporates in a rigorous way the interplay between phase equilibria (solubility) and kinetics, and the results obtained demonstrate that albeit the simplifications introduced there is a good qualitative and quantitative agreement between the experimental and calculated extraction yields at the SCEs operating conditions examined.

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Unbiased solar charging of an organic-inorganic redox flow battery with a tandem photoelectrode

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Today, one of the challenges in the renewable energy industry is to store electrical energy in a cost-efficient way. Redox flow batteries have shown great potential, being cost-effective compared to other technologies. In this work, a solar redox flow cell (SRFC) has been developed to store sunlight directly as chemical energy in liquid electrolyte solutions. An innovative near-neutral organic-inorganic aqueous SRFC is demonstrated using organic and low-cost redox pair anthraquinone-2,7-disulfonate disodium (2,7-AQDS) on the negative side and iodide/iodine (I/I2) redox couple on the positive side. The SRFC yielded a cell potential of ~ 0.8 V. Hematite (α-Fe2O3) was used as a photoelectrode and photoelectrochemical characterizations showed a stable performance of α-Fe2O3 in contact with iodide/iodine (I/I2) redox couple at pH 5.5, although it is less stable for pH < 7. To charge the SRFC unbiasedly, a tandem system composed of a dye-sensitized solar cell (DSSC) and a α-Fe2O3 photoanode was used. The tandem system provided 1.6 V of photovoltage.

Introduction

Renewable energies such as wind and sun are an abundant and clean sources of energy. However, due to the intermittent nature of these energy sources and mismatch between production and consumption, the utilization of the renewables in the grid in large extent depends on the availability of low-cost electrical energy storage technology. Amongst many different technologies such as pump hydrop, compressed air, thermal, and future energy efficient hydrogen energy storage, redox flow batteries (all vanadium, zinc bromine, etc.) have shown the lowest potential cost[1]. Their biggest advantage is the possibility of independent scaling up of power and capacity. Their price will further decrease in the following years due to large scale production, improved engineering and reduced use of high cost materials. Integrating a redox flow battery (RFB) with a photoelectrochemical (PEC) cell results in a solar redox flow cell (SRFC), to directly convert solar energy to chemical energy in the liquid electrolytes. In this work, a new SRFC operating with an organic compound on the negative side - anthraquinone-2,7-disulfonate disodium (2,7-AQDS) - and inorganic iodide/iodine (I/I2) redox couple on the positive side of the solar battery was studied. Different techniques such as cyclic voltammetry, two/three-electrode characterization and electrochemical impedance spectroscopy (EIS) were employed to characterize and improve the performance of the 2,7-AQDS/iodide SRFC.

Methods, results and conclusions

Cyclic voltammetry tests showed the reversibility of the electrochemical reactions occurred on the both side of the SRFC (Figure 1). The cell potential is as much as 0.8 V, higher than the similar cell with acidic electrolytes[2]. α-Fe2O3 photoelectrodes prepared by spray pyrolysis, as described elsewhere[3, 4], were characterized in a three-electrode configuration using the electrolyte containing NH4I and I2 to determine the effect of donor concentration (I–/iodide), acceptor concentration (I2, iodine) and pH of electrolyte on the photovoltaic and photoelectrochemical performance. The obtained results revealed the initial increase in the photocurrent by increasing the donor concentration, which quickly remained constant for concentrations above 0.3 M. This behavior demonstrated that the photocurrent is limited by the hole collection length to the photoanode/electrolyte interface[5]. On the other hand, the photocurrent remained unchanged by increasing the acceptor concentration. In addition, photovoltage remaining constant, shows that back electron recombination is not the limiting mechanism[5]. By increasing the pH of electrolyte, both photovoltage and photocurrent were raised; photovoltage increased 27 mV/pH. The photocurrent increment was due to the larger voltage drop across the photoanode and larger electric field, which consequently causes larger photocurrent at high pH values. Furthermore, EIS analysis was performed to obtain: i) a general equivalent circuit for the photoelectrode/electrolyte interface; ii) the charge transfer mechanism to the photoanode interface; and iii) flatband, conduction and valence band potential using Mott–Schottky analysis. EIS data indicated that the hole transfer occurs via surface states[3]. The existence of surface states at the α-Fe2O3 photoanode surface was confirmed by cyclic voltammetry analysis in dark condition. Stability tests were ran for 60 min for electrolytes contain 0.25 mM I2 in 1 M NH4I at pH 5.5 and buffer solution (0.5 M Na2SO4 & 0.1 M

Figure 1. Cyclic voltammetry of 0.025 mM I2 in 0.25 mM NH4I (orange curve) and 0.25 mM Na2AQDS in 1 M NH4I (pink curve) at scan rate of 100 mV/s.
K$_2$PO$_4$ at pH 5.5. Hematite proved to be quite stable in the first solution, delivering the same initial photocurrent after 60 min, while being unstable in the buffer solution. This is consistent with high kinetics of iodide/iodine (I$^{-}$/I$_2$) electrochemical reaction at the α-Fe$_2$O$_3$ interface compared to water oxidation. Finally, to charge the solar battery without an external bias, a tandem configuration based on a α-Fe$_2$O$_3$ photoanode and a dye-sensitized solar cell (DSSC) in series was tested. The tandem system yielded 1.6 V of photovoltage. The unbiased photocurrent of solar battery was increased from 0.024 mA/cm$^2$ using only α-Fe$_2$O$_3$ photoanode to 1 mA/cm$^2$ using a tandem system. Overall, we demonstrate a solar redox flow cell using Hematite as photoanode. Hematite showed stable performance in an aqueous iodide/iodine (I$^{-}$/I$_2$) solution at pH 5.5. We also studied effect of donor&acceptor concentration and pH on photoelectrochemical performance. Integrating Hematite with a photovoltaic dye synthesized solar cell, increases photovoltage up to 1.6 V which is quite sufficient to unbiasedly charge the AQDS|iodide SRFC.

Figure 2. Photocurrent density-voltage (J-E) curve for a bare hematite sample under 150 mW cm$^{-2}$ illumination in contact with an electrolyte containing (a) 0.25 mM iodine (I$_2$) and different concentrations of NH$_4$I; (b) 1 M NH$_4$I and different concentrations of iodine (I$_2$); Photocurrent-voltage (J-E) curve under 100 mW cm$^{-2}$ illumination and dark for a SRFC using (c) bare Hematite and (d) tandem Hematite-DSSC as photoanode on the positive side in contact with 0.25 mM I$_2$ in 1 M NH$_4$I and carbon felt electrode on the negative side in contact with 0.05 M Na$_2$AQDS in 1 M NH$_4$I.

References
Optimization of a passive direct methanol fuel cell with different current collector materials

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Passive direct methanol fuel cell (DMFCs) are promising devices to replace conventional batteries in portable applications as they use a liquid fuel and offer high energy densities, instant recharging and longer runtimes than batteries. However, to introduce these systems in the market it is mandatory to achieve an optimum balance between its cost, efficiency, reliability and durability. In fuel cells, the current collectors (CCs) ensure the electrons collection, give structural support to the membrane and are responsible for about 80% of the cell total weight. Consequently, to achieve the passive DMFC technology goals (small, compact, low cost and height and high durability and power), CCs should have: high electrical conductivity, corrosion resistance and mechanical strength and low weight and cost. In this work, the effect of the CCs on the DMFC performance is evaluated through the polarization and power density curves and using the electrochemical impedance spectroscopy (EIS).

Introduction

Conventional batteries are becoming inadequate for the increasing power requirements of portable electronic devices, like cell phones and tablets, since these devices incorporate graphics and games, internet service, instant messaging and are helpful even to find a restaurant or a museum, requiring higher autonomies. Passive direct methanol fuel cells (DMFCs) are promising candidates to replace the conventional batteries in portable applications, since they use a liquid fuel, methanol, and offer high energy densities and longer runtimes than batteries [1]. In addition, the refuelling of a passive DMFC is fast and the fuel can last several months. Due to these unique properties, they can be used either in a plane, train, or car or even in remote areas where there is no electricity/electric grid. However, for its massive use, a passive DMFC system must be small and compact and have an attractive cost. Therefore, to introduce these systems in the market it is mandatory to achieve an optimum balance between its cost, efficiency, reliability and durability [2].

In fuel cells, the current collectors (CCs) ensure the electrons collection at the anode side and its conduction through the external circuit to the cathode side and give structural support to the membrane. Thus, they must have a high electrical conductivity, low thermal conductivity and mechanical resistance. In addition, in passive systems, the CCs are responsible for the fuel and oxidant supply to the reaction zone and products removal from the cell. Therefore, the CCs should be build-up with channels, of a specific design, covering the entire area or have a porous structure, with different open ratios and sizes [3]. The current collectors used in passive DMFCs are mainly made of stainless steel, but this material after a long-term operation suffers from corrosion, increasing the contact resistance between the CC and the membrane and leading to the presence and accumulation of the corrosion products on the different fuel cell layers, poisoning them [2]. The presence of these compounds on the membrane leads to a decrease of the membrane conductivity and hinders the protons transport towards the cathode side [4]. Therefore, to avoid corrosion, the CCs are usually coated with precious metals such as platinum, gold and titanium. However, this coating increases significantly the CC costs and consequently the system overall costs. The development of low-cost materials and an optimization of the CCs coating in order to ensure an optimal balance between the corrosion resistance and its cost are key factors towards the commercialization of passive DMFC systems.

In the present work, an experimental study of the effect of the anode and cathode current collector materials on the performance of an «in-house» passive DMFC with an active area of 25 cm² is described. The CC materials were selected based on its unique properties and towards a cost and weight reduction. Bearing in mind that the electrodes used on both anode and cathode sides also represent a significant fraction on the system overall costs, lower catalyst loadings (3 mg/cm² Pt/Ru and 1.3 mg/cm² of Pt) than the conventional ones (4 mg/cm² Pt/Ru and 4 mg/cm² of Pt), were used in this work. The effect of the CCs materials on the DMFC performance was evaluated through the polarization and power density curves and using the electrochemical impedance spectroscopy (EIS). The EIS measurements, as a complementary diagnostic technique in fuel cell systems, were carried out to identify and quantify the different losses that affect the passive DMFC performance (activation, ohmic and mass transfer losses) [5].

Materials and Methods

The passive DMFC used in this work is composed by two acrylic end plates (opened at the cathode side and with a fuel reservoir at the anode side), two isolating rubber plates, two current collector plates, two diffusion layers, two catalyst layers and a Nafion 117 membrane. The experiments were carried out using carbon cloth with a micro porous layer (MPL) as anode diffusion layer (thickness of 0.410 mm) and carbon cloth as cathode diffusion layer (thickness of 0.400 mm). The catalyst used at the anode and cathode side were, respectively, 3 mg/cm² of Pt/Ru and 1.3 mg/cm² of Pt. The passive DMFC has an active area of 25 cm² and was operated at ambient conditions (room temperature and pressure). The experimental tests were performed with different methanol concentrations and different current collector materials on both anode and cathode sides. The experiments were performed with a commercial electrochemical test station (Zahner Elektrik GmbH &Co. k.), the polarization measurements were conducted galvanostatically and the impedance ones were performed potentiostatically. For the impedance tests, it was employed a frequency range from 10 mHz to 100 kHz with an amplitude of 10 mV. EIS measurements were conducted at different fuel cell voltages and at each voltage, the spectrum recording was performed after a period of time of 3 minutes, which was...
applied to ensure that the system is under stable operation. An electric equivalent circuit (EEC), presented in Fig. 1, was used to fit the impedance plots, by the complex non-linear regression least squares fitting, using the Thales software from Zahner.

Figure 1. Equivalent electric circuit (EEC).

As shown in Fig. 1, the equivalent circuit used in this work consists of a resistance (R1) connected in series with three circuits, each one comprising a resistance (R2, R3 and R4) in parallel with a constant phase element (CPE1, CPE2 and CPE3). In the EEC, R1 represents the ohmic resistance, R2, R3 and R4 indicates the major losses from the anode and/or cathode side that affect the cell performance, and CPE1, CPE2 and CPE3 are associated with the capacitance properties of the double-layer interfaces.

Acknowledgements
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References
Minimizing non-ideal discharge effects in tangential multicyclone systems

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The dust used to feed the TM cyclone was talc, which was widely tested before and can reach high feed rates. The same batch was used in all experiments, with a real density of 2802 ± 7 kg/m³. The median diameter of the bulk sample was 23.12 μm and there was a small ducting deposition before entering the cyclone (Fig. 1).

Table 1. Experiments performed in the pilot cyclone.

<table>
<thead>
<tr>
<th>Dipleg Length (mm)</th>
<th>Leaks in the Hopper</th>
<th>Vortex Breaker</th>
<th>Cyclone Pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No</td>
<td>Yes</td>
<td>Negative</td>
</tr>
<tr>
<td>680</td>
<td>No</td>
<td>Yes</td>
<td>Negative</td>
</tr>
<tr>
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<tr>
<td>680</td>
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<td>Positive</td>
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<tr>
<td>1080</td>
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</table>

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To evaluate overall and grade efficiencies, experimental values were obtained from offline sampling mass

Introduction
Cyclones are gas/solid separator devices, highly used for the recovery of raw materials and/or to comply with particle emission limits. To design the most compact solution for a multicyclone, a specific optimized geometry was developed, with main focus on scalability, keeping pressure drop and footprint kept to the minimum possible. This geometry, before being tested on a matrix kind setup (as a typical multicyclone), was tested isolated to evaluate its performance and its sensitivity to non-ideal discharge effects: the dust dipleg length, hopper leakage and the presence of a vortex breaker. The results showed that non-ideal discharge effects may be significant, stressing that air leakages must be avoided. The vortex breaker cannot be neglected, even if the discharge dipleg is kept at an ideal length.

Methodology and Experimental work
The pilot-scale experiments were performed using a tailor-made (TM) cyclone with D = 400 mm i.d.. This optimized cyclone, mainly used for ceramics recovery, has approximately a 2D/2D cylinder-to-cone length ratio and is characterized by a rectangular gas entry. The inlet velocity and flow rate were kept constant through the experiments at 20.7 ± 0.3 m/s and 812 ± 17 Nm³/h, respectively. The concentration at the cyclone inlet was 889 ± 41 mg/Nm³. Three lengths of the dipleg were under study (0 mm, 680 mm and 1080 mm), and the effect of leakages, pressure and the presence of a crosshead on the hopper were analyzed. Table 1 sums up the experiments.

This work presents the results from the experiments performed for three collector type setups. The first setup has the cyclone cone directly connected to a cylindrical dust collector (hopper), with the following dimensions: diameter and height of 200 mm, and the leakage valve positioned on the side of the hopper. The second setup has the cyclone bottom first connected to a tube (dipleg) of 680 mm in length and 125 mm diameter. Next, the dipleg connects to a cylindrical hopper, with diameter and height of 300 mm, with the leakage valve positioned on top of the hopper. The third setup has the cyclone connected to a tube of 125 mm of diameter, and 1080 mm of length. The tubes are then connected to the same hopper described above.
concentrations and particle size distributions (PSD). The PSD were determined by a Beckman Coulter Lasersizer LS230 using ultrasounds and ethanol as dispersant media.

Results and Conclusions
A first test was made with the standard dipleg (1.7D) to verify the influence of the vortex breaker on global efficiency. As the efficiency increased from 97.1% to 98.5%, decreasing emissions by ≈ 50%, further tests were performed with the vortex breaker on.

Figure 2 show the major results of this study, where all series follow the nomenclature given in Table 2.

Figure 3 shows typical grade efficiency curves obtained for the system with and without leakages, for the system at negative pressure and without dipleg. It is clear that leakage is damaging collection in the range of 0.3-5µm.

Table 2. Experiments performed in isolated cyclone.

<table>
<thead>
<tr>
<th>Series</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>No Vortex Breaker</td>
</tr>
<tr>
<td>NL</td>
<td>No Leak</td>
</tr>
<tr>
<td>L</td>
<td>Leak</td>
</tr>
<tr>
<td>P&gt;0</td>
<td>Inlet Positive Relative Pressure</td>
</tr>
<tr>
<td>P=0</td>
<td>Inlet Neutral Relative Pressure</td>
</tr>
<tr>
<td>P&lt;0</td>
<td>Inlet Negative Relative Pressure</td>
</tr>
</tbody>
</table>

Figure 2. Overall Efficiency vs. Dipleg Length.

Conclusions
The major conclusions of this study are shown below, taking into account that the complete set of information will be presented in detail afterwards:

Vortex breaker effect. The presence of a vortex breaker increased collection efficiency. However, care must be taken when using a vortex breaker under heavy load and sticky particles, as dust build up may compromise efficiency.

Dipleg length effect. The best results were obtained with a dipleg with a 1.7D length, as increasing further its length decreases global efficiency. This happens under both negative and positive pressures.

Leak impact. The best results were obtained without leakage from the dust hopper. With leakage results worsen with negative as compared to positive pressure.

Future work will involve the quantification of hopper leakage, which will be critical when using high efficiency cyclones and multicyclones currently under development.

Acknowledgements
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References
Introduction
Solar energy is a major source of inexhaustible free energy. In 1991 M. Grätzel [1] proposed for the first time dye-sensitized solar cells (DSSCs) as a new and promising photovoltaic technology. Today DSSCs are viewed as a promising technology for the building integrated photovoltaic (BIPV) market due to their special aesthetic appearance [2]. A DSSC device comprises three elements: photoelectrode, electrolyte and counter-electrode, encapsulated by two TCO (transparent conducting oxide) coated glasses. The most traveled routes to high-efficiency are normally related to increasing effective light harvesting [3] and surface treatment of photoanode to improve crystallinity and dye absorption [4], which usually include time-consuming processes and many high-end resources. Khatani et al. [5] showed the importance of determining the optimum thickness of the photoanode, to reduce the internal resistance of the cell and to increase electron transport, achieving a PCE of 4.54 % for a 18 µm photoanode. Vesce et al. [6] treated the printed photoanode with a solution of TiCl₄ to enhance the electron transfer at the TiO₂ mesoporous layer, by filling the gaps among TiO₂ nanoparticles, and reported a PCE of 7.56 % for the treated photoanodes. Cameron et al. [7] examined the advantages of adding a blocking layer to hinder the recombination of the electron hole at the interface between the conductive substrate and the redox mediator. This work reports a highly efficient DSSCs both under solar and artificial illumination, by optimizing the whole manufacturing process but using commercially available materials, mainly focusing on simple and easy procedures to improve the performance of the device. The strategies utilized to develop the DSSC involved photoanode thickness optimization, including different types of titanium dioxide pastes, post-treatments to the photoanode, application of light reflective materials, addition of bulky groups to the sensitizer, optimization of dye adsorption time, minimizing the distance between photoelectrode and electrical contacts, and addition of blocking layer.

Methods
To assemble the device, first the photoanodes were prepared by screen printing a commercially available titanium dioxide paste (30NR-D Transparent Titania Paste, and 18NR-AO Active Opaque Titania Paste from Greatcell Solar) and sintered at 500 °C for 1 h. After sinterization, the photoanodes were treated with a 40 mM TiCl₄ aqueous solution at 70 °C for 30 minutes, rinsed with distilled water, dried with nitrogen, and sintered again (500 °C for 1 h). The samples were dipped in a dye solution (5 mM of N179 from Solaronix®) to enable complete dye adsorption by the porous TiO₂ photoelectrode. The counter-electrodes were drilled with two holes with 1 mm diameter each and the platinum paste (Platisol T/SP from Solaronix®) was applied by doctor blade technique and sintered at 460 °C for 30 min. Both electrodes were sandwiched and sealed with thermoplastic sealant with 60 µm thick (Surllyn® from Solaronix®) in a hot-press at 160 °C for 15 s. After cooling the DSSC was injected with electrolyte (EL-HPE High Performance Electrolyte from Greatcell Solar). The injection holes were sealed with Surlryn® and a circular coverglass. For the characterization of the devices, the current-voltage (I-V) curves were recorded under simulated solar light (AM1.5 G) driving with intensity of 1000 W·m⁻²; the electrochemical impedance spectra were collected in the dark at potential 10 mV less than the open circuit potential of the cell in frequency range 100 kHz - 0.10 Hz by applying 10 mV sinusal perturbation. The incident-photon-to-electron conversion efficiency (IPCE) spectra were recorded at a light chopping frequency of 8.3 Hz, at 2 nm wavelength interval between 290 and 800 nm. To evaluate the indoor light application, two artificial interior light sources were used: a LED lamp (Osram, Class A+, 60 W, 2700 K) and a Fluorescent lamp (Osram, Class A, 86 W, 2700 K). The light flux intensity of the lamps

Commercially available materials were used in the optimization of a high energy efficiency dye-sensitized solar cell (DSSC). The type and thickness of titanium dioxide pastes were optimized to 13 µm of mesoporous paste and 7 µm of scattering paste, post-treated with TiCl₄; the combined effect of using a scattering layer and TiCl₄ post-treatment made Jsc and Voc to increase by 55 % and 6.6 % respectively. Dipping time, for an efficient sensitization, and DSSC configuration assembly were also optimized; adding chenodeoxycholic acid (CDCA) to the dye and a blocking layer to the photoanode decreased the charge recombination. The final PCE improved from 2.55 % to 9.84 %, under AM 1.5 G one sun light intensity (1000 W·m⁻²). The best performing DSSC was also characterized under artificial light displaying a PCE of 28.7 % at a light intensity of 1580 µW·cm⁻² and a PCE of 23.3 % when the light intensity was 225 µW·cm⁻². These results are amongst the best performances ever reported.
was appraised in W·m⁻² using a radiometer (Delta Ohm, HD 2102.2).

Results

In a DSSC the thickness of the photoanode is crucial for a good performance; there must be a balance between a high dye uptake, provided by a large area of titanium dioxide layer, and a minimum recombination effect at the surface of TiO₂ nanoparticles. The thickness of mesoporous layer, which comprised nanoparticles of 30 nm, was optimized concerning the power conversion efficiency (PCE). It was also added a scattering layer, which contained active nanoparticles with an average size of 20 nm and scatter nanoparticles of 450 nm. The increasing the thickness of the mesoporous layer yielded an increment in the short circuit current density ($J_{SC}$), especially from 7 to 13 µm. The open circuit potential ($V_{OC}$) decreased as the thickness of the mesoporous layer increased. Increasing the thickness of the mesoporous layer led to a better absorption of light. However, the resistance of photoelectrode transport through the film was also increased. The addition of the scattering layer proved to be effective as the $V_{OC}$ increased slightly, indicating lower recombination rates. The TiCl₄ treatment improved both $J_{SC}$ and $V_{OC}$, and thus had a very positive impact on the overall performance. This treatment rendered the TiO₂ mesoporous layer more electrically conductive thus the $J_{SC}$ increased by 10 %. To improve the performance of the cells even further (with TiCl₄ treatment), reflective layers of aluminum foil and white tape were placed in the backside of the DSSC, i.e., the cathode side. Analyzing the current density, with the aluminum reflector the $J_{SC}$ increased 38 % and with white reflector 50.1 %. The optimal time for sensitization in dye solution was 21 h; then the PCE decreased 5.6 %, stabilizing until 49 h, after which there was a noticeable decline of 22.6 %. As the dye loading time increased, the hindrance effect of the dye aggregates increased, which led to lower photocurrent density. The blocking layer was used to minimize the back reaction of the photoinjected electrons with the oxidized state of the redox mediator; it acted as an electron extraction layer. The application of an optimized blocking layer had a huge impact in the $J_{SC}$ (Graphical Abstract Figure). To confirm the blocking layer role, electrochemical impedance spectroscopy spectra (EIS) were recorded at -0.70 V under dark conditions (Fig. 1). The electron recombination resistance at the photoanode ($R_k$) increased 87 % and the electron lifetime ($\tau_n$) increased from 17 to 36 ms with the addition of the blocking layer, confirming its critical role. The champion cell delivered a PCE of 9.92 % under simulated solar light, and 28.7 % under artificial light (at 1580 µW·cm⁻²).

Conclusions

A systematic approach was used to improve the efficiency of a DSSC employing only commercially available materials and simple manufacturing processes. Factoring all optimization steps, the champion device displayed a PCE of 9.92 % under simulated solar light. Under artificial light (LED lamp) DSSC produced demonstrated a PCE of 28.7 % at a light intensity of 1580 µW·cm⁻² and a PCE of 23.3 % when the light intensity is 225 µW·cm⁻², showing its great potential for indoor applications.

Acknowledgements

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References

Intensifying heterogeneous TiO$_2$ photocatalysis for bromate reduction: A static mixer as catalyst support

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This study focuses on the application of TiO$_2$ photocatalysis for the reduction of bromate (BrO$_3^-$) in aqueous solutions using a stainless steel Kenics® static mixer (SM) as support for TiO$_2$. The SM was assembled in a tubular photoreactor coupled to a compound parabolic collector (CPC) under simulated sunlight. The reuse of TiO$_2$ films and the effect of the following parameters on the BrO$_3^-$ reduction were assessed: (i) SM pre-treatment, (ii) number of TiO$_2$ layers deposited on the SM by dip coating, (iii) SM position during coating, (iv) solution pH, (v) solution temperature, (vi) dissolved oxygen (DO) content, and (vii) addition of formic acid (HCOOH) as organic sacrificial agent. TiO$_2$ films showed a high stability during consecutive reactions. BrO$_3^-$ reduction was favorable under the following conditions: 6 TiO$_2$ layers, vertical position of the SM during coating, acidic pH and 25 ºC. DO had a negative effect on BrO$_3^-$ reduction for pH higher than 5.5. HCOOH had null or negative effect.

Introduction

Bromate (BrO$_3^-$) is a by-product that commonly occurs as a result of disinfection processes, such as ozonation, applied to the treatment of waters containing bromide (Br$^-$). Since BrO$_3^-$ is possible carcinogenic to humans, its content in drinking waters is regulated at a strict standard of 10 µg L$^{-1}$ by various entities [1-4]. As a result, advanced methods to reduce BrO$_3^-$ levels in drinking waters are required.

The photocatalytic reduction of BrO$_3^-$ to Br$^-$ is one way to reduce its levels. The reduction of inorganic species using heterogeneous photocatalysis can occur by two main mechanisms [5]: (i) direct photoReduction by electrons of the conduction band (e$_{cb}$), and (ii) indirect photoReduction by intermediates species generated during the oxidation of electron donors present in the media by holes of the valence band (h$_{vb}$) or hydroxyl radicals (HO$^-$) produced from water oxidation.

To bring heterogeneous photocatalysis closer to the market, it is necessary to design new photoreactors employing immobilized catalysts and able to overcome mass and photon transfer limitations.

Objectives

In this study, heterogeneous TiO$_2$ photocatalysis was applied to the reduction of BrO$_3^-$ in aqueous solution (200 µg L$^{-1}$ = 1.56 µM) using a commercial stainless steel Kenics® static mixer (SM) as support for the catalyst titanium dioxide (TiO$_2$)-P25. To the best of our knowledge, this is the first study regarding the use of a SM as catalyst support for the photocatalytic reduction of an inorganic pollutant. The coated SM was assembled in a tubular photoreactor under simulated sunlight coupled to a compound parabolic collector (CPC). The Kenics® SM provides intense mixing even under laminar flow, permitting to overcome mass transfer limitations. Furthermore, the use of the CPC to reflect sunlight ensures a uniform illumination of the entire photocatalyst surface area. According to the SM geometry, the radiative transport occurs through front side illumination (FSI) mechanism, maximizing the illuminated catalyst area since a higher film thickness can be used when compared to back-side illumination (BSI) mechanism.

The effect of various operational parameters on the BrO$_3^-$ photocatalytic reduction rates was assessed: SM treatment before coating, number of catalyst layers deposited on the SM by dip coating, position of the SM during the coating procedure, solution pH, solution temperature, dissolved oxygen (DO) content, and addition of formic acid (HCOOH) as organic sacrificial agent. The reusability of the photocatalytic films was also assessed.

Methods

The Kenics® SM was made of plain polished 304 stainless steel, composed of 66-74% of iron, 18-20% of nickel, among other minor constituents. The photocatalytic system mainly comprised: (i) a jets photoreactor of 0.271 L holding the SM, (ii) a 1.6 L capacity recirculation cylindrical glass vessel thermostatically controlled and magnetically stirred, (iii) a solar radiation simulator (Suntest XLS + from Atlas) equipped with a 1700 W air cooled xenon arc lamp at the top, and (iv) a CPC placed below the jets photoreactor. This system is comprehensively described by Díez et al. [6].

Before TiO$_2$ deposition, the SM was subjected to: (i) sandblast abrasion, (ii) impurities removal, and (iii) thermal treatment or thermal treatment followed by chemical treatment. The thermal treatment was carried out in a furnace using a temperature ramp rate of 2 ºC min$^{-1}$ until reaching 500 ºC and this temperature was maintained for 4 h. Afterwards, the furnace was cooled down up to reach room temperature. The chemical treatment was executed by immersing the SM in a sulfuric acid solution (10 wt. %) for 3 h. In order to eliminate acid traces, the SM was immersed two times in ultrapure water under sonication for 30 min. Finally, the SM was dried at 100 ºC for 1 h.

TiO$_2$ deposition was performed by the dip coating method using a TiO$_2$-P25 aqueous suspension of 2% (w/v), and it was carried out in an automatic dip coating unit using a speed of insertion and drawing of 50 mm min$^{-1}$ and a dipping time of 30 s. After each dip, the SM was dried at room temperature for ca. 3 min and at 60 ºC for 30 min.

BrO$_3^-$, Br$^-$ and HCOO$^-$ were quantified by ion chromatography.

Results and Discussion

SM pre-treatment affected BrO$_3^-$ photocatalytic reduction, showing higher efficiencies when simple thermal treatment was applied rather than thermal and chemical treatments. This can be attributed to a negative modification of the stainless steel surface, likely at the nano-scale level, when applying the chemical treatment. Using the thermal SM pre-treatment, the TiO$_2$ films could be used in consecutive trials (maximum of 12
reuses were tested) without changing the process efficiency. This indicates a very good adherence and stability of the TiO$_2$ films deposited by dip coating on the stainless steel surface of the SM.

BrO$_3^-$ reduction was improved with increasing number of TiO$_2$ layers up to 6, with similar results for 6 and 12 layers. This indicates improved absorption of incoming photons up to a given thickness of the TiO$_2$ film. From this point, the reaction rate remained constant with further film thickness increase since the diffusional length of the charge carriers to the catalyst–liquid interface does not change (FSI mechanism). Vertical and horizontal positions of the SM during the dip coating procedure presented different film homogeneities. The one achieved when coating the SM in the vertical position contributed to a higher BrO$_3^-$ photocatalytic reduction.

The effect of solution pH on BrO$_3^-$ reduction was assessed at pH 3.0, 4.0, 5.5, 6.5 and 7.0 (Figure 1). Higher BrO$_3^-$ reduction rates were achieved for more acidic pH values, with quite similar rates for pH 3.0 and 4.0. This can be mainly ascribed to the TiO$_2$-P25 surface charge at the different pH values – the point of zero charge (PZC) of TiO$_2$-P25 is 6.2 [7]. The catalyst surface was negatively charged at pH 6.5 and 7.0, occurring an electrostatic repulsion between the TiO$_2$-P25 nanoparticles and BrO$_3^-$, negatively affecting the reaction rate. At pH 3.0, 4.0 and 5.5, the catalyst surface was positively charged, favoring the adsorption of BrO$_3^-$ in the TiO$_2$-P25 nanoparticles and increasing reaction rates.

BrO$_3^-$ photocatalytic reduction slightly increased from 15 °C to 25 °C likely due to an enhanced desorption of the products from the TiO$_2$ surface. Similar efficiencies were attained at 25 °C and 30 °C, suggesting a detrimental effect of higher temperature likely due to a weak adsorption of BrO$_3^-$ and a higher recombination of e$^-$/h$^+$* promoted by the thermal agitation.

Two DO ranges were tested: 212-239 µM and <3.1 µM. The presence of O$_2$ in the solution affected negatively the reduction rates for pH 6.5 and 7.0, and had a null effect for pH 3.0, 4.0 and 5.5. These results can be mainly attributed to a stronger adsorption of BrO$_3^-$ onto the TiO$_2$ surface at pH values below 6.2, competing more efficiently with O$_2$ for e$^-$/h$^+$*. The effect of adding HCOOH as organic sacrificial agent was assessed for different HCOOH:BrO$_3^-$ molar ratios, DO ranges and solution pH values. For a molar ratio of 3:1 and pH 6.5, the BrO$_3^-$ photocatalytic reduction decreased for both DO ranges when compared to the reaction without addition of HCOOH. This can be attributed to an excess of HCOOH that possibly saturates the TiO$_2$ surface and hinders BrO$_3^-$ reduction. For molar ratios of 1:1 and 0.5:1 at pH 6.5 and 5.5, the addition of HCOOH did not improve the reduction rates for both DO ranges, possibly due to the weak adsorption of BrO$_3^-$ onto the TiO$_2$ surface.

Acknowledgements
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References
Introduction
Nowadays, the increasing energy demand and the limited reserves of the conventional energy sources have raised the interest on alternative renewable energy resources. An alternative non-fossil source for electrical energy and heat generation is woody biomass [1]. In Portugal, 200 thousand tons of woody biomass ash are used to produce energy annually.

However, the use of woody biomass for energy leads to the production of ashes during the combustion process that should be properly treated. Today, the increasing number of environmental regulations allow ash producers in Europe to face the choice of recycling their ashes. In addition, the incorporation of ashes in construction materials could lead not only to the reduction of the atmospheric emissions, but also to energy cost savings [2].

The aim of this work is to evaluate the environmental impacts of bottom ash management valorisation in bituminous asphalt in comparison with a base scenario of landfilling. Different scenarios were assessed considering ashes produced with two different types of energy conversion technologies: vibrating grate furnaces and fluidised bed.

Methods
The environmental impacts evaluation are made using life cycle assessment (LCA) methodology. The functional unit used in this study is the disposal/valorisation of 1 t of woody biomass ashes. The bottom ashes under study are generated from the combustion of eucalypt and maritime pine in a vibrating grate furnace (BA1) and in a fluidised bed furnace (BA2).

A cradle-to-grave approach was applied to assess the environmental impacts. The system boundaries include the transport of the woody biomass ash from the power plant to its destination (landfilling or valorisation), the on-site processes and the production of materials/energy consumed in each scenario, as shown in the Graphical Abstract. Furthermore, when a material is recycled the product system is credited with an avoided burden based on the reduced requirement for virgin material production. In the valorisation scenarios, the avoided burdens are also included in the system boundaries through the substitution approach.

The characterisation factors used in this study for the impact assessment were those suggested in the International Reference Life Cycle Data System (ILCD) [3]. The impact categories selected for the analysis were, namely, climate change, freshwater eutrophication and mineral and fossil depletion.

- Landfilling
The base scenarios are the landfilling of BA1 and the landfilling of BA2. Data for these scenarios were taken from the Ecoinvent database [4]. During landfilling, there are two main pollutant flows – landfill gas and leachate. The landfill gas is partially captured and used to produce electricity that is consumed during the short-term leachate treatment in wastewater treatment plant.

- Bituminous asphalt production
The raw materials to produce bituminous asphalt are: asphaltic bitumen, filler and coarse aggregate (crushed stone and sand) [5]. The aggregates and the bitumen are mixed and heated with temperature varying from 130 to 180 °C. No chemical reactions occur during the production and the bitumen is already delivered with the temperature needed for working [5]. The manufacturing process to produce bituminous asphalt was taken from Ecoinvent database [4].

When woody biomass ashes are used in bituminous asphalt production, they are mixed with the remaining raw materials without pre-processing. The proportion and type of ash most appropriate for asphalt production was determined based on a previous study conducted by Dias [6]. According to this study, the replacement of filler by BA1 up to 20 % resulted in a mixture with similar properties to the reference mixture (with no ashes incorporated). However, for BA2, it has been found that mixtures only up to 10 % have similar properties as the reference mixture. Therefore, the valorisation scenarios are as follows:

- Bituminous asphalt with BA1 (replacing the filler by 20 %) with ash pre-treatment (drying). In this scenario, the ashes are dried as their moisture content is high (21.3 %).
electricity consumption of the drying process is 0.2 MJ/kg of ash [8].

- Bituminous asphalt with BA2 (replacing the filler by 10%).
  In this scenario, drying is not required.

Results

Figure 1 presents the net impact of the valorisation scenarios (grey) and base scenarios (black traces). The net impacts depicts the difference between the environmental impacts of bituminous asphalt production with ashes and the avoided burdens associated with the substitution of conventional raw materials.

The results show that the environmental impacts caused by ash processing and transportation are lower than the impacts which would be caused to make a virgin material (avoided burdens), for most of scenarios under study. In the impact category climate change, it was observed that the impact could be avoided by the valorisation scenarios.

The net impacts in climate change category were -2.6 kg CO2-eq, while the disposal in landfill produces an impact of 47 kg of CO2-eq. The total impact in landfill scenarios is related to the fugitive methane emissions that are released from the biogas that are not burned. In BA2, although the substitution percentage was lower, the non-need of pre-treatment ensured a larger net impact.

Since the bitumen is a semi-solid material, it is necessary to be heated through an oil-heater (at 130-180 °C) to become fluid and thus can be mixed with the other aggregates. This process is intensive regarding the use of resources and fuel consumption, which contributes with 93% of the total impact. Nevertheless, the use of ashes minimizes the filler extraction and the net impacts were 1.3 g Sb-eq. in the mineral and fossil depletion category, for BA1 and 1.4 g Sb-eq for BA2.

The only exception is in the impact category freshwater eutrophication for scenario BA1. However, although the environmental impacts are higher than the avoided burdens (2.15 g P-eq > 0) for BA1, the impacts related to the ash transportation and pre-treatment (drying) do not exceed the impacts of landfill disposal (2.39 g P-eq). The net impact obtained for BA2 was equal to 5.94 g P-eq.

The results show that the conversion technology (grate furnace and fluidised bed) and the need of pre-processing interferes in the environmental impacts. The production of asphalt appears to be a good alternative for both type of ashes, better than landfilling, but the avoided impacts are higher using BA2.

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References

Comparison of radical driven technologies applied for parabens mixture degradation

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Parabens (esters of p-hydroxybenzoic acid) are a group of commonly used ingredients acting as a preservative in cosmetics and personal care products. Although it was found that butylparaben can be synthesized by marine bacteria [1], only produced synthetically commercial parabens are used for industrial purpose [2]. Furthermore, for the last 10 years, the EU regulation about parabens content in cosmetics products has been restricted. Isopropylparaben, isobutylparaben, phenylparaben, benzylparaben and pentylylparaben, according to Regulation (EU) No 358/2014 are prohibited in cosmetic products [2]. As reported by Regulation (EU) No 1004/2014 propylparaben and butylparaben (also their isomers and salts) were banned from cosmetic products based on those substances potential endocrine activity [2]. Additionally, the maximum concentration allowed for these two parabens were reduced [2].

Parabens can be considered as endocrine disruptors and carcinogens [2,3]. Moreover, the toxicity and estrogenicity of benzylparaben is comparable to bisphenol A [4]. Therefore, it is important to find not only effective but also ecofriendly methods for their removal from aqueous environment. From all well-known water and wastewater treatments technologies - Advanced oxidation processes (AOPs) are not only important, efficient, and ecofriendly but also promising methods that allow to remove persistent aqueous contaminants.

Objectives
The goal of this paper is the comparison of several radical driven technologies for parabens mixture degradation. H2O2/Fe2+, H2O2/UVC, O3/H2O2, O3/UVA, UVACat, O3/Cat, O3/UVA/Cat were selected from AOPs to degrade parabens from aqueous environment. As catalysts noble metals (Pt, Pd, Au, Ag) modified TiO2 were applied and compare with pure TiO2.

Methods
A solution of five parabens: MP (methylparaben), EP (ethylparaben), PP (propylparaben), BuP (butylparaben) and BeP (benzylparaben) was used as model effluent. The pollutant solutions were prepared with MilliQ (MQ) water using 10 mg/L of each compound.

H2O2/Fe2+: Reaction was carried out in a batch stirred glass reactor. Ferrous ion (Fe2+) was added in the form of iron sulphate and the reaction started at the desired amount of H2O2 was injected. pH was left to run freely (at the beginning 6±0.7 and after 120 min of reaction the final pH value falls to 3.5±0.3). The influence of water matrix was investigated (MQ and natural water).

H2O2/UVC: The reaction set-up was composed by quartz test tubes (10 mL) placed between two exposure panels using a merry-go-round device at a distance of 15 cm from its centre. Each panel was composed by three low-pressure mercury lamps (7.2W each) with a maximum emission for 254 nm. The pH of the reaction medium was 7, adjusted by a phosphate buffer.

O3/H2O2: A semi-batch reactor of 500mL was used to perform ozonation experiments. Ozone was produced in situ by an ozone generator that was fed by a pure oxygen stream (99.9%) supplied by Praxair. Ozone concentration in the gas entering ([O3]in) and leaving ([O3]out) the reactor was measured by a gas ozone meter. To ensure chemical regime, the reactor was agitated at the approximate speed of 700 rpm by means of a magnetic stirrer. The pH of the reaction medium was 7, adjusted by a phosphate buffer.

O3/UVA, UVA/Cat, O3/Cat, O3/UVA/Cat: Experiments were carried out in a 2L glass photoreactor equipped with 3 lamps for UVA irradiation (main emission 365 nm). To test the adsorption capacity of catalyst it was placed in the reactor with the solution of parabens 5 min before ozone being fed and turning on the light irradiation. The stirring speed was previously optimized at 700 rpm to ensure chemical regime. Along the reaction, samples were withdrawn and immediately centrifuged at 3500 rpm to remove the catalyst. pH was left to run freely (at the beginning 5.7). For ozone based technology, ozone was produced from pure oxygen stream (99.9%) and the inlet ([O3]in) and outlet ([O3]out) ozone concentration were measured by ozone analysers.

The toxicity assessment was investigated by luminescence inhibition tests with V. fischeri using a LUMIStox 300 apparatus. The bioassays with C. fluminea were conducted using adult clams. Before clams were used as bioindicator, they were maintained at least one week under continuous aeration at constant temperature and photoperiod (16-h light and 8-h dark) prior use. The laboratory culture water was fully renewed once a week. Mortality tests were conducted under static conditions. Blank controls with dechlorinated municipal water were applied along with a dilution series of untreated and treated model effluent with exposure lasting 72 h.

Results
Chemical quality improvement after oxidation was evaluated by following the Chemical Oxygen Demand (COD). Bears in mind that the decrease of COD value, does not have to indicate an improvement of the effluent toxicological profile, the investigation was complemented by the toxicity assessment. Toxicological analyses were performed to assess whether the
treatment process would actually decrease the effluent toxic potential rather than for formal risk assessment purposes. As can be seen in Table 1 almost all processes led to complete degradation of parabens concentration after 120 min. Moreover, when some processes have been applied the removal of each paraben was achieved much faster. Only during photocatalytic oxidation, the degradation after 3h was incredible low. And the decontamination has been influenced by the catalysts. For all effluents (containing parabens mixture) before treatment the initial COD value as well as V. fischeri luminescence inhibition after 15 min of exposure was 102±7.5 mgO2/L and 95.57±0.04%, respectively.

Table 1. Maximum Parabens Concentration, COD removal and V. fischeri luminescence inhibition (15 min of exposure (LI15 min)) after 120 min of treatment

<table>
<thead>
<tr>
<th>Process</th>
<th>Max. Cparabens</th>
<th>COD</th>
<th>LI15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>H2O2/Fe2+</td>
<td>100%</td>
<td>40%*</td>
<td>Nd#</td>
</tr>
<tr>
<td>H2O2/UVC</td>
<td>100%</td>
<td>81%</td>
<td>Nd#</td>
</tr>
<tr>
<td>UVA/TiO2</td>
<td>12% BuP</td>
<td>10%V</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>UVA/TiO2-Pt</td>
<td>42% BuP</td>
<td>18%V</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>UVA/TiO2-Pd</td>
<td>73% BuP</td>
<td>19%V</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>UVA/TiO2-Ag</td>
<td>50% All</td>
<td>17%V</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>UVA/TiO2-Au</td>
<td>16% BuP</td>
<td>8%V</td>
<td>&gt;80%</td>
</tr>
<tr>
<td>O3/H2O2</td>
<td>100%</td>
<td>70%</td>
<td>57.1±2.8</td>
</tr>
<tr>
<td>O3/UVA</td>
<td>100%</td>
<td>27%</td>
<td>59.9±1.8</td>
</tr>
<tr>
<td>O3/TiO2</td>
<td>100%</td>
<td>28%</td>
<td>26.5±0.5</td>
</tr>
<tr>
<td>O3/TiO2-Pt</td>
<td>100%</td>
<td>32%</td>
<td>32.9±1.0</td>
</tr>
<tr>
<td>O3/TiO2-Pd</td>
<td>100%</td>
<td>32%</td>
<td>33.7±1.1</td>
</tr>
<tr>
<td>O3/TiO2-Ag</td>
<td>100%</td>
<td>23%</td>
<td>35.2±3.9</td>
</tr>
<tr>
<td>O3/TiO2-Au</td>
<td>100%</td>
<td>23%</td>
<td>31.4±1.4</td>
</tr>
<tr>
<td>O3/UVA/TiO2</td>
<td>100%</td>
<td>39%</td>
<td>36.3±0.0</td>
</tr>
<tr>
<td>O3/UVA/TiO2-Pt</td>
<td>100%</td>
<td>50%</td>
<td>61.4±1.2</td>
</tr>
<tr>
<td>O3/UVA/TiO2-Pd</td>
<td>100%</td>
<td>41%</td>
<td>44.2±1.4</td>
</tr>
<tr>
<td>O3/UVA/TiO2-Ag</td>
<td>100%</td>
<td>44%</td>
<td>43.4±1.4</td>
</tr>
<tr>
<td>O3/UVA/TiO2-Au</td>
<td>100%</td>
<td>38%</td>
<td>55.4±1.0</td>
</tr>
</tbody>
</table>

*after 60 min of treatment, \(^\dagger\) after 180 min of treatment; \(^\#\) Nd- Not determined. The toxicity after treated time was too low to inhibit luminescence, \(^\ast\) For photocatalysis, the maximum removal is indicated (the paraben considered is given as superscript).

The best results of COD removal have been obtained when H2O2/UVC was applied, in spite of the lowest time of treatment (1h). Contrarily the worst COD abatement was obtained when photocatalytic oxidation when the treatment time was the longest one (3h). It should be noticed, that during this process no more than 50% removal of each paraben concentration was achieved.

While, during others processes the degradation of parabens were obtained before 120 min. The initial parabens mixture was highly toxic to V. fischeri. During reaction the toxicity has been reduced. According to literature, the sample can be considered nontoxic when LI is below 30% [5]. Taking this value into account, for the Fenton process and H2O2/UVC the nontoxic effluents has been achieved. Similar effect has been obtained for O3/H2O2 and O3/UVA (irrespective to COD removal). When the processes with catalysts were considered large differences have been noticed. It was found that the photocatalytic oxidation gave the worst results (LI15min>80%). According to literature when LI is higher than 80% the bioassay test is not sensitive to changes, which indicates still very high toxicity towards V. fischeri [5]. Catalytic ozonation gives much better toxicity results than photocatalytic ozonation, in spite of lower COD removal.

These results can indicate that the degradation pathway is different or different transformation products (TPs) could be formed. The next explanation is that the same TPs, that are formed at the last stage of degradation, are in higher concentration. This finding can be with agreement that lower COD removal gives better toxicity results. However, it should be also noticed that application of pure TiO2 leads to lower toxicity and lower COD removal in comparison to catalysts modified by noble metals. Furthermore, from all metals used for TiO2 modification platinum seems to be the best.

In the case of Asian clams, for the same conditions, for the initial mixture of parabens 100% mortality was achieved after 72 h of contact. After photocatalytic oxidation the behaviour and mortality of C. fluminea was dependent of the catalysts. However, for Pt-TiO2, Ag-TiO2 and Pd-TiO2 the reduction of the parabens toxicity of towards C. fluminea after the treatment was observed. While after all catalytic ozonation experiments (except for TiO2) no mortality was observed. For the samples obtained after photocatalysis and photocatalytic ozonation with different catalysts no mortality was verified for all concentrations tested after 72 h of exposure.

Conclusions

The toxicity of the parabens mixture was determined by extremely high toxicity of BeP, 100 times higher than those showed by the others. For all applied processes the same trend was observed. The BeP was the fastest degraded, then followed in order BuP>>Pp>>Ep>Mep. The treatments reduced not only COD but also toxicity towards V. fischeri. Furthermore, the treatments applied to the initial mixture of parabens clearly reduced the toxicity towards C. fluminea despite the fact that the total COD removal was not achieved. This indicates that the refractory by-products produced are less toxic than parabens.
Bioenergy from wastewater in a microbially-charged redox flow cell

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Wastewater is a valuable renewable energy source to generate newest approaches for electrochemical energy conversion in bioelectrochemical system (BES) for energy storage devices. A BES to generate a bio-charging redox pair to be use as negalyte in a redox flow cell (RFC) was studied. In BES 2,6-antraquinone was introduced as new redox pair (catholyte), where wastewater mixed communities were the anolyte for the generation of a bio-charged redox pair. The BES results indicated the reduction of the quinone (ca. 50 %), promoting power density ca. 10 mW m⁻². A RFC with the bio-charged redox pair as negalyte and potassium hexacyanoferrate (III) as posilyte was tested, reaching ca. 100 % of coulombic efficiency, with potential and energy efficiencies ca. 60 %. Wastewater in the new microbially-charged redox flow cell generate a clean energy that can be stored in a new landmark system.

Redox flow batteries (RFB) are electrochemical energy storage devices where the energy is stored in the redox pairs dissolve in a aqueous solution of supporting electrolyte [1]. Several works describing different types of redox pairs in aqueous supporting electrolyte solutions are well-known. Organic redox species, such as anthraquinones were a step forward for use in RFB, been projected as promising for their low cost and lower toxicity substituting ion metals as redox pairs. [2]. RFB exploring solar energy as renewable energy source was designed in a solar redox flow cell [3]. In these systems solar energy is converted into storable electrochemical fuels. These advance open doors for new ways to store energy and coupling processes with RFB.

Wastewaters are an attractive source of organic compounds, been an accessible and low cost renewable energy source for the recovery of different biochemical products, such as scarce metals, methane or hydrogen and their use for bioenergy production [4]. Bioelectrochemical systems (BES) are innovative microbial technologies that are commonly used for applications in wastewater treatment, nutrients recovery, biosensing, bioremediation and renewable energy generation [5]. BES are not yet able to reach the same maximum power densities as an RFB and BES electrochemical energy generated is not safeguarded in the system.

The purpose of this work is to harnessing wastewater for bioenergy storage in a microbially-charged redox flow cell, assessing their capacity to produce storable electrochemical fuels in BES, which can be used to produce electricity in a redox flow cell (as shown in the Graphical Abstract). The 2,6-anthaquinone was the redox chemical specie used in the BES as catholyte for their electrochemical reduction with wastewater (anolyte). The BES were operated with an external resistance (1000 Ω) in batch conditions, the potential was measured over time. In a second step, a redox flow cell (RFC) was using, with potassium hexacyanoferrate (III) as posilyte and as the negalyte the 2,6-anthaquinone charged by the BES.

The results from the BES pointed that the electrochemical reduction of 20 mM 2,6-AQDS (50 cm³) in BES with wastewater mixed communities was observed after 27 days, fact assessed by a visually distinctive color change (from yellow to dark red), characteristic of the 2,6AQDS. UV-Vis spectrophotometry at 330 nm allowing estimating the electrochemical conversion of the quinone, which was approximately 50 %.

The BES maximum power density was ca. 10 mW·m⁻² at 80-100 mA·cm⁻² and display ca. 40 % of coulombic efficiency. The maximum power density and coloumbic efficieny were obtained with wastewater mixed communities and complex carbon sources are in the range of previous reports with BES operated with wastewater [6, 7].

The RFC operated over time with a constant current density of 3.75 mA·cm⁻² for 10 cycles resulted in ca. 100 % coulombic efficiency and ca. 60 % potential efficiency and ca. 60 % energy efficiency. The RFC promoted similar result efficiencies to previous works [8].

Conclusions
In this study the wastewater potential as renewable energy source to convert into storable electrochemical fuels was assessed, and the bio-charged redox pair was tested in a redox flow cell (2,6-AQDS || Fe(CN)₆³⁻) with ca. 100 % coulombic efficiency. The integration of the BES and a RFC resulted in a half microbially-charged redox flow cell that is a novel promising approach of converting wastes into storable and easy to use green energy and a way to promote circular economy.
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Photo-electro-Fenton process for the treatment of highly polluted effluents. Importance of the operational variables

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Introduction
Advanced Oxidation Processes (AOPs) are powerful remediation techniques which are based on the generation of oxidizing species, mainly and most powerfully the hydroxyl radical (HO·) (2.6 eV vs SHE). The classical AOPs to obtain these radicals is the Fenton process, where hydrogen peroxide (H₂O₂) and Fe³⁺ react following equation 1, producing HO·, HO₂⁻ and Fe²⁺ [1].

\[ \text{H}_2\text{O}_2 + \text{Fe}^{3+} \rightarrow \text{HO·} + \text{HO}_2^- + \text{Fe}^{2+} \] (Eq. 1)

The main drawbacks of this process is the necessity of working at acid pH to avoid the iron precipitation, the stipulation of eliminating the iron after the treatment, the necessity of constant addition of reagents and the generation of Fe(OH)³⁺ and Fe(C₂O₄)²⁻ complexes which diminish the available quantity of Fe³⁺ and HO₂⁻ [2].

Applying light radiation favors the breakage of the iron complexes and thus augmenting the availability of Fe, HO· and degradable organic matter. The application of an electric field is also necessary in order to obtain the Fe³⁺ regeneration and the H₂O₂ generation (Eq. 2 and 3). This cyclic Fe³⁺ regeneration avoids the necessity of high iron concentrations and then all the iron usage disadvantages. Thus, these processes are combined in a synergistic way, shown in the Graphical Abstract: the photo-electro-Fenton process (PEF).

\[ \text{Fe}^{3+} + e^- \rightarrow \text{Fe}^{2+} \] (Eq. 2)
\[ \text{O}_2 + 2\text{H}^+ + 2e^- \rightarrow \text{H}_2\text{O}_2 \] (Eq. 3)

However, PEF can be a highly expensive process if not properly optimized. In this context, not only some variables such as voltage or iron concentration are important, but also the reactor configuration which should enhance the radiation receipt or the electricity efficiency.

This well performant process can be used for the treatment of effluents which are not suitable for being remediated under the typical biological treatment. This is the case of winery wastewater effluents as they have high and variable organic matter content and their industries work in batches. All of that is a challenge when working with microorganisms [3].

Objectives
Therefore, the main objective of this work is to optimize the PEF process for the treatment of a real winery wastewater effluent. For that, the main variables would be distance between electrodes (gap), applied voltage, radiation source and type of cathode.

Methods
A cubic reactor of 400 mL of capacity was set up (Figure 1), which allowed a separation of two compartments, the electro-Fenton and the photo-radiated one. The size of both of them is variable thanks to the movable support inserted (piece 5 of Figure 1) The electrode are was fixed at 38 cm, the aerated at 1 L/min, the electrolyte support at 10 mM (Na₂SO₄) and the treatment time at 3 h. The other aforementioned variables were evaluated considering the winery effluent discoloration and its mineralization. Preliminary tests were done with simulated wastewater (wine diluted 1:10) whereas the validation of the procedure was accomplished with real matrices.

Results
First of all, and taking into advantage the reactor configuration which allowed the modification on the gap, its optimization was accomplished. Therefore, at 5 V with graphite sheet electrodes, the degradation of the simulated winery wastewater was
performed (Table 1) and, as expected, the decrease on the gap enhanced the performance of the process, as it helps the electrochemical processes.

The effect of the voltage was also assessed (Table 1) as increasing it may assist the electrochemical degradation and the Fe\textsuperscript{2+} and H\textsubscript{2}O\textsubscript{2} evolution. However, increasing it too much may favor parasitic reactions [4]. Actually, the winery wastewater degradation was astonishingly enhanced when augmenting the voltage to 10 V but was slightly reduced when testing 15 V.

Table 1. Winery wastewater degradation after PEF process.

<table>
<thead>
<tr>
<th>GAP (cm)</th>
<th>Voltage (V)</th>
<th>Discoloration (%)</th>
<th>Mineralization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>8</td>
<td>5</td>
<td>48.19</td>
<td>20.01</td>
</tr>
<tr>
<td>6</td>
<td>5</td>
<td>69.05</td>
<td>29.24</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>80.68</td>
<td>36.08</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>77.68</td>
<td>37.61</td>
</tr>
<tr>
<td>2</td>
<td>10</td>
<td>97.08</td>
<td>67.08</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
<td>92.81</td>
<td>64.47</td>
</tr>
</tbody>
</table>

Afterwards, and in order to reduce the operational costs, the comparison between a mercury lamp (80 W) and a low emitting diode lamp (40 W) was carried out. Under this latter low-consumption lamp, the results were 6 % improved, reducing then the electric consumption to a half.

Then, other cathodes were tested to use more porous material which enhance the Fe\textsuperscript{2+} and H\textsubscript{2}O\textsubscript{2} regeneration and generation [5]. Thus, the performance of graphite sheet, nickel-foam and a synthetized PTFE (polytetrafluorethylene) composite cathode was compared for the H\textsubscript{2}O\textsubscript{2} generation (Figure 2).

The nickel-foam cathode performed apparently better, however, after 75 min, the H\textsubscript{2}O\textsubscript{2} started to be electrically decomposed whereas the PTFE composite was able to keep the oxidant concentration constant. In order to select properly the best cathode, the actual winery wastewater degradation was carried out and the PTFE composite enhanced the mineralization 17 %.

Figure 2. H\textsubscript{2}O\textsubscript{2} generation with graphite sheet (black symbols), PTFE composite (dark grey symbols) and nickel-foam (light grey symbols) cathodes.

Under the optimal conditions, the treatment of real wastewater matrixes (originating from white and red wine production industries) was accomplished. The white effluent was more easily degraded due to showing a lower light scattering. In both cases 60 % of mineralization was attained, which, taking into account the high complexity of the effluent, is a good result. Moreover, the PTFE composite showed a high stability and the effluent can be treated until complete mineralization.

Conclusions

An original reactor configuration was set and the optimal variables optimized for the PEF treatment of a winery wastewater effluent. Under optimal conditions (2 cm of gap, 10 V, low emitting lamp and PTFE composite cathode) the process was suitable for the remediation of real winery wastewaters.

Acknowledgements

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References

Valorization of spent coffee grounds as biosorbent for the retention of fluoxetine from water – a cost-effective alternative to activated carbon

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The goal of this study is to evaluate and to compare the effectiveness of spent coffee grounds (SCG), a waste-based biosorbent, and granular activated carbon (GAC) for the removal of fluoxetine, a widely consumed psychiatric pharmaceutical. Equilibrium measurements performed in batch experiments allowed to determine the adsorption capacity of the materials under evaluation. GAC had by far the highest adsorption capacity, 215.0 mg/g, while SCG showed a maximum uptake of 14.31 mg/g. The cost analysis performed revealed that SCG, although presenting lower adsorption capacity, is the most economically feasible adsorbent, with a cost of 0.77 € per gram of fluoxetine removed, that is quite lower when compared to that of GAC, 1.16 €/g. This study demonstrates that SCG is a waste-based biosorbent that may be successfully applied and be cost-effective for the removal of fluoxetine from water.

Introduction
Pharmaceuticals and their metabolites have been introduced in aquatic ecosystems, mainly from effluents of WWTP [1]. According to the Directive 2013/39 EU [2], there is an urgent need to evaluate new ways of reducing their input into the environment. The removal of pharmaceuticals by adsorption is one of the most attractive techniques for the treatment of wastewater [3–5]. Although commercial adsorbents, such as activated carbon, provide high removal rates, their high cost is a drawback to their application in large scale systems. Thus, the search for alternative low-cost and biodegradable adsorbents is an urgent need. The use of wastes derived from agriculture has attracted the attention of the scientific community due to their abundance in nature, low price, good mechanical, chemical resistance and biodegradability. Spent coffee grounds (SCG), the solid residues obtained from the preparation of coffee, is an agro-based waste that has been used as low-cost biological adsorbent in wastewater treatment. The European Union is by far the biggest importer and consumer of coffee, where most of the SCG is currently being incinerated or disposed of in landfills [6]. The conversion of waste materials into adsorbents has, therefore, a double environmental benefit, including improved waste management and environmental protection. The goal of this study is to measure and compare the sorption capacity of activated carbon and spent coffee grounds, as well as to perform a cost analysis in order to select the most economical adsorbent.

Experimental
Fluoxetine-HCl (>98%) was purchased from Sigma-Aldrich. HPLC-grade acetonitrile (Fisher Chemical), phosphoric acid (85%, Merck) and ultra-pure water, obtained from a Milli-Q Millipore system, were used to prepare the mobile phase for fluoxetine quantification by ultra-high performance liquid chromatography (UHPLC). Granular activated carbon, supplied by MERCK, was ground with a mortar and pestle, sieved (< 1 mm) and dried at 105 °C for 12 h. SCG was collected at a local coffee shop, dried and sieved through a 1 mm mesh in order to uniform particle size. The soluble materials of SCG were removed in contact with a 0.1 M NaOH solution for 3 h in an orbital incubator operating at 40 °C and 100 rpm. After that, the SCG was washed repeatedly with deionized water and finally was dried during 24 h at 70 °C. Different masses of SCG and GAC were added to amber Erlenmeyer flasks containing 50 mL of 5 mg/L fluoxetine-HCl solution. The suspensions were kept in an orbital incubator under moderate agitation, 170 rpm, at 25°C, for the period of time needed to attain the equilibrium. Adsorbent doses between 0.01 and 0.1 g/L were used for GAC while adsorbent doses in the range of 0.1–1.5 g/L were used for SCG. After the equilibration time, samples were taken, filtered and analysed by UHPLC with diode array detection for the determination of fluoxetine-HCl concentration.

Adsorption isotherms
The amount of fluoxetine adsorbed in the equilibrium, $q_e$ (mg/g) was calculated using Eq. (1):

$$ q_e = \frac{(C_0 - C_e)V}{w} $$

where $C_0$ (mg/L) is the initial concentration of fluoxetine-HCl solution, $C_e$ (mg/L) is the equilibrium concentration, $V$ (L) is the volume of the fluoxetine-HCl solution and $w$ (g) is the mass of sorbent.

The experimental data were fitted by the Langmuir and Sips isotherm models (Eqs. (2), and (3), respectively) in order to determine the equilibrium parameters of the systems. The models are as follows:

$$ q_e = \frac{q_{\text{max}}K_1C_e}{1 + K_1C_e} $$

Eq. (2)

where $q_{\text{max}}$ represents the maximum adsorption capacity (mg/g) and $K_1$ is a constant (L/mg).

$$ q_e = \frac{q_{\text{max}}K_2C_e^{1/m}}{1 + K_2C_e^{1/m}} $$

Eq. (3)

where $K_2$ is the affinity constant (L/g) and $m$ is a parameter related with the heterogeneity of the system.

Results and discussion
The results obtained from the equilibrium adsorption experiments and the fittings with isotherm models are shown in Table 1 and Figure 1. According to $R^2$ values presented in Table 1, the Sips model is the one that best fits the adsorption isotherm onto SCG, while for GAC both models fit adequately the adsorption data. GAC was the adsorbent with the highest
Adsorption capacity, 215.0 mg/g, being fifteenfold the value determined for SCG, 14.31 mg/g. The highest value of the Sips coefficient, $K_s$, was attained for GAC, which points to a higher affinity of fluoxetine to this adsorbent in comparison with SCG.

Although activated carbon is one of the most common and efficient adsorbent used for the removal of micropollutants, such as fluoxetine, its high cost is a significant disadvantage. Therefore, in the present work, an attempt is made to compare the cost of commercial activated carbon and spent coffee grounds, a waste-based biosorbent. The cost for the preparation of SCG biosorbent was calculated based on the performed pretreatments (washing, heating and drying procedures). For GAC it was only considered its purchase cost. Taking into consideration the values of $q_{\text{max}}$ obtained, the total costs were calculated for the removal of 1 g of fluoxetine hydrochloride from water (Figure 2).

The cost for the removal of 1 g of fluoxetine using GAC is 1.16 €, while SCG has a cost of 0.77 €/g. These results indicate that the costs associated with SCG are quite smaller when compared to that of commercial activated carbon.

**Conclusions**

This study demonstrates that SCG, a waste-based biosorbent, may be successfully applied and be cost-effective for the removal of fluoxetine from water. Adsorption technology employing wastes has a double environmental benefit, namely pollution mitigation and valorization of residues that otherwise would have to be disposed. The use of alternative biosorbents capable to compete with commercially available adsorbents is still an emerging field of research that requires further exploitation.

**Acknowledgements**

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**References**


Carbon dioxide conversion into renewable synthetic fuels, through an electrochemical process

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The environmental sustainability can be supported by the RENEFUEL project, aiming to produce synthetic fuels, such as methane, methanol and di-methyl ether (DME), which can be a renewable alternative to fossil fuels and coal as solutions for mobility. The work, currently under development, is directed to the production of methane from electrochemical technologies, namely through a specific water electrolysis process. The tests carried out, until now, have shown the high potential of this electrochemical process to produce renewable synthetic fuels, an alternative to fossil fuels, with several possible applications, such as rural electrification, in remote locations (for example, islands and mountain villages) and, as an alternative for energy storage or as fuel for mobility.

Introduction
The challenges of environmental sustainability require the decarbonization of the current energy mix with the progressive elimination of fossil fuels and their replacement by renewable energies.

Objectives
The environmental sustainability can be supported by the RENEFUEL project, aiming to produce synthetic fuels, such as methane, methanol and di-methyl ether (DME), which can be a renewable alternative to fossil fuels and coal as solutions for mobility.

Methods
The work, currently under development, is directed to the production of methane from electrochemical technologies, exploring two alternative routes, A and B.

Alternative route A – Production of synthesis gas by electrolysis of water in an innovative process using graphite electrodes, and without gas separation [1]. In this configuration the obtained oxygen, produced in the anode, directly oxidizes the (consumable) electrode producing carbon monoxide and carbon dioxide, which combines with the hydrogen generated at the cathode of the electrode, as shown in the Graphical Abstract (1).

Operational parameters, such as temperature, pressure, current, number of cells, concentration and type of electrolyte, were studied and optimized in a large range. The obtained synthesis gas (a mixture of hydrogen, carbon dioxide, carbon monoxide and small amounts of oxygen) is subjected to a methanation reaction, named Sabatier Process, in a tubular catalytic reactor [2], as shown in the Graphical Abstract (2).

Alternative route B – Another line of work developed in parallel is the production of these same synthetic renewable fuels, passing the gases obtained from the electrolysis of water (where metal electrodes are present without separation of the produced gases – hydrogen and oxygen), as shown in the Graphical Abstract (3 and 4), by liquefied biomass (of different origins, for example, cork and eucalyptus shale), with oxidation and hydrogenation of the carbonaceous matter for the production of carbon monoxide, carbon dioxide and methane. An in situ methanation reaction, named Sabatier Process, is observed, which is significantly enhanced with the use of catalysts (namely acidified Y zeolite – HY) inside the batch catalytic reactor, as shown in the Graphical Abstract (2).

Results
Alternative route A – A nickel catalyst supported on calcium oxide and aluminum oxide was tested and the influence of temperature (between 100 and 250 °C), at 1 bar of pressure, for methane production was studied, with high efficiencies (> 50 %) and selectivities (> 96 %) for a temperature of 200 °C. Interesting results where shown at low temperatures (in the order of 150 °C), and at atmospheric pressure [3].

Alternative route B – This solution was tested at various operating temperatures of the batch catalytic reactor (between 150 °C and 300 °C), at atmospheric pressure [4].

Conclusion
The current studies contemplate the testing of other catalysts (namely a nickel catalyst supported on acidified Y zeolite, a nickel/magnesium catalyst supported on acidified Y zeolite, a nickel catalyst supported on calcium oxide and aluminum oxide, and a nickel catalyst supported on carbon nanoparticles) and the redesign of the experience layout for the study of pressure influence, into a more resistant stainless-steel tubular catalytic reactor and a more resistant stainless-steel batch catalytic reactor, for both alternative route A and B, respectively.

The tests carried out, until now, have shown the high potential of this electrochemical process to produce renewable synthetic fuels, an alternative to fossil fuels, with several possible applications, such as rural electrification, in remote locations (for example, islands and mountain villages) and, as an alternative for energy storage or as fuel for mobility [1].

Acknowledgements
The authors acknowledge the SECIL’s support in providing the liquefied biomass and for the collaboration provided in the project. The authors acknowledge also, to the Instituto Politécnico de Lisboa (IPL), for funding support, through the IDI&CA research project “Biosyngas”.

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References


Intensifying heterogeneous photocatalysis for bromates reduction using the NETmix Photoreactor

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The main goal of this study was the intensification of the heterogeneous TiO$_2$ photocatalytic process for bromate reduction, using a multi-photoreactor, based on the NETmix technology, irradiated by UVA-LEDs. The BrO$_3^-$ reduction rate was assessed as a function of: i) illumination mechanism, front-side (FSI) vs. back-side (BSI) irradiation; ii) catalyst film thickness; iii) solution pH; iv) solution temperature; and v) absence/presence of oxygen. Higher reduction rates were obtained using the FSI mechanism, mainly associated with a 3-fold increase of a catalyst surface area per reactor volume, when compared to BSI. Temperature (15-30°C) had a negligible effect on the reduction rate. Under the best conditions (stainless steel slab as catalyst support, pH=5.5, 15 mL of a 2% wt. TiO$_2$ suspension, 25°C), the reduction rate remained similar for consecutive reactions and almost complete reduction of 200 µg BrO$_3^-$ L$^{-1}$ was achieved after 90 minutes, resulting in [BrO$_3^-$] < 10 µg L$^{-1}$ (guideline value).

Introduction
Bromate (BrO$_3^-$) is an o xoanion classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (group 2B), which led to the establishment of a guideline value of 10 µg BrO$_3^-$ L$^{-1}$ in water intended for human consumption. Since BrO$_3^-$ might be formed during the treatment of water containing bromide ions (Br$^-$), through ozonation or chlorination processes, it is necessary to find efficient solutions for its removal from water [1]. Advanced reduction processes (ARPs), such as heterogeneous photocatalysis, can be a promising technology for bromate removal from water. Heterogeneous photocatalysis is a photoinduced reaction accelerated by the presence of a catalyst, a semiconductor material (e.g. Titanium dioxide – TiO$_2$), which is activated by absorption of a photon with an energy equal or higher than the band-gap energy ($E_{bg}$), inducing the transference of a negative electron ($e^-$) with reducing capacity from the valence band (VB) to the conduction band (CB) of the catalyst, thus generating a positive hole ($h^+$) in the valence band with oxidative properties [2].

The main barriers associated with the application of photocatalytic processes for water decontamination are mass and photon transfer limitations. To overcome these barriers, it is necessary to enhance the photocatalytic reaction rates by the use of innovative photoreactors and illumination sources [2]. The multi-photoreactor, based on the NETmix technology, consists of a network of cylindrical chambers interconnected through a series of rectangular channels that allows a high degree of mixing under laminar regime [3]. In this reactor, the catalyst can be fixed in a support medium, avoiding the need of extra treatment steps to remove the catalyst from the solution. The use of microscale illumination such as light-emitting diodes (LEDs) has a great potential in heterogeneous photocatalysis due to several advantages, such as: i) flexible design, ii) high energy efficiency; and iii) longer lifetime expectancy [2].

The main goal of this study was to intensify the heterogeneous TiO$_2$ photocatalytic process for BrO$_3^-$ reduction, by using the NETmix multi-photoreactor and UVA-LEDs as a light source. The effect of several process variables on bromate reduction rate was evaluated, such as: i) illumination mechanism; ii) catalyst film thickness; iii) solution pH; iv) solution temperature; and v) absence/presence of oxygen.

Materials and Methods
The NETmix multi-photoreactor used in this work has been fully described elsewhere [4]. Two different configurations were used: i) an acrylic structure containing a stainless-steel slab (SSS) imprinted with a network of channels and chambers where the catalyst was deposited (10-20 mL of a 2% wt. TiO$_2$ suspension, which corresponds to 193-381 mg), and then covered with a borosilicate slab (BS) without catalyst, being possible to analyze the mechanism of FSI (catalyst surface area per reactor volume of 989 m$^2$ m$^{-3}$); ii) an acrylic base imprinted with the network of channels and chambers and covered with a BS coated with 10-40 mg of catalyst, thereby analyzing the mechanism of BSI (catalyst surface area per reactor volume of 333 m$^2$ m$^{-3}$). The radiation source, placed on top of the BS, consisted in 9 UVA–LEDs (270 mW, $\lambda_{peak}=365$ nm). TiO$_2$-P25 nanoparticles were used to prepare a 2% wt. aqueous suspension for the spray deposition technique. Before deposition, SSS and BS were washed with alkaline detergent and ultrapure water (UPW) and dried in an oven. During the spray deposition process, the slabs were over a heating plate (SSS: $T_{plate}=150^\circ$C, BS: $T_{plate}=200^\circ$C).

The experimental procedure consisted of the following steps: i) 1.5 L of BrO$_3^-$ solution (200 µg L$^{-1}$ in UPW) was added to a jacketed glass vessel, connected to a thermostatic bath, in order to maintain the solution temperature at the intended value (15, 20, 25 and 30°C); ii) the solution pH was adjusted to the desired value (5.5, 6.5 and 7.5); iii) the recirculation pump was turned on (at 75 L h$^{-1}$). When dissolved oxygen (DO) levels lower than 0.1 mg L$^{-1}$ were required, nitrogen was also injected into the solution. Then, the initial sample was collected immediately after the reaction was initiated (t=0) by turning on the UVA-LEDs. Samples were collected at predefined times, during the 2-hour reaction period, to quantify BrO$_3^-$ and Br$^-$ concentrations.

Results and Discussion
Preliminary tests showed that, during 2-hour reaction period, BrO$_3^-$ was not: i) adsorbed on the catalyst surface, in the absence of light; ii) photoreduced in the absence of catalyst.
Regarding the BSI mechanism (Figure 1), a 2.8-fold increase on the reduction rate was observed with the increment of catalyst amount from 10 to 40 mg (film thicknesses of 0.21 and 0.83 μm, respectively). A further increase on the catalyst film thickness showed a negligible improvement on the reaction rate. As expected in a BSI mechanism, the reaction rate reaches a maximum value, and then an additional increase on the catalyst film thickness led to lower reaction rates since the charge carriers are generated far from the catalyst-liquid interface, enhancing the electron/hole recombination.

![Figure 1. Influence of the BSI (closed symbols) and FSI (open symbols) mechanisms on BrO₃⁻ photoreduction using different TiO₂ amounts: (■) 10, (○) 20 and (▲) 40 mg, for BSI; and (■) 10, (○) 15 and (▲) 20 mL of a 2% wt. TiO₂ suspension, for FSI. Operating conditions: pH = 5.5; T = 25 °C; Q = 75 L h⁻¹.](image)

As concerns the FSI mechanism (Figure 1), an increment on catalyst film thickness from 2.0 to 3.0 μm (film density from 0.7 to 1.1 mg cm⁻²) led to a 1.3-fold increase on the reduction rate, and after that, the reaction rate remained constant. This performance is in good agreement with the FSI mechanism, since after a certain catalyst film thickness the diffusion length of the charge carriers to the catalyst-liquid interface does not change. Comparing the FSI with BSI mechanism, under the best conditions (film thicknesses of 3.0 and 0.83 μm, respectively), one can conclude that the catalyst reactivity in combination with the photoreactor was improved 2.8 times from 41 to 113 μmol BrO₃⁻ m⁻² illuminated volume s⁻¹. This is in agreement with the 3-fold increase on the catalyst surface area per reactor volume when comparing FSI and BSI mechanisms. Furthermore, on both catalyst supports, the TiO₂ films showed good adherence and stability after 13 consecutive reactions.

The effect of operating conditions, on bromate reduction rate, was evaluated using the FSI mechanism, resulting in the following conclusions: i) the lower the pH the higher the reaction rate, since the TiO₂ point of zero charge (PZC) is 6.4 [5]; this means that, above PZC, the negative charges will prevail on the TiO₂ surface and will lead to an electrostatic repulsion of BrO₃⁻, whereas bellow PZC, positive charges are predominant and will favor the adsorption of BrO₃⁻ onto TiO₂ surface, thereby increasing the reaction rates; ii) the temperature (15-30°C) had no influence on the photoreduction kinetics; iii) the injection of nitrogen did not improve the BrO₃⁻ reduction rate, since it was not possible to completely purge the DO from the NETmix milli-photoreactor, as result of the eight inlets that might enhance air dissolution into the reactor. This is contradictory with the preliminary tests carried out in a batch reactor.

Conclusions
The TiO₂-driven photocatalytic reduction of BrO₃⁻ using the NETmix milli-photoreactor was successfully accomplished. The FSI mechanism, where the network of channels and chambers imprinted in back stainless steel slab were coated with a thin film of catalyst, proved to be the best configuration due to a 3-fold increase on the catalyst surface area per reactor volume when compared to BSI. For the best photocatalyst thickness (15 mL of a 2% wt. TiO₂ suspension on the SSS), the catalyst thin film showed to be very stable and reusable for at least 13 times. Temperature (15-30°C) had a negligible effect on the reduction reaction rate. Under the best conditions (SSS: pH=5.5, 15 mL of catalyst suspension, 25°C), almost complete reduction of 200 μg BrO₃⁻ L⁻¹ into Br⁻ was achieved in less than 90 minutes, resulting in [BrO₃⁻] < 10 μg L⁻¹ (guideline value).

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References
Zeolite-based catalysts could be promising catalysts for power-to-gas due to their tunable properties, potentially interesting for the Sabatier reaction. As CO₂ conversion into CH₄ has been mainly performed over Ni catalysts, in the present work a complete study regarding the optimization of Ni-based zeolites for this reaction was carried out. Thus, the effects of the preparation conditions, the zeolites composition and even the framework type on the catalysts’ properties and performances were studied. Then, a promoter of the catalytic activity (Ce) was incorporated to the most promising Ni-Zeolite and the obtained performances in terms of CO₂ conversion and CH₄ selectivity were compared to those of a commercial Ni/γ-Al₂O₃ catalyst. The optimized Ni-Ce/Zeolite from the present work (with CH₄ yield ~80% at 300 ºC) was more active and selective than the commercial catalyst (with CH₄ yield ~30% under the same reaction conditions), proving the suitability of zeolite-based materials for this application.

Introduction

Power-to-gas constitutes a relevant strategy for storing the temporary over-production of renewable electric energy through the conversion of carbon dioxide into synthetic natural gas by CO₂ methanation reaction. This approach could lead to a more efficient electric energy network and also to the mitigation of the CO₂ emissions, not only due to the usage of CO₂ as a raw material, but also due to the reduction of the intrinsic limitations of renewable sources utilization for energy production [1,2].

The efficiency of power-to-gas plants strongly depends on the CO₂ methanation catalysts, being the core of the methanation process the development of active, selective and stable catalytic systems. Ni-based systems supported in Al₂O₃, SiO₂, CeO₂, ZrO₂ mesoporous materials or even zeolites have mainly been reported in the literature [3–5], being the last ones promising materials due to their tunable properties.

Consequently, in the present work a complete study regarding the optimization of Ni-based zeolite catalysts for CO₂ methanation was performed, being the results of the best prepared sample compared to those obtained for a commercial Ni/γ-Al₂O₃ catalyst.

Methods

Catalysts preparation. In this work several Ni-based samples have been prepared using as support different commercial zeolites. Firstly, the effect of the Ni incorporation method (ion exchange or incipient wetness impregnation of 2 wt.% Ni over a USY zeolite followed by calcination at 500 ºC under air flow), the Ni loading (5, 10, 15 and 25 wt.% Ni incipient wetness impregnation over an USY zeolite followed by calcination under the same conditions indicated above) and the pre-reduction temperature (470, 550, 600 and 700 ºC for a 15%Ni/USY zeolite prepared by impregnation) were evaluated. Also, the compensating cation nature effect was tested by preparing H-, Li-, Na-, K- and Cs-USY zeolites by ion-exchange method. Then, 15 wt.% Ni was impregnated over all the supports followed by calcination. The Si/Al ratio effect was also studied by using three USY zeolites with ratios of 3, 15 and 38 and with H⁺, Na⁺ and Cs⁺ as compensating cations. Again 15 wt.% Ni was impregnated over all the supports and samples were finally calcined. The framework type effect was also verified by preparing Na- and Cs-Zeolites (USY, BEA, MOR and ZSM-5) with similar Si/Al ratios. As in previous cases, 15 wt.% Ni was impregnated over the supports and samples were finally calcined. Finally, the most active zeolite support was co-impregnated with Ni and Ce (15 and 20 wt.%, respectively) and calcined giving rise to the Ni-Ce/USY sample. A commercial catalyst with ~25 wt.% Ni was also used for comparison purposes.

Characterization techniques. Samples from the present work were characterized by XCP analysis, TGA-DSC, XRD, Ni participation, CO₂ adsorption, H₂-TPR and UV-Vis spectroscopy. Additionally, reduced samples were characterized by TEM.

Catalytic tests. Reactivity tests were carried out in a fixed bed reactor using ~0.177 g of catalyst. Pre-reduction treatment was performed under a 250 ml/min H₂/N₂ flow at a specific temperature during 1 h and, later, reaction was carried out from 250 to 450 ºC (by 50 ºC steps) keeping a CO₂:H₂ ratio of 1:4 and using a 250 ml/min of total flow.

Results and discussion

Preparation conditions effect. As a first step, the Ni incorporation method (ion-exchange or incipient wetness impregnation) was optimized, being obtained better results for impregnated samples due to the more favorable reducibility properties of the Ni species [6]. In addition, the effect of Ni loading indicated that higher Ni contents favor the performances due to the higher number of Ni⁺ active sites in the samples after the pre-reduction process and 15 wt.% Ni was found as the most favorable loading [6]. Finally, it was observed that increasing the pre-reduction temperature in the studied range does not lead to enhancements in the performances, being 470 ºC identified as the most favorable reduction temperature [7].

Zeolites composition effect. The most promising compensating cation was also identified by comparing 15 wt.% Ni/USY samples containing H⁺, Li⁺, Na⁺, K⁺ and Cs⁺ on their structure. Larger cations were found to enhance the interaction with CO₂ weaken the interaction with H₂O (found as an inhibitor of this reaction) and enhance the reducibility properties of Ni species [8]. Indeed, Cs⁺ was found as the most favorable cation (Table 1) [8]. Regarding the effect of the zeolites Si/Al ratio, when comparing three 15%Ni/USY samples with ratios of 3, 15 and
38, it was found that, both with Na⁺ or Cs⁺ as compensating cation, higher ratios lead to a relevant decrease in the interaction of the samples with water (Table 1), reducing the inhibitory role of water in the reaction [9]. This effect in the interaction with water was suggested as a key and predominant parameter affecting the observed performances, since other catalysts' properties, such as the interaction with CO₂, normally pointed as beneficial for CO₂ methanation reaction, varied in the opposite direction of the performances.

Table 1. Catalytic performances (T\text{reaction} = 350 °C) of the most representative samples of the composition effect study.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Si/Al</th>
<th>X\text{CO}_2 (%)</th>
<th>S\text{CH}_4 (%)</th>
<th>Y\text{CH}_4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%Ni/H-USY</td>
<td>3</td>
<td>18</td>
<td>82</td>
<td>15</td>
</tr>
<tr>
<td>15%Ni/Li-USY</td>
<td>3</td>
<td>27</td>
<td>87</td>
<td>23</td>
</tr>
<tr>
<td>15%Ni/Na-USY</td>
<td>5</td>
<td>28</td>
<td>85</td>
<td>24</td>
</tr>
<tr>
<td>15%Ni/K-USY</td>
<td>8</td>
<td>28</td>
<td>85</td>
<td>24</td>
</tr>
<tr>
<td>15%Ni/Cs-USY</td>
<td>15</td>
<td>48</td>
<td>90</td>
<td>43</td>
</tr>
<tr>
<td>15%Ni/Ce-USY</td>
<td>38</td>
<td>51</td>
<td>92</td>
<td>47</td>
</tr>
</tbody>
</table>

Framework type effect. Also, four 15%Zeolites with the same compensating cation (Na⁺ or Cs⁺) and similar Si/Al ratios (~40) were compared in terms of catalysts properties and performances. From the obtained results (Table 2) it was concluded that USY remains the most promising zeolite support for the CO₂ methanation reaction, mainly due to the higher hydrophobic character of these samples and the lower negative effect of water in the reaction [10].

Table 2. Catalytic performances (T\text{reaction}=350 °C) of the most representative samples of the framework type effect study.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>X\text{CO}_2 (%)</th>
<th>S\text{CH}_4 (%)</th>
<th>Y\text{CH}_4 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%Ni/Na-USY</td>
<td>55</td>
<td>95</td>
<td>52</td>
</tr>
<tr>
<td>15%Ni/Na-BEA</td>
<td>43</td>
<td>92</td>
<td>40</td>
</tr>
<tr>
<td>15%Ni/Na-MOR</td>
<td>40</td>
<td>87</td>
<td>35</td>
</tr>
<tr>
<td>15%Ni/Na-ZSM-5</td>
<td>40</td>
<td>85</td>
<td>34</td>
</tr>
</tbody>
</table>

Incorporation of Ce as an activity promoter. Finally, and after being obtained the best results for the 15%Ni/Cs-USY(38) zeolite, the Ni-Ce/USY sample whose preparation was described in the Methods section was carried out, being its catalytic performances presented in Figure 1. As observed, Ce strongly enhances the performances towards CO₂ methanation, leading to CH₄ selectivity higher than 98% for all the studied reaction temperatures. This effect could be attributed, according to our findings [6,11,12], to the enhancement of the Ni species dispersion and reducibility and the higher CO₂ activation with the incorporation of CeO₂ due to the intrinsic ability of cerium oxide to activate carbon dioxide and the establishment of interactions between Ni and Ce species [11].

Figure 1. Catalytic performances obtained for the best Ni/USY sample from the present work, the promoted sample and the commercial catalyst.

Conclusions
To conclude, in the present work the suitability of zeolite-based materials for CO₂ methanation has been proved. For this purpose, a complete study was carried out step by step, evaluating the effects of the preparation conditions, zeolite composition and framework type in the performances of the prepared samples. Thus, an optimization of the samples composition was attempted being obtained the best results in terms of Ni/Zeolites for the 15%Ni/Cs-USY(Si/Al=38) sample. Finally, a promoted Ni-Ce/USY sample was prepared by co-impregnating Ni and Ce over the best USY support (Cs-USY(Si/Al=38)). Results were then compared to the obtained for a commercial Ni+y-Al₂O₃ catalyst, being the bimetallic zeolite the one leading to the highest CH₄ yield (~80%), especially at lower reaction temperatures.

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References
Simultaneous desulfurization and denitrogenation of fossil fuels

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Removal of sulfur and nitrogen compounds from fossil fuels is a key issue, given the stringent legislation and increasing demands for gasoline and diesel quality, as well as the impact on the environment and public health. Hydrotreating process is the current technology used for removal of such compounds in the oil refineries. Nevertheless, it presents several drawbacks, such as, high cost of operation and reduced efficiency in the removal of refractory sulfur compounds. Thus, the search for new and sustainable alternatives or complementary processes is mandatory. Ionic liquids and recently Deep Eutectic Solvents (DESs) have been explored as alternative key players for the removal of sulfur and nitrogen from fossil fuels, according several approaches. The aim of this work is focused in the application of new DESs-based systems for the simultaneous extractive desulfurization and denitrogenation of fuels.

Energy, as an essential factor for growth and development, has become the driving force of modern economy. Although changes to include a greater contribution from renewable energies are predictable, fossil fuels will remain dominant over the next decade. By 2040, fossil fuels are still expected to meet 77% of total energy demand. The road transportation sector is a main responsible for the fossil fuels demand and concomitantly, for the increasing air pollution, which has become an important issue around the world. In particular, the effect of fuel’s impurities in air quality has been an important subject [1].

The sulfur present in highway fuels leads to SOx emission to air, inhibits the performance of pollution control equipment on vehicles and affects numerous refinery processes. Nitrogen is also a problem once it leads to NOx emission and affects sulfur elimination. Hydrotreating processes are the current technology used for removal of sulfur and nitrogen compounds in the oil refineries. However, they present several drawbacks, such as high operating cost and low efficiency in the removal of refractory sulfur compounds [2]. Thus, the search for new sustainable alternative processes for the removal of such compounds is mandatory.

To date, extractive desulfurization/denitrogenation (EDS/EDN) according a liquid-liquid approach or solid-liquid approach (adsorption) is one of the most promising desulfurization processes due to its simple operation, low cost and benign effects on the final fuel quality. Ionic liquids (ILs) have been widely explored as alternative solvents for sulfur and nitrogen removal and although they have many desirable properties, such as non-flammability, non-volatility and high tunability, they also present major disadvantages like, high cost and, in some cases, toxicity. To overcome these limitations, Deep Eutectic Solvents (DESs) have been proposed as alternative to ILs, since DES can be seen as a low cost and more environmentally friendly ionic solvent [3,4].

Recently, our group published a work in which a wide range of DESs were tested and ranked according to their effectiveness in the removal of different refractory sulfur compounds from a model fuel [5]. At this point, and taking into account the knowledge already acquired, we are interested in the development of a method capable of simultaneously removing the sulfur compounds and also nitrogen compounds. In this way, several systems have been tested, among them liquid systems (namely DES) and also solid systems (adsorbents).

In a first phase of the work, the extraction capacity of each system was determined for four different fuel pollutants (namely: thiophene, dibenzothiophene, pyridine and carbazole) dissolved in hydrocarbons, in order to mimic a fuel. After finding a suitable system, we are challenged to solve some issues, such as, the recycling of our system, and its use in different real fuel matrices.

Acknowledgements

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References

New ecofriendly cheap spacers for efficient monolithic dye sensitized solar cells
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Monolithic configuration is one of the most promising dye sensitized solar cells (DSSCs) architectures; this architecture is easier to fabricate and more compact. Monolithic DSSCs were assembled using commercial electrical spacer layers based on ZrO2 and new cost-effective SiO2 and SiO2-TiO2 insulating layers. For deposition of silica spacers, a water-based formulation (volatile organics free) of silica paste was developed. Porosity and reflectance of the new spacer layers were adjusted to render a monolithic DSSC with high photocurrent conversion efficiency. The best energy performance of DSSCs comprehends a TiO2 blocking layer deposited by spray pyrolysis. 20-25 μm TiO2 photoanode coated with a SiO2-TiO2 (70 wt. % SiO2, 30 wt. % TiO2) spacer layer and platinum metal counter electrode of ca. 100 nm applied by DC magnetron sputtering. The cell delivered a PCE of 8.4 % without aperture mask and 8.3 % with mask – the highest PCE ever reported for monolithic DSSCs with liquid electrolyte.

Dye sensitized solar cells (DSSCs) are budding technological solution for direct photoelectrochemical conversion of solar light energy to electricity [1]. Monolithic architecture of DSSCs was first reported by Kay and Grätzel [2]. The highest reported PCEs for monolithic DSSC is 10.2 % (8.5 % with an aperture mask) when solid state electrolyte is used [3] and 9.5 % (8.0 % with an aperture mask) for liquid electrolyte [4]. Device stability, however, has never been reported. Monolithic DSSCs display lower manufacturing costs since require only a single conductive substrate. Moreover, the production of large area photovoltaic modules using monolithic structures is far more straightforward, when compared with Z-type connected conventional DSSCs [5]. Monolithic DSSC consists of a single transparent conductive substrate on which the conductive coating is divided into two to separate the photoanode and the cathode. The electrodes are configured in a triple layered structure, composed of i) photoanode - nanocrystalline/mesoporous TiO2 sensitized with dye; ii) electrical spacer and iii) counter electrode. This triple arrangement is encapsulated using a non-conductive material (usually glass); the cell is then filled with electrolyte and sealed.

The spacer layer in a monolithic DSSC has significant impact on overall efficiency of the cells [5]. Electric spacer should meet the following criteria: be fairly insulating to block electron flow from TiO2 conduction band to the counter electrode; to have sufficient porosity for unimpeded transfer and electrochemical reaction of redox shuttle on counter electrode; should be reflective to send unabsorbed light back to the photoanode for good light harvesting. As electrical spacers between photoanode and counter electrode, porous layers of ZrO2 is commonly used. Spacer is applied spreading zirconia paste on top of the photoanode and sintering. Commercial pastes are heavily loaded (~ 60-70%) with volatile organics: α-terpineol, acetic acid and ethanol. In addition to the high cost of zirconia itself, the need for capturing the harmful volatiles in large-scale industrial production leads to the drastic increase of the process cost. This work presents efficient and stable monolithic DSSCs assembled using advantageous cost effective SiO2-based electrical spacers derived from water-based paste, no volatile organics were used in the paste formulation. To achieve high power conversion efficiency (PCE) of the monolithic DSSCs, porosity and reflectance of the SiO2 layers were modified adding TiO2 (anatase) nanoparticles to the formulation. DSSCs were assembled on blank FTO-coated glasses (7.1cm2) and coated with ca. 80 nm of TiO2 blocking layer (by spray pyrolysis). Circular shaped photoanodes (0.13 cm2) were prepared by sequential screen printing of a transparent mesoporous TiO2 layer and opaque atop layer using 18NR-T and 18NR-AO pastes respectively, both from GreatCell Solar, and post-treated in 40 mM aqueous solution of TiCl4. The overall thickness of the photoanodes was 25±2 μm comprised from 17±1 μm of mesoporous and 8±1 μm of opaque TiO2 layers. Silica paste for deposition of electrical spacers was prepared from an aqueous suspension of colloidal silica (LUDOX® AS-40, monodispersed 22 nm particles) using hydroxypropyl cellulose (HPC, Mw ~ 100 000 au) as a structure-forming agent. For SiO2-TiO2 pastes, titania (PC 500, Lyondell, ~20 nm anatase) was added to the formulation in the amounts of 10, 20 and 30 wt % with respect to SiO2 (denoted as SiO2@10TiO2, SiO2@20TiO2, SiO2@30TiO2 correspondingly). Mass fraction of the solids in the pastes was adjusted to 20 wt.% by adding the required amount of H2O; the mass fraction of HPC was maintained at 15 wt.%. Ammonium hydroxide was used to prevent pastes gelation. Typical spacer layers of ZrO2 were prepared from commercial pastes (Zr-Nanoxide, Solaaronix®), ZTS/P for semi-transparent layer of ZrO2 and ZR/SP for opaque ZrO2. All insulating layers were deposited by doctor-blading originating after sintering a 13±2 μm layer. The counter electrode was ca. 100 nm of platinum metal applied by DC magnetron sputtering. Sensitization was made using a solution of 0.5 mM N719 dye + 5 mM chenodeoxycholic acid in absolute ethanol. High-performance electrolyte (EL-HPE) from GreatCell Solar was used to fill the cells. The photovoltaic performance of the cells was characterized under simulated solar light (AM1.5G) driving from a solar simulator MiniSol (LSH-7320, Newport) with intensity of 100 mW·cm−2 using an electrochemical workstation Zennium (Zahner). The electrochemical impedance spectra (EIS) were collected using Autolab (PGSTAT 302 N) in the dark at potential 20 mV less then open-circuit potential of the DSSC. The incident photon to current conversion efficiency (IPCE) spectra were recorded using a PC-operated setup consisting of light source, a Monochromator and a Lock-in Amplifier (Newport).
All monolithic DSSCs assembled using different spacer layers demonstrate typical for photodiode current-voltage (I-V) response. From the I-V curves the key parameters of the photovoltaic performance: open-circuit voltage (V_{oc}), short-circuit current (J_{sc}), filling factor (FF) and power conversion efficiency (PCE) were calculated and summarized in Table 1. Charge transfer resistance (extracted from EIS) in counter electrode/electrolyte interface (R_{CE}), serving as an indicator of the porosity of the spacer layer, and reflectance at 520 nm (corresponds to the maximum in absorbance spectra of N719 dye) of the produced spacers are presented in Table 1.

Cells assembled using commercial spacer layers of ZrO₂ demonstrate 6.1 and 6.7 % of PCE for semi-transparent and opaque zirconia respectively. Due to the presence of big particles (up to 5 μm) in the opaque ZrO₂ film, these layers have higher porosity, while semi-transparent ZrO₂ layers are less porous, made of 20-40 nm particles. Higher porosity of opaque ZrO₂ leads for larger surface area available for electrochemical reaction in the interface spacer/counter electrode and R_{CE} decreases; lower R_{CE} favors higher PCE. Also, the reflectance of opaque ZrO₂ spacer is 3 times higher than semi-transparent one. This contributes to the effective reflection of unabsorbed light back to the photoelectrode, which leads to an increase in PCE.

Elaborated silica spacers originated a PCE of 6.4 % even without further modifications of the formulation. However, reflectance of SiO₂ layer is very low and R_{CE} in the cells prepared using SiO₂ insulating layer is slightly higher when compared with the best ZrO₂ spacer. Further modification of the porosity and reflectivity of SiO₂ spacer layer was achieved by addition polydisperse TiO₂ nanoparticles into the film.

Increasing the concentration of TiO₂ in the film leads to gradual decrease of the R_{CE} more likely due to loosening of compact SiO₂ structure normally formed from monodispersed 22 nm SiO₂ particles. Noteworthy, films with 10 and 20 % of titania show practically the same reflectance as blank SiO₂. A sudden increase of reflectivity to 60 % was observed when 30 wt.% of TiO₂ was added. DSSC device with SiO₂@30TiO₂ spacer show PCE of 7.1 %. The impact of the reflectivity of the spacer layer on overall efficiency manifests itself as increase of IPCE in spectral region above 500 nm (Fig.1).

The efficiency of the best performing DSSC was further improved using a thin pinhole-free blocking layer (BL) of TiO₂, between FTO-substrate and photoanode. Application of BL gives rise to IPCE (Fig.1) at wavelengths below the absorption edge of sensitizer (N719) pointing to effective suppressing of photoelectron recombination reactions; J_{sc} and V_{oc} increase yielding 8.4 % of PCE (8.3 % with an aperture mask). The PCE history of the prepared cells was followed for 1000 h and revealed negligible (0.1%) drop of efficiency during natural ageing.

In conclusion, new electrical spacers based on SiO₂ for monolithic DSSCs were developed ecofriendly, entirely water-based formulations, rendering a device that for the first time ever demonstrate high efficiency of 8.4 % (8.3 % with an aperture mask) and full stability.

Table 1. Photovoltaic parameters of the cells, charge transfer resistance in Pt/electrolyte interface and reflectance of spacer layer at 520 nm.

<table>
<thead>
<tr>
<th>Semi-transparent ZrO₂</th>
<th>V_{oc}/V</th>
<th>J_{sc}/mA·cm⁻²</th>
<th>FF</th>
<th>PCE/%</th>
<th>R_{CE}/Ω·cm⁻²</th>
<th>Reflectance/%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Opaque ZrO₂</td>
<td>0.73</td>
<td>12.0</td>
<td>0.70</td>
<td>6.1</td>
<td>33.1</td>
<td>21.5</td>
</tr>
<tr>
<td>SiO₂</td>
<td>0.71</td>
<td>13.0</td>
<td>0.73</td>
<td>6.7</td>
<td>10.8</td>
<td>68</td>
</tr>
<tr>
<td>SiO₂@10TiO₂</td>
<td>0.71</td>
<td>12.7</td>
<td>0.70</td>
<td>6.4</td>
<td>13.9</td>
<td>9</td>
</tr>
<tr>
<td>SiO₂@20TiO₂</td>
<td>0.68</td>
<td>11.2</td>
<td>0.70</td>
<td>5.3</td>
<td>8.9</td>
<td>8</td>
</tr>
<tr>
<td>SiO₂@30TiO₂</td>
<td>0.72</td>
<td>13.8</td>
<td>0.70</td>
<td>6.9</td>
<td>7.7</td>
<td>9.5</td>
</tr>
<tr>
<td>SiO₂@30TiO₂</td>
<td>0.72</td>
<td>14.5</td>
<td>0.69</td>
<td>7.1</td>
<td>6.9</td>
<td>60</td>
</tr>
<tr>
<td>BL-SiO₂@30TiO₂</td>
<td>0.73</td>
<td>17.3</td>
<td>0.67</td>
<td>8.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>BL-SiO₂@30TiO₂ (with an aperture mask)</td>
<td>0.72</td>
<td>15.9</td>
<td>0.73</td>
<td>8.3</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

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References
Beating the performance of solar charging redox flow batteries based on a hematite photoelectrode

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Portugal presents one of the highest solar incidences in Europe, making development of sunlight harvesting and storage technologies very relevant. A solar redox flow cell (SRFC) combines the direct conversion of solar energy into chemical fuels, through the integration of a photoelectrochemical (PEC) cell, for sunlight conversion into both electrochemical and thermal energy, and a redox flow battery for electricity generation. This work reports the optimization of an aqueous alkaline SRFC based on low-cost hematite photoelectrodes and electrolytes consisting of the redox couples ferrocyanide and anthraquinone-2,7-disulphonate in NaOH. Performance improvement strategies are demonstrated for the ferrocyanide-hematite junction, allowing to reach a photocurrent of ca. 1.4 mA cm\(^{-2}\) and a photovoltage of 0.8 V. Different hematite surface treatments and cell arrangements were studied and allowed the design of an innovative and very promising SRFC device.

Introduction

Sustainable development directives for the 21\(^{st}\) century recommend that all countries must have access to affordable and clean energy, requiring technologies for its harvesting, storage and release on demand. Solar energy is the largest and most widespread source of renewable energy; at any instant the solar power striking the Earth’s surface is 89 000 TW. However, the intermittent and diurnal nature of sunlight makes developing a suitable energy storage technology for load leveling and stable power on demand imperative. Photoelectrochemical (PEC) devices offer the promise of directly producing solar fuels through artificial photosynthesis, but most of these require high overpotentials and complex systems. Redox flow batteries (RFB) store electricity as electrochemical fuels, being considered one of the most suitable technologies for stationary applications for their high charge-discharge energy efficiency, fast response time, modularity, flexibility and low-cost, promising the lowest cycle storage costs of 3 €/kWh per charging cycle by 2050\[1\].

The combination of a RFB with a PEC cell, which results in a solar redox flow cell (SRFC), is a very promising approach, allowing to directly convert sunlight into charged electrochemical fuels and heat. At a SRFC, the PEC cell where a semiconductor, in contact with the electrolyte in what is called a semiconductor-liquid junction, promotes the photocharging of redox pairs, which are discharged at the RFB for producing electricity, as shown in the Graphical Abstract. The SRFC concept was described for the first time in the 80s; however, research gained a new impulse with a TiO\(_2\) photoelectrode, but it absorbs a small fraction of the solar spectrum limiting the efficiency\[2\]. To bring this technology closer to industrialization, the development of efficient and stable photoelectrodes matching with redox pairs are crucial. Hematite is one of the most promising, yet challenging, materials for this application, mainly due to its abundance, low-cost, excellent stability and maximum solar-to-fuel efficiency of 15 % with a bandgap of 2.1 eV\[3\]. Previously reported SRFC, by the research team, built from an hematite-ferrocyanide junction reported lower photocurrents (\(< 0.2\) mA cm\(^{-2}\)) and a photovoltage of 0.6 V\[4\]. This work aimed at improving the performance of an aqueous alkaline SRFC based on a hematite photoelectrode, in terms of photocurrent, stability and photovoltage. Several cell arrangements were studied to understand what is limiting the most the performance of a SRFC.

Methods

Hematite thin films were deposited by spray pyrolysis on 2.2 mm thick, 7 Ω cm\(^{-1}\) fluorine-doped tin oxide (FTO) coated glass substrates (Solaronix, Switzerland), as described elsewhere\[3\]. First, the glasses were adequately cleaned and pre-treated with 1.5 mL of a diluted TEOS (tetraethyl orthosilicate) solution (10 % volume in ethanol) onto heated substrates at 450 °C. Then, the substrates were sprayed with 10 mM of Fe(acac)\(_3\) (99.9 %, Aldrich) in EtOH (99 %, Aga), delivered by an automatic syringe pump (Cronus Sigma 2000) adjusted to a flowrate of 12 ml min\(^{-1}\). A design of experiments was performed to optimize the deposition conditions: the substrates temperature of 425 °C, and a total volume of 42 ml reached through deposition of discrete volumes of 1 ml with a time gap of 45 s between sprays. These samples were then annealed for 30 min at 550 °C. The surface of hematite was treated performing an annealing at 800 °C to remove the surface states\[5\] and coating an efficient co-catalyst of FeNiO\(_x\) by photo-electrodeposition to enhance the kinetics of oxidation reaction\[6\].

The SRFC comprised an hematite photoanode immersed in an aqueous solution of ferrocyanide (K\(_2\)Fe(CN)\(_6\)) and NaOH and a carbon felt counter electrode immersed in an aqueous anthraquinone-2,7-disulphonate (2,7 AQDS) and NaOH solution separated by a cation conductive Nafion-117 membrane\[4\]. While half-cell characterizations were performed using a PEC cell device known as “cappuccino” filled with a 0.2 M K\(_2\)Fe(CN)\(_6\) in a three-electrode configuration (counter electrode of 99 % pure platinum wire and Ag/AgCl/Sat. KCl reference electrode), the full solar charging of the RFB was performed in an innovative SRFC device in-house designed (made of PEEEK\(^{®}\)) in a two-electrode configuration.

PEC characterization (J-V, EIS and long-term stability tests) were conducted using a Zahner Zennium workstation controlled by Thales software package. A class-B solar simulator equipped with a 150 W Xe lamp (Oriel, Newport, USA) and an AM 1.5 G filter (100 mW cm\(^{-2}\), 25 °C, Newport, USA) was used and the light beam was calibrated with a c-Si photodiode (Newport, USA). An appropriate electrical analog
was then fitted to the EIS data using a ZView software (Scribner Associates Inc., USA).

### Results

Figure 1 shows the \( J-V \) curves of bare hematite (BH), hematite annealed (H800) and treated with a FeNiOx co-catalyst (BHFeNiOx) tested in an aqueous alkaline solution of NaOH containing ferrocyanide ([Fe(CN)\(_6\)]\(^{4-}\)). Upon illumination of the hematite photoelectrode, electron-hole pair generation takes place in the hematite and holes move to its surface oxidizing ferrocyanide through reaction:

\[
4\text{Fe(CN)}_6^{3-} + h^+ \rightarrow \text{Fe(CN)}_6^{4-}.
\]

In the photo-induced redox reaction, the photon energy is converted and stored directly as electrochemical energy, and the process produces the charged ferricyanide ([Fe(CN)\(_6\)]\(^{3-}\)).

Hematite coated with an abundant co-catalyst (BHFeNiOx) showed the best performance, i.e. a photocurrent-density of ca. 0.67 mA cm\(^{-2}\) before dark current onset. Also, a cathodic shift of the photocurrent onset potential was observed (0.5 V RHE), which allows to achieve a high photovoltage of 0.8 V. The performance of BH sample was also evaluated illuminating through the FTO/glass substrate - backside illumination - such that there was no competitive light absorption from the adjacent electrolyte. In fact, the color of ferrocyanide solution was responsible for the lower photocurrents obtained when compared with values reported elsewhere[5].

Figure 1. \( J-V \) characteristics of BH, H800 and BHFeNiOx samples obtained in the dark (dashed lines) and under 1-sun AM 1.5 G illumination (solid lines) conditions.

The next step aimed at connecting multiple bare hematite thin films to take advantage of the internal space inside the cell and extend light harvesting for further efficiency enhancement. Figure 2 shows a scheme of the photoelectrodes configuration inside the PEC cell and their performance. Through the use of six photoelectrodes connected in series a photocurrent of ca. 1.4 mA cm\(^{-2}\) was obtained, corresponding to an increase higher than 100 % - Figure 2. In this configuration, the first photoelectrode (illuminated from the frontside) is responsible for ca. 60 % of the final device efficiency.

A new and easy-assemble SRFC device was therefore designed for accomplishing all the requirements observed during this study, such as an illumination from the backside, a photoelectrode that is a window of the cell, suitable electrolyte recirculation and better diffusion of the electrolyte close to the counter electrode. The stability of the alkaline SRFC was then evaluated over 1000 h to show the viability of this technology.

Figure 2. Top: PEC cell combining 6 BH samples connected in series, where photoelectrodes 1, 3 and 5 are illuminated from the frontside and 2, 4 and 6 from the backside. Bottom: Their \( J-V \) characteristics obtained in NaOH with and without ferrocyanide.

### Conclusion

The performance of an alkaline SRFC based on hematite photoelectrodes was evaluated through different hematite-ferrocyanide junctions by employing an annealing treatment and using FeNiOx as a co-catalyst. The presence of the catalyst improved the cell performance, reducing the electron-hole recombination. Through the combination of six photoelectrodes in a series, it was obtained a photocurrent of ca. 1.4 mA cm\(^{-2}\).

Acknowledgements

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### References

Evaluation of different pre-treatment systems for the energy recovery of greenhouse agriculture wastes in a clinker production plant.

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This study shows the evaluation and selection of the best sequence of operations for the pre-treatment of the wastes derived of the intensive agriculture of greenhouse before recovering their potential energy in the industry. In this research is defined a set of operations aimed at reducing the chlorine percentage and the moisture content present in this kind of wastes for using like an alternative fuel in a clinker production plant. A washing of wastes under optimal conditions could reduce the chlorine content around 60% of the original value and increase the lower calorific value in an 8%. A drying of these wastes with a hot gas stream could produce a recoverable waste with a moisture lower than 3.0 %, which enable the temporary storage avoiding the degradation. The partial substitution of the traditional fossil fuel employed in the clinker production plant involves the reduction of emissions associated with the decrease of the fossil fuel consumption, improvements of the plant operation and economic saving of up to 2.9 € per tonne of clinker produced.

Introduction
The combustion process is one of the most known and employed technology for the energy recovery of wastes or biomass. This recovery method could be known as waste to energy (WTE) or energy-from-waste (EfW) in the field of the energy recovery from waste [1] by the possibility to generate energy in the form of electricity and/or heat from the combustion of wastes. Despite the robustness and flexibility of this technology, a lot of studies are focused on increase the efficiency of this process and make it more competitive. These studies include the increase of the recovery energy of the biomass or wastes used [2]. In this regard, the development of the pre-treatment system is necessary for adapting the properties of wastes to the conditions required for the industry where they are going to be recovered.

Wastes derived of the intensive agriculture of greenhouses show a high content of chlorine and a high content of moisture for being directly burned in an industrial process. The heterogeneity and seasonality associated with them make difficult the search of energy recovery alternatives [3,4], being currently reused as animal feed, compost production or deposited in landfills [5,6].

Figure 1. Appearance of the agriculture wastes of greenhouse evaluated in this work.

Objectives
The main objective of this work is get the best pre-treatment system for wastes derived of the intensive agriculture of greenhouse for obtaining a suitable fuel for his use in the rotatory kiln of a clinker production plant. For it, different sequences of operations are defined and evaluated in the laboratory facilities of the School of Engineering of Seville (ETSI). The aim pursued is to get a fuel from the agriculture wastes of greenhouse with the properties demanded by the clinker plant where they are going to be recovered.

Table 1. Properties demanded for the combustion of an alternative fuel in the clinker production plant.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Limits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lower calorific value (kcal/kg)</td>
<td>&gt;1,500</td>
</tr>
<tr>
<td>Chlorine content (% wt.)</td>
<td>&lt;0.8</td>
</tr>
<tr>
<td>Moisture content (% wt.)</td>
<td>&lt;8.00</td>
</tr>
</tbody>
</table>

Methods
The evaluation methodology of the different pre-treatment operations shows in the figure 2. These operations are defined after evaluating the properties of the gross waste received in the ETSI laboratory facilities.

A total of eight samples are received over a year with an initial moisture content between 50.0 and 85.0 % wt. The analysis of each samples allows evaluating the heterogeneity and seasonality of these wastes to select the most efficient treatment method based on the final properties that they present. Table 2 show the sequences of operations defined for the pre-treatment of each samples received in the laboratory.

Figure 2. Methodology for the evaluation of the best pre-treatment system for agriculture wastes of greenhouse.

Results
Each pre-treatment samples are analyzed in the laboratory using the corresponding Biofuel Standards. The chlorine content, the moisture content and the final ash percentage present in each pre-treatment samples are evaluated and compared with the
values of the gross waste samples (T02) for the selection of the best pre-treatment system. The lower calorific value exceeds 1,500 kcal/kg in all sequences of operations tested.

Table 2. Sequences of operations defined for the pre-treatment of agriculture wastes of greenhouse.

<table>
<thead>
<tr>
<th>ID</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>T02</td>
<td>Reference. Gross waste (only drying operation). Sequences of operations with washing and drying of wastes in different operation conditions.</td>
</tr>
<tr>
<td>T04</td>
<td>Sequence of operations with an additional pressing of wastes.</td>
</tr>
<tr>
<td>T09</td>
<td></td>
</tr>
</tbody>
</table>

Figure 3. Evaluation of the chlorine content [% Cl] in the different pre-treatment waste samples.

Wastes with an initial moisture content up to 80.0 % should be treatment through the sequence of the operations defined by the T05 pre-treatment system. When this parameter value is higher, the best system would be the T09 pre-treatment operations. The moisture degree corresponds with the harvesting period defined in the table 3 for the kind of waste evaluated in this work. The lower calorific value associated to these two pre-treatment systems is an 8.00% higher than the original value, being the final chlorine content lower in the T09 pre-treatment systems than the T05. To both the final moisture content is less than 3.0%.

Conclusions

The energy recovery of agriculture wastes of greenhouse could be possible like an alternative fuel in a clinker production plant. For it, it is necessary adequate their properties to the limits demanded by the particular plant. In this case, two sequences of operations are selected for the pre-treatment of these wastes due to the heterogeneity and seasonality associated to these wastes. Through each of these pre-treatment systems is possible to obtain a green fuel for the industry, decreasing the chlorine content around 60% of the initial value, achieving a moisture content less than 3.0%, and increasing the lower calorific value in an 8.0%, which involve considerable advantages for the process (lower emissions, important economic saving and the improvement of the operation plant). In this case, the substitution of up to 51.0 % of the thermal energy given by petroleum coke consumed in the clinker production plant could provide savings of up to 2.9 € per tonne of clinker produced.

Acknowledgements

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References

Oral Session

INNOVATIVE MATERIALS AND APPLICATIONS
Development of bio- and eco-composites for the footwear industry

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Cork composites are often used in several applications including footwear components. The traditional agglomeration process uses a reactive polyurethane-based binder (containing TDI), generating composites with low flexibility and poor water absorption/desorption characteristics, resulting in products with low comfort properties. Furthermore, the presence of TDI, is another important drawback due to its high toxicity. Therefore, the present work is devoted to the development of cork composites, where the TDI-based binder was replaced by an aqueous PUD reinforced with Melissa officinalis L. extract. Cork-composites were produced using different adhesive/cork ratios, and characterized in terms of dimensional stability, water absorption/desorption properties, and mechanical behavior. After the selection of the best adhesive content, composites reinforced with sugarcane bagasse, malt bagasse, coconut fibers, and chia seeds were also produced and characterized.

Introduction

Traditionally, the cork agglomeration process uses a reactive polyurethane-based adhesive, generating composites with low flexibility and poor water absorption/desorption characteristics, resulting in products with low comfort properties. Additionally, this type of binder is based on toluene disiocyanate (TDI), a highly toxic compound. Efforts are being made to replace this adhesive by more environmentally friendly products. In this context, the use of water-based polyurethane-urea dispersions free of NMP (N-methyl-2-pyrrolidone), and exempt of volatile organic compounds (VOC), can be envisaged. Therefore, the present work had as main objective the production of cork composites, where the traditionally used adhesive was replaced by a water-based polyurethane. This former adhesive was synthesized by a process developed at our research group, which generates a final product, both NMP and VOC-free. Moreover, in order to improve composites performance, the adhesive formulation was modified with Melissa officinalis L. extracts [1]. Also, water absorption/desorption properties, as well as mechanical properties, were tested as a function of the added biomass residue.

This work is organized according to the following stages: (1) synthesis and characterization of the water-based polyurethane-urea dispersions. Three formulations were produced: base PUD (PUD), and modified PUDs using 3 (PU3C) and 5% (PU5C) (w/w, prepolymer-basis) of Melissa officinalis L. extract. (2) Testing of the adhesive content for composite production. Contents of 20, 30 and 40% (w/w, cork basis; adhesive weight based on its solids content). Following the characterization results, the best adhesive content was selected for further studies; (3) testing of the biomass content: for that composites incorporating 5 and 10% (w/w, cork-basis) of biomass residues (sugarcane bagasse, malt bagasse, coconut fibers and chia seeds), were produced and characterized in terms of stress at break (σb) and strain at break (εb), dimensional stability and water absorption/desorption behavior. The main objective was to select the composites with the best properties, and validate their use in the production of footwear insoles.

Materials and Methods

The Melissa officinalis L. dry plant used to produce the extract was purchased in a local market. For the production of the PUD adhesives the following raw-materials were used: Polypropylene glycol with molecular weight 2000 (PPG 2000) as the macrodil, isophorone disiocyanate (IPDI) as the disiocyanate, 2,2-Bis(hydroxymethyl) propionic acid (DMPA) as the internal emulsifier, triethylamine (TEA) as the neutralizing agent, dry acetone as a co-solvent, 1,2-Ethylene diamine as the chain extender and tin (II) 2-ethylhexanoate (SO) as the catalyst. PPG2000 and DMPA were dried under vacuum at 50°C overnight previously to the synthesis process. The tested biomasses were sugarcane bagasse, malt bagasse, coconut fibers and chia seeds.

Melissa officinalis L. extracts. The extract of Melissa officinalis L. was obtained by the infusion method from dry plant material. Briefly, 20 g of gridded plant was added to 800 mL of boiling distilled water during 5 minutes. The obtained solution was then filtered and freeze-dried, in order to recover the extract in powder form.

PUD synthesis process. PUDs were produced using the modified prepolymer method, developed by Fernandes and co-workers [2]. Three different formulations were produced: base formulation (PUB), and modified formulations with 3 and 5% of Melissa officinalis extract (PU3C and PU5C, respectively). The used NCO/OH ratio was 1.7, and DMPA content was 5% (w/w, prepolymer basis). The PUD adhesives (100 g of prepolymer) were synthesized by using a 500mL four neck jacketed reactor equipped with heating, thermocouple and mechanical stirring. The synthesis was carried out under nitrogen atmosphere and the reaction progress was monitored by the dibutylamine back titration method, according to ASTM D 2572-97. Briefly, the required amounts of PPG2000, SO and IPDI were added to the reactor, heated at 80°C under stirring until the theoretical NCO value was reached. Thereafter, the mixture was cooled to 50°C to add the pre-neutralized DMPA in acetone solution. The reaction was left to proceed until the NCO conversion reached the theoretical value. Then the NCO-terminated prepolymer was cooled to 25°C, and distilled water containing the required amount of Melissa officinalis added dropwise under vigorous
stirring (prepolymer dispersion phase). The resultant dispersion was heated to 35°C, and EDA aqueous solution (concentration dependent on the NCO conversion after the dispersion stage) added at a flow rate of 0.3 mL/min. Finally, acetone was removed in a rotary evaporator (40°C, 350 mbar).

Cork composites production. The composites were produced using a hydraulic press equipped with heated plates and a scale based on 10 g of cork. Two cork granulometries (0.2-0.5 mm and 0.5-1 mm) were used at a 50:50 ratio. The tested adhesive content (w/w, cork basis; adhesive weight based on its solids content) was 20, 30, and 40% (this content was reflected in the composites nomenclature, e.g., the sample PUD3C20 is the composite with PUD3 adhesive at a content of 20%). For composites production, first both corks were weighed (5 g of each granulometry) and hand mixed. When biomass was added, the applied amount was also mixed with the cork. After, the required amount of adhesive was weighted and added to the previously prepared mixture, and mixed during 10 minutes using a mechanical stirrer. Then, the obtained mixture was transferred to the mold and pressed at 15 bar (135°C), during 2 minutes. After cooling, the mold was open and the composite removed. The samples were stored at ambient temperature during one week, before testing. After the selection of the best adhesive content, cork composites added with residual biomass (contents of 5% and 10%) were produced. PUD and composites characterization. PUD adhesives were characterized in terms of the produced dispersions and films. For the dispersion, solid content, pH, viscosity, and mean particle size were determined. Films were evaluated concerning chemical structure (FTIR) and thermal behavior (DSC and TGA). Composites were evaluated in what concerns water absorption/desorption behavior and dimensional stability according to the standards ISO 22649:2016 and ISO 22651:2002, respectively. The stress-strain curves and the stress at break ($\sigma_b$) and the strain at break ($\varepsilon_b$) of the composites were obtained by using a Shimadzu Autograph AGS-X series Europa testing machine.

Results

Table 1 summarizes the properties of the obtained dispersions. The pH values for all the dispersions was around 7, reflecting the absence of free chain extender, showing the good efficiency of the chain extension process. The viscosity values were influenced by the solids content, once a viscosity increment was noticed as the extract content increases. The effect of the extract is also perceptible on the particle size increment, once the PUD presented higher mean particle size among the three produced PUD formulations (Table 1).

The dimensional stability is evaluated by measuring the length and width of the sample specimen before and after conditioning at 37°C for 24 hours. The established specification for shoe soles requires a variation inferior to 2% (ISO 22651:2002). The obtained results show a change from 0.21% (PUD3C20) to 2.33% (PUD3C30) for cork composites without biomass, and between 0.21%, for the PUB30 with 5% of malt bagasse, and 2.09% for the PUB30 containing 10% of sugarcane bagasse, for the biomass containing composites. Biomass addition improves the dimensional stability. For the water absorption/desorption tests, the ISO 22649:2016 standard specifies a water absorption higher than 70 mg/cm² and a minimum water desorption of 80%. The water absorption results for the cork composites without biomass varied from 75.12 mg/cm² (PUB20) to 171.38 mg/cm² (PUD3C40), while water desorption was between 49.31 (PUD3C30) and 80.03% (PUD5C20). When biomass is added, absorption values varied from 67.78 mg/cm² (PUB30 with 5% of sugarcane bagasse) to 174.59 mg/cm² (PUD3C30 with 10% of coconut fiber), while water desorption varied between 25.72% (PUB30 with 10% of chia seed) to 73.32% (PUD5C30 with 10% of sugarcane bagasse). Considering these results, the biomass giving the best effect on water absorption is the coconut fiber at a content of 10%, while for desorption, 10% of chia seeds gave the best results.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\mu$ (mPa.s)</th>
<th>Solids (% w/w)</th>
<th>Average particle size (μm)</th>
<th>Number</th>
<th>Volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUB</td>
<td>7.75</td>
<td>18.55</td>
<td>38.8</td>
<td>0.0157</td>
<td>0.145</td>
</tr>
<tr>
<td>PUB3C20</td>
<td>5.55</td>
<td>61.5</td>
<td>39.7</td>
<td>0.0161</td>
<td>0.107</td>
</tr>
<tr>
<td>PUB3C30</td>
<td>7.55</td>
<td>1878.7</td>
<td>36.59</td>
<td>0.132</td>
<td>2.510</td>
</tr>
</tbody>
</table>

Stress at break ($\sigma_b$) and strain at break ($\varepsilon_b$) were determined from stress-strain curves of the composites produced with the different adhesive contents. The results are described in Table 2. The highest $\sigma_b$ values were registered for the composite samples containing 30 and 40% of the adhesives PUD and PUD3C, while for the PUB5C lower values were obtained. The highest value for $\sigma_b$ was 0.99 MPa (PUD3C30 composite). Based on these results, the selected adhesive content was 30%, once it gave rise to the highest $\sigma_b$ with both PUB3C30 and PUB5C30 adhesives.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$\sigma_b$ (MPa)</th>
<th>$\varepsilon_b$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUB20</td>
<td>1.10</td>
<td>12.45</td>
</tr>
<tr>
<td>PUB30</td>
<td>1.12</td>
<td>15.40</td>
</tr>
<tr>
<td>PUB40</td>
<td>1.76</td>
<td>14.56</td>
</tr>
<tr>
<td>PUB3C20</td>
<td>0.93</td>
<td>14.84</td>
</tr>
<tr>
<td>PUB3C30</td>
<td>1.13</td>
<td>18.03</td>
</tr>
<tr>
<td>PUB3C40</td>
<td>1.10</td>
<td>22.11</td>
</tr>
<tr>
<td>PUB5C20</td>
<td>0.57</td>
<td>13.52</td>
</tr>
<tr>
<td>PUB5C30</td>
<td>0.99</td>
<td>21.55</td>
</tr>
<tr>
<td>PUB5C40</td>
<td>0.85</td>
<td>25.45</td>
</tr>
</tbody>
</table>

Conclusions

In a general way, it was observed that water-based polyurethane adhesives presented suitable properties for the production of cork-based composites. The adding of residual biomass increases water absorption/desorption properties.

Acknowledgements

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References


Catalytic mineralization of formaldehyde by molybdovanadophosphate polyanions supported on cellulose-silica hybrids

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Introduction

The diminishing of indoor formaldehyde levels is a key activity to reduce public health risks [1]. Besides passive measures (e.g. removal of indoor sources or increased ventilation), active actions have been proposed such as, for example, air purification by advanced catalytic systems targeting formaldehyde degradation [2]. The utilization of ultraviolet photocatalytic oxidation (UVPCO) systems, where degradation of pollutants is performed over a solid TiO2 catalyst with UV light of high intensity, is economically feasible to degrade volatile organic compounds (VOCs) of low concentration (<10 ppm), but provides incomplete mineralization [2,3]. Thermal catalytic oxidation (TCO) systems using different metal oxide particles and operating at high temperatures (100-1200 °C) mineralize VOCs at relatively high concentrations under aerobic conditions. However, for low concentrations of VOCs the technical and economic feasibility of TCO systems is much lower than that of UVPCO systems [3].

Recently, highly effective redox catalysts based on transition metal complexes, such as polyoxometalates (POMs), have been proposed for room temperature aerobic oxidation of VOCs [4,5]. In particular, the efficiency of Keggin type heteropolyanions [PMO12·nV2O40]3+n (n=1,2) for VOCs oxidation is comparable to that usually reported for UVPCO systems. Thus, cellulose/silica hybrids doped by POMs such as [PMO12·nV2O40]3+n (n=1,2) (POM-CSH) were synthesized and assessed for the room temperature oxidation of VOCs present in real samples of polluted urban air[4]. The main goal of this work was to evaluate the efficiency of POM-CSH for the gas phase aerobic oxidation of formaldehyde (H2C=O).

Experimental

Heteropolyanions series [PMO12·nV2O40]3+n—(n=1,2) were synthesized and anchored on cellulose-silica hybrid (CSH) material via electrostatic interactions with protonated propylamine groups of modified silica (Graphical Abstract) according to previously described methods [4]. The catalysts supported on CSH were packed in 180 x 4 mm (i.d.) Teflon tubes sealed on both sides with a fiberglass plug. The packed tubes contained ca. 245 mg of POM-CSH material. In reference experiments, the tube was packed with similar amounts of CSH material without supported POM. Supported catalysts were analyzed, before and after being used in the reaction with CH2O, either by diffuse reflectance (Evolution 220 UV-Vis spectrophotometer in the range of 200-800 nm) or by Raman spectroscopy (BRUKER RFS 100/S Raman spectrometer @ 1064 nm under backscattering configuration in the range of 200–1800 cm⁻¹ and 4 cm⁻¹ resolution). The experimental setup for in-situ generation of formaldehyde and its oxidation over the POM-CSH catalyst packed tubes along with the CH2O quantification method (by reaction with NH2OH.HCl in absorber units) was previously described [5]. The outlet gases soluble in water (gasometer system) were analyzed by HPLC. The amounts of CO2 and H2O formed were evaluated by sorption with Ackarite® and Anhydride®, respectively.

Results and Discussion

The CSH materials doped with PMO11V1 and PMO10V2 were packed into Teflon tubes and subjected to catalytic tests at room temperature. For each run, 5.00 dm³ of polluted air (ca. 550 ppm of CH2O) passed through the catalytic bed at constant linear velocity (ca. 0.33 m/s) with a residence time ca. 0.5 s. The amounts of CH2O converted by the two CSH-POM catalysts and by CSH (control experiments) were quantitated by chemosorption method and are summarised in Table 1. The reference material (CH2O without POM) removed CH2O by chemisorption whereas the CSH-POM materials removed higher amount of CH2O by sorption and degradation (Table 1, 1st cycle).

Chemosorption of CH2O by CSH can be explained by reaction with amine moieties of modified silica and, to some extent, by reaction with the free hydroxyl groups of cellulose:

\[
\text{C}_{\text{CSH}} - \text{OH} + \text{CH}_2\text{O} \rightarrow \text{C}_{\text{CSH}} \text{N} - \text{CH}_2\text{H}_2 + \text{H}_2\text{O}
\]

(1)

Cell-\text{OH} + \text{CH}_2\text{O} \leftrightarrow \text{Cell-O-CH}_2\text{OH}

(2)

The amount of CH2O removed by CSH became negligible after 3 consecutive cycles (ca. 15 dm³ of CH2O/air mixture passed) due to eventual “saturation” of CSH material (Table 1). However, after the same number of cycles, “saturation”
was not detected for the CSH-POMs, which was assigned to CH$_2$O oxidation catalysed by POMs. In fact formaldehyde removal was 100 % during the first two cycles and ca. 75 % in the 3rd cycle. During the 4th and 5th consecutive cycles, CSH-POM materials still removed CH$_2$O (Table 1). Noteworthy silica-supported PMo$_{11}$V$_1$ shows better activity of than the parent PMo$_{10}$V$_2$ material.

Table 1. Experimental results for CH$_2$O removal by CSH and CSH-POM catalysts, and turnover numbers (TON).

<table>
<thead>
<tr>
<th>Cycle</th>
<th>CH$_2$O removed (mg)</th>
<th>CH$<em>2$O-PMo$</em>{11}$V$_1$</th>
<th>CH$<em>2$O-PMo$</em>{10}$V$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of initial amount</td>
<td>total (mg) / degraded</td>
<td>TON$^*$ / degraded (mg)</td>
</tr>
<tr>
<td>1</td>
<td>2.23 / 65</td>
<td>3.39/1.16</td>
<td>3.96/1.73</td>
</tr>
<tr>
<td>2</td>
<td>1.01 / 30</td>
<td>3.34/2.33</td>
<td>3.15/2.14</td>
</tr>
<tr>
<td>3</td>
<td>0.1 / 3</td>
<td>2.47/2.37</td>
<td>1.71/1.70</td>
</tr>
<tr>
<td>4</td>
<td>0.0 / 0</td>
<td>1.61/1.61</td>
<td>1.18/1.18</td>
</tr>
<tr>
<td>5</td>
<td>0.0 / 0</td>
<td>1.03/1.03</td>
<td>0.71/0.71</td>
</tr>
<tr>
<td>6</td>
<td>0.1 / 4</td>
<td>1.37/1.25</td>
<td>1.27/1.15</td>
</tr>
<tr>
<td>7</td>
<td>0.1 / 3</td>
<td>1.41/1.31</td>
<td>1.33/1.23</td>
</tr>
</tbody>
</table>

$^*$ All experiments refer to the same amount of hybrid materials and 5.00 dm$^3$ of gaseous formaldehyde (ca. 3.4 mg) passing through the catalyst bed per cycle. Degraded amount was calculated by subtracting the amount removed by CSH in the control experiment.

The oxidative catalytic activity of POMs was visualised by a characteristic change in color of the CSH-POM materials from yellow (ca. 400-420 nm) to deep green (ca. 650-670 nm) due to the reduction of vanadium atoms (V(V) → V(IV)) upon oxidation of CH$_2$O (reflectance spectra are not shown). Assuming that PMo$_{11}$V$_1$ and PMo$_{10}$V$_2$ intensively oxidise CH$_2$O, high concentrations in air (> 500 ppm) should difficult the re-oxidation of these POMs by oxygen which is probably the limiting step. To check this hypothesis, the packed-bed reactors with CSH-POM were regenerated after the 5th cycle by purging the packed bed with purified air (ca. 0.25 dm$^3$/min), during 4 h. After performing the 6th cycle, in the case of CSH a small increase of CH$_2$O removal from air was detected. This was assigned to desorption of CH$_2$O from the hybrid materials occurring during the regeneration step due to the shift in reaction equilibrium depicted by Eq. (2). In the case of CSH-POM the conversion of CH$_2$O increased almost 60 % (Table 1). The repeated oxidation/catalyst regeneration cycle (7th cycle) revealed ca. 40% removal of CH$_2$O from polluted air. Similar features were observed at least in 5 repeating cycles (8th-12th cycles, results are not presented).

These results were interpreted in terms of redox catalysis with POMs and its regeneration by air purging. It is known that V(V), present in the composition of PMo$_{11}$V$_1$ and PMo$_{10}$V$_2$, usually oxidises aldehydes to the corresponding carboxylic acids under aerobic atmosphere being in turn re-oxidised by molecular oxygen [6]. Hence, on could expect a slightly better catalytic activity with PMo$_{11}$V$_1$ than with PMo$_{10}$V$_2$ due to the higher content of active V(V) centres in the former POM and also due to easier re-oxidation of V(IV) to V(V) with molecular oxygen. However, this was not the case since CH$_2$O conversion results were higher with PMo$_{11}$V$_1$ than with PMo$_{10}$V$_2$ (Table 1). This can be partially explained by the higher redox potential of PMo$_{11}$V$_1$, which is important in a typical rate-controlled one-electron oxidation of aldehydes [6]. However, this is not the only possible explanation if one considers the observed reaction products of CH$_2$O degradation. Only trace amounts of HCOOH were found among the reaction products, with the majority (> 95 %) being carbon dioxide. Although the water balance was not very accurate, its formation during the oxidation experiments was confirmed. This fact indicates a complex mechanism involving mixed electron transfer and free radical reactions. The last reactions may include the one-electron oxidized substrate with dioxygen:

\[
\text{'CHO + O}_2 \rightarrow \text{CO + HOO}^+ \quad (3)
\]

\[
\text{HOO}^- + \text{CH}_2\text{O} \rightarrow \text{CHO} + \text{H}_2\text{O}_2 \quad (4)
\]

The eventual formation of H$_2$O$_2$ as oxidation intermediate was confirmed by analysis of CSH supported PMo$_{10}$V$_2$ after being used in the catalytic tests. Raman spectroscopy confirmed the presence of peroxy-molybdate complexes by showing characteristic bands at ca. 590 and 880 cm$^{-1}$ assigned to v (Mo=O) and v (O=O), respectively.

Conclusions

[PMo$_{12-n}$V$_n$O$_{40}$]$^{3+n-}$ (n=1,2) supported on cellulose-silica hybrid (ca. 2% w/w) exhibited noticeable catalytic activity in gaseous heterogeneous aerobic oxidation of formaldehyde at room temperature. The oxidation products were carbon dioxide and water. The V(V) active centres in POMs were largely responsible for the catalytic activity and demonstrated reversible V(IV)→V(V) turnover during catalyst regeneration with purified air. The relative rate of CH$_2$O oxidation with PMo$_{11}$V$_1$ was higher than that with PMo$_{10}$V$_2$ being 8.8×10$^{-3}$ and 5.3×10$^{-3}$ mol/(mol$_{catalyst}$ s), respectively. The continuous sustainable oxidation of CH$_2$O in polluted air would be possible at the steady state conditions at concentrations below 50 ppm.

Acknowledgements

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References

Hybrid ionic liquids/metal organic frameworks – IL@MOFs - for gas separation

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Introduction
Global warming is arguably the major environmental and societal threat of the 21st century. Efforts have been made to develop environmentally-friendly chemical processes to mitigate the impact of CO₂ emissions to the atmosphere. Gas adsorption-based processes fit into this category, as they are low-energy demanding, cost-effective and make use of absorbent materials with high capacities for the species to be captured or separated [1]. Recently, a novel type of microporous absorbent materials, Metal-Organic Frameworks (MOFs), have appeared. These crystalline materials consist of metal atoms or small metallic clusters linked to organic functional groups, thus possessing a high degree of tunability in their composition and shape, and interesting properties for adsorption processes, such as very high specific surface area and porosity [2].

In parallel, ionic liquids (ILs) are another class of materials that have been used for CO₂ capture. They are conventionally defined as salts with low melting point (below 373 K) and consist of organic, asymmetric cations and organic or inorganic anions. ILs exhibit distinctive properties such as negligible vapor pressure, nonflammability, high thermal and chemical stabilities and high CO₂ solubility [3,4]. The high level of tunability of both cation and/or anion can be used to enhance CO₂ solubility. Since both MOFs and ILs show high CO₂ sorption, several combinations of ILs-MOFs were synthesized in this work, where the IL is impregnated into the MOF structure (IL@MOF). These new composite materials were extensively characterized and CO₂ and CH₄ adsorption equilibria measurements were performed at 303 K.

Objectives
The main objective of this work was the assessment of the adsorption capacities of CO₂ and CH₄ in the produced composites compared with the pristine MOF, and relating them with the presence of the IL. A second objective was the validation of the experimental impregnation methodology adopted and the determination of synergistic effects by exhaustive characterization of the composite materials.

Materials and Methods
The MOF used in this work was ZIF-8 (2-methylimidazole zinc salt), chosen due to its microporosity, high thermal stability and high specific surface area [5]. Nine imidazolium-based ILs and one phosphonium-based IL were chosen to be impregnated in ZIF-8, due to their structure and high CO₂ solubility. Acetone was used as solvent for impregnation, which was accomplished with overnight stirring at room temperature.

![Figure 1. Experimental method for the preparation of IL@ZIF-8 materials.](image)

The characterization techniques used for these composites include Helium Pycnometry, N₂ Adsorption-Desorption at 77 K, Fourier Transform Infrared Spectroscopy (FT-IR), Powder X-Ray Diffraction (PXRD) and Scanning Electron Microscopy (SEM). CO₂ and CH₄ adsorption-desorption equilibria measurements were performed between 0-16 bar and at 303 K using a previously reported standard static gravimetric method [6].

Results
FT-IR results confirm the presence of impregnated IL in ZIF-8, since the spectra of the composites show IL-related bands.
PXRD results show that IL impregnation does not significantly change the structure of ZIF-8. SEM analysis reveals that composites, depending on the IL structure, present different morphologies, confirming at the same time the presence of impregnated IL. From Helium Picnometry it was recorded a change in the skeletal density of IL@ZIF-8 composites, when compared to the pristine ZIF-8. These differences indicate IL-MOF interactions that are consistent with FT-IR results. N$_2$ adsorption-desorption equilibria at 77 K measurements show BET specific surface area and total pore volume losses due to the IL partial occupation/blockage of the ZIF-8 pores. Moreover, the obtained adsorption isotherms and pore size distributions confirm the microporous nature of the produced composites. CO$_2$ and CH$_4$ adsorption equilibria measurements at 303 K reveal that composites adsorb less than the pristine ZIF-8, particularly at high pressure, which is consistent with the N$_2$ adsorption-desorption equilibria at 77 K. Ideal CO$_2$/CH$_4$ selectivities were calculated for ZIF-8 and IL@ZIF-8 materials. Composites, due to their IL structure, show up to 40% selectivity increase between 0-1 bar. At high pressure, selectivity increases are not so pronounced.

Conclusions
Ten ILs were impregnated into ZIF-8 using a direct contact method, with acetone as the solvent. The produced IL@ZIF-8 composites were extensively characterized, confirming the IL impregnation of IL-MOF interactions. The impregnation method does not significantly change the structure of ZIF-8. Textural properties were also determined, with composites showing reduced BET specific surface area and total pore volume, when compared to the pristine ZIF-8. CO$_2$ and CH$_4$ adsorption capacity of these IL@ZIF-8 materials were determined between 0-16 bar at 303 K. IL@ZIF-8 materials in general show inferior adsorption capacity than the pristine ZIF-8 in all pressure ranges, due to partial IL occupation/blockage of the ZIF-8 pores. However, the ideal CO$_2$/CH$_4$ selectivities indicate that these composites, by tuning the IL structure, can have high selectivities between 0-1 bar, being a potential alternative adsorbent material for CO$_2$/CH$_4$ adsorption-based gas separation processes at sub-atmospheric pressures.

Acknowledgements
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References
A novel continuous production of melamine based microcapsules with a skin-hydrating active principle for textile applications using the NETmix technology

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In this work the production of microcapsules for textile applications was developed by adapting the NETmix technology for the steps more commonly made by batch processes: making an oil-in-water emulsion and then curing the membrane around the oily droplets forming microcapsules. Microcapsules were formed through a polycondensation process by coating a skin-hydrating oily active principle with a melamine resin which forms a resistant shell material with a smooth surface. Different temperatures and recirculation times were tested in the cure step. Microcapsules show diameters around 20 µm, within the ideal size range for impregnation on fabrics, and were successfully impregnated on cotton fabrics by a padding process. Integrity and flexible behavior of these microcapsules in the textiles was observed. With the NETmix reactor it is possible to reduce the cure time relative to traditional batch processes, producing microcapsules with similar characteristics in a continuous process.

Introduction

Microencapsulation processes consists of coating an active principle by a membrane of another material, and thus protecting the active principle, the functional material, from the surrounding environment. The active ingredient is referred as the core and the surrounding material forms the shell [1]. The textile industry is an important area of interest for microencapsulation, since functionalized textiles with microcapsules can promote comfort and protection. Carrier oils such as triglycerides are non-irritating skin emollients, and are encapsulated due to their skin hydrating properties, and good skin absorption [2]. Melamine resin was used in this work as the shell material due to its good resistance against water and heat, mechanical robustness, and a surface smoothness which is an advantage for textile applications [3]. Emulsification, with the formation of a stable emulsion, is the key step in microencapsulation, since the diameters of the oily droplets define the microcapsules diameter [4]. Droplet size control is particularly difficult when the emulsification process is scaled from laboratory to industrial scale, commonly resulting in polydisperse emulsions [4, 5]. Furthermore, in batch processes, care must be taken to obtain a homogeneous and stable emulsion in order to produce microcapsules with similar properties [5]. To obtain a good emulsion, various types of stirring systems can be used, and it must be ensured that conditions such as the power of the stirrer promotes good mixing through the whole volume of the vessel [4].

Traditional batch methods to produce microcapsules with melamine resin as the wall material result in a total manufacturing time of around 4 h, where the cure step for the resin takes from 2 to 3 hours [3].

The NETmix static mixer is a patented technology [6] developed at LSRE-LCM/FEUP and it consists of a network of interconnected chambers and channels where chambers operate as mixing zones and channels behave as plug flow. Above a critical Reynolds number of 150, this system evolves to a self-sustained oscillatory laminar flow regime inducing local strong laminar mixing [7].

The main goal of this work is using a NETmix static mixer to produce, in a continuous mode, a stable oil-in-water emulsion and to cure the microcapsules membrane. A carrier oil is used as the core material and melamine resin is used as the shell material. The oily droplets were encapsulated by a melamine resin through an interfacial polycondensation process. The microcapsules were then impregnated into cotton fabrics by a padding process to obtain functionalized textiles.

Methods

Both emulsification and cure steps were performed in the NETmix reactor. The emulsion was produced by mixing a melamine-based pre-polymer and a carrier oil at room temperature with a pre-mixing injection scheme. The cure step was obtained by recirculating the emulsion at 80 °C for 30 minutes. Both steps were performed using a Reynolds number of 200. The cured microcapsules were then submitted to coating and stabilizing steps. The resulting microcapsules were characterized by optical microscopy and SEM to evaluate morphology and size. Particle size distribution was also analyzed. After impregnation with microcapsules, textiles were analyzed by SEM.

Results

Emulsions were obtained with either a single pass or by recirculating the mixture through the NETmix, whereby it was shown that recirculation promotes a decrease of the diameter of the oily droplets to circa 20 µm. Microcapsules were then cured by recirculating at 80 °C for 30 minutes. In this step, microcapsules were formed (Figure 1a).

After coating and stabilizing steps, microcapsules remain stable, do not disintegrate, and show a smooth surface with no membrane fragments (Figure 1b). Noteworthy is that the cure step is reduced from 150 minutes, typically observed in batch process, to 30 minutes.

The microcapsules produced by the continuous process present mean diameters of circa 23 µm, which is within the preferred size for textile applications. SEM images (Figure 2a) show the microcapsules with a smooth surface and well-defined shape. A few broken microcapsules or deformed can be observed but there is a total absence of fragmented membrane.
Microcapsules were impregnated on cotton fabrics by a padding process with a pressure of 4 bar. Figure 2b shows the preservation of microcapsules shape and the good fixation on the fabrics. No damaged membranes were observed and consequently the integrity the microcapsules after impregnation proves the durable and robust membrane produced with NETmix. A flexible shell, characteristic of a melamine resin, was observed. This characteristic is due to its viscoelastic behavior at a small deformation [3].

Conclusions
Microcapsules of melamine resin with an oily active principle were produced in a continuous mode using the NETmix technology. It was observed that an emulsion with recirculation promotes the decrease of droplet size to around 20 µm, which is in the ideal size range for application on fabrics. The cure step was also performed by recirculation in the NETmix. The resulting microcapsules are stable, with the required morphology and size range. Microcapsules were well fixated to the cotton fabric fibers and they present a viscoelastic behavior. The NETmix technology allows a stable emulsion production and the reduction of cure time by 80% compared with the traditional batch manufacturing process.

Acknowledgements
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References
Novel alginate-chitosan aerogel fibres for potential wound healing applications

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Wound healing is a complex and dynamic biological process. In recent years, the interest and development of new wound dressing products from marine sources has been increasing due to their unique properties such as antimicrobial activity (e.g., chitosan) or the capacity to provide a moist environment (e.g., alginate). Since alginate (polyanion) and chitosan (polycation) are oppositely charged, it is possible to create the conditions for the formation of polyelectrolyte complexes, which has been a growing area of study due to the combination of their physicochemical characteristics and high biocompatibility [1]. Moreover, aerogels are porous structures with large surface area in which it is possible to achieve high drug loadings.

The aim of this work was to produce aerogel fibres with different alginate-chitosan proportions and, therefore, evaluate the potential of these new fibres for wound healing applications. The aerogel fibres were prepared with different mass ratios of alginate-chitosan: 99:1, 19:1 and 9:1 (w/w). To produce them, a hydrogel of both polymers was made by the emulsion-gelation method. Through solvent exchange an aerogel was obtained, removing the water from the hydrogel by ethanol, which was then dried with supercritical CO₂ leaving behind a solid structure that greatly resembles original wet gel.

Once the fibres were produced, some techniques were done in order to perform the solid-state characterization. The morphology was studied by Scanning Electron Microscopy (SEM) whereas the specific surface area and the pore volume were obtained through Brunauer–Emmett–Teller (BET) and Barrett–Joyner–Halenda (BJH) methods, respectively. Since chitosan was used as alginate gelling agent, due to their opposite charges, Fourier-transform infrared spectroscopy (FTIR) was performed in order to evaluate the ionic interactions between both polymers. After the fibres production, Elemental Analysis was performed in order to verify the fibres nitrogen content, thus allowing the quantification of chitosan, and therefore confirming that the final concentration of both polymers was as expected.

In order to compare with a consumer product already on the market, cell and antibacterial assays were made for the fibres and for Kaltostat®, a dry calcium-sodium alginate wound dressing.

Since the aim of this work was to evaluate the fibres potential for wound healing, it was essential to validate the fibres biocompatibility with skin cells as well as the ability of stimulating cell migration, one of the key steps in wound healing process [2]. As such, in vitro tests were performed. The cellular biocompatibility was evaluated using NCTC clone 929 (mouse fibroblasts) by the direct contact method described in ISO 10993-5 (methods to assess the in vitro cytotoxicity of medical devices). Cell viability was quantified spectrophotometrically by MTS cytotoxicity test. Cell migration was measured by scratch assay, mimicking cell migration during wound healing in vivo.

One of the important steps in wound treatment is the prevention or elimination of pathogenic microorganisms. Therefore, to evaluate the fibres antimicrobial activity, Staphylococcus aureus and Klebsiella pneumoniae were the species selected as representative of gram-positive and gram-negative bacteria, recognized as skin commensals as well as nosocomial pathogens [3,4]. ASTM E 2149-01, a method designed to evaluate the resistance of non-leaching antimicrobial treated specimens to the growth of microbes under dynamic contact conditions, and the absorption method described in ISO 20743:2013, an evaluation method in which the bacterial suspension test is inoculated directly onto specimens.

As can be seen in Figure 1, the fibres that were produced showed no cytotoxicity against the fibroblasts, as well as the medical device tested. In the migratory assay, it is verified in Figure 2 that the fibres comparatively to the control accelerated the wound closure, as Kaltostat® which also presented statistically significant differences compared to control. Moreover, produced fibres did not show statistically significant differences comparing to Kaltostat®.

The objective of this study was to produce new alginate-chitosan aerogel fibres and to perform the solid-state characterization, biocompatibility and bioactivity evaluation in order to realize their potential for wound healing applications. The aerogel fibres were prepared by emulsion-gelation method and further dried with supercritical CO₂. The morphology, specific surface area and pore volume as well as the ionic interaction between both polymers and the chitosan content were evaluated. Finally, possible cytotoxicity, in vitro cell migration and the antimicrobial activity of these new aerogel fibres were also evaluated. The results of this work demonstrated that the alginate-chitosan aerogel fibres can be considered as having a high potential for wound healing applications.

Figure 1. Cytotoxicity assay using MTS reagent: samples were incubated, at a concentration of 1.7 mg/mL, in NCTC clone 929 cell line during 24h at 37 °C and 5% CO₂ humidified atmosphere (mean ± SD, n=3). Solution of 10% (v/v) of DMSO in cell culture media was used as a positive cytotoxic control. If viability is reduced to <70% of the control, samples have a cytotoxic potential. Statistically significant differences when
compared to control conditions are indicated by **** (p < 0.0001).

Figure 2. Scratch assay: samples were incubated, at a concentration of 1.7 mg/mL, in NCTC clone 929 fibroblasts, during 8h at 37 °C and 5% CO₂ humidified atmosphere (mean ± SD, n=4). Statistically significant differences when compared to control conditions are indicated by **** (p < 0.0001).

Regarding the antimicrobial activity, in the dynamic shake flask test (Figure 3) as in the standard method of antimicrobial evaluation of textile products (Table 1) the fibres presented a clear antimicrobial activity when compared with the control and with the tested medical device, Kaltostat®.

![Figure 3. Percent reduction of S. aureus and K. pneumoniae](image)

Table 1. Efficacy of antibacterial property of the tested material against S. aureus and K. pneumoniae from contact with samples, at a concentration of 2 g/mL, during 24h at 37 °C (mean ± SD, n=3). Efficacy defined as significant (2 ≤ antibacterial value < 3) or strong (antibacterial value ≥ 3).

<table>
<thead>
<tr>
<th>Strain</th>
<th>Sample</th>
<th>Antibacterial Value</th>
<th>Efficacy</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>alg:chit (99:1)</td>
<td>5,8</td>
<td>Strong</td>
</tr>
<tr>
<td></td>
<td>alg:chit (19:1)</td>
<td>5,5</td>
<td>Strong</td>
</tr>
<tr>
<td></td>
<td>alg:chit (9:1)</td>
<td>6,6</td>
<td>Strong</td>
</tr>
<tr>
<td></td>
<td>Kaltostat®</td>
<td>1,6</td>
<td>N/A</td>
</tr>
<tr>
<td>K. pneumoniae</td>
<td>alg:chit (99:1)</td>
<td>5,4</td>
<td>Strong</td>
</tr>
<tr>
<td></td>
<td>alg:chit (19:1)</td>
<td>6,2</td>
<td>Strong</td>
</tr>
<tr>
<td></td>
<td>alg:chit (9:1)</td>
<td>7,1</td>
<td>Strong</td>
</tr>
<tr>
<td></td>
<td>Kaltostat®</td>
<td>2,1</td>
<td>Significant</td>
</tr>
</tbody>
</table>

In summary, produced alginate-chitosan aerogel fibres showed no cytotoxicity against the tested fibroblast cell-line, presented similar behaviour as Kaltostat® on NCTC clone 929 cell promoting cell migration and even stronger antimicrobial activity, suggesting to have promising application on wound healing treatment.

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References

The interest in carotenoids for food and pharma applications has been growing due to its antioxidant activity and its colorant properties. The encapsulation of these compounds to increase its stability and bioavailability has been a target of investigation.

In the present work, an emulsion-coacervation technique for carotenoids encapsulation was studied, using β-carotene as a model compound. Coacervation between lysozyme:pectin (L:P) and lysozyme:arabic gum (L:AG) was optimized at different mass ratios and pH values. Coacervates of 4:1 L:P (pH 4) and 2:1 L:AG (pH 8) were selected for encapsulation. Oil-in-water emulsions with lysozyme (aqueous phase) and β-carotene in olive oil (oil phase) were produced and coacervated with the pectin/arabic gum to produce particles. Formation of solid rigid particles was confirmed by scanning electron microscopy and optical microscopy. Load capacities of 0.38 and 1.36 µg β-carotene/mg particle were determined for L:AG and L:P particles, respectively.

Introduction

The incorporation of added-value products from natural sources into food and pharma systems is being exponentially explored, aiming to replace chemical additives and to add or enrich certain properties, as antioxidant, antimicrobial, etc [1,2].

The interest in carotenoids has been growing due to its bioavailability in natural sources, as tomato, carrot, marine crustaceous, etc, and to its dye and antioxidant properties [3].

The field of nano/micro formulation is known by its potential contribution in modulating release of compounds, solubility improvement and bioavailability enhancement [4].

Complex coacervation is a phenomenon that occurs by electrostatic complexation of biopolymers with opposite charge. The combined emulsion-coacervation technique is commonly applied for the entrapment of lipophilic and thermolabile compounds, as the carotenoids. In terms of process, it is a good option for food and pharma applications, since it does not require the use of organic solvents [5].

Lysozyme is a protein with strong positive charge and reported antimicrobial and anti-inflammatory properties. Pectin and arabic gum are commonly used as negative polysaccharides for complex coacervation [5,6].

In the present work, emulsion-coacervation method was studied for the encapsulation of carotenoids.

Methods

First, coacervation between lysozyme:pectin and lysozyme:arabic gum was optimized, by investigating the pH (3-11) and protein:polysaccharide mass ratios (1:1, 2:1, 4:1 and 1:2), through turbidimetric and zeta-potential analysis. The encapsulation of carotenoids was further studied by emulsion-coacervation technique. Oil-in-water (O/W) emulsions 3:50 (v/v) with β-carotene dissolved in olive oil (oil phase) and lysozyme (water phase) were produced. Solid particles were then formed by mixing the emulsion with the polysaccharide (pectin or arabic gum) solution at the optimal pH for coacervates formation. Physicochemical characterization of the particles was made by optical microscopy (OM) and scanning electron microscopy (SEM).

The load capacity of the produced particles was determined by UV-vis spectrophotometry. Antimicrobial evaluation of the produced particles is being optimized.

Results

Regarding complex coacervation, maximum coacervates formation occurred for lysozyme:pectin mass ratio of 4:1 at pH 4 and lysozyme:arabic gum mass ratio of 2:1 at pH 8. The formation of complex coacervates results into higher turbidity and thus higher values of absorbance (figure 1).

The zeta potential of these complexes was close to zero (data not shown), which translates the complexation of the polymers opposite charges in the same proportion (maximum complexation), supporting the turbidimetric results. The 4:1

Figure 1. Turbidimetric analysis of lysozyme:pectin (above) and lysozyme:arabic gum (below) at different pH and protein:polysaccharide ratios.
L:P (pH 4) and 2:1 L:AG (pH 8) were the coacervation parameters used for the encapsulation of β-carotene through emulsion-coacervation. The formation of spherical rigid particles by this process was observed by OM (data not shown) and SEM (figure 2). The presence of some non-spherical structures in the SEM images may result from non-coacervated polymers or particles degradation from the drying process.

**Conclusion**

In conclusion, coacervates of lysozyme:arabic gum 2:1 (w/w) and lysozyme:pectin 4:1 (w/w) at respective pH’s of 8 and 4, through emulsion-coacervation method. The lysozyme:arabic gum particles presented higher load capacity than lysozyme:pectin.

**Acknowledgements**

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**References**

Insights into polymer-silica aerogel composites from a molecular modelling and simulation approach

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Introduction
Silica-based aerogels, due to their remarkable properties such as very low density and high porosity and surface area, have found wide applications, particularly in the fields of space engineering and environmental science. Nevertheless, the poor mechanical resistance of these materials, as well as issues associated with particle shedding, limit their production on a commercial scale. The mechanical reinforcement of the silica matrix by the addition of polymers is a promising method to circumvent these issues [1,2]. It is often necessary to modify the silica aerogel matrix with functional groups that can promote stronger interactions with the polymer, either by surface modification or by synthesis with appropriate co-precursors. It has been found that these organically-modified silica (ORMOSIL) aerogels and their composites with polymers have improved structural and mechanical properties when compared to bare aerogels.

The systematic design of these composites requires fundamental knowledge about the chemical and physical interactions that take place during the growth of the silica matrix (especially the first stages of polycondensation), and that support the adhesion between that matrix and the polymer chains. However, the nature of these interactions is not completely understood, and experimental data alone is not sufficiently detailed to provide a clear picture of processes occurring at the nanoscale. Molecular modelling and simulation (MM&S) tools provide a unique systematic approach to answer these questions, complementing the limited information from experimental data with calculations based on physical models. In the last decades, with the exponential growth in computing power, MM&S has established itself, on both academic and industrial circles, as an invaluable methodology for product design, effectively contributing for setting a new paradigm in Chemical Engineering [3].

Although there are already several computational studies of unmodified silica aerogels (i.e. those derived from tetramethyl/tetraethylorthosilicate), there are very few of such studies regarding ORMOSIL aerogels [4,5] or their composites with polymers. The objective of this work is therefore to gain insights, at the microscopic level, on the formation of several aerogels and their composites with polydimethylsiloxane, and the nature of the interactions between the organic and inorganic phases, by employing well-established quantum mechanics and molecular dynamics methods.

Methods
Our studies begun at the atomic scale, by using density functional theory (DFT) calculations (B3LYP/6-311+G(d,p)), preformed with the Gaussian™ software suite, to model ORMOSIL aerogel silica-based oligomeric entities (primary particles) of various compositions: 100% tetramethylorthosilicate (TMOS), 100% vinyltrimethoxysilane (VTMS), 50% TMOS/50% VTMS (henceforth designated TMVT) and 50% TMOS/50% aminopropyltrimethoxysilane (APTMMS) (henceforth designated TMAP). For each composition, a large set of possible clusters that can be obtained at the initial stages of the condensation of silica precursors was considered based on previous work [4]. The theoretical IR spectra derived from these calculations have been compared with their experimental counterparts in order to obtain a microscopic model for these materials. Such a model was then used as a basis for molecular dynamics (MD) simulations at the nanoscale, aimed at modelling the silica-polymer interactions and the effects of the polymer on the aggregation of silica-based primary particles. All-atom GROMOS96 force field parameters were automatically derived from quantum mechanical calculations using the online platform ATB [6]. Then, systems of silica and silica-polymer were simulated for 100 ns, under an NPT ensemble (1 bar and 300 K), using the software GROMACS 2018.1 software package.

Results and main conclusions
IR frequencies and intensities were calculated at the B3LYP/6-311+G(d,p) level of theory for each silica-based oligomer under study. Given the multitude of possible species, a theoretical spectrum was calculated for each aerogel composition by employing a numerical fitting procedure, whereby the percentage contribution of each oligomeric entity to the overall theoretical spectrum was optimized by minimizing the mean squared difference between the theoretical and experimental spectra. Figure 1 gives an example of a theoretical IR spectrum obtained in this manner, which is very close to its experimental counterpart in terms of the frequencies of the respective bands. This numerical procedure allowed the initial set of silica-based oligomers studied by quantum mechanics based calculations to be reduced to just a few species, which were therefore assumed.
to be the most representative of the chemical composition of the silica aerogels they aim to model.

Figure 1. Experimental and optimized theoretical IR spectra of a silica aerogel derived from a mixture of 50% TMOS and 50% VTMS.

Once obtained the representative silica-based oligomers for all of the major aerogel compositions studied, these were used to setup MD simulations after deriving appropriate force fields. Six reference systems were simulated constituted only by the silica clusters and solvent. In addition, composite systems with polydimethylsiloxane (PDMS) and aerogels based on TMOS, VTMS and TMVT were simulated (with 10% and 50% PDMS). The results showed that the addition of the polymer PDMS significantly changes the aggregation behavior of silica primary particles during the formation of the respective composites with silica aerogels that were studied in this work. A representative example of this phenomenon can be seen in Figure 2, for the composite system based on the TMVT aerogel mixture. The extent of the aggregation process is better in the systems with a lower PDMS percentage (almost in the same extent as in the corresponding reference cases). The difference between those cases can be explained by the strength of the hydrogen bonding between silica-based species and between silica and PDMS. The better adhesion of PDMS to the silica aggregates in systems with 10% PDMS was also confirmed by an RDF analysis.

Figure 2 - Aggregate size distribution at the beginning and at the end of the simulation for two systems based on the silica precursor mixture 50% TMOS/50% VTMS: (a) reference system (silica only) and (b) 90% silica and 10% PDMS composite.

Acknowledgements
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References
Microencapsulation of marine *Chlorella* by spray drying

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**Microalgae technology** is a growing field that supports food, feed, pharma and cosmetic industry. Despite their positive effects on health, microalgae products have not gained much importance on food sector, mainly due to their sensorial characteristics (strong color and smell). To overcome this drawback the ability of inulin, a natural prebiotic polysaccharide, as encapsulation matrix is explored. Microparticles of *Chlorella*-inulin were produced by spray drying at different inlet temperatures (120-200 °C), solid content (5-25%) and wall:core ratios. The optimized microencapsulation process, with a yield of 66%, generated a light powder with a much less intense green tone than the original lyophilized biomass (ΔE=30,35). Microalgae encapsulation led to smaller particles (10 microns) and lower moisture content. In addition, SEM analysis revealed spherical capsules with smooth surfaces, indicative of a good encapsulation efficiency, corroborating inulin’s ability to act as a wall agent.

**Materials and methods**

Encapsulation by spray drying consists on a liquid feedstock containing solids in solution, suspension or emulsion that is converted to a dry powder by atomization into a flowing stream of hot gas. Parameters such as inlet temperature, feed rate, spray air flow, aspirator flow, solid content in the liquid feedstock and wall:core materials ratio define the characteristics of the final product. Inulin was selected as wall component as it is a nontoxic natural polysaccharide found in more than over 3000 vegetables.

Marine *Chlorella* biomass was produced in close tubular photobioreactors by Buggypower Lda. (Portugal) and supplied as a freeze dried powder. Commercial spray-dried inulin ‘Orafti® Synergy1’ was supplied by BENEÖ (Germany).

**Introduction**

Microalgae are known for decades, but this biotechnology only began to develop in the middle of the last century. Nowadays, there are numerous commercial microalgae applications which range from biomass production for food and feed to valuable products for ecological and pharmaceutical applications. *Chlorella*, a unicellular microalgae, is characterized by high productivity and high valuable components [1]. Nutritional studies have demonstrated that its proteins are of high quality and extracts of *Chlorella* have been documented as showing diverse antitumor, antioxidant, anti-inflammatory and antimicrobial activities. It also decreases blood pressure and cholesterol levels, accelerates wound healing and enhances the immune system. However, despite its health benefits, the use of *Chlorella* as food or food substitute is not as common as it could be expected. Being commonly commercialized as powder, the major obstacles are the powder-like consistency, its dark green color and strong fishy smell, which limit the incorporation of algal material into conventional food. Consumers associate color with food safety and quality since an inappropriate color is often related to spoilage, bad processing or faulty transportations. In this scenario, microencapsulation appears as a solution to the problem. This technology not only provides barriers between sensitive bioactive materials and the environment, but also allows to mask bad tasting and smelling. Moreover, it stabilizes food ingredients, avoiding evaporation and degradation of volatile compounds and preventing reactions between the microencapsulated product with oxygen and water. Although encapsulation can be achieved by several processes spray drying is the most commonly applied technology. Economic viability, reproducibility and process flexibility contributed to make this process the predominant method for producing encapsulated food ingredients [2].

In the present study, the viability of encapsulating freeze dried marine *Chlorella* powder by spray drying is evaluated.

**Materials and methods**

Encapsulation by spray drying consists on a liquid feedstock containing solids in solution, suspension or emulsion that is converted to a dry powder by atomization into a flowing stream of hot gas. Parameters such as inlet temperature, feed rate, spray air flow, aspirator flow, solid content in the liquid feedstock and wall:core materials ratio define the characteristics of the final product. Inulin was selected as wall component as it is a nontoxic natural polysaccharide found in more than over 3000 vegetables.

Marine *Chlorella* biomass was produced in close tubular photobioreactors by Buggypower Lda. (Portugal) and supplied as a freeze dried powder. Commercial spray-dried inulin ‘Orafti® Synergy1’ was supplied by BENEÖ (Germany).

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**Table 1. Prediction of theoretical responses compared to the obtained values.**

<table>
<thead>
<tr>
<th>Factors</th>
<th>200 °C</th>
<th>Inlet temperature</th>
<th>2,66:1</th>
<th>Ratio (1:C)</th>
<th>10,98%</th>
<th>Solid content in the solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Spray Dryer Conditions</td>
<td>100%</td>
<td>Aspirator</td>
<td>246 L/h</td>
<td>N₂ Flow</td>
<td>30%</td>
<td>Outlet temperature</td>
</tr>
<tr>
<td>Outlet temperature</td>
<td>120°C</td>
<td>Feed Rate</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted yield</td>
<td>66,96%</td>
<td>Obtained</td>
<td>66,21%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predicted color</td>
<td>63,04</td>
<td>Obtained</td>
<td>61,44</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Results
A design of experiments was used to provide an efficient means to optimize the spray drying process, using the software MODDE Pro 12.0 (Umetrics AB). Based on the encapsulation yield and color of the final particles, the optimized conditions of the process are summarized in Table 1. Yield was calculated as (collected powder weight / inulin and Chlorella mass in feedstock) × 100. The yield achieved under the conditions described in Table 1, is 66%. Color was assessed by the CIELAB method using the value of the parameter L*, representative of the luminosity of the sample [3]. While the raw microalgae has an L*=36.50, this parameter almost doubles the value for the encapsulated microalgae (L*61.44), revealing a rather significant increase in color clarity (Table 2).

Surface morphology directly impacts on key characteristics of spray dried powder such as particle size distribution, moisture content, bulk and particle density, flowability and friability. Figure 1 shows the comparison between the surface morphology of commercial spray dried inulin raw Chlorella and Chlorella microcapsules with inulin as wall material by SEM. Inulin particles present smooth walled capsules while freeze dried Chlorella powder has a rather rough surface. Inulin-Chlorella microcapsules show less rough surfaces when compared to the raw microalgae. This may be an indicative of a good encapsulation efficiency, corroborating inulin’s ability to act as a wall agent. Moreover given the size discrepancy of the microalgae cells, the microcapsules also have slightly distinct diameters. However, the vast majority appears to have a diameter of approximately 10 microns, a value much smaller than that of the non-encapsulated cells. Particle size distribution of microcapsules was verified by laser diffraction (D0.5 = 9.04 ± 0.29 μm). Additionally, water content was measured in raw Chlorella (6%) and microparticles (0.16%) which confirms the potential of this technique to obtain encapsulated products of high quality and stability.

Conclusions
The presented microencapsulation process shows high yield, generating light powder with a much less intense green tone than the one of original microalgae biomass. Besides Chlorella microencapsulation led to a more stable powder, as a result of smaller particles with homogeneous size distribution and lower moisture content.

Table 2. Color properties of the optimized spray dried microcapsules compared to those of the raw Chlorella powder.

<table>
<thead>
<tr>
<th></th>
<th>L*</th>
<th>∆E</th>
<th>RGB color</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw Chlorella</td>
<td>36.5</td>
<td>0.00</td>
<td></td>
</tr>
<tr>
<td>Optimized microcapsules</td>
<td>61.44</td>
<td>30.35</td>
<td></td>
</tr>
</tbody>
</table>

Acknowledgements
The authors acknowledge the financial support received from the Portuguese Fundação para a Ciência e Tecnologia (FCT) through the PEst-OE/EEQB/LA0004/2011 grant. iNOVA4Health - UID/Multi/04462/2013, a program financially supported by FCT/Ministério da Educação e Ciência, through national funds and co-funded by FEDER under the PT2020 Partnership Agreement is also acknowledged. A.A. Matias thanks for the IF Starting Grant – GRAPHYT (IF/00723/2014). The authors are grateful to BUGGYPOWER for providing the raw material.

References
Cr(III) removal from aqueous solution by activated carbons obtained through the co-pyrolysis of wastes from rice production

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* da.dias@campus.fct.unl.pt

Rice husk and polyethylene were mixed (50 % w/w each) and submitted to pyrolysis. Four physical activation processes with CO₂ were performed on the resulting co-pyrolysis char (PC). The activation at 800 °C, for 4 h, generated the activated carbon (PAC3) with the best textural properties. PAC3 and a commercial activated carbon (CAC) were submitted to Cr(III) removal assays under two S/L: 5 and 10 g L⁻¹. PC had a high volatile matter content that was removed after the physical activation, resulting in more available pores in PAC3. In Cr(III) removal assays, PC did not remove Cr(III) from the solution, but PAC3 presented similar results to CAC. At the S/L of 5 g L⁻¹, Cr(III) removal was of 58.5 % for PAC3 and 62.5 % for CAC, both by adsorption mechanism; at the S/L of 10 g L⁻¹, Cr(III) removal was almost complete due to precipitation caused by pH increase. The highest uptake capacities were of 7.92 mg g⁻¹ for PAC3 and 8.71 mg g⁻¹ for CAC, at the S/L of 5 g L⁻¹.

Introduction

Rice is the second most produced cereal in the world and its production generates great amounts of wastes, mostly rice husk (RH), rice straw (RS) and plastics (mainly polyethylene – PE). Due to their calorific values, these wastes can be used in thermochemical processes, such as pyrolysis, generating liquids and gases that can be used as renewable energy sources. Char is also produced in pyrolytic and gasification processes and can be used as alternative feedstocks to produce activated carbons [1]. On the other hand, chromiu m is one of the priority raw materials for Europe; therefore, its recovery from wastewaters is a topic of utmost importance [2].

The objectives of this work were (i) to produce activated carbons from char obtained in the co-pyrolysis of wastes from rice production, and (ii) to use them in Cr(III) removal from aqueous solution.

Materials and Methods

50% (w/w) RH and 50% (w/w) PE were blended and submitted to pyrolysis at 390 °C and 6 bars for 35 min. In order to improve its adsorption capacity, the pyrolysis char (PC) was physically activated (Table 1) with a CO₂ flow of 150 ml min⁻¹.

Table 1. Activation conditions of the pyrolysis char

<table>
<thead>
<tr>
<th>Activation</th>
<th>Code</th>
<th>Time (h)</th>
<th>Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>PAC1</td>
<td>2</td>
<td>800</td>
</tr>
<tr>
<td>2</td>
<td>PAC2</td>
<td>2</td>
<td>850</td>
</tr>
<tr>
<td>3</td>
<td>PAC3</td>
<td>4</td>
<td>800</td>
</tr>
<tr>
<td>4</td>
<td>PAC4</td>
<td>4</td>
<td>850</td>
</tr>
</tbody>
</table>

The pyrolysis activated carbons (PAC’s) and PC were texturally characterized (surface area and pore volume distribution were determined through N₂ adsorption-desorption isotherms at 77 K, with previous sample degasification under vacuum conditions at 150 °C); afterwards, both PC and PAC samples with the best textural properties were characterized for: (b) Proximate analysis – moisture content (M) (EN 14774-1), volatile matter (VM) (EN 15148), ashes (Ash) (EN 14775) and fixed-C (100% – M– VM– Ash) were determined by gravimetric method; (b) pHpzc – the adsorbents were placed in 0.1 M NaCl solutions with an initial pH (pHᵢ) between 2 and 12, at a solid/liquid ratio (S/L) of 5 g L⁻¹. The solutions were stirred in a roller-table device, at 150 rpm, for 24 h. At the end of agitation time, final pH (pHₑ) was measured. The pHpzc value corresponded to the point where pHᵢ = pHₑ.

The removal assays were performed under the following conditions: stirring – 150 rpm; contact time – 24 h; initial concentration of Cr(III) – 70 mg L⁻¹; initial pH – 4.5; S/L – 5 and 10 g L⁻¹.

For comparison purposes, a commercial activated carbon (CAC) (Nori GAC 1240) was also characterized and used in the adsorption assays.

Discussion and Results

The textural characterization (Table 2) showed that PC had no porous structure, as it is a non-activated material. After the physical activation, the surface areas and pore volumes increased, as expected. PAC3 had the highest surface area and total volume of all co-pyrolysis activated carbons. Still, these results were lower than for CAC. Considering these results, PAC3 was the activated carbon selected to be further characterized and used in adsorption assays. Regarding the proximate analysis (Table 3), PC was mainly composed by fixed-C, but still with a high percentage of volatile matter and ashes. PAC3 was mainly composed by fixed-C and ashes, as the physical activation removed almost all the volatile matter present in the precursor. As expected, CAC was mainly composed by fixed-C.

Concerning pHᵢₑ, PC presented a neutral to slightly acidic pHᵢₑ, but after the physical activation (PAC3), the pHᵢₑ increased significantly to 9.9, which characterized PAC3 sample as an alkaline material. This happened because the activation removed most volatile matter, thus concentrating the ashes which are responsible for the alkalinity of the adsorbent. pHᵢₑ of CAC was also alkaline although slightly lower than for PAC3. Cr(III) removal assays (Figure 1) revealed that PC removed almost no Cr(III) from the solution (Figure 1a) because this is a non-porous material (Table 2); consequently, PC uptake...
capacity for both assays (Figure 1b) was very low. However, after the physical activation, the resulting material (PAC3) showed interesting results for Cr(III) removal assays, comparable to those obtained for CAC (Figure 1). At a S/L of 10 g L⁻¹, both activated carbons (PAC3 and CAC) removed almost all Cr(III) present in the solution due to precipitation (final pH > 5) (Figure 1a), showing uptake capacities of 6.53 and 6.09 mg g⁻¹ for PAC3 and CAC, respectively (Figure 1b). At a S/L of 5 g L⁻¹, both assays presented a final pH < 5, indicating that precipitation mechanism was not dominant, and adsorption could have explained Cr(III) removal (Figure 1a). Although the surface area and pore volume of PAC3 were much lower than for CAC (Table 2), the performance of both adsorbents was very similar: PAC3 removed 58.8 % of Cr(III) from the solution and CAC removed 62.5 %, showing uptake capacities of 7.92 and 8.71 mg g⁻¹, respectively, which were even higher than those at the S/L of 10 g L⁻¹ where all Cr(III) was removed. These results suggest that the adsorption of Cr(III) by PAC3 was not only by physical adsorption, but also by chemical adsorption, namely by ion exchange, due to the high ash content of PAC3, composed by minerals that can exchange ions with Cr, such as K, Ca, Na and Mg [3].

Table 2. Textural properties of the adsorbents

<table>
<thead>
<tr>
<th>Samples</th>
<th>$S_{BET}$ (m² g⁻¹)</th>
<th>$V_{total}$ (cm³ g⁻¹)</th>
<th>$V_{micro}$ (cm³ g⁻¹)</th>
<th>$V_{meso}$ (cm³ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PC</td>
<td>&lt; 5.0</td>
<td>n.d.</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>PAC1</td>
<td>190</td>
<td>0.11</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>PAC2</td>
<td>223</td>
<td>0.12</td>
<td>0.07</td>
<td>0.05</td>
</tr>
<tr>
<td>PAC3</td>
<td>325</td>
<td>0.18</td>
<td>0.10</td>
<td>0.08</td>
</tr>
<tr>
<td>CAC</td>
<td>1030</td>
<td>0.56</td>
<td>0.30</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Table 3. Proximate analysis and pHₚₑₑₑ of the adsorbents

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PC</th>
<th>PAC3</th>
<th>CAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (% w/w ar)</td>
<td>1.38</td>
<td>4.36</td>
<td>13.1</td>
</tr>
<tr>
<td>Volatile matter (% w/w ar)</td>
<td>22.7</td>
<td>4.25</td>
<td>7.04</td>
</tr>
<tr>
<td>Fixed-C (% w/w ar)</td>
<td>46.0</td>
<td>51.0</td>
<td>74.2</td>
</tr>
<tr>
<td>Ashes (% w/w ar)</td>
<td>30.0</td>
<td>40.4</td>
<td>5.70</td>
</tr>
<tr>
<td>pHₚₑₑₑ (Sørensen scale)</td>
<td>6.35</td>
<td>9.89</td>
<td>9.13</td>
</tr>
</tbody>
</table>

ar: as-received basis

The difference in the uptake capacities for both activated carbons was not significant. Therefore, it can be concluded that, under these conditions, PAC3 showed good properties to be an efficient renewable alternative to the commercial activated carbon tested in this study.

Conclusions

PC was a non-porous material due to its high percentage of volatile matter, which was blocking the char pores. For that reason, Cr(III) was not removed by PC sample. The physical activation at 800 °C for 4 h originated a PAC with the best textural properties (PAC3) (surface area of 325 m² g⁻¹ and a total volume of 0.18 cm³ g⁻¹). This material was composed mainly by fixed-C and ashes, indicating that the volatile matter was removed during the activation. The Cr(III) removal assays proved the good performance of PAC3. At the S/L of 5 g L⁻¹, PAC3 removed 58.3% of Cr(III) by adsorption, almost the same as CAC (62.5 %). At the S/L of 10 g L⁻¹, both PAC3 and CAC removed almost all Cr(III) from the aqueous solution by precipitation. The highest uptake capacities were found at the S/L of 5 mg L⁻¹, with a value of 7.92 mg g⁻¹ for PAC3 and 8.71 mg g⁻¹ for CAC. Although with less porosity than CAC, PAC3 obtained very similar results to CAC on Cr(III) removal assays, which can be attributed also to its high mineral content.

Acknowledgements

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References

Overcoming the mass transport limitations of amino acid-based ionic liquids in CO$_2$ chemical absorption by using Encapsulated Ionic Liquids

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During last decade ionic liquids (ILs) have awoken great attention due to its unique properties such as very low vapour pressure, good thermal and chemical stability and high polarity$^1$. In CO$_2$ capture, ILs can be applied to absorb CO$_2$ by either physical or chemical absorption$^2$. This work is focused on amino acid-based ILs (aa-ILs) promising materials because of their low cost, abundant availability, and nontoxic biodegradability$^3$. However, they present high kinetic limitation due to their high viscosity. Encapsulated ionic liquids (ENILs) are novel materials composed by hollow carbon submicrospheres (C$_{cap}$)$^4$ filled by a large amount of IL (up to 80% in weight) that increase drastically the gas-liquid contact surface enhancing the CO$_2$ sorption rates$^5$$^6$. In this work three ENIL materials were prepared filling high loads (55–60% w/w) of aa-ILs: namely, 1-butyl-3-methylimidazolium proline [Bmim][Prol], 1-butyl-3-methylimidazolium methionite [Bmim][MET] and 1-butyl-3-methylimidazolium glycinate [Bmim][GLY]. Sorption experiments were carried out using ILs and ENILs at 303, 318 and 333K, for partial pressure of CO$_2$ in the range of 0.3-20 bar. The influence of the encapsulation and chemical nature of the amino acids was analysed at different operating conditions.

The performance of three amino acid-based ionic liquids (aa-ILs) has been evaluated in CO$_2$ capture by mean of gravimetric measurements. The tested aa-ILs were Bmim with Glycinate, Prolinate and Methionite. First, the CO$_2$ chemical absorption process was experimentally analyzed by in situ FTIR-ATR, comparing them with theoretical measurements obtained by quantum chemical calculations. This study let us suggest a mechanism of CO$_2$ chemical absorption on aa-ILs. Then, gravimetric experiments were carried out to characterize the CO$_2$ capture by aa-ILs. It was found that CO$_2$ absorption quantification of these ILs was rather slow, because of their high viscosities, so other methodologies had to be employed to quantify the CO$_2$ capture. In this sense, aa-ILs were encapsulated in porous carbon capsules (aa-ENIL) in order to solve this problem, promoting chemical absorption. The aa-ILs showed considerable higher CO$_2$ solubility compared to amine cation-based ILs or non-functionalized ILs.

![Figure 1. TEM image of carbon capsule (C$_{cap}$).](image)

The aa-ILs used in this study were synthesized by Iolitec at 95% of purity, all the aa-ILs were dried and degassed at 333 K under vacuum ($10^{-4}$ mbar) during 24 hours prior to their use. Carbon dioxide (CO$_2$) and nitrogen (N$_2$) were supplied by Praxair, with a minimum purity of 99.999 %. The hollow microcapsules (C$_{cap}$) synthesized for their use as ENIL support were prepared following the methodology reported by our group in previous works$^7$. The ENIL materials were prepared by incipient wetness impregnation of an IL-acetone solution over C$_{cap}$. After impregnation acetone was removed by evaporation. The measurements of CO$_2$ solubility in ILs and ENILs were performed in a gravimetric high-pressure sorption analyzer (ISOSORP GAS LP-Flow, Rubotherm) equipped with a magnetic suspension balance (MSB).

![Figure 2. Kinetic curves of CO$_2$ uptake in neat aa-ILs and aa-ENIL based material at 301.5 K and p$_{CO2}$ = 0.3 bar, using [Bmim][MET].](image)

The C$_{cap}$ prepared as support ca be observed on Figure 1, spherical capsules were obtained containing high carbon content (94% w/w), homogeneous morphology (~700 nm of diameter and ~150 nm of shell thickness), high pore volume (> 3.5 cm$^3$ g$^{-1}$) and BET surface area (1644 m$^2$ g$^{-1}$). Regarding the CO$_2$ sorption behaviour of the neat and encapsulated aa-IL, Figure 2 shows the CO$_2$ kinetic sorption curves recorded for the neat and encapsulated [Bmim][MET] at 301.5 K and 1 bar of CO$_2$ partial pressure, using a similar sorbent sample mass (~200 mg). The CO$_2$ sorption rate is drastically enhanced using the aa-derived ENIL material, since these ILs turned solid when the CO$_2$ was absorbed. This methodology of IL encapsulation not only enhances the mass transport properties of the neat IL, but also let us to use ILs that would not reach the thermodynamic equilibrium. This huge improvement is due to the large increase of the contact surface between the gas phase and the aa-IL, upon dispersion of the absorbent into microdrops using ENIL system.

The CO$_2$ capture in three aa-ENIL materials was measured gravimetrically at 303, 318 and 333 K and CO$_2$ partial pressures from 0.3 to 20 bar. The resulting absorption isotherms were fitted to a model to obtain the corresponden
physical ($K_H$) and chemical ($K_{eq}$) equilibrium constants. Table 1 collects CO$_2$ solubility at P$_{CO2}$ of 1 bar, equilibrium constants and reaction enthalpy derived from Van’t Hoff equation using adjusted data. CO$_2$ solubility follows the subsequent trend $[MET] < [PRO] < [GLY]$, being possible to achieve maximum molar fraction of uptake CO$_2$ corresponding to 0.50, 0.52 and 0.53, respectively at 303 K and 20 bar. An increase in the operating temperature displaces the chemical equilibrium toward the reactant (aa-IL), reducing the amount of CO$_2$ chemically bonded. The same effect occurs for the physical sorbed CO$_2$. Reaction enthalpies range between -16.3 and -34.4 kJ·mol$^{-1}$ values in good agreement with those for a reversible chemical capture of CO$_2$.

Summarizing, the encapsulation of aa-ILs greatly enhance the mass transfer in the CO$_2$ absorption process, allowing to reach sorption capacities higher than for the neat IL due to solidification of the media during the chemical reaction.

Table 1. CO$_2$ solubility at 1 bar, equilibrium constants ($K_{eq}$ and $K_H$) and reaction enthalpy for aa-ILs.

<table>
<thead>
<tr>
<th>ILs</th>
<th>$T$ (K)</th>
<th>molCO$_2$/molIL</th>
<th>gCO$_2$/gIL</th>
<th>$K_{eq}$</th>
<th>$K_H$ (bar)</th>
<th>$\Delta H$ (kJ/mol)</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Bmim][PRO]</td>
<td>303</td>
<td>0.18</td>
<td>0.031</td>
<td>7.65</td>
<td>137</td>
<td>-16.30</td>
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<tr>
<td></td>
<td>318</td>
<td>0.13</td>
<td>0.023</td>
<td>4.70</td>
<td>186</td>
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<tr>
<td></td>
<td>333</td>
<td>0.12</td>
<td>0.021</td>
<td>4.50</td>
<td>236</td>
<td></td>
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<tr>
<td>[Bmim][MET]</td>
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<td>0.13</td>
<td>0.020</td>
<td>2.40</td>
<td>112</td>
<td>-34.37</td>
</tr>
<tr>
<td></td>
<td>318</td>
<td>0.10</td>
<td>0.015</td>
<td>1.69</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td></td>
<td>333</td>
<td>0.07</td>
<td>0.010</td>
<td>0.69</td>
<td>156</td>
<td></td>
</tr>
<tr>
<td>[Bmim][GLY]</td>
<td>303</td>
<td>0.19</td>
<td>0.038</td>
<td>9.25</td>
<td>139</td>
<td>-19.16</td>
</tr>
<tr>
<td></td>
<td>318</td>
<td>0.15</td>
<td>0.032</td>
<td>7.28</td>
<td>195</td>
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<tr>
<td></td>
<td>333</td>
<td>0.12</td>
<td>0.024</td>
<td>4.82</td>
<td>277</td>
<td></td>
</tr>
</tbody>
</table>

Acknowledgements
The authors are grateful to Comunidad de Madrid (project S2013-MAE-2800) and Ministerio de Economía y Competitividad of Spain (project CTQ2017-89441-R) for financial support.

References
The release of air pollutants into the environment causes severe environmental and health problems. Volatile organic compounds (VOCs) are one class of air pollutants known for contributing to several environmental problems such as, stratospheric ozone depletion, formation of photochemical smog and increase in the particulate matter [1]. As such, the treatment of these air pollutants before discharge into the atmosphere is imperative.

The most common process used for the treatment of air contaminated with VOCs is thermal oxidation. Using high temperatures (between 600 and 1200 °C) in an oxygen containing atmosphere, the carbon and hydrogen present in these pollutants are converted into CO₂ and H₂O [2]. However, a high amount of energy is needed during the treatment, due to the very high temperatures, which will increase the costs. The composition of the feed gas also needs to be thoroughly controlled since, due to the high temperatures, the concentration of the VOCs could approach the explosion range. Harmful by-products can also be formed if the temperature is not sufficiently high.

In order to avoid or diminish these problems, new and more efficient technologies started to be developed. The utilization of a catalyst in the thermal oxidation of VOCs increases significantly the efficiency of this process, allowing the removal of these pollutants at much lower temperatures (generally bellow 400 °C) and typically without formation of harmful by-products [3]. However, the application of catalysts in this process presents some challenges such as, possible deactivation due to the formation of coke deposits, thermal degradation of the material or presence of poisons [4]. The preparation of an active and stable catalyst is imperative for the successful application of this technology. Cryptomelane-type manganese oxides present high activity for the oxidation of VOCs, normally ascribed to the mixed valence state of manganese (slightly below 4) and to the high mobility of the lattice oxygen [5].

Most studies of VOC oxidation are performed with powder catalysts; however, the macro-structure of these powder catalysts have several advantages such as, a lower cost of operation (due to lower pressure drop), good mass transfer, safer operating conditions and easier separation [6]. In this work, ceramic and metallic (FeCrAlloy®) monoliths with different cpsi were coated with cryptomelane-type manganese oxide prepared by a solvent free method. These monoliths were tested in the oxidation of ethyl acetate.

The powder cryptomelane was synthesized by a solvent free method. Manganese acetate and potassium permanganate were mixed and milled for 1 h. The resulting material was placed in an oven at 80 °C for 4 h, washed with distilled water and dried at 120 °C.

The ceramic monoliths (400 cpsi) were pretreated at 900 °C in air for 1 h to remove impurities. The metallic monoliths (between 289 and 1330 cpsi) were pretreated at 900 °C in air for 22 h to increase surface roughness [11].

The catalytic slurry was prepared by mixing the powder catalyst in distilled water and adding colloidal alumina. The monoliths were immersed in the solution for 1 min, the excess liquid was removed by centrifugation and then the monoliths were dried at 120 °C.

This process was repeated until the desired catalyst loading was achieved. The monoliths were then calcined at 450 °C for 2 h. Some monoliths (both ceramic and metallic) were previously coated with a layer of alumina prior to the coating with the active phase. This coating was performed similarly to the one with the active phase. The monolith was immersed in a 1:4 colloidal alumina/ water solution, dried at 120 °C and calcined at 500 °C.

The zeta potential of the powder was measured and an isoelectric point of 8.5 was found. pH below 6 presented a zeta potential above 30 mV which is sufficient to allow a stable slurry [12]. A pH of 6 was chosen to prepare the catalyst slurry since at lower pH the particle size of the cryptomelane increased significantly. Several slurries were prepared with different solids content (between 12 and 20 %). It was found that slurries with a solid content higher than 18 % presented very high viscosity, which led to a non-uniform coating of the monolith. However, below 18 % the viscosity was low, which led to a low amount of mass adhered to the monolith. Therefore, a solid content of 18 % was chosen to prepare the optimized slurry. In order to increase the amount of mass adhered to the monolith, without decreasing the homogeneity of the coating, milling of the powder material previously to the preparation of the slurry was performed. The milling decreased the particle size in the slurry, increasing the viscosity and the stability of the slurry allowing a higher deposition of the active phase on the monolith with high homogeneity.
It was found that the initial layer with colloidal alumina improved the adherence of the active phase on the ceramic monoliths. However, for the metallic monoliths the alumina layer had a negative impact. Most likely, the alumina layer is covering the whiskers formed during the pre-treatment of the metallic monoliths [13], decreasing surface roughness. For the preparation of the different monoliths, a different number of immersions were needed to achieve 350 mg of active phase adhered to the ceramic and metallic monoliths [13], decreasing surface roughness. For example, with 2 immersions the mass adhered per lateral area (specific loading) obtained was 0.8 and 0.4 mg cm⁻² for the ceramic and metallic monoliths, respectively.

Comparing the different metallic monoliths it is noticeable that those with higher cpsi retain a higher amount of active phase, which was expected since the lateral area of these monoliths is higher. Since the specific loadings per immersion were the same for all metallic monoliths, and the lateral area was different, to achieve the 350 mg of active phase, higher total specific loadings were achieved by the monoliths with low cpsi, which correlates with higher average layer thickness (ranging from 5 to 10 µm) that can affect the mass transfer.

The textural properties of the prepared materials were determined by N₂ adsorption isotherms. Both ceramic and metallic monoliths, as well as the powder cryptomelane and the slurried catalyst (powder obtained after drying and calcination of the catalytic slurry) presented similar BET surface areas (150 ± 10 m² g⁻¹) and pore volume (0.47± 0.02 cm³ g⁻¹). This shows that the formation of the slurry and its coating onto the monolith does not significantly change the textural properties of cryptomelane. The X-ray diffraction (XRD) spectra of the powder samples present the main peaks of cryptomelane. However, in the spectra of the slurried catalyst, small amounts of impurities were detected at a 2θ angle of 47°. This impurity could be due to the colloidal alumina or the presence of Mn₃O₄ since both present a peak at this angle.

Temperature programmed reduction (TPR) analysis was performed on the materials prepared. The results are summarized in Table 1.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Max peak (°C)</th>
<th>H₂ consumption (cm³/g cryptomelane)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptomelane</td>
<td>297</td>
<td>233</td>
</tr>
<tr>
<td>Slurred catalyst</td>
<td>313</td>
<td>228</td>
</tr>
<tr>
<td>Metallic monolith</td>
<td>336</td>
<td>204</td>
</tr>
<tr>
<td>Ceramic monolith</td>
<td>306</td>
<td>207</td>
</tr>
</tbody>
</table>

The TPR spectra of cryptomelane presents a large peak near 300 °C and a shoulder at low temperatures. A clear shift in the peak to higher temperatures is observed for the powdered slurry. Most likely, the alumina in the solution is decreasing the availability of cryptomelane. A secondary peak near 400 °C is also observed for this sample, which suggests the presence of Mn₃O₄ as already seen by XRD. The monoliths, both metallic and ceramic, present a similar profile to the powdered slurry. However, the metallic monolith is shifted to higher temperatures. This is due to the different thermal conductivities of the monoliths. Since the metal has a higher thermal conductivity than cordierite, and taking into account that the temperature registered by the equipment is measured in the center of the monoliths, for the same reading in the equipment, the temperature in the periphery will be higher for the ceramic monolith, which will result in an apparent displacement of the two profiles.

The materials prepared presented high activity in the oxidation of ethyl acetate.

Acknowledgements
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References
Novel hydroxyapatite-TiO$_2$ composite material for photocatalytic degradation of diclofenac

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Diclofenac (DCF) is one of the most widely prescribed non-steroidal anti-inflammatory drugs. Since conventional wastewater treatment does not achieve its complete removal, it has been detected in the environment. Advanced oxidation processes such as photocatalysis received increasing attention as effective technologies for environmental remediation. In this study, degradation of DCF was investigated by photocatalysis using a new synthesized photocatalyst based on hydroxyapatite obtained from cod fish bones and TiO$_2$. Degradation of 95% of the target compound was achieved in 24h. Investigation of photo-transformation products was performed by means of UPLC-QTOF/MS/MS, their chemical structure was proposed. The toxicity evaluation of final solution was performed using different assays. Overall, the toxicity of the water samples obtained from the photocatalytic experiment decreases, showing the potential applicability of this catalyst for the removal of DCF and detoxification of water matrices.

Introduction

Pharmaceuticals have emerged as environmental contaminants for which concern is increasing. Conventional wastewater treatment plants (WWTPs) have a limited capability to remove these compounds, resulting in their release into the environment. Diclofenac (DCF) is a widely prescribed non-steroidal anti-inflammatory drug. Nowadays, this drug is ubiquitously present in the aquatic environment [1] due to its continuous release from WWTPs, being considered a pseudo-persistent pollutant [2]. DCF is one of the substances on the watch list for European Union-wide monitoring in the Decision 2015/495/EU.

Advanced oxidation processes (AOPs) such as photocatalysis has being pointed out as effective technologies for pollutants degradation. In this context, materials showing photocatalytic activity have been widely investigated, specially heterogeneous photocatalysts composed of titanium dioxide (TiO$_2$) [3]. The combination with hydroxyapatite (Ca$_5$(PO$_4$)$_3$(OH)$_2$, HAP), which also possess photocatalytic activity, seems very promising. Previous work demonstrates the possibility to produce high-value Hap-based materials from cod fish bones by-products and its photocatalytic activity [4,5]. A multiphasic material constituted of HAP and TiO$_2$ (HApTi) showed to be effective for degradation of pharmaceuticals. The objective of this work was the use of this novel synthesized HApTi material to photodegrade DCF; investigate the formation of phototransformation products, and to assess the toxicity of the photocatalysis treatment samples in comparison with the parent compound and photolysis without catalyst.

Material and Methods

A detailed description of the photocatalytic material preparation and characterization was previously published [5]. Briefly, washed and dried cod fish bones were treated in titanium sulphate and then calcinated at 800 °C. Photocatalysis experiments were performed as previously described [6]. Briefly, DCF solution (5 mg/L) in distilled water was placed with 0.2 g of HApTi (4 g/L) and irradiated from the top with a XX-15 BLB UV lamp (λ: 365 nm; 1.80 mW/cm$^2$). Controls without HApTi were performed to evaluate DCF degradation by photolysis. DCF concentration was determined by HPLC [7]. The investigation of intermediates was performed using UPLC-QTOF/MS-MS [8]. Evaluation of the toxicity of photodegradation samples was performed by: Daphnia magna acute toxicity test, Toxi-ChromoTest and Lactuca sativa germination inhibition test.

Results

Results of photodegradation of DCF showed that in the photolysis treatment without catalyst, it was observed about 60% of DCF degradation, while in the photocatalytic experiment with HApTi, it was achieved 95% degradation of the compound in 24h [9]. The DCF photodegradation rate constants were well fitted to the pseudo-first order kinetics (the value of R$^2 = 0.97$). According to the obtained results, the presence of the catalyst resulted in higher reaction rate and, consequently, half-life times revealed an inverse trend (Table 1). These results indicate that the HApTi is an effective catalyst for the degradation of DCF.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>$k$ (h$^{-1}$)</th>
<th>$t_{1/2}$ (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UV light</td>
<td>0.034</td>
<td>20.39</td>
</tr>
<tr>
<td>UV light + HApTi</td>
<td>0.122</td>
<td>5.68</td>
</tr>
</tbody>
</table>

In previous experiments, TOC measurements showed that not all DCF removed was completely mineralised [6]. In this perspective, samples collected at different times during photodegradation experiments were analysed for phototransformation products (TPs) and toxicity evaluation. Major TPs were identified using UPLC/QTOF/MS-MS. For each potential TP, the elucidation of the structure was assessed based on the accurate mass of the MS/MS fragments and considering all the reaction mechanisms likely to occur. A pathway of DCF degradation in presence of HApTi was proposed. The time-profiles of TPs revealed that higher concentrations were detected at 4h or 6h and then decreased at the end of the experiment, being the extent of reduction much higher in the samples from photocatalysis with HApTi [9].

At the beginning of the experiments, original DCF solutions (5 mg/L) exhibit high toxicity on D. magna (total inhibition)
while the toxicity for the other tested organisms was absent (mutant bacteria) or very low (L. sativa). The toxicity of the DCF samples for D. magna drastically decreased after 4h of photocatalytic experiment with HApTi and completely disappeared at the end of the experiment. While in the case of the photolysis without catalyst, the toxicity did not vary significantly. D. magna was the most sensitive testing organisms for DCF but other tests revealed the same trend - reduced toxicity of the samples resulted from the photocatalytic experiment in relation to the photolysis. Is important to notice that for other organisms the toxicity of the samples increased with the treatments, especially in the case of photolysis without catalyst, probably due to the accumulation of TPs more toxic for these organisms that the parent compound [9].

Conclusions
The present study demonstrated the effectiveness of the novel synthesized multiphasic HAp-TiO₂ material, the former obtained from codfish bones, for the photocatalytic degradation of a pollutant of major concern - DCF. Major transformation products were detected and the chemical structure elucidated. Despite the mineralization not being complete, a reduced toxicity was observed in the samples treated with the photocatalytic experiment in comparison to the photolysis without catalyst and in comparison to the original DCF sample. HApTi photocatalyst was effective for the detoxification of samples containing DCF and DCF transformation products and represents an interesting and eco-safe technology for DCF degradation. It is important to notice that HApTi has a size mesh much higher than conventional TiO₂ catalyst, which facilitates its removal at the end of the wastewater treatment. These results confirm that the valorisation of food by-products such as fish bones could lead to the development of high added value products, which can be used for environmental remediation.

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References
P-doped glucose-derived carbon/carbon nanotubes hybrids for oxygen reduction reaction

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P-doped carbon materials were prepared by the hydrothermal carbonization of glucose in the presence of carbon nanotubes (CNTs), followed by impregnation with phosphoric acid. The materials were used as electrocatalysts in the oxygen reduction reaction (ORR) in order to evaluate the effect of the incorporated functionalities on their catalytic activity. The optimal thermal treatment temperature was found to be 800 °C, leading to the highest BET surface area and largest incorporation of heteroatoms. Moreover, the combination of chemical and physical activation originated a material with enhanced activity towards the ORR, indicating that impregnation with phosphoric acid improves the electrochemical performance of glucose-derived carbon electrocatalysts.

Introduction
Nowadays, with the continuously rising global energy demands, cheaper, greener and more efficient ways to convert chemical energy into electrical energy are required without using fossil fuels. One interesting system is the fuel cell (FC), which is considered an outstanding conversion device and represents an interesting path towards greener electrical energy [1]. However, FCs have two main drawbacks that prevent their commercialization: i) the most commonly used electrocatalyst is a platinum-based material that greatly increases the total cost of a FC; and ii) the oxygen reduction reaction (ORR) that takes place at the cathode is a slow process that hinders the overall reaction kinetics [2]. To address these concerns, many studies have been focused on the replacement of Pt-based electrocatalysts by different types of carbon materials, such as carbon nanotubes, graphene-based materials and activated carbons. Among them, activated carbons are interesting materials, as they can be obtained directly from biomass, which is an abundant, environmentally friendly and low-cost source. Notwithstanding, biomass-derived carbons must be properly tailored to obtain electrocatalysts with enhanced performance for ORR. Some strategies include the modification of the textural and chemical properties, and more specifically the effect of introducing heteroatoms such as oxygen, nitrogen, sulphur and boron [3-5]. However, the effect of phosphorus has not yet been studied in depth. Accordingly, in the present study, biomass-derived carbons have been doped with phosphorus to evaluate its effect on the ORR.

Objectives
In this work, glucose-derived carbon/carbon nanotube hybrids doped with phosphoric acid were prepared to evaluate the effect of the phosphorus species on the catalytic activity of the carbons towards ORR.

Methods
Carbon materials were prepared by hydrothermal carbonization of glucose in the presence of CNT. First, glucose and CNTs were dispersed in deionized water under sonication for 30 min. The mixture was then introduced in a Teflon-lined autoclave, sealed and heated at 180 °C for 12 h. The polymerized material was then washed with distilled water and dried at 80 °C overnight. The dried material was impregnated with phosphoric acid and left in contact for 24 h to ensure full impregnation of the carbon. The impregnated sample was then washed and dried under the same conditions previously mentioned. The impregnated samples were then thermally treated under an inert atmosphere of N2 (100 cm3 min-1) or a CO2 atmosphere (80 cm3 min-1 g-1). The samples treated under the inert atmosphere were heated at 700, 800 and 900 °C for 2 h (samples labelled CG_CNT_P_X, where X is the treatment temperature) while the sample that underwent treatment in CO2 atmosphere was heated to 800 °C for 4 h (sample AG_CNT_P_800). For comparison, non-impregnated samples were also prepared. One was treated under CO2 atmosphere at 900 °C for 4 h (sample AG_CNT) and the other under N2 at 700 °C for 2 h (sample CG_CNT).

All the materials obtained were characterized by nitrogen adsorption at -196 °C, energy dispersive X-ray spectroscopy (EDS), X-ray photoelectron spectroscopy (XPS), elemental analysis and temperature programmed desorption (TPD). For the evaluation of the catalytic activity towards the ORR, the prepared carbon materials were first dispersed in a mixture of Naion, ethanol and water, and then deposited on a glassy carbon electrode. The performance as electrocatalyst in the ORR was evaluated in a three-electrode cell immersed in 0.1 M KOH electrolyte solution, saturated with N2 or O2. Cyclic voltammetry and linear sweep voltammetry (LSV) were performed using an Autolab GSTAT 302N potentiostat/galvanostat to evaluate the catalytic efficiency of the samples on the ORR.

Results
The chemical properties allowed to understand the effect of temperature on the incorporation of phosphorus and oxygen on the sample surfaces. Sample CG_CNT_P_700 showed the smallest incorporation of the studied heteroatoms. Phosphorus and oxygen contents of 2.3 % and 8.0 %, respectively, were obtained, all the species being attributed to phosphate groups. An increase in the temperature to 800 °C shifts the type of phosphorus groups created from phosphate to metaphosphate. Besides, sample CG_CNT_P_800 showed the highest content of P and O (10.3 % and 12.8 %, respectively). However, a further increase of the temperature results in a loss of phosphorus, as sample CG_CNT_P_900 had a content of P of 7.0 %. Moreover, the amount of phosphates also decreased regarding sample CG_CNT_P_800. The activated sample also exhibited smaller amount of oxygen and phosphorus due to the larger time used for activation. Non-impregnated samples, CG_CNT and AG_CNT, exhibited 4.2 % and 1.6 % of oxygen content, respectively.

Nitrogen adsorption/desorption isotherms of all samples are shown in Figure 1. Among the samples treated under inert atmosphere at different temperatures, sample CG_CNT_P_800
exhibited the largest surface area, suggesting a direct relationship between the amount of phosphorus and the BET surface area obtained. As expected, the largest microporosity was observed for activated samples (Figure 1). However, the impregnated sample exhibits larger surface area due to the presence of P when compared to the AG_CNT sample.

Figure 1. Nitrogen adsorption/desorption isotherms at -196 °C

All the samples were evaluated by their LSV curves for ORR (Figure 2). The introduction of P and O into the samples proved to be beneficial to the catalytic activity of the carbon materials. Comparing the samples treated in N2 at different temperatures with the pristine sample (CG_CNT), a shift on the onset potential of 110 mV to more positive values can be observed. The pristine sample exhibits similar BET surface area, suggesting that the increase in the potential is due to the incorporation of heteroatoms. A slight increase in the kinetic current density obtained with the increase in BET surface area was also observed. This effect is even more notorious for activated samples, as the kinetic current density greatly increases with the BET surface area. However, the difference in the kinetic current density between impregnated (AG_CNT_P_800) and non-impregnated (AG_CNT) samples is larger than the difference in their BET surface areas, indicating once again that impregnation of carbon materials with phosphoric acid is an interesting strategy to enhance the catalytic activity of carbons towards ORR. Moreover, activated samples exhibit a mechanism closer to the four-electron pathway (number of electrons = 3.3) at lower potentials, while CG samples stay at the two-electron pathway for all potentials.

Figure 2. LSV curves for ORR in O2 saturated 0.1 mol dm-3 KOH solution (at 5 mV s⁻¹) at 1600 rpm.

Conclusion
Glucose-derived carbon/carbon nanotube hybrids doped with phosphoric acid were prepared and used as electrocatalysts for the ORR. A treatment temperature of 800 °C resulted in an increase of the BET surface area and phosphorus and oxygen contents, compared to the pristine carbon, which provided an increase of the onset potential. The activation of the samples gave rise to the highest values of the BET surface area, which led to significant improvements of the kinetic current density and number of electrons involved in the ORR mechanism. These results demonstrate that impregnation of carbon materials with phosphoric acid is an interesting strategy to enhance their catalytic activity towards ORR, paving the way for further research using P-doped carbon materials.

Acknowledgements
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References
Hybrid polysaccharide membranes for dehydration processes

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New hybrid polysaccharide (FucoPol) membranes were prepared by incorporation of a SiO₂ network homogeneously dispersed by a sol-gel method with GPTMS as crosslinker. The hybrid membranes were tested for ethanol dehydration by pervaporation and also for gas dehydration. To mimic industrial dehydration processes, namely flue gas and biogas dehydration, permeation of water vapor, pure gases (CO₂, CH₄ and N₂) and gas mixtures containing 20 vol.% CO₂ + 80 vol.% N₂ and 70 vol.% CH₄ + 30 vol.% CO₂, at different conditions of relative humidity, was monitored by mass spectrometry. The membranes exhibited high water selectivity values, similar to PERVAP® 4101, however they lost their stability when exposed to solutions of 10.0 wt. % water in ethanol. In contrast, they were stable when applied in gas dehydration with a low gas permeability, while exhibiting high water permeability and selectivity.

Solvent dehydration processes have a high economic and environmental relevance in the pharmaceutical, fine-chemistry and chemical industry. In particular ethanol dehydration, is one of the most important applications. Bioethanol has an important advantage over other biofuels, since it can be directly integrated in existing fuel systems, typically as a mixture with gasoline, not requiring modifications of the current engines, ensuring environmental sustainability and process economy.

Gas dehydration can be used for the dehydration of natural gas, drying of gases for packaging purposes and humidity control in closed spaces, such as air conditioning in buildings, aviation and space flight. In particular, dehydration of flue gas, originated in the production of electricity by coal-fired power plants, has a great interest due to the energy saving in power plants and reduction of diffusion of pollutants through water. Other potential application is biogas dehydration which, after purification, can be used as an alternative to natural gas and be distributed as power supply in rural and urban areas.

When compared to other dehydration methods, membrane-based dehydration has numerous benefits. Membrane technology involves a lower energy consumption, since the only energy consumed is the one required to maintain a partial pressure difference across the membrane and smaller footprint. Additionally, this technology is usually rather flexible and involves a compact modular design, easy to maintain and control.

The critical issue is to achieve membranes with high permeability and selectivity combined with good chemical and mechanical stability at high temperatures, with a reasonable cost. Hydrophilic polymers, such as, polyvinyl alcohol (PVA), polysulfone (PS), polyamides (PA), among others have been selected as membrane materials for the dehydration of various solvents. In gas dehydration, hydrophilic polymers, such as polyacrylonitrile (PAN), sulfonated polyetherketone (SPEEK) and poly(vinyl alcohol) (PVA) are usually used. Their drawback is that they are petrochemical based polymers and involve the use of solvents in their manufacture.

Therefore, developing environmentally friendly materials for the preparation of membranes is attracting much attention. Particularly, polysaccharides, such as alginate and chitosan have been tried for solvent dehydration due to their high affinity for water, solvent resistance and high separation performance. However, for gas dehydration, these are rarely reported, being cellulose acetate (CA) the most common biopolymer used for drying of compressed air since it presents an adequate H₂O:N₂ selectivity.

Hybrid membranes have become an important research topic in membrane science because they combine the easy processing and low-cost of polymeric membranes with the high permeation fluxes and mechanical properties of inorganic membranes. This work evaluates new membrane materials obtained from a renewable source, for ethanol and gas dehydration. The novel hybrid polysaccharide membranes were prepared with FucoPol - produced by Enterobacter A47, using as carbon source the glycerol by-product of the biodiesel industry [1]. This biopolymer was purified using a solvent free method (dia-ultrafiltration) in order to reduce the environmental impact and increase the membrane process sustainability. In order to reinforce the mechanical and thermal properties incorporation of a SiO₂ network homogeneously dispersed was obtained by using a sol-gel method with GPTMS as a crosslinker silica precursor. These membranes were applied in ethanol dehydration with a high transport performance (similar with PERVAP® 4101 commercial membrane), but an irreversible swelling in the presence of water was noticed, compromising long term operation. Table 1 shows the results obtained for consecutive pervaporation experiments performed using wet membranes in a solution of 10 wt% of ethanol in water with an interval of 130 h, T=30 °C and downstream pressure 1.0 mbar. From these results it is clear that the hybrid polysaccharide membrane is not stable during repeated use. After 7 h, the membrane selectivity decreases drastically (from 570 to 182) and, after that, the membrane continues losing selectivity [2].

For gas dehydration, in order to simulate real industrial applications, a N₂/CO₂ mixed gas with a proportion of 80/20 v/v% and a CH₄/CO₂ mixed gas with a proportion of 70/30 v/v% were prepared to mimic dehydration of flue gas and biogas, respectively. The experiments were carried out under constant humidified conditions and monitored by mass spectrometry. On-line monitoring (MS) has proved to be an efficient tool allowing...
to obtain the composition of the permeate stream at one data point each second (or less, if required), making possible to perform real-time monitoring during the whole permeation process. From Error! Reference source not found., it is important to note that the hybrid polysaccharide membrane showed to be effective in flue gas and biogas dehydration. Moreover, this membrane presented high water selectivity for the two mixtures analyzed, and gas permeabilities were always below 3.0 barrer. The hybrid polysaccharide membranes showed that, in real situations, they have the ability to dehydrate mixtures, due to the low gas permeability characteristic of polysaccharides and also the introduction of inorganic particles in the polymer matrix by the sol-gel technique used, which increases the gas barrier properties of the polymer. In addition, for other relevant industrial dehydrations, such as natural gas (which presents 600-1200 ppm of water vapour, the hybrid polysaccharide membranes may have a high potential, with the advantage of not losing CH₄, due to the low permeability values of this gas. To analyze the stability of the membrane, the same membrane was operated during 20 consecutive experiments (during approximately 7 h each experiment, taking into account the purge and testing time-length), with pure and humidified gases. Afterwards, the pure gas permeation experiments of new membranes were compared with “used membranes” in consecutive experiments, after the membrane are exposed to water vapour. For CO₂ permeation there was no significant increase in the permeability value. In contrast, the N₂ and CH₄ transport behaviour was slightly different when using a fresh or a repeated used membrane. Still, it is worth mentioning that, despite increasing, the permeability values for these gases are always lower than 3.0 barrer. This means that the membrane maintains its gas barrier characteristics after long-term exposure to water vapour [3].

In conclusion, hybrid polysaccharide membranes, prepared from a low-cost substrate and developed by a sol-gel method, were evaluated for their potential use in dehydration processes. In ethanol dehydration by pervaporation the membranes exhibited high water selectivity values, similar to PERVAP® 4101, however they lost their stability when exposed to solutions of 10.0 wt. % water in ethanol. For gas dehydration, two relevant industrial dehydration processes were selected: flue gas and biogas dehydration. The FucoPol hybrid membranes developed revealed to be an excellent gas barrier to all gases studied with permeability values below 1.0 barrer and presented high selectivity for water vapour transport.

In close-to-real conditions, the hybrid polysaccharide membranes showed the ability to dehydrate gas mixtures (binary and ternary mixtures), with the advantage of not losing gases to the permeate stream, due to their low permeability for the gases studied (CO₂, CH₄ and N₂). This characteristic makes these membranes potential alternatives for other relevant dehydration processes in industry, such as natural gas and air dehydration.

Table 1. Stability of the hybrid polysaccharide membrane in three pervaporation consecutive experiments.

<table>
<thead>
<tr>
<th>Exp.</th>
<th>$P_v \times 10^{12}$ (mol/m.s.Pa)</th>
<th>$P_v \times 10^{13}$ (mol/m.s.Pa)</th>
<th>Selectivity (w-et)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (3 h)</td>
<td>7.7 ± 1.1</td>
<td>0.1(4) ± 0.0(2)</td>
<td>570 ± 117</td>
</tr>
<tr>
<td>2 (7 h)</td>
<td>9.7 ± 1.4</td>
<td>0.5(3) ± 0.0(8)</td>
<td>182 ± 37</td>
</tr>
<tr>
<td>3 (130 h)</td>
<td>14.7 ± 2.1</td>
<td>6.2 ± 0.9</td>
<td>24 ± 5</td>
</tr>
</tbody>
</table>

Table 2. Transport performance of the hybrid polysaccharide membrane for synthetic flue gas and biogas dehydration.

<table>
<thead>
<tr>
<th>Mixtures</th>
<th>Gas content (wt. %)</th>
<th>$P_{gas}$ (barrer)*</th>
<th>$P_{CO_2}$ (barrer)</th>
<th>$P_{H_2O}$ (barrer)</th>
<th>Sel (H₂O/CO₂)</th>
<th>Sel (H₂O/gas)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flue gas (N₂/CO₂)</td>
<td>0.0</td>
<td>1.6 ± 0.1</td>
<td>0.9 ± 0.0(5)</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.7</td>
<td>1.9 ± 0.0(3)</td>
<td>2.6 ± 0.1</td>
<td>565.0 ± 23.0</td>
<td>218.3 ± 11.6</td>
<td>294.4 ± 12.7</td>
</tr>
<tr>
<td>Biogas (CH₄/CO₂)</td>
<td>0.0</td>
<td>0.6 ± 0.0(3)</td>
<td>1.3 ± 0.1</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>0.8</td>
<td>0.4 ± 0.0(4)</td>
<td>2.0 ± 0.2</td>
<td>1766.3 ± 43.6</td>
<td>888.4 ± 76.9</td>
<td>4041.9 ± 344</td>
</tr>
</tbody>
</table>

*gas represents N₂ or CH₄, respectively for flue gas or biogas mixtures

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References

Production of biomaterial composed by natural polymers

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Introduction

Biopolymers are biodegradable polymers or copolymers generated from renewable raw materials that are obtained on a short time compared to fossil sources such as petroleum cause low impact on the environment. There are several advantages to these materials, such as being biodegradable, renewable, easy to produce, low cost, among others, and can be applied in the areas of health, cosmetics, packaging and food [1,2]. Fibroin is a light chain protein found in the silk filaments of cocoons of various insects. In Bombyx mori, its content ranges from 70 to 80%. The material its high mechanical strength and traction [3]. Glaconacetobacter xylinus is an obligate gram-negative aerobic rod-shaped bacterium belonging to the family Actobacteraceae. The microorganism excretes bacterial nanocellulose for its survival. Its chemical composition is similar to vegetable cellulose, but its fibers are organized in nanometric dimensions, which make the materials different [4]. Both fibroin and bacterial nanocellulose can be used as bio-curatives in their simple form or as composites for diverse medical applications and in the field of tissue engineering. It may also serve as drug delivery in some cases [5,6].

When the material is only composed of fibroin, it does not have good mechanical properties, but when a reinforcing material is added, this frame is modified and there is an improvement of the product. With this in mind, fibroin films with cut bacterial nanocellulose were developed in order to improve the mechanical properties of the material. However, the films did not have good mechanical structure [7].

For this reason, this study aimed to develop a biomaterial with fibroin and bacterial nanocellulose, since, it is highly applicable in chemical, cosmetics, food, medical, pharmaceutical fields (aiming at recovering cutaneous injuries), among others.

Material and Methods

For fibroin extraction, this is the protocol established by Rockwood et al. (2011), where Bombyx mori cocoons were used. For BC production, the Glaconacetobacter xylinus ATCC 53582 in Hestrin and Schramm broth (HS) was utilized as described in Jozala et al.(2014) and Ataide et al. (2017). The BC-FB films that were produced increased the ratio of BC in larger sizes (20 cm diameter), utilizing erlenmeyers, and FB. The BC-FB films were dried in laminar flow at room temperature for 48 h. The Fourier Transform Infrared Spectroscopy (FTIR) technique was performed on the Shimadzu IRAfinity-1S equipment, ranging from 4500 cm⁻¹ to 500 cm⁻¹. The test was performed in triplicate and the data were analyzed in the program Origin 7.0. The FTIR allows the identification of the compounds contained in the samples, confirming the presence of the material through the vibration of the atoms of the molecule upon receiving radiation, forming a graph with specific spectra. The microstructure of the films were monitored by scanning electron microscopy. The films were freeze-dried, and they were previously fractured in liquid N2 and fixed to the stub with double-sided adhesive tape. Microphotographs were taken using electron beams with energy and acceleration speeds of 50pA e 15kV. The samples were randomly scanned and photomicrographed at magnifications x5000.

Results and Discussion

The samples were dry at room temperature and formed translucent films as expected. In the freeze-dried samples, brittle structures were formed. The BC-FB films presented great appearance and formed translucent films. The infrared spectra of the analyzed samples presented the characteristic bands that prove the presence of fibroin and bacterial nanocellulose in the films, as indicated in the graphs.

When comparing the standard bacterial nanocellulose (control) with films containing fibroin, similar bands were identified in the range of 1250-1000/cm-1, proving the presence of bacterial nanocellulose in the characterized material. The presence of fibroin was confirmed by three characteristic spectra indicated in Figure 1. Each of them corresponds to the amide group, amide I (1700-1600 cm⁻¹) part of the C = O stretch, amide II (1600-1500 cm⁻¹) is given by the combination of N-H and C-H, and amide III (1300-1200 cm⁻¹) refers to N-C stretching and C = O flexing [6].

The spectra presented bands similar to the control of bacterial nanocellulose in the range of 1250-1000 cm⁻¹, but some unidentified bands were present. According to Amaral (2013), the main spectra that indicate the presence of bacterial nanocellulose are: 3399 cm⁻¹ (stretch O-H); 2988 cm⁻¹, (C-H...
stretch), 1653 cm⁻¹ (OH-deformation) and 1044 cm⁻¹ (CO-deformation).

* Fibroin Characteristical spectra
* Bacterial nanocellulose Characteristical spectra

CM- Bacterial Nanocellulose, FB-Fibroin

Figure 1. Results of FTIR Spectroscopy

The SEM analyses done at x5000 indicated that the BC and fibroin formed a structure with randomly distributed voids throughout the sample. (Figure 2). The structure resembles a bee hive, a similar structure was observed by Chen et al. (2017). However, Chen and collaborators, prepared silk fibroin and bacterial nanocellulose nanoribbon composite scaffolds utilizing a multi-staged freeze-drying method.

Figure 2. SEM analyses

Conclusion

It is possible to produce bacterial nanocellulose with fibroin in different concentrations utilizing simple assay, immersion contact. Both show great biocompatibility, considering their biodegradability, moldable characteristic and excellent mechanical and chemical properties, making them a viable option for different applications.

Acknowledgements

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References
Oral Session

MODELING, SYNTHESIS AND INTEGRATION OF CHEMICAL PROCESSES
Introduction

Urea-formaldehyde (UF) resins are one of the most widely used adhesives in modern wood-based panel industry. The good binding strength, low price, and low press time for full curing are the main reasons for their wide applications. There are also some limitations such as low moisture resistance and formaldehyde (F) emission during manufacturing, and service life. So, a better understanding of the mechanisms of the synthesis process is crucial to improve the performance of these resins. Nowadays, two industrial procedures are mainly used: one is alkaline-acid-alkaline three-step procedure, another one is alkaline-acid-alkaline two-step procedure. However upon, and after reviewing the literature, we realized that some issues related to the evaluation of synthesis process have not been studied: a comparison between different processes and specifically the assessment of resin evolution during the synthesis using different techniques seems to be limited [1]. Nevertheless, several methods have already used trying to understand the structure of the polymer formed during the synthesis process [2,3]. In this work, two resins were produced using the alkaline-acid process (three-step Resin A and two-step B). The synthesis of the resins was off-line monitored by $^{13}$C-NMR, GPC/SEC and HPLC. To assess the effect of the synthesis conditions particleboards (PBs) were prepared and analyzed according to European standards. The differences between the two synthesis and along the synthesis are notorious from the different characterization techniques. The study allows to better understand the structure of the polymer and to evaluate the two different synthesis processes.

Methods

The production of the resins was carried out in 2.5 L round bottom reactor, equipped with mechanical stirring and thermometer. The reactor was heated with a mantle and the temperature was controlled with a thermometer. Both resins were produced according to the alkaline-acid process (one three-step and another two-step) and two final F/U molar ratios were presented (1.10 and 1.25). The graphical abstract presents the main characteristics of the resins synthesized. The boards produced were tested according to the European standards for density (D) (EN 323), moisture content (MC) (EN 322), internal bond strength (IB) (EN 319) and thickness swelling (TS) (EN 317). The F content of all samples was determined according to the perforator method (EN 12460-5).

Results

The samples were collected at critical moments of the synthesis: end of methylolation stage; start, middle and end of condensation stage; start and end of the last U addition and one day after the synthesis.

Table 1. Identification of different stages during the synthesis of Resin A.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Reaction step</th>
<th>T (ºC)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>At the end of methylolation step</td>
<td>&lt;80</td>
<td>8.50-9.50</td>
</tr>
<tr>
<td>2</td>
<td>At the beginning of condensation step</td>
<td>&lt;80</td>
<td>4.00-6.00</td>
</tr>
<tr>
<td>3</td>
<td>During condensation step</td>
<td>&gt;80</td>
<td>4.00-6.00</td>
</tr>
<tr>
<td>4</td>
<td>At the end of condensation step</td>
<td>&gt;80</td>
<td>4.00-6.00</td>
</tr>
<tr>
<td>5</td>
<td>At the beginning of addition of last load of U</td>
<td>&lt;80</td>
<td>7.50-9.50</td>
</tr>
<tr>
<td>6</td>
<td>At the end of last load of U (RM 1.10)</td>
<td>&lt;80</td>
<td>7.50-9.50</td>
</tr>
<tr>
<td>7</td>
<td>At the end of last load of U (RM 1.25)</td>
<td>&lt;80</td>
<td>7.50-9.50</td>
</tr>
<tr>
<td>8</td>
<td>Day after (RM 1.10)</td>
<td>25</td>
<td>7.50-9.50</td>
</tr>
<tr>
<td>9</td>
<td>Day after (RM 1.25)</td>
<td>25</td>
<td>7.50-9.50</td>
</tr>
</tbody>
</table>

The GPC/SEC chromatograms presented in Figure 1 were obtained for the different samples from Resin A and were similar to those of Resin B (not presented here). The presence
of low and high molecular weight chains formed during the reaction is clearly evident.

Figure 1. Chromatograms of samples taken at different stages during the synthesis of Resin A.

The fraction of free U and methylolureas present in each stage was analyzed by HPLC and the corresponding chromatogram is presented in Figure 2. Three different elution times can be observed corresponding to: unreacted U (U – 5 min), MMU (MMU – between 6 and 7 min) and DMU (DMU – between 8 and 9 min). Similar chromatograms were obtained for Resin B (not presented here).

Figure 2. HPLC analysis taken at different stages during the synthesis of Resin A.

The influence of the synthesis process on the resins was also assessed by preparing PBs using the final resins with two different F/U ratios. For the same F/U ratio, the IB values are similar (Resin A 1.10 - 0.52 N mm² and Resin B 1.10 - 0.55 N mm², Resin A 1.25 - 0.71 N mm² and Resin B 1.25 - 0.66 N mm²) and higher than the minimum acceptable for panels type P2 (IB > 0.35 N mm²) according to EN 312. As regards F emission, the panels prepared using resins with F/U=1.10 are below the limit for class E1 (E1 < 8 mg/100 g oven dry board) (EN 13986+A1). However, the panels prepared using resins with F/U ratio of 1.25 are out of the limit allowed for E1.

![Figure 3. Ratio of methylene/methylol and methylene ether/methylene for different stages of Resin A and B determined by 13C-NMR.](image)

The ratios of methylene/methylol and methylene ether/methylene groups obtained from 13C-NMR are presented in Figure 3 for different stages of Resin A and B. From these ratios, it is possible to conclude that the methylene linkages are more abundant than the methylene linkages at the beginning of synthesis, but this feature is reversed at the end of condensation step.

Conclusions

This work describes two main synthesis processes for the production of UF resins. The GPC/SEC chromatograms present the same behavior for both resins. From HPLC, it is notorious the progress and the difference between chromatograms with the duration of the synthesis. However, the growth is also similar for both resins. As regards the 13C-NMR, some differences between the processes are observed. The results obtained using this technique corroborate the GPC/SEC analysis in what concerns the growth of the polymer. Resin A and Resin B present differences between the polymer in all synthesis.

As regards, the final product, the PBs produced show a difference on the IB depending on the F/U ratio which was expected. The F emission for the same F/U ratio is similar. With this work, it was possible to understand the impact of the two main synthesis process on resin properties, evaluated using different techniques and to measure the effect of these resins on the final product.

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References


From the traditional packed-bed reactor to the sorption-enhanced membrane reactor: a step towards H₂ production optimization through glycerol steam reforming

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Introduction

The production of H₂ through biodiesel-derived by-product glycerol steam reforming (GSR) has been a growing target of research during the last decade not only due to its potential to benefit the economic competitiveness of biodiesel, but also due to the environmental and processual advantages [1, 2]. The GSR consists of two major steps: decomposition of glycerol into syngas, Eq. 1, followed by water-gas shift (WGS) reaction (Eq. 2).

\[ C_3H_8O_3 \rightarrow 3CO + 4H_2 \quad (\Delta H_{298}^\circ = 251 \text{ kJ} \cdot \text{mol}^{-1}) \] (1)

\[ CO + H_2O \rightarrow H_2 + CO_2 \quad (\Delta H_{298}^\circ = -41 \text{ kJ} \cdot \text{mol}^{-1}) \] (2)

This process is described by the following global reaction (sum of Eq. 1 and Eq. 2):

\[ C_3H_8O_3 + 3H_2O \rightarrow 7H_2 + 3CO_2 \quad (\Delta H_{298}^\circ = 128 \text{ kJ} \cdot \text{mol}^{-1}) \] (3)

The GSR is normally accompanied by secondary reactions such as CO and/or CO₂ methanation, liquid compounds and coke formation [3], the last being associated to catalyst deactivation. The use of intensified reaction-separation process concepts with the aim of surpassing both thermodynamic and kinetic limitations has been considered for steam reforming reaction having CO₂ and H₂ as main products. The removal of CO₂ is normally carried out in a sorption-enhanced reactor – SER (combining the catalyst and a CO₂ sorbent) and the removal of H₂ in a membrane reactor – MR (by using a H₂-permeselective membrane) [1].

Experimental analysis of a combined sorption-enhanced and membrane reactor (see graphical abstract scheme) system has only been reported a few times [4] and up the authors knowledge it has never been reported for GSR. In this work, the GSR reaction was carried out in both SER and SEMR, where a Rh supported on alumina catalyst was combined with a commercial hydrotalcite, K-MG30, in the SER. A dense Pd-Ag membrane (25 wt.% silver with wall thickness of 100 μm, and 120 mm of length), in the SEMR. Since there are restrictions of materials compatibility of the CO₂ sorbent with the H₂-permeselective membrane, the best option for in situ CO₂ capture are hydrotalcites; both sorption and regeneration can be carried out at temperatures compatible with Pd-Ag membranes while maintaining reasonable sorption capacities and kinetics [1]. The obtained results were not only compared between the SER and SEMR, but also with the results obtained in a traditional reactor – TR.

Experimental

A 120 long stainless steel reactor, filled with an homogeneous mixture of 800 mg of Rh-based catalyst from Johnson Matthey and 1.6 g of K-MG30 hydrotalcite from Sasol, was used in the TR and SER. In the case of the SEMR, the membrane tube (120 mm long and 10 mm thick) was filled with the same mass of catalyst and CO₂ sorbent. The SEMR tests were performed at 673 K, 4.5 bar and a feed of 0.1 mL min⁻¹ (7.5 mL h⁻¹ g⁻¹) of a solution of glycerol (water to glycerol molar ratio of 19). Prior to the catalytic tests, the SEMR was heated to 673 K under N₂ atmosphere (80 mL N₂ min⁻¹) and membrane activation was carried out under approximately 5 bar of H₂ (200 mL N₂ min⁻¹) while the permeating H₂ was swept by 200 mL N₂ min⁻¹ of N₂. Atmospheric pressure was maintained in the permeate side. In general, the tests were performed with different time-on-stream (circa. 8, 16, 12 or 20 h). Moreover, oxidative regeneration of the catalyst, sorbent and membrane (performed after 4 h of reaction) was carried out under 2.5 mL N₂ min⁻¹ of reconstituted air diluted with 97.5 mL N₂ min⁻¹ of N₂ for 15 h and always at 673 K.

Results and Discussion

As for the TR, high deactivation due to coke formation was observed. The combination of GSR with oxidative regeneration (carbonizing coke) allowed reaching better performance compared to the case where no oxidative regeneration was carried out (data not shown).

A test consisting of 6 reaction periods of 2 h in the SER and 13 h in the TR was carried out, being that, in the case of SER, between each reaction stage the sorbent was regenerated with N₂;
While complete conversion of glycerol was observed during the whole test in the SER, a decrease started being observed at around 8 h in the TR. The post-breakthrough conversion into gaseous products started at around 50-55% and suffered an absolute decrease below 20% during the six 2 h reaction cycles in the SER. Even though similar post-breakthrough glycerol conversions into gaseous products were initially observed in the TR and SER, a more pronounced loss of activity over time was observed in the first. When comparing both SER and TR experiments, higher H2 yield and purity were obtained during the whole duration of the test with the hybrid SER.

A comparison between the SER and SEMR in terms of the yields of H2, CO2, CO and CH4 is established in Fig. 2. More H2 was produced in the SEMR than in the SER during both reaction cycles. Methanation inhibition and WGS enhancement occurred during the breakthrough and post-breakthrough using the SEMR. During the pre-breakthrough, maximum H2 yields of approximately 3.6 mol·mol−1 fed glycerol were attained for both cycles when using the Pd-Ag membrane, while the maximum H2 yields obtained in the SER during the pre- and breakthrough were around 1.6 mol·mol−1 fed glycerol (enhancement of 125%).

Figure 1. Comparison of the evolution of both total glycerol conversion and CO2 post-breakthrough glycerol conversion into gaseous products over time between the TR and SER. The vertical dashed lines represent the instants of sorbent regeneration with pure N2.

Figure 2. Comparison of the evolution of the yields of a) H2, b) CO2, c) CO and d) CH4 between the SER and SEMR. The vertical solid line represents the instants of oxidative regeneration.

Conclusions
Further enhancement of H2 production was obtained by combining both catalyst and CO2 sorbent with an H2-permselective membrane in a SEMR. The removal of both CO2 and H2 avoided the occurrence of methanation in the pre-breakthrough region, which allowed to reach higher H2 yields during that period comparatively to the SER. This allowed a simultaneous production of highly pure H2 in the retentate and ultra-pure H2 in the permeate. Moreover, since there was no CO in the pre-breakthrough, poisoning of the membrane due to its presence did not occur. Therefore, if the SEMR is applied in a multi column system (thinner membranes would be required) it would allow continuous operation in the pre-breakthrough region, while ultra-pure (depending on the thinner membranes selectivities) and highly pure H2 would be continuously obtained in the permeate and retentate streams, respectively, and at higher flow rates.

Acknowledgements
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References
Window-based feature methods for end-of-batch quality prediction

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Introduction

Batch processes are ubiquitous in modern industry and batch data analysis is an important task for improving process operation. In this context, feature-oriented methods are effective methodologies that have the key advantage of not requiring complex data preprocessing, such as synchronization. However, these methods lack time-resolution since features are extracted for the full duration of the batch or batch stage. Thus, in this work, we propose a class of methods based on features with time localization, called window-based features (WiF). The underlying idea consists in extracting features from small windows of data, thus enhancing the time-resolution capabilities of feature-oriented methods. Two simulated case studies are considered to test the proposed approach. In both cases, WiF presents similar or better prediction performance when compared to other benchmarks and also presents an improved ability to easily identify relevant process variables and locate important batch periods.

Methods

Feature-oriented methods are a rather recent class of methods employed for batch data analysis and, therefore, there are many opportunities for research to exploit their potential. As the method proposed in this work is a modification/extension of available feature-oriented methods, we first present a short description of two feature-oriented methods.

SPA [2] is based on the statistical moments of a given variable over the batch. Typically, the mean, variance, kurtosis, and skewness are computed for each variable over the batch or batch stage. The covariance between all process variables is also included as additional features.

TIME [3] is based on the wavelet transform and decomposes each variable in a set of detail and approximation coefficients at different decomposition scales. The median value of the squared detail coefficients at a given scale corresponds to the energy content at that scale, summarizing the main dynamic patterns observed in the data. Therefore, TIME maps the measured variables to a set of energy features that can be used for quality prediction.

The proposed window-based approach modifies the aforementioned SPA and TIME methods in order to increase their time-resolution. To achieve this goal, each variable is divided into contiguous intervals and features are computed for each window separately (see the Graphical Abstract). The number of intervals is defined based on process knowledge and may be fine-tuned by cross-validation or similar techniques. In our experience, the results do not vary greatly for different interval lengths (we have divided the batch length into 4 segments in all the case studies considered). Two newly proposed techniques are tested: window-based SPA (Wi-SPA) and window-based TIME (Wi-TIME). These features capture understanding can be achieved. This is particularly relevant for end-of-batch quality prediction since it can uncover periods that are critical for batch quality, helping practitioners to better control the batch operation at these periods.

In the next section, we briefly describe the proposed methods. Afterward, the case studies considered are presented and the comparison results discussed. Lastly, the main conclusions of this work are summarized.

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relevant characteristics in each window, which are later on used for model building.

Results
Two case studies are considered to test the proposed approaches. The first one considers a semi-batch reactor where an exothermic first-order reaction occurs. The reactor is equipped with a cooling jacket that maintains the reactor temperature at 25 °C. During process operation, 6 process variables are measured and 200 batches are available for model building. All batches are extended by repeating the last measurement until their duration is 608 time points. This ensures that each window has the same number of data points (since 4 segments are considered, each window has 152 samples). This simulated process is called Semiex, and the quality parameter to be predicted is the product concentration. The second case study is the well-known Pensim process where penicillin is produced. The reactor operates in a semi-batch mode and is equipped with both a temperature and pH controller. For this case study, 16 process variables are measured and 400 batches are available for model building. Similar to Semiex, all batches are extended to have 512 points by repeating the last measurement. The quality parameter of interest is the penicillin concentration at the end of the batch. For both case studies, partial least squares (PLS) models are built. These models use, as predictors, features obtained from the process variables and the response is the quality parameter of interest. The models are built using a randomly selected set of training batches (150 batches in Semiex and 300 batches in Pensim) and their performance is assessed in an independent test set (50 batches in Semiex and 100 batches in Pensim). The results are presented in Table 1, where one can note the improvements in prediction performance obtained from the proposed methods (Wi-SPA and Wi-TIME) compared to their standard versions. This stems from the ability of these methods to extract more detailed features and, although the models have a larger number of parameters, these features are more local and highlight information contained in shorter time periods.

Table 1. Prediction performance in terms of R^2 in a test set for both the Semiex and Pensim processes

<table>
<thead>
<tr>
<th>Methods</th>
<th>Semiex</th>
<th>Pensim</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPA</td>
<td>0.90</td>
<td>0.78</td>
</tr>
<tr>
<td>Wi-SPA</td>
<td>0.95</td>
<td>0.8</td>
</tr>
<tr>
<td>TIME</td>
<td>0.94</td>
<td>0.78</td>
</tr>
<tr>
<td>Wi-TIME</td>
<td>0.94</td>
<td>0.85</td>
</tr>
</tbody>
</table>

Besides prediction ability, another important advantage of these window-based approaches is the ability to identify important features. Fig. 1 presents the variable importance in projection (VIP) that conveys information regarding important predictors for the Semiex case study (only 3 variables are presented due to space constraints).

Analyzing Fig. 1, one can note that variable #2 is the most relevant variable and is especially important at the beginning of the batch (in windows 1 and 2). This fact is also verified for the other two variables, revealing that much of the batch quality is dictated at the onset of process operation. This is critical information for process operators that can now devise better control strategies for that time period. The vip for the Pensim case study (not shown due to space constraints) identifies variable #1 at all stages as the most important variable. Nevertheless, the other process variables reveal a pattern suggesting that they become more important at later stages of the batch. These results demonstrate the advantages of window-based methods. By assessing feature importance for the standard methods (SPA and TIME), the same variables would be identified. However, they are not able to provide information relative to the time periods where a particular feature is relevant.

Conclusions
In this work, we have proposed and tested variants of two feature-oriented methods for end-of-batch quality prediction: window-based statistical pattern analysis (Wi-SPA) and window-based translation-invariant multiscale energy-based (Wi-TIME) features. These methods are based on extracting features for short time intervals, which are later utilized for model building. This extension enhances the time-resolution capabilities of these methods, allowing the identification of both important variables and periods that are critical to quality. Two case studies were considered: a simple semi-batch reactor and the well-known Pensim simulator. The results demonstrated that the proposed modification is effective and provides good/better prediction performance as well as improved process knowledge by identifying important periods.

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References
Nitration process intensification

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The liquid-liquid nitration of benzene to mononitrobenzene (MNB) still faces restriction environmental regulations related to the formation of by-products such as nitrophenols (NP) and dinitrobenzenes (DNB). Microreactors have proven their potential to conduct hazardous and/or multiphasic reactions limited by mass and heat transfer with high-performance results. Benzene nitration was carried out in a Corning® Low-Flow Reactor (LFR) microreactor to study the influence of the main operating variables on conversion and by-products formation. The most influenced were the initial composition of the aqueous phase in sulfuric (SA) and nitric (NA) acids and the reactor outlet temperature (Tout). The use of a strongly acidic media, higher operating temperature, an intermediate residence time (τ) and a lower stoichiometric excess of benzene to HNO3 (F_Bz/F_HNO3) promoted high conversion (< 99.9 %). Lower NP and similar or higher DNB concentrations were achieved, being most of them lower than 1000 ppm.

Introduction

Process Intensification (PI) was first described as “devising an exceedingly compact plant which reduces both the ‘main plant item’ and the installation’s costs” [1]. Since then this vision has undergone evolution widening its scope to include chain efficiency, wastes reduction, process safety, and more. The potential of PI has been recognized by the chemical industry, even though known industrial applications are fewer than the reports on equipment development and assessment at bench or pilot scale. Microreactors (MR) are among the PI promising technologies.

The MR compact inner geometry, with channels or orifices between 0.1 - 1000 µm, reduces the paths for heat transport and molecular diffusion leading to an increase in the rate of these phenomena and thus lower reaction times (up to 1 order of magnitude). The small inner volume and the improved heat and mass transfer allow a better control of the process. As a result, process yield and selectivity may be increased using extreme operating conditions at a low risk. MR are particularly suitable to handle multiphase systems [2], as the interface area between phases may be up to 3 orders of magnitude higher than in the conventional reactors.

Liquid-liquid (L-L) nitration reactions of organic compounds are among the best candidates for PI using MR [3]. One example is benzene (Bz) nitration to yield mononitrobenzene using mixed acid (an aqueous mixture of nitric and sulfuric acids), where H2SO4 acts as catalyst and heat sink. A major concern in the industrial process is the formation of by-products, such as, dinitrobenzene isomers and nitrophenols (namely 2- and 4-mononitrophenol (MNP), 2,4- and 2,6-dinitrophenol (DNP), and trinitrophenol (TNP)), mainly due to environmental impact.

The main goal of this work was the experimental assessment of microreactors, and PI, on by-products formation during benzene nitration. The study was carried out in a MR Low-Flow Reactor (LFR) from Advanced-Flow™ Reactors of Corning®. The LFR numbers up glass modules, in series, to increase residence time, integrating two independent circuits, one for the reacting mixture (1.6 ml) and one external circuit (20 ml) where the heat transfer fluid flows. In the internal circuit, mixing intensification is achieved by separation and recombination of the phases in mixture elements that intercalate a microchannel with an internal diameter of 550 µm. To assess the performance of the LFR the operating conditions were selected according to industrial practice. Previous studies [5-6] pointed out the most relevant operating parameters to improve selectivity of this process and these were further investigated in this study.

Methods

Benzene (≥ 99.7 wt%, Riedel-de Haën) and a mixed acid solution previously prepared with H2SO4 (95-97 wt%, Scharlau), HNO3 (≥ 65% wt%, Panreac) and pure water (Interlab.UV-10 system) were the organic and aqueous phases. The setup consisted in a Corning® microreactor LFR with four modules in series, a thermostatic bath CC-K6 (Huber) and a 505U pump (Watson Marlow) for heating and circulating the heat transfer fluid (HC-50 from Dynalene) in counter current flow. The inlet and outlet temperatures of the heating fluid were measured with type K thermocouples. Benzene and preheated mixed acid were fed separately making use of two REGLO-CPF digital pumps from ISMATEC®. Temperature and pressure block sensors from Mikroglas (up to 110 °C and 6 bar) were used to measure the operating conditions of the reactor inlet and outlet streams. Data acquisition was done with a system from Mikroglas using LabVIEW™ 2016 software (version 16.012).

The operation of the experimental setup was monitored in real time. Benzene and mixed acid reservoirs were weighed (analytic balance AS220/S/2 from RADWAG, ± 0.0001 g). Before starting reaction, the experimental setup was warmed up by circulating the pre-heated mixed acid. After reaching a stable temperature, the mixed acid flow was stopped and then benzene was fed at the selected flow rate followed by a gradual increase of the mixed acid flow up to the desired conditions. After attaining steady state, samples of the reacting mixture were collected and quenched to stop the reaction. After settling, the two phases were separated, weighed and the samples were stored for analysis. At the end of the experiment, reagents reservoirs and effluent flasks were weighed again.
Total acidity and nitric acid content in aqueous phase were accessed by titration with NaOH (1.00 M) and a FeSO₄ solution (0.25 M) in a 736 GP and 794 Basic titrators from Metrohm, respectively [4]. The NP content in the organic phase was determined in an Elite LaChrom HPLC from VWR Hitachi and the other organic compounds concentration was measured in a 7890A GC-FID from Agilent Technologies [4].

The main operating variables were: the inlet aqueous phase composition (weight fractions of sulfuric acid, $W_{SA,I}$, and nitric acid, $W_{NA,I}$), the molar ratio between benzene and nitric acid ($F_{BZ}/F_{NA}$), the residence time ($\tau$ = [0.17-1.04] min, limited by the total flow rate $Q_I$ range of LFR) and the product outlet temperature ($T_{out}$). The operating variables tested ranges were: $50 < W_{SA,I} < 70$ wt%; $1.75 \leq W_{NA,I} < 4.55$ wt%; $1.04 < F_{BZ}/F_{NA} < 3.15$; $2 \leq Q_I < 10$ ml/min; $50 < T_{out} < 110$ °C.

### Results

The runs carried out to evaluate the performance of the LFR microreactor followed a design of experiments which accounted the main effects and the second order interactions of the main factors $W_{SA,I}, W_{NA,I}, F_{BZ}/F_{NA}, Q_I$ and $T_{out}$. The relationships between each operating variable and the production of MNB and formation of by-products (DNB and NP) are shown in the Graphical (where red lines represent density ellipses covering 90 % of the data. In this scatterplot (obtained with software JMP® Pro 13.2.1), the influence of aqueous phase initial composition and of outlet temperature ($T_{out}$) in the distribution of all reaction products is quite clear, mainly for H$_2$SO$_4$. An increase in molar ratio $F_{BZ}/F_{NA}$ can be relevant in by-products formation, namely DNB. The residence time did not have a clear association with products concentration. Higher MNB concentrations with lower by-products formation (DNB < 1000 ppm; mostly, NP < 1000 ppm) were reached with a concentrated mixed acid, higher operating temperature, lower molar excess of BZ, and an intermediate residence time.

The scatterplots (graphical abstract) are informative but oblivious to interactions between variables, which may stand out from the data summarized in Table 1. The highlighted results for run-d were very satisfactory with 98.8 % conversion of HNO$_3$ ($X_{NA}$), high yield (MNB/(MNB + DNB + NP)), a good selectivity (MNB/(DNB + NP)) and low by-products formation. In run-d the concentration of DNB is lower than in similar studies (0 < DNB < 1500 ppm [5]) although NP are in the same range 10 - 980 ppm [5], despite the much higher molar ratio $F_{BZ}/F_{NA}$ and much lower residence time in this work ($F_{BZ}/F_{NA}$ ≤ 1.023; $\tau$ = [1.47; 2.95] min [5]). Other data in Table 1 indicate that DNB formation can be reduced with higher results for yield and selectivity (run-i), and conversion (run-b), but with an increase of NP concentration. The distribution of NP reveals that TNP formation is systematically low and that DNP concentrations are higher for high conversions (run-b). Low by-products concentration (run-c) was attained with a concentrated mixed acid, an intermediate $F_{BZ}/F_{NA}$, a low residence time and a high $T_{out}$. A $W_{SA,I} < 61$ wt% is not recommended (run-a) – reaction rate decreases and higher residence times are needed. Additionally, high conversion with lower $W_{NA,I}$ is rather to be used with intermediate residence time and $F_{BZ}/F_{NA}$, and a higher $T_{out}$ (run-e versus run-f).

### Conclusion

The intensification of benzene nitration using a Corning® LFR was studied under different operating conditions in a range representative of the industrial process. A high-performance was attained for complete conversion of HNO$_3$ (98.8 %), with 99.8 % yield and good selectivity, with 726 ppm of DNB and 970 ppm of NP. It was also observed that strongly acidic media, higher operating temperature, intermediate residence time and a lower stoichiometric excess of benzene can benefit this reaction providing high conversion and low by-products formation, compared to similar studies reported in the literature. The use of LFR for MNB production can reduce significantly the NP (mostly, < 1000 ppm) with similar or higher DNB formation (< 1000 ppm) with low residence times (< 1.04 min).

### Table 1. Results for benzene nitration in LFR microreactor ($u_{total}$ = [323-1944] cm/min; $P_i$ = 1.0-4.5 [ bar].

<table>
<thead>
<tr>
<th>Run</th>
<th>$W_{SA,I}$ (wt%)</th>
<th>$W_{NA,I}$ (wt%)</th>
<th>$F_{BZ}/F_{NA}$</th>
<th>$\tau$ (min)</th>
<th>$T_{out}$ (°C)</th>
<th>$X_{NA}$ (%)</th>
<th>Yield (%)</th>
<th>Selectivity ($\times 10^{-4}$)</th>
<th>By-products (ppm)</th>
<th>NP distribution (wt%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>61</td>
<td>3</td>
<td>2.6</td>
<td>1.04</td>
<td>93</td>
<td>81.5</td>
<td>99.8</td>
<td>5.56</td>
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<tr>
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<tr>
<td>c</td>
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<tr>
<td>d</td>
<td>68</td>
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<td>0.89</td>
<td>96</td>
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<tr>
<td>e</td>
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<td>17.3</td>
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<tr>
<td>f</td>
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<td>0.75</td>
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</tr>
</tbody>
</table>

### Acknowledgements

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### References


From CUF to Bondalti

In more than a century and a half, CUF has gone from making soap to nanotechnology. In 2018, the time has come to assume a differentiated identity, a new brand: Bondalti. The new Bondalti brand, which succeeds the historic CUF brand (chemical industry business area of the José de Mello Group), is the culmination of a strategic repositioning process, which now enters a phase of more growth, more ambition, more innovation and, most of all, more internationalization. The new brand translates a business strategy more geared towards the global market, which is made of change, innovation and challenges. The new brand is one of the variables in the process of business transformation and growth and assumes the commitment on strengthening the company’s status as a reference partner in the international chemical industry.

The strategic repositioning of the company allows Bondalti to reinforce its status as the main non-integrated European producer of aniline and nitrobenzene and to create the conditions for a leadership position in the chlorine market, which has also been reflected in the recent acquisition of a manufacturing unit in the Spanish region of Cantabria.

R&D+i at Bondalti

Research, Development and Innovation (R&D+i) activities in the chemical sector are the main driving force for development and competitiveness. In this sense, Bondalti systematically promotes an atmosphere of innovation across the entire organization.

Innovation at Bondalti is based on integration and effective working of the processes comprising the R&D+i Management System, and these include managing Interfaces, Business Intelligence, Intellectual Property, R&D+i Projects, Ideas and Knowledge. The R&D+i Management System (NP 4457:2007) is certified since 2013 at Bondalti.

Some of the input and output indicators of Bondalti’s R&D+i activity for 2017, are summarized in Table 1.

Table 1. R&D+i main inputs and outputs (2017).

| Employees involved in R&D+i activities | 26% |
| PhD or Post-Doctorate fellows | 13 |
| R&D+i projects | 68 |
| R&D+i partnerships | 36 |
| Patent Families | 12 |
| Scientific publications/external presentations | 25 |
Since 2006 PRIO distributes and commercializes liquid fuels and LPG, having a tank terminal located in Aveiro (for stockage and independent primary logistics) as well as its own biodiesel factory. It is 100% funded by Portuguese capital and is the only Iberian gas company with the triple certification for quality, safety and environment. PRIO’s network includes over 200 fuel stations across Portugal. With a continuous focus on more sustainable options for mobility, PRIO has been sharing its challenges with the scientific community along the years. More recently, PRIO has launched JUMP Start, an open innovation programme especially designed for startups. In PRIO we believe that a more sustainable mobility is possible, and that open innovation is the quickest way to achieve it.

In the biofuels’ business area, the main challenges that PRIO addresses today are related to more sustainable raw materials, such as used cooking oils; other biofuels from biomass and other ways of reducing the carbon footprint of the product and logistics process.

Within our gas stations’ business area, PRIO faces different challenges, such as electrification of transport, the energetic mix for the mobility of the future and the customer relation digitalization.

In PRIO we believe that a more sustainable mobility is possible, and that open innovation is the quickest way to achieve it.
AMS-1B is a borosilicate molecular sieve that has an MFI framework topology like Mobil’s ZSM-5 aluminosilicate sieve. Unsupported AMS-1B is not active. It becomes active when placed in an alumina matrix. Mobil claimed that activation takes place by aluminum from the matrix replacing boron in the sieve framework. Aluminum in the framework of ZSM-5 gives rise to a band in IR spectra at 3610 cm\(^{-1}\) associated with an Si-OH-Al bridging hydroxy, which is the active acid site in ZSM-5. As far back as the 1980’s we showed that this band is not observed in active AMSAC catalysts. While, IR indicated that the active AI species in AMSAC catalysts is different than that in ZSM-5, it did not indicate what it is. That is a problem that bothered me for 30 years. Thirty years later I received a grant from BP to work with Dr. Luis Mafra at the University of Aveiro to revisit the problem with modern Solid State NMR techniques, which only became available very recently. These include cross polarization techniques that can probe interactions between Al or B and neighboring atoms. I will describe results from our efforts which led to two publications in the Journal of Catalysis.

I retired from industry in 2017 after 35 years at BP Amoco Chemical Company R&D. My years were spent mainly on paraxylene (pX) catalyst and process R&D. pX is the hydrocarbon intermediate that is oxidized and esterified to make polyester, which is used to make clothing, film, and plastic bottles. I worked on all aspects of pX manufacture. I am a chemical engineer, and as such did reactor modeling, chemical plant simulation, design, and economics. I was on the design team for the BP pX unit at Geel, Belgium, and the recently commissioned pX unit at the Reliance export refinery in Jamnagar, India, based on BP pX crystallization technology and licensed through a partnership between BP and Lummus (CBI). This is the largest pX unit in the world. I spent 5 months in India in 2016 transferring the technology and starting up that unit.

While an engineer per my degrees, my passion has always been more chemistry related, i.e., catalyst development and characterization, which was the subject of my Ph.D. dissertation. When I first joined Amoco in the 1980’s, I was on a team characterizing Amoco’s AMS-1B Molecular Sieve on Alumina (AMSAC) pX catalysts in defense against a law suit from Mobil. AMS-1B is a borosilicate molecular sieve that has an MFI framework topology like Mobil’s ZSM-5 aluminosilicate sieve. The unsupported AMS-1B borosilicate is not active. It becomes active when placed in an alumina matrix. Mobil claimed that activation takes place by aluminum from the matrix replacing boron in the sieve framework. Aluminum in the framework of ZSM-5 gives rise to a band in IR spectra at 3610 cm\(^{-1}\) associated with an Si-OH-Al bridging hydroxy, which is the active acid site in ZSM-5. As far back as the 1980’s we showed that this band is not observed in active AMS-1B on alumina catalysts.

While, IR indicated that the active AI species in AMSAC catalysts is not the same as that in ZSM-5, it did not indicate what it is, which left me unsatisfied. In the ‘80’s, we applied the rudimentary Solid State (SS) NMR techniques available at the time to look for the active AI species. We observed tetrahedral Al, which Mobil claimed but did not prove was in the framework in conflict with the IR data. Thirty years later I obtained a grant from BP to work with Dr. Luis Mafra at the University of Aveiro to revisit the problem with modern SS NMR techniques, some of which have been developed very recently. These new techniques can probe interactions between AI, B, and neighboring atoms, something that could not be done in the ‘80’s. This was one of my final projects at BP, a return to my passion: catalyst characterization.

A successful University-Industry project should provide a win-win for both sides. For BP, it was the ability to access expensive NMR equipment and an expert in SS NMR that it did not have in-house. To get our academic partner interested in the project, we knew we had to be able to carve out some project material that could be published, while protecting sensitive information about an important proprietary BP catalyst. We were able to do this by focusing part of the research on the nature of the unsupported AMS-1B sieve, which led to two publications in the Journal of Catalysis. Sensitive information that we learned about the nature of the AI species in active AMSAC catalysts remains unpublished. Of course, the other thing that industry can bring is financial support. This can multiply as the success of Industry-University cooperation can lead to success in securing new government grants, leveraging our industrial support.

Since retiring, I have been invited to Aveiro to work with Dr. Mafra on unrelated catalyst research projects. I will also pass some of my industrial experience to students by teaching a course on refining and petrochemicals this fall and lecturing on other subjects such as biofuels.

Acknowledgements
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P.L.C. We thank the support from the Univ. of Aveiro and CICECO-Aveiro Institute of Materials (Ref. FCT UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when applicable co-financed by FEDER under the PT2020 Partnership Agreement. The Portuguese NMR Network (RNRMN) is also acknowledged for financial support. We also acknowledge the Portuguese NMR Network (RNRMN) for funding. LM greatly thanks FCT for the awarded development grant (IF/01401/2013) and R&D research project PTDC/QEQQAN/6373/2014.
Oral Session
MULTISCALE AND MULTIDISCIPLINARY ENGINEERING EDUCATION
Introduction to Systems Thinking

These materials aim to familiarize the learner with the basic methodology of creative problem solving using the systems approach such as the “Systems CUBE” methodology which is made up of a $[3,3]$ Matrix of the steps, phases and life-cycles together with the places of the individual activities taken in the execution of an engineering project; see for example, Sage and Armstrong [2].

Each life-cycle (R&D, Planning and Marketing, Acquisition and Procurement) is executed through the combination of phases (definition, development and deployment) each comprising the steps (formulation, analysis and interpretation). Within the matrix of activity schedule, activities 1-3 for example represent the three steps of the first phase (definition) whilst 6-9 represent the steps within the last phase (deployment) of each of the life-cycles; see Figure 1. Here, the order within which the life-cycles are considered depends on the type of engineering project being executed based on the principle of “reducing the possibility envelope”. The learner is expected to demonstrate the systematic process of “narrowing down” of the options and choices through a decision-making process starting off with the lead activity life-cycle which defines the outermost boundary of the “possibility envelope”. For example, in assessing the potential market share of a novel product that could help to consolidate a company’s position against the competitors, the lead activity life-cycle is the “planning and marketing” used to scope the possible options to maximize sales whilst maintaining and rewarding consumer loyalty. Decisions and activities related to R&D and Acquisition, Procurement and Manufacturing life-cycles will then follow to define the final shape of the possibility envelope.

The scope of possible actions is then reduced successively by the introduction and consideration of all other life-cycles with the metrics and tools available in the decision-making process being introduced to progress along the decision pathway which integrate the considerations made under each of the life-cycles.

Assessment of Risk and Risk Management

Concepts of risk and risk management strategies are introduced in activities involving definition, development and deployment phases of an engineering project. For example, specification of the mutual issues involving both stake-holder and share-holder interests such as plant location, access to public utilities, environmental impacts could introduce delays in the implementation of the deployment phase. Early marketing information from the potential customer base could reveal risks associated with potential sales not previously accounted for by planning and prototype design. Assessment of risks associated with customer preferences impacting on likely sales and on the extension of the lead-time to market penetration are aspects considered under risk management for chemical product design; see for example, Cussler and Moggridge [3]. For the pharmaceutical and foodstuffs sectors, mandatory field trials of new products for any potentially hazardous side effects also constitute essential parts of the risk assessment and management exercise. Compliance assessment with environmental and employment legislation involving Hazards and Operability studies are also introduced during the project development and deployment phases. The systems engineer will broker information from all the interested parties in each of these management protocols of the organizational activities of the engineering project. Design heuristics and simple graphical tools that allow the determination of systems performance targets are covered first followed by design integration strategies involving grassroots as well as retrofit design scenarios. Two case studies are used to illustrate the environmental design integration to abate and eliminate Atmospheric Greenhouse Gas Emissions and integrated Industrial Water Systems Design to illustrate environmental pollution reduction/elimination through re-use, re-cycle and regeneration strategies. Here the learner is expected to produce the respective information flow diagrams for each of the functional systems configurations marking out the primary (feed-forward), secondary (iterative) and cascading (cumulative) information flows within the process flow sheet hierarchy integrating the environmental impact considerations for different scenarios. These concepts could further be developed numerically to facilitate their use within commercial process chemical process optimization software.

Sustainability Design Integration

Examples of the metrics that can be used include the methodology to establish PURPOSE, FUNCTION and STRUCTURE with a range of system management and support tools incorporating SURVEY, DESIGN and CASE related software, computer modeling, resource and personnel
scenarios, feed the information iteratively to the project executive and co-ordinate feedback and cascading flows of secondary information which will result in corrective actions to minimize risks to the success of an engineering project. These activities, whilst largely technical, define the systems engineer’s role in risk management.

Micro-Macro Focus on Systems Integration
The above approach is also extended to integrate the micro (molecules, unit operations, processes) to the macro (industrial sites, value chains, regions and the globe) by way of an ecological systems framework encapsulating three main categories of social, economical and environmental impact applications: i) Resource Efficiency and Chemical Manufacturing and Supply Chains, ii) Implementation of Green Technology Strategies in SME’s and the concept of cleaner production, iii) By-Product Synergy Networks: Driving Innovation through waste reduction and carbon mitigation. The current challenge is to extend the micro-formulation of the systems thinking described above to the integrated and interactive macro systems through innovation steps and phases that connect and protect the triple bottom line: planet, people and prosperity also referred to as the three P’s; see [4].

Acknowledgements
The teaching framework introduced and discussed in this paper has been developed and delivered as part of 4th year Chemical and Process Engineering MEng program at the University of Surrey, UK (2006-2010) and some of the contents have been used further as part of the 4th year MEng program teaching in Chemical Engineering at the Department of Engineering Science, University of Oxford, UK (2013-2015).

References
Virtual labs: Tools to enhance students autonomy in Chemical Engineering education

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Engineered educators have been driven to develop different approaches to supplement scientific background and further develop the capacity for autonomous and critical thinking in students. In 2009, the University of Coimbra has made available on-line a virtual platform with a wide scope, directed towards Chemical Engineering education. The platform is divided into four different educational topics: Unit Operations and Separations, Chemical Reaction, Process Systems Engineering and Biological Processes. These sections include simulators, applications and case studies to help understanding chemical/biochemical processes. The ultimate objective was to help students in their autonomous study and, thereafter, in developing their capacity of autonomy. This paper presents an assessment of the use of that platform by two different groups of students: a group from the 3rd year of the 1st cycle of Chemical Engineering, and another one from a Project Design course (2nd cycle, MSc of Chemical Engineering).

Introduction
The continuous development and spreading of information technologies, created opportunities for educators to expand teaching methodologies and learning environments outside the traditional classrooms, and to enhance students’ learning outcomes. With the improvement of communication technologies and the sophistication of web tools, it became reliable to remotely operate laboratories or simulators [1,2], stream instructional multimedia [3], establish comprehensive virtual platforms to provide content using content management systems (CMS) [4] or to administrate courses using learning management systems [5].

Recently, the increasing computing and graphics processing power, made possible the development of more complex environments for process simulation [6], serious games [7,8] and 3D learning environments [9], with deeper levels of immersion that provide more realistic experiences.

For the educators, the inclusion of these information technologies in their teaching methods can pose some challenges too, especially the concern with a careful design, oriented by pedagogical practices, to better anticipate the students’ possible needs, expectations, and engagement, considering the specific educational goals of the curriculum.

Throughout Europe, engineering education was transformed by the Bologna process, which led to a reduction of lecturing hours, allowing the students more time to enhance their critical thinking, self-regulated learning, and teamwork. With fewer lecturing hours, web-based tools assume a greater importance for educators to support didactic activities that help the students’ learning and, consequently, also contribute and favour their autonomy.

Within this context, in 2009 it was launched a virtual platform called LABVIRTUAL (http://labvirtual.eq.uc.pt), as the result of a joint collaboration between the Chemical Engineering departments of both universities of Coimbra and Porto. It is a broad scope platform dedicated to the teaching of chemical processes, covering several subjects associated with unit operations, chemical reaction, biological processes and process systems engineering. LABVIRTUAL uses a synergy of pedagogical methods (e.g., fundamentals, case studies, simulators, virtual laboratories), which, in close connection with classroom lectures, provide to the students a better understanding of the different topics they have to tackle during their education and thus increase their engagement.

In this presentation, we will describe LABVIRTUAL with an emphasis on the pedagogical methods used in the platform. Also, results of an assessment directed to Chemical Engineering students from the University of Coimbra, enrolled in the disciplines of Separation Processes and Project Design, respectively from the 3rd and 5th years of the Chemical Engineering Master’s degree, from that same university, will be presented, in order to better understand how the students are using the virtual platform and if it is contributing to increase their autonomy and critical thinking.

Description of the platform
The structure of LABVIRTUAL is presented in Figure 1. The platform is constituted by four main components: chemical processes, dissemination and outreach, pedagogical methods and web application framework. The Chemical Processes section includes subjects encompassing unit operations, chemical reaction engineering, biological processes and process systems engineering, that are essential in any Chemical Engineering curriculum. Regarding pedagogical methods they were designed to fulfill the instructional objectives of the courses related to the contents of the platform. These may be fundamental concepts, models and applications, simulators, case studies, virtual laboratories or interactive videos. All these components are glued together by a web application framework that displays the materials through a graphical user interface (GUI) and sets the communication between the GUI and the computational platform, where all web services are handled and the mathematical calculations performed.

![Figure 1. Scheme of the virtual platform LABVIRTUAL.](image-url)

As aforementioned, there are computer simulators also available in the platform, programmed according to the context in which the subjects are taught in the classrooms, aiming at a good...
balance between fundamental topics and applications. Each simulator was designed with the flexibility needed to enable the students to explore various operational conditions, being complemented by a description of its capabilities and the mathematical models herein contained. The computer codes of the simulators are open to the students that are challenged during the classes with team/individual projects to modify or create new programs, stimulating their teamwork, computational and creative thinking skills. Additionally, several case studies are provided to guide the students in the use of the simulators, through worked examples, which demonstrate how the fundamentals, mathematical models, and computer applications can be used to solve real-world problems. This way, the students can appreciate better the value of the knowledge he/she should assimilate, an important factor to promote their self-regulated/autonomous learning [10].

Virtual experiments is an area of the website that approaches some subjects within Chemical Processes from an experimental viewpoint, to support the students in their laboratory classes. Each experiment includes a demonstration video, sometimes an interactive video, together with the theoretical background, a description of the experimental setup, and the corresponding experimental procedures. Nevertheless, a careful selection of contents and the strategy of presentation is required to match the user’s needs, delivering the right medium, at the right time, to ensure relevant and self-contained contents.

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References


Final remarks

This work focused on the pedagogical methods in LABVIRTUAL, a virtual platform oriented to the teaching of Chemical Engineering subjects. The web platform contains fundamentals, case studies, simulators, virtual laboratories and interactive videos related to the main topics in a Chemical Engineering curriculum: unit operations; chemical reaction; biological processes; and process systems engineering.

The results of questionnaires presented to students in two disciplines of the Masters course in Chemical Engineering degree at the University of Coimbra show that about 60% of them used LABVIRTUAL often or very often, mainly to perform equipment sizing, support their self-regulated/autonomous study, and simulate chemical processes. Furthermore, the survey revealed that the students score LABVIRTUAL as friendly to use and a valuable learning tool, which we believe is due to the synergy of pedagogical tools existing in the platform, complementing well the face-to-face classroom lectures.

Thinking about possible ways of enhancing still further the use of the platform by the students, one possibility is rewarding the students that make use of the platform capacities to solve design problems or others. This strategy is already followed in the Separation Processes course referred to in this work, even if those tasks are not compulsory.

Finally, we stress that LABVIRTUAL is freely available online and has been having a continuous high number of visits worldwide, especially from Portuguese-speaking countries.
Understanding the effect of oxygen on microbial growth – teaching bioprocess engineering to biotechnology students

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The study of metabolic behavior of cell cultures along with the operation of bioreactors is of extremely importance for students of Biotechnology and Chemical Engineering. The objective of this lab work was to consolidate the theoretical concepts acquired by students, and understand how bacterial growth, nutrients consumption and oxygen uptake can be correlated in biological systems. A batch bioreactor with controlled temperature, agitation and aeration was used to grow a culture of *Saccharomyces cerevisiae*. The measurement of oxygen concentration was carried out in a reactor connected to a respirometer in which was introduced an oxygen sensor. This work required around 8 h and ran continuously throughout a day. Students divided their tasks and shared their experimental results with colleagues for further analysis and data processing to determine kinetic and stoichiometric parameters. The balance of this practical work was very positive, since students apprehended the link between the metabolic functions and process variables, as well as they learnt methods of calculation and organization of information, in addition to acquisition of team work skills.

Introduction
Bioprocess engineering demands for practical courses where students can actively correlate biology and engineering. This requires a carefully shaped program to ensure that students are not overwhelmed by the complex content of the area; especially those belong to a non-engineering field as students of BSc. in Biotechnology of University of Aveiro. A practical laboratory work that follows the growth of *Saccharomyces cerevisiae* was developed as part of the curriculum of Microbial Biotechnology of 3rd year of BSc. Biotechnology. This work also requires knowledge from other two subjects also related to Bioprocess Engineering: Introduction to Process Engineering (2nd year) and Biochemical Engineering 1 (3rd year). The general concept of the experiment performed was adopted, modified and improved to be performed on a smaller laboratory-scale, and during the time predicted for two practical classes (4h+4h). Due to time limitation, the preparation of the seed culture and the setup and installation of the bioreactor are already done and students only prepare the medium, inoculate the reactor, conduct and control the experiment, perform online analysis of oxygen consumption, and take samples for offline analysis (pH, biomass, and sugars). Despite the simplicity of the setup of the bioreactor (Figure 1), this work provides an extensive view on the principles of bioprocess engineering, since students will be able to determine kinetic and stoichiometric parameters of the process with the experimental data obtained. In general, literature on experiments in bioreactors designed and evaluated specifically for application in teaching is scarce [1]. Therefore, the purpose of this work is to show that a simple setup and protocol allows the practical teaching and students deepen understanding of Bioprocess Engineering. The lab course as presented in this study is not limited or restricted to this particular process and can be adapted to other biological processes.

Methods
A batch bioreactor (670 mL) with controlled temperature (29°C), agitation (200 rpm) and aeration (0.5vvm) was used to grow a pure culture of yeast *Saccharomyces cerevisiae*. The measurement of oxygen concentration was carried out in a smaller vessel (45 mL) called respirometer in which an oxygen sensor was introduced horizontally in order to avoid air spaces inside. The reactor and the respirometer are connected and the reactional mixture is recirculated between them by a peristaltic pump working at its maximum capacity. The growth medium is composed yeast extract (6.0 g/L) and glucose (3.0 g/L) diluted in tap water. The oxygen concentration throughout the test was read along the operational time and, simultaneously, samples were taken to quantify cell and sugar concentrations. Optical density at 650 nm was measured after appropriate dilution with medium and converted to dry weight concentration using a calibration curve determined by students in a previous class. The consumption of glucose was determined by the method of DNS for reducing sugars [2]. Values of biomass and glucose concentration were used to determine parameters as specific growth rate (μ), growth yield (Yx/s), and the parameters of Monod equation, the maximum specific growth rate (μmax) and the affinity constant (Ks) [3]. These parameters were determined using the Regression function of MS Excel. Periodically, in order to cut the air supply to the respirometer, the peristaltic pump was switched off and the decrease on oxygen concentration was followed for 1-2 minutes. The values of oxygen were plotted against time and the slope corresponded to oxygen uptake rate, OUR [4]. The specific oxygen uptake rate, qO2, can be obtained after dividing the OUR by the correspondent biomass concentration. By integrating the curve that corresponds to the variation of OUR against time, the total amount of oxygen consumed by the culture could be determined and the respiration yield (YyO2) was determined. Before inoculation the volumetric mass transfer coefficient (kLa) was determined by degasifying the medium with nitrogen and follow the oxygen increase during the subsequent aeration [4].

Results
The practical work is being performed since 2009. Each practical class of Microbial Biotechnology lasts 4 h and is divided in four groups of 3-4 students. The experiment takes two classes, one in the morning and other in the afternoon with a lunch break of 1 h, accounting for a total of 9 h of experiment. The morning class usually starts and inoculates the

Figure 1 – Experimental setup
bioreactor and the afternoon class finishes the work when the stationary phase was reached or, at least a significant period of time of exponential phase was observed. Samples were taken every 15 minutes, and all elements of the group participate on that. Organization between them is crucial for the success of the experimental work and for the quality of the results of the groups that followed that experiment. In some cases the different experimental points are a consequence of different people handling the samples. The different groups share their experimental results with the other groups for further analysis and data processing. The e-learning is used for sharing data in Excel worksheets and for comparison and discussion of results. The professor monitors the progress of all data sharing, as well as, all calculations of each group with access to all information. Finally, each group presents and discusses the results in a final report to be delivered two weeks after the experiment.

Figure 2 shows a typical evolution of OUR, \( q_{O_2} \), biomass and glucose values along the experimental work.

![Figure 2. Typical evolution of OUR (dark green), \( q_{O_2} \) (brown), biomass (orange) and glucose (light green) values along the experimental work.](image)

Table 1 shows some of the typical values obtained during the experimental work.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biomass (g/L)</td>
<td>1.44</td>
</tr>
<tr>
<td>( \mu_{\text{max}} ) (h(^{-1}))</td>
<td>0.35</td>
</tr>
<tr>
<td>Glucose consumed (g/L)</td>
<td>1.89</td>
</tr>
<tr>
<td>( k_{La} ) (min(^{-1}))</td>
<td>0.76</td>
</tr>
<tr>
<td>( Y(X/S) ) (mol/mol)</td>
<td>0.94</td>
</tr>
<tr>
<td>( Y(O_2/S) ) (mol/mol)</td>
<td>0.04</td>
</tr>
</tbody>
</table>

There was a clear relation between the parameters obtained and the different growth phases of the microbial culture. Also, it was possible to establish the amount of substrate used for growth and for respiration. Moreover, the sum of the storage and respiration yields account for 0.98 g/g, meaning that most of the carbon was accounted. The values obtained were not very different from those described in the literature for the metabolism of \( S. \) cerevisiae using glucose as carbon source under fully aerobic conditions [5].

**Conclusions**

The balance of this this practical work is very positive, since students understand the practical link between the metabolic functions and process variables, as well as they learn methods of calculation and organization of information, in addition to acquisition of team work skills.

**Acknowledgements**

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Poster Session

BIOLOGICAL ENGINEERING
AND BIOTECHNOLOGY
Centrifugal partition chromatography on the separation of phenolic compounds derived from lignin depolymerization

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Phenolic compounds are ubiquitous biomass building blocks. They are considered as one of the most versatile and important industrial organic chemicals [1], widely used in the food, pharmaceuticals, chemicals and cosmetics. Due to their wide range of applications, these products are economically attractive when compared with petrochemical phenolic compounds. In 2015, the price of synthetic phenols achieved values around 1000 $ per tonne [2], while the price of phenolic compounds derived from the lignocellulosic biorefinery ranged from 1000–12 000 $ per tonne depending on the phenolic compound. Their high price is a result of significant drawbacks regarding the lignocellulosic biorefinery processing, especially considering the need for more effective purification methods and downstream processes.

The alkaline oxidative process [3] and the hydrothermal processing [4] of lignin are the conventional depolymerisation platforms used to produce monomeric aromatic compounds, mainly due to their greener and cheaper characteristics. Nevertheless, one of the main concerns is the difficulty to fractionate the heterogeneity of phenolic compounds resulting from the lignin depolymerisation process. In literature, supercritical carbon dioxide extraction, ionic liquid (IL) extraction, and adsorption in specific polymeric resins are the main fractionation techniques already described for lignocellulosic products (e.g. vanillin, syringaldehyde and p-hydroxybenzaldehyde). However, the high cost and difficult scale-up are the main disadvantages of these techniques. Therefore, there is an effective and crucial need to develop more efficient, yet scalable, fractionation processes, operating under mild conditions with minimal waste formation, in order to selectively separate and purify each phenolic compound produced by lignin depolymerisation, in which aqueous biphasic systems (ABS) can be envisioned as a promising alternative.

Previous successful studies used ABS to purify structurally similar biomolecules,[5] including phenolic compounds [6]. However, none of these studies reported the scale-up of the technique. Amongst these, sodium polyacrylate (NaPA)-PEG-based ABS appeared as a promising combination of phase-forming components that have been successfully applied in the purification of a wide variety of biomolecules. These novel polymer-based aqueous systems have some important advantages when compared to other polymer combinations, namely a lower viscosity and a faster separation rate, beneficial to reduce energetic inputs and improve mass transfer. Moreover, PEG-NaPA-based ABS exhibit a remarkable high water content, with phase separation occurring at very low concentrations of the polymer (3–5 wt% of each polymer). However, one of the major drawbacks associated with the use of polymer-based ABS is the difficulty to transpose the high yields of extraction and the purity levels obtained in the lab, to continuous processes at a larger scale, culminating in an industrial process. To this end, centrifugal partition chromatography (CPC) can enhance the resolution of the separations and convert liquid–liquid extractions based on ABS into processes scalable to large flow rates [7]. This chromatographic downstream technology operates with liquid stationary and mobile phases, which, in this work, will correspond to the two phases of the polymer-based ABS.

The downstream processes, representing more than half of the cost of the final products, are following the same trend. New strategies to minimise the consumption of solvents and energy, as well as the production of wastes, are being followed aiming for an enhanced sustainability. [8] Therefore, it is important to provide green metrics for these processes to evaluate their sustainability and identify opportunities for decreasing their environmental impact. In this work, PEG 8000 + NaPA 8000-based ABS, using ionic liquids or inorganic salts as electrolytes, were studied in the separation of five model phenolic compounds: three phenolic acids (caffeic, ferulic and protocatechueic acids) and two aldehydes (vanillin and syringaldehyde), derived from lignocellulosic depolymerisation. The selection of the best ABS and its optimization was performed, followed by its application in CPC to reinforce the technique scale-up. After the development and characterization of the integrated process to fractionate the mixture of phenolic compounds, an environmental evaluation was done considering the carbon footprint as the main parameter/output.
Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145- FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors are grateful for the financial support of the Portuguese Foundation for Science and Technology (FCT) for the doctoral grant of SFRH/BD/102915/2014 of J. H. P. M. Santos. S. P. M. Ventura acknowledges for contract IF/00402/2015. M. G. F. acknowledges the European Research Council (ERC) for the grant ERC-2013-StG-337753. The authors acknowledge the FCT funding through the project “Multipurpose strategies for broadband agro-forest and fisheries byproducts valorisation: a step forward for a truly integrated biorefinery (PAC – Programa de atividades Conjuntas) ref: SAICTPAC/0040/2015. A. C. R. V. Dias acknowledges FCT/MCTES for a contract under Investigador FCT 2013 contract number IF/00587/2013, and for the financial support to CESAM (UID/AMB/50017), through national funds, and the co-funding by FEDER, within the PT2020 Partnership Agreement and Compete 2020.

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Phototrophic enhanced biological phosphorus removal: A solution for reducing the aeration necessities in conventional enhanced biological phosphorus removal systems

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An innovative Phototrophic - Enhanced Biological Phosphorus Removal (Photo-EBPR) system, consisting of a consortium of photosynthetic organisms and polyphosphate accumulating organisms (PAOs), was studied in this work. A sequencing batch reactor fed with a mixture of acetate and propionate was subjected to dark/light cycles in order to select a photo-EBPR system containing PAOs and photosynthetic organisms, the system oxygen providers. The results showed that the photo-EBPR culture can perform P release in the dark and has high capacity of P removal in the light phase in the absence of air or another electron acceptor. These results indicated that a photo-EBPR system can be a potential solution for P removal with low COD/P ratios and in the absence of aeration, prospecting the use of natural sunlight as illumination, which would reduce the costs of EBPR operation regarding aeration.

Introduction
Due to the wide use of phosphorus (P) in agriculture, chemical and food industries, P can be frequently found in water streams in concentrations susceptible of causing environmental problems [1]. Therefore, it is essential to treat the P containing streams and, ideally, recover this element and reuse it. Enhanced Biological phosphorus removal (EBPR) is a sustainable process used to remove P from wastewaters but requires aeration, which contributes to the high energetic costs of the treatment process, since aeration is the most energy-intensive operation in wastewater treatment [2]. The novel photo-EBPR configuration presented in this study is based on sequential dark/light periods. In the dark phase, as no oxygen will be produced by photosynthetic microorganisms, the system will be anaerobic and Polyphosphate accumulating organisms (PAOs) can-take up volatile fatty acids (VFAs) and convert them into polyhydroxyalkanoates (PHA), using the energy from poly-P and glycogen hydrolysis. In the light phase, the photosynthetic microorganisms use the light to produce ATP while generating oxygen, which can be used by PAOs to oxidize PHA and obtain ATP for P uptake. Algae may also contribute for P uptake and for CO2 capture. The proposed system may not only reduce the operational costs but is also an environmental friendly alternative for wastewaters treatment.

Methods
A sequencing batch reactor was seeded with sludge from an EBPR reactor, already enriched in PAOs, and operated in 8 hour cycles (3 h dark, 4 h light and 1 h idle period, including 0.5 h of settling). The sludge retention time was 10 days, the hydraulic retention time was 16 hours, temperature was controlled at 20ºC and pH at 7.5. The reactor was fed with a solution (75%-25% COD concentration in the feed was 60 mg/L. Phylogenetic analysis of the bacterial community was done through Fluorescence in situ hybridization (FISH) as described elsewhere [3]. The oligonucleotide probes used were the fluorescein isothiocyanate (FITC)-labelled EUBmix (EUB338, EUB338II, EUB338III) for all bacteria, applied with the cyanine 3 (Cy3)-labelled probes: PAOmix (PAO651, PAO462, PAO846) for Candidatus Accumulibacter phosphatis and CPB_654 for Candidatus Competibacter phosphatis [4].

Results
The culture optimization process started with the SBR being fed with COD concentration of 160 mg/L, in which 3 sequential phases were imposed to the reactor: dark anaerobic; light without external air supply and light with aeration. With a light intensity of 328 W/m² two COD concentrations in the feed were tested (stage1: COD=160; stage 2: COD= 200 mg/L). In stage 1 a total P uptake of 85 mg/L was obtained, of which 40 mg/L were taken up in the light phase period with no external aeration, contributing to a net P removal of 27 mg/L (table1). These results indicated the contribution of the photosynthetic microorganisms for, at least, the system oxygenation during the period with no external aeration, supporting the ability of the proposed system to perform EBPR. When COD was increased to 200 mg/L (stage 2), as a result of the high VFAs concentration, more PHA was produced during the dark phase, contributing to an increase of energy availability in the light phase for P uptake by PAOs. Thus, the total P uptake in the light phase increased to 94 mg/L with 52 mg/L being removed in the period without aeration, being achieved a net P removal of 41 mg/L (table1).

With the aim of simulating the typical summer light intensity in Portugal, the light intensity was increased to 600 W/m² (stage 3), which contributed to an increase of the algae amount and, consequently, to the increase of chlorophyll concentration (table 1). This modification led to a P uptake of 76 mg/L, with 54 mg/L being removed in the light phase without aeration, achieving a net P removal of 43 mg/L. As the goal was to implement a photo-EBPR totally independent of air supply, the system was operated in the same conditions as mentioned above, but the external aeration was completely removed (stage 4). Under these conditions a total P uptake of 64 mg/L was achieved in the light phase, contributing to a net P removal of 34 mg/L, which is a value comparable or exceeding those of other EBPR studies. These results show the potential of using photosynthetic microorganisms as oxygen providers in a photo-EBPR system.
Also, due to the high level of P uptake and net P removal obtained, even higher than some values obtained in other studies regarding EBPR systems, it is likely that photosynthetic microorganisms (algae, cyanobacteria) may not only contribute for oxygen production but also for P removal in the light phase. It is noteworthy that the P release decreased with the increase of light intensity, likely due to an increase of GAOs (table 1).

Comparing the presence of PAO microorganisms in the biomass in stage 2 and in stage 4, FISH results (table 1 and figure 1) indicate the same amount of PAOs in both stages. However, the amount of GAOs increased in stage 4, perhaps due to the increase of the oxygenation of the EBPR provided by the photosynthetic microorganisms.

### Conclusions

The proposed photo-EBPR system is a good option for phosphorus removal and reuse, as it presents good P removal efficiency in the absence of external aeration, reducing the energetic costs. Also, it opens up the possibility of directly using the biomass as fertilizer due to the improved characteristics of algae-based sludge during direct application in soils. Therefore, photo-EBPR can be a better and more sustainable alternative to conventional EBPR systems.

### Table 1. Stoichiometric and kinetic parameters, FISH and Chlorophylls results for the photo-EBPR during stage 1, 2, 3 and 4 (Prelease, Puptake, Pnet and in mg-P/L; TSS in g/L) [5].

<table>
<thead>
<tr>
<th>Stage</th>
<th>Dark phase</th>
<th>Light phase</th>
<th>Total</th>
<th>FISH</th>
<th>Chlorophyll</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Non-aerated</td>
<td>Aerated</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TSSi</td>
<td>Puptake</td>
<td>Puptake</td>
<td>Puptake</td>
<td>Pnet</td>
<td>PAOs</td>
</tr>
<tr>
<td>1</td>
<td>57 (2)</td>
<td>2.24 (0.47)</td>
<td>40 (6)</td>
<td>53 (5)</td>
<td>85 (1)</td>
</tr>
<tr>
<td>2</td>
<td>53 (15)</td>
<td>3.79 (0.45)</td>
<td>52 (13)</td>
<td>43 (17)</td>
<td>94 (4)</td>
</tr>
<tr>
<td>3</td>
<td>32 (10)</td>
<td>4.21 (0.82)</td>
<td>54 (13)</td>
<td>22 (2)</td>
<td>76 (11)</td>
</tr>
<tr>
<td>4</td>
<td>30 (2)</td>
<td>3.39 (0.23)</td>
<td>64 (5)</td>
<td>n/a</td>
<td>64 (5)</td>
</tr>
</tbody>
</table>

Figure 1. Microscope image of the selected culture with a) 328 W/m² and b) 600 W/m². (a.1 and a.2: FISH image from the culture labelled with PAO mix probe. b.1: bright field image and b. 2: FISH image from the culture labelled with PAO mix probe.)

### Acknowledgements

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### References

Biodegradation of a sulfonated azo dye in anaerobic-aerobic bioreactors treating a simulated textile wastewater investigated by liquid chromatography-tandem mass spectrometry

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This study compared two anaerobic-aerobic sequencing batch reactors (SBRs) operated under different hydodynamic regimens, regarding the treatment performance of a synthetic textile wastewater, with a focus on azo dye (Acid Red 14, AR14) biodegradation. Liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) was used for structural characterization of AR14 biodegradation metabolites, their molecular formulae being confirmed by high resolution MS/MS. The SBRs shared most of the identified compounds (32 molecules), but differences were observed in their metabolite aerobic profiles. Biodecolorization via AR14 anaerobic reduction was confirmed by the identification of the sulfonated aromatic amine 4A1NS, which was further aerobically biodegraded, involving aromatic ring deamination and hydroxylation. The other aromatic amine (1N2A4S) was not detected, probably undergoing autoxidation reactions. A new AR14 biodegradation pathway was observed upon supplementation with nitrate.

Introduction

Textile industry wastewater represents one of the main sources of pollution worldwide, carrying high organic loads and recalcitrant dyes, with azo dyes as the main type used [1]. The urgent need for efficient and environmentally friendly dye removal processes led to the study of biological wastewater treatment systems. Generally, these involve mixed bacterial cultures and include an anaerobic treatment stage for the reductive cleavage of azo dyes, followed by an aerobic stage for degradation of potentially toxic, colorless breakdown aromatic amines (AA) [2]. In fact, the use of flocculent activated sludge in anaerobic-aerobic sequencing batch reactor (SBR) systems has been studied in the past years [3], with notable success in the anaerobic decolorization stage. Yet, the scarce information on the fate of the breakdown AA during the aerobic stage revealed that most of them are not degraded, thus constituting a health hazard [2,3]. Sulfonated AA are particularly resistant to biodegradation due to the hydrophilic nature of the sulfonate group [4]. Biodegradation has mostly been shown for relatively simple sulfonated amino-benzene/naphthalene compounds [3]. This limitation has generally been attributed to the lack of an adequate aerobic microbial population capable of metabolizing such compounds. Yet, the operation of SBRs using the innovative aerobic granular sludge (AGS, [5]) was recently shown to be advantageous in terms of azo dye degradation, as compared to conventional activated sludge [6]. In fact, the excellent biomass retention capacity of AGS systems can favor the establishment of a more diverse microbial population, namely slow-growing bacteria, with the potential ability to biodegrade recalcitrant aromatic amines, such as 4-amino-naphthalene-1-sulfonic acid (4A1NS; graphical abstract) [6]. Accordingly, 4A1NS biodegradation was also accomplished through the use of biofilm reactors, which allow high biomass retention [7]. Moreover, the use of a plug-flow feeding regimen in AGS SBR systems was shown to play an important role in the selection of slow-growing bacteria [5,8].

The aim of this study was to compare two anaerobic-aerobic AGS SBRs using different feeding regimens (statically fed, anaerobic-aerobic SBR1 vs anaerobic plug-flow fed, anaerobic-aerobic SBR2; graphical abstract) regarding azo dye degradation performance. The SBRs were fed with a synthetic textile wastewater supplemented with the azo dye Acid Red 14 (AR14; graphical abstract) and specific changes were introduced in the feeding solution along the 224-day experimental run, including nitrate supplementation. Samples representative of different operating periods were analyzed by liquid chromatography coupled with electrospray ionization tandem mass spectrometry (LC-ESI-MS/MS) for the structural characterization of AR14 reductive metabolites and degradation products in the two SBRs.

Materials and Methods

AGS, previously used in SBRs and subsequently stored at 4°C for 18 days, was used to inoculate two 1.5-L SBRs (H/D=2.5). The SBRs were operated for 224 days using different feeding regimens: statically fed (top), anaerobic-aerobic SBR1 vs anaerobic plug-flow fed (from bottom), anaerobic-aerobic SBR2. They were operated in 6-h cycles: 30-min or 1-h feeding, followed by 1.5- or 1-h stirred anaerobic phase in SBR1 or SBR2, respectively, 3.5-h aeration, 5-min settling, 1-min drain and idle. A synthetic textile wastewater [6] containing a hydrolyzed starch-based size agent (Emsize E1), nutrients, pH buffering phosphates and AR14, was supplied to the SBRs at a 12-h hydraulic retention time and an organic loading rate, as chemical oxygen demand (COD), of 2.0 kg O₂ m⁻³ d⁻¹ as COD. Six experimental periods were defined, according to the changes introduced in the SBRs feed composition: I (days 0-35) – the reactors were supplemented with 10 mg AgNP L⁻¹; II (days 36-118) – AgNP (silver nanoparticles) addition was stopped from day 36 on; III (days 119-134) – a three-fold increase in dye concentration to 120 mg AR14 L⁻¹ was applied; IV (days 135-146) – 40 mg AR14 L⁻¹ was restored; V (days 147-153) and VI (days 154-224) – the feed was supplemented with nitrate, specifically 60 and 120 mg NO₃⁻ L⁻¹, respectively. Samples were harvested from the SBRs mixed liquor at the end of the anaerobic/aerobic phases (samples A/B, respectively) of selected treatment cycles (periods II, III, VI) and centrifuged.
Supernatant clean-up was performed by solid-phase extraction, using the generic Oasis HLB (Waters) method without the wash step. LC-MS/MS and LC-high resolution MS/MS analyses were conducted on a Dionex Ultimate 3000 HPLC system coupled inline to an LCQ Fleet ion trap mass spectrometer equipped with an ESI source (Thermo Scientific), and on an LC (Elute OLE UHPLC) interfaced with a Q-TOF Impact II mass spectrometer equipped with an ESI source (Bruker Daltoniks), respectively. The mass spectrometers were operated in the ESI positive and negative ion modes. LC was performed on a C18 reversed-phase Cortecs column (Waters) using a gradient elution of 0.1% formic acid in water and acetonitrile (ACN): 0-18 min, linear gradient to 50% ACN; 18-20 min, linear gradient to 90% ACN; 20-23 min, isocratic 90% ACN; and 23-24 min, linear gradient to 0% ACN.

Results and Conclusions

Thirty-two molecules detected in the SBRs samples (examples in Figure 1 and Table 1) were suggested to be associated with AR14. In fact, by comparing samples from periods II and III, it was observed that the abundance of [M-H] species with m/z values 174, 189, 222, 239, 378 and 391 significantly increased in the SBRs upon the three-fold increase in AR14 concentration. The relative abundance of the 32 selected ions was followed along the aerobic stage. Although both SBRs shared most of the identified compounds in each experimental period, specific differences were observed in their metabolites’ aerobic profiles. Overall, a decrease in ion abundance along the aerobic stage was more often observed in SBR2 than in SBR1, indicating a potential advantage in using a plug-flow feeding regimen.

The identification of 4A1NS (m/z 222, [M-H]-) in sample A confirmed that AR14 (m/z 457, [M-H]-) was reduced in the anaerobic phase. Moreover, the absence of 4A1NS in sample B indicated it was further transformed during the aerobic stage in both SBRs. Based on the identified metabolites (Figure 1), 4A1NS aerobic biodegradation is proposed to follow deamination and aromatic ring hydroxylation. On the contrary, the other AA derived from AR14 azo bond reduction (1N2A4S; graphical abstract) was not detected, probably owing to its expected unstable character [9]. In fact, complex aromatic structures containing sulfonate groups were identified, potentially resulting from 1N2A4S autoxidation forming dimeric, stable products. Finally, 4A1NS was no longer detected in sample A harvested from both SBRs on period VI. Instead, a new metabolite emerged (m/z 207, [M-H]-), suggesting that a different AR14 biodegradation pathway took place in the presence of nitrate, possibly involving direct deamination of 4A1NS upon AR14 reduction. Although the presence of nitrate probably stimulated nitrogen metabolism in the SBRs, further investigation is needed to understand the underlying mechanism. Overall, these results represent a relevant contribution towards the elucidation of AR14 biodegradation pathways, involving mechanisms whose understanding may be crucial for efficient textile wastewater treatment.

Figure 1. Molecular formula and proposed chemical structure for some of the ions (m/z) detected by LC-MS/MS.

<table>
<thead>
<tr>
<th>RT (min)</th>
<th>Measured m/z</th>
<th>Ion Formula</th>
<th>m/z</th>
</tr>
</thead>
<tbody>
<tr>
<td>10.0</td>
<td>207.0143</td>
<td>C_10H_15NO_S</td>
<td>207.0121</td>
</tr>
<tr>
<td>3.7</td>
<td>222.0237</td>
<td>C_16H_28NO_5S</td>
<td>222.0230</td>
</tr>
<tr>
<td>6.7</td>
<td>239.0078</td>
<td>C_10H_15NO_S</td>
<td>239.0020</td>
</tr>
<tr>
<td>12.3</td>
<td>251.9979</td>
<td>C_16H_28NO_5S</td>
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<td>12.8</td>
<td>377.0599</td>
<td>C_16H_28NO_5S</td>
<td>377.0602</td>
</tr>
<tr>
<td>15.5</td>
<td>378.0446</td>
<td>C_16H_28NO_5S</td>
<td>378.0442</td>
</tr>
<tr>
<td>12.7</td>
<td>457.0252</td>
<td>C_16H_28NO_5S</td>
<td>457.0170</td>
</tr>
<tr>
<td>13.7</td>
<td>470.0636</td>
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<td>470.0637</td>
</tr>
</tbody>
</table>

Acknowledgements

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References

Technological aspects of beverage production using rice processing by-products: Bran and broken rice

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Introduction

Rice is a cereal consisting essentially of starch (90%) appreciated and consumed all over the world [1]. About 600 Mton are produced annually worldwide, being Asian region the largest producer [2]. In Portugal, rice is the second most cultivated cereal (169 289 ton) only exceeded by corn crop (710 634 ton) [3]. Rice by-products, like broken rice and rice bran are generated during the industrial rice processing, accounting of 14% and 8%, respectively [4]. Nowadays their most common destination is animal feed [2]. However, their recognized high nutritional properties comparing with polished rice [2] give them a high potential for valorization, especially for vegetable-based drinks development for human consumption. The increasing concerns about health, allergies and intolerances has stimulated the demand of these type of drinks, that compete directly with the dairy market [5]. The sweet taste of “rice milk” can be obtained using enzymatic hydrolysis. The starch breaks down into simple sugars making the sugar/sweeteners addition unnecessary [6].

Methods

Bran and broken rice, supplied by Cooperativa Agrícola Montemor-o-Velho (Portugal), were first characterized and ground to be used in the form of flour for the development of the rice beverages. Thereafter a 1:10 dilution with water was made to each flour. During the enzymatic hydrolysis two commercial enzymes supplied by Novozymes (Denmark) were used: AMG 300L and TERMAMYL 120L. Trials were performed in order to study the combined activity of both enzymes (in rice bran and broken rice flours) and their individual activity (only in broken rice flour). The performance of both enzymes in the starch hydrolysis was conducted sequentially according to their optimum reaction temperature [7]. Namely the mixture was first heated to 90 °C, TERMAMYL 120L was added and allowed to react for 45 minutes under stirring. AMG 300L was then introduced, after cooling down the mixture to 60 °C, and the reaction takes place for more 180 minutes. In the case that the enzymes were tested separately, the optimum reaction temperature of each enzyme was maintained constant for 120 minutes. The starch hydrolysis and enzymes activity were monitored by UV-vis spectroscopy (Hach, XION 500 - LPG385, Germany). The soluble reducing sugars were evaluated, in duplicate, by dinitrosalicylic (DNS) colorimetric assay and by refractometry (° Brix). Weende scheme was used to determine moisture, protein, fiber, fat and ash contents in rice by-products. Starch content was determined according to [8].

Results

According to Table 1, broken rice consists essentially of starch and protein, while rice bran is characterized by high content of fiber, fat and ash.

Table 1. Chemical composition of rice bran and broken rice

<table>
<thead>
<tr>
<th>Composition (% dry basis)</th>
<th>Rice bran</th>
<th>Broken rice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture</td>
<td>3.46 ± 0.01</td>
<td>6.37 ± 0.01</td>
</tr>
<tr>
<td>Dry extract</td>
<td>96.54 ± 0.01</td>
<td>93.63 ± 0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>22.32 ± 0.26</td>
<td>10.90 ± 0.35</td>
</tr>
<tr>
<td>Fiber</td>
<td>12.24 ± 0.10</td>
<td>0.37 ± 0.08</td>
</tr>
<tr>
<td>Fat</td>
<td>19.84 ± 0.12</td>
<td>0.50 ± 0.11</td>
</tr>
<tr>
<td>Ash</td>
<td>9.32 ± 0.01</td>
<td>0.60 ± 0.03</td>
</tr>
<tr>
<td>Starch</td>
<td>12.36 ± 5.77</td>
<td>60.50 ± 2.76</td>
</tr>
</tbody>
</table>

This composition is somehow expected because it is intrinsically related with the part of the rice grain from where it was extracted.

Figure 1 shows the combined enzyme activities for rice hydrolysis. In the case of broken rice, it was observed that the reducing sugars concentration doubles over the reaction. However, after AMG 300L addition the hydrolysis slows down maybe due to the reaction temperature decrease (60 °C). In the case of the rice bran, the reducing sugars concentration practically did not change over time. This result can be explained by the low quantity of starch present in this by-product (Table 1). It was also possible to inferred that the

![Diagram of rice processing and by-products](image-url)
Commercial enzymes used in this study were not appropriated for the rice bran starch hydrolysis. For that reason, only the broken rice was used to test the enzymes individually (Fig 2).

Figure 1. Combined enzymes activity in broken rice and rice bran.

Using only TERMAMYL 120L, the reducing sugars profile was similar to the one found where both enzymes were applied, indicating that this enzyme was the main responsible for the hydrolysis. This behavior was confirmed by refractometry (data not shown). In the case of AMG 300L there was no variation in reducing sugars over the reaction time. These results confirm the lack of activity of this enzyme under the tested conditions (time and temperature) and by-products.

Figure 2. Reducing sugars concentration during the hydrolysis of broken rice using AMG 300L or TERMAMYL 120L.

Conclusion
This study allowed to conclude that TERMAMYL 120L is appropriated for the production of broken rice-based beverages leading to higher reducing sugar concentrations. Refractometry is a simple and expeditious method to monitor the starch hydrolysis. It was also found that AMG 300L can be disposed in this case allowing to a processing costs reduction. The smaller amount of starch in rice bran and the absence of enzymes activity in this by-product demonstrated that it is not appropriate for the production of rice-derived beverages.

Acknowledgements
Authors would like to thanks to Cooperativa Agrícola Montemor-o-Velho for supplied the broken rice and rice bran and also the financial support to FEDER, through the partnership agreement Portugal2020 - CENTRO2020, under the project CENTRO- 01-0145-FEDER-023631 SoSValor.

References
Bioethanol production from Kraft pulp in a circular economy perspective

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Bioethanol is a potential alternative to fossil fuels, which have negative impact in health and environment. Bioethanol is mainly produced from food crops, leading to food-fuel competition. Second generation bioethanol is produced from lignocellulosic biomass (LCB), requiring a costly and technically difficult pretreatment. Kraft pulping removes lignin, releasing cellulose, and can be considered as a pretreatment. Kraft pulping followed by hydrolysis of polysaccharides and fermentation is a promising approach to valorise wastes resulting from pulp and paper industry and can be integrated in a biorefinery. This work studied the production of bioethanol from unbleached Kraft pulps of Eucalyptus globulus. Hydrolysis of the Kraft pulp allowed a hydrolysis yield of 95.6 ± 2.6 %. The sequential co-culture of Saccharomyces cerevisiae and Sheffersomyces stipitis was the most effective assay, with a maximum ethanol concentration of 20.0 ± 0.6 g.L⁻¹, and an ethanol yield of 0.464 ± 0.009 g.g⁻¹.

Eucalyptus globulus (Figure 1) to access the feasibility of producing bioethanol from pulp and paper industry wastes. In this way, integrated biorefineries can be implemented in the existing Kraft pulp mills.

Figure 1. Unbleached Kraft pulp of Eucalyptus globulus.

Enzymatic hydrolysis was used to convert polysaccharides of Kraft pulp into its monomers. The hydrolysis had the duration of 24 h with mechanical stirring of 100 rpm, temperature of 45-50 °C, and pH 4.5-5.5. Hydrolysates with 65.4 ± 0.8 g L⁻¹ of glucose and 16.0 ± 1.8 g L⁻¹ of xylose were obtained, with a hydrolysis yield of 95.6 ± 2.6 %.

Saccharomyces cerevisiae PYCC 5246 and Sheffersomyces stipitis NRRL-7124 were used to study the fermentation of the hydrolysate at two scales: Erlemeyer assays with a working volume of 100 mL, and bioreactor assays with a working volume of 3 L. The Erlemeyer assays were carried out at 28 °C and 180 rpm, without pH control. Table 1 shows the results of the Erlemeyer assays. In these assays, fermentation with S. cerevisiae was firstly studied and lead to an ethanol yield of 0.450 ± 0.009 g.g⁻¹ and a maximum ethanol concentration of 19.8 ± 0.1 g.L⁻¹. Although glucose was fully consumed, most of the xylose was almost no consumed. Fermentation with S. stipitis, a hexose- and pentose-fermenting yeast, was tested. However, a lower ethanol concentration and yield were obtained, and a high concentration of xylose was still present at end of fermentation, since S. stipitis preferred to consume glucose. Then, a sequential co-culture was evaluated, with S. cerevisiae being inoculated at the beginning of the fermentation and S. stipitis inoculated only after glucose exhaustion. Both glucose and xylose were fully consumed by

Fossil fuels, which represent 79.4 % of the global final energy consumption, have negative impact on health and environment [1]. Due to the much lower GHG emissions, biofuels emerge as a potential renewable energy sources in replacing fossil fuels [2]. Bioethanol is currently the most produced biofuel, corresponding to about 73 % of the total biofuels produced, being almost entirely of first generation, since it is produced from food crops, e.g. sugarcane, corn, and sugar beet [1,3]. Bioethanol can be alternatively produced from lignocellulosic biomass (LCB), like agricultural residues, organic portion of municipal solid wastes, or woody biomass. This so called second generation bioethanol does not raise concerns about food availability [4]. However, its production is still not economically feasible on a large scale, as it faces several technical barriers, namely the need for pretreatments, still costly and technically difficult due to the recalcitrance of LCB [5].

Pulp and paper industry is the major global consumer of woody biomass and, consequently, pulp and paper mills already possess the infrastructures required to handle LCB [6]. The pulp and paper industry is of great importance for the Portuguese economy, being responsible for 5 % of the country’s exportation. Most of the pulp is produced through Kraft pulping, a process that removes lignin and targets hemicelluloses to some extent, and therefore can be considered as a LCB pretreatment [7,8]. Kraft pulps, composed essentially by cellulose and few hemicelluloses, can be hydrolyzed to fermentable sugars, which can be converted by microorganisms into ethanol. Kraft pulping and subsequent hydrolysis and fermentation to ethanol seem like a promising approach to valorise wastes resulting from this industry, such as low-quality wood, bark and other rejects, as well as low quality and excess pulp. This would contribute to diversify the products and increase the profitability of pulp and paper industry, converting the already existing pulp and paper mills into integrated biorefineries. Biorefining is a crucial part of circular economy, as it closes loops of biomass, water and carbon through co-production of food/feed ingredients, biobased products and bioenergy from renewable resources, maximizing biomass valorization [9]. The aim of this work was to study the production of second generation bioethanol from unbleached Kraft pulps of
the end of the fermentation and higher ethanol yield, 0.464 ± 0.009 g.g⁻¹, and maximum ethanol concentration, 20.0 ± 0.6 g.L⁻¹, were achieved.

Table 1. Erlenmeyer fermentation assays.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Ethanol [g.L⁻¹]</th>
<th>Ethanol yield [g.g⁻¹]</th>
<th>Conversion efficiency [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. cerevisiae</td>
<td>19.8 ± 0.1</td>
<td>0.450 ± 0.009</td>
<td>88.3 ± 1.7</td>
</tr>
<tr>
<td>S. stipitis</td>
<td>17.5 ± 0.1</td>
<td>0.333 ± 0.011</td>
<td>65.3 ± 2.2</td>
</tr>
<tr>
<td>Co-culture</td>
<td>20.0 ± 0.6</td>
<td>0.464 ± 0.009</td>
<td>91.0 ± 1.8</td>
</tr>
</tbody>
</table>

In the bioreactor assays, temperature, stirring and pH were automatically controlled and set to 28 °C, 180 rpm and 5.5, respectively. In these assays fermentation with S. cerevisiae mono-culture and S. cerevisiae and S. stipitis sequential co-culture were studied (Figure 2). In the bioreactor assays, the results were expected to be better, since pH was controlled to 5.5 throughout the fermentation, a value considered optimal for the process.

Acknowledgements
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References
Polyhydroxyalkanoates production from agricultural wastes and domestic wastewater with phototrophic purple bacteria

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Abstract
Polyhydroxyalkanoates (PHAs) are biodegradable biopolymers with physical-chemical properties similar to conventional plastics. They can be produced by several microorganisms including phototrophic purple bacteria (PPB). Currently, two Horizon 2020 European projects, INCOVER and NoAW are studying operating strategies to valorize agricultural wastes and domestic wastewater via PHA production with mixed cultures of PPB. The strategies developed thus far, led to PHA accumulation values of up to 20% PHA/VSS in the INCOVER selector reactors, and up to 30% in accumulation tests. NoAW attained a PHA content of 15 % PHA/VSS with synthetic feed simulating fermented manure. It is expected that the studies developed under these two projects will provide answers to address the request for a sustainable plastic production system that comprises the utilization of renewable feedstocks and a cost-effective production of biodegradable plastics.

Introduction
In the last decades, polyhydroxyalkanoates (PHAs) production using aerobic mixed microbial cultures (MMC) has been thoroughly studied. Several systems have been optimized in order to increase mixed culture’s PHA accumulation capacity, while decreasing operational costs. With the goal of further decreasing PHA production costs, a new PHA producing system has been recently proposed. It consists in operating phototrophic mixed cultures (PMC) and enriching them in PHA producing phototrophic bacteria. These phototrophic bacteria can obtain energy from light and therefore, do not require aeration. Since aeration is the parameter that most strongly contributes to operational costs in MMCs, its elimination from PMCs operation can lead to a more cost-effective PHA production system.

Thus far, studies with PHA producing PMCs occurred mostly at laboratory scale with very promising results. Indeed, whether by applying the typical selection strategy of carbon feast and famine used of aerobic MMCs or by applying a permanent carbon feast strategy specifically designed for PMCs, PHA accumulation levels up to 30% [1] and 60 % [2], have been respectively achieved. Currently, studies with PHA producing PMCs are occurring under two Horizon 2020 European projects: INCOVER and NoAW. The two projects approach the utilization of PMCs from two different perspectives. INCOVER is run at demonstration scale and aims to do a retrofitting of already existing algae pond facilities used for domestic wastewater treatment, adapting their operation for PHA production using PMCs, under local outdoor conditions. Regarding the NoAW project, it is being determined, at laboratory scale, what are the optimal operational conditions for valorising fermented agricultural wastes (manure and maize silage) through PMCs PHA production.

Methods
INCOVER project. Domestic wastewater from El Torno Wastewater treatment plant in Chiclana, was mixed with molasses residues (1% molasses in wastewater) and fermented in laboratory scale anaerobic reactors (UASB) to obtain a volatile fatty acid (VFA) rich stream. This effluent was fed to two laboratory scale photo bioreactors (6L) simulating two different High Rate Algae Ponds (HRAP) operated at El Torno. The laboratory tests would allow the development of operating strategies, specifically for each HRAP, that enable the conversion of the currently operated algae cultures into photosynthetic mixed cultures (PMC) capable of producing PHA. Therefore, one of the photo bioreactors (PBR1) was operated under a permanent carbon feast strategy while the second photo bioreactor (PBR 2) was operated under a feast and famine strategy. The photo bioreactors were operated under controlled conditions of temperature and illumination (12h dark/12h light) simulating the average summer conditions at El Torno.

NoAW project. NoAW PBR (4 L) was inoculated with sludge from a wastewater treatment plant and fed with synthetic effluent simulating the organic acids and nutrients present in the fermented stream of manure and maize silage mixture. The synthetic effluent contained acetic, propionic, butyric and valeric acids with a total VFA concentration of 12372 mg COD/L. The photo bioreactor was operated under a permanent carbon feast strategy at 30°C and transient illumination (12h dark/12h light) in order to simulate a day cycle.

Results
INCOVER project. The fermentation of the 1% molasses in wastewater led to an effluent mostly composed of acetic acid (one third), propionic acid and butyric acid (Table 1). By feeding this VFA rich stream to PBR 1 and 2, it was possible to convert the initial algae culture into PMCs enriched in PHA accumulating purple bacteria (Fig 1). In PBR 1 and 2 it was possible to obtain PHA accumulation levels up to 20% PHA/VSS in the selector reactors, and up to 30% in accumulation tests using sludge from PBR 2 operated under improved illumination conditions.

NoAW project. Starting with wastewater sludge and synthetic feed, the NoAW PBR became enriched with a PMC capable of accumulating PHA up to 15 % PHA/VSS (See Graphical Abstract). Currently, operating conditions are being adjusted to
introduce the real fermented agriculture waste as influent and improve the culture PHA production capacity.

**Figure 1.** Photo bioreactors enriched in PHA accumulating purple bacteria. Left: PBR 1 operated under permanent carbon feast and simulating a HRAP with low sun exposure and anaerobic conditions. Right: PBR 2 operated under feast and famine and simulating a paddle wheel HRAP with high light availability and aeration.

**Conclusions**

Regarding the INCOVER project, the best operating strategies developed at laboratory scale will be implemented at Chiclana ponds. Demonstration results will allow to conclude on the advantages of using already installed facilities and replacing the currently used algae communities by PHA producing photosynthetic bacterial communities.

As for the NoAW project, the ongoing operating strategies development are providing results that will allow to design photobiorreactors specifically for PHA producing PMCs from industrial waste and wastewater streams that best suit their operational requirements for a maximized PHA production.

It is expected that the PMC studies developed under these two projects will provide answers to address the request for a sustainable plastic production system that comprises the utilization of renewable feedstocks and a cost-effective production of biodegradable plastics.

**Table 1.** Average composition of the UASB fermented effluent. OA – organic acids.

<table>
<thead>
<tr>
<th>Lactate</th>
<th>Acetic</th>
<th>Propionic</th>
<th>Butyric</th>
<th>Isovaleric</th>
<th>Valeric</th>
<th>Ethanol</th>
<th>Nutrients (mg/L)</th>
<th>Ethanol + OA (mM C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 ± 5</td>
<td>34 ± 6</td>
<td>24 ± 14</td>
<td>16 ± 4</td>
<td>1 ± 1</td>
<td>8 ± 2</td>
<td>2 ± 4</td>
<td>N-NH₄</td>
<td>73 ± 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>179 ± 60</td>
<td>P-PO₄</td>
<td>8 ± 2</td>
</tr>
</tbody>
</table>

**Acknowledgements**

The authors would like to acknowledge the Fundação para a Ciência e Tecnologia (Portugal) for funding through SFRH/BPD/101642/2014. UCIBIO acknowledges financing by national funds from FCT/MEC (UID/Multi/04378/2013) and co-financed by ERDF under PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728). INCOVER and NoAW projects have received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement n° 689242 and 688338, respectively).

**References**


Optimization of operating conditions in accumulator reactors for improved polyhydroxyalkanoates production with phototrophic purple bacteria

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Phototrophic mixed cultures (PMCs) were recently proposed as new microbial systems for polyhydroxyalkanoates (PHAs) production. These cultures can be selected under the permanent presence of carbon and the PHA production can be enhanced in a subsequent accumulation step. To optimize the PHA production in accumulator reactors, this work evaluated the impact of two different operating conditions on the culture performance: initial acetate concentration and light intensity. Results indicate that the highest PHA production rates can be obtained when operating the accumulator reactors at low acetate concentration (< 30 CmM) and specific light intensities around 20 W/g X. Also, it was shown for the first time, the capability of a PMC to use a real waste, fermented cheese whey, to produce PHA with an HV content of 12%. These results confirm that cheap VFA-rich fermented wastes can be used as substrates for PHA production with PMCs and prospect the operation under direct sunlight illumination.

Introduction

In the last decade, polyhydroxyalkanoates (PHAs) production using aerobic mixed microbial cultures has been thoroughly studied. However, to overcome the high costs associated with system’s aeration, a new PHA production system that uses phototrophic mixed cultures (PMC) has been recently proposed. This system does not require aeration since phototrophic purple bacteria can obtain energy from light. Though very promising results have already been obtained, such as PHA contents of 60% [1] and production of HB-HV co-polymers, process efficiency needs further optimization. Hence, this work investigates the effect of substrate initial concentration (acetate concentration from 30 to 70 CmM) and light intensity (from 1 to 20 W/g X) in the PHA production efficiency during accumulation tests. Also, it was evaluated for the first time the capability of a PMC to use a real waste, fermented cheese whey, for PHA production.

Methods

The selector photo bioreactor (PBR) was operated under continuous illumination in a 4.4 L reactor with 24h cycles and internally illuminated with a volumetric light intensity of 1.3 W/L of culture broth. The SBR was fed with acetate and at the end of each cycle, one third of the continuously stirred PMC was wasted, resulting in a hydraulic and sludge retention time of 3 days. Temperature was controlled at 30ºC and argon was continuously sparged to assure anaerobic conditions.

To evaluate the effect of acetate concentration on the PHA production in the accumulator reactors, sludge from the SBR was collected at the end of the cycle, placed in separate accumulator reactors and in case A, fed with normal carbon medium (1/3 of fresh medium to 2/3 of sludge, leading to [Acet] = 70 CmM) and in case B fed with medium lacking acetate (1/3 of fresh medium to 2/3 of sludge, leading to [Acet] = 30 CmM coming from residual acetate in the sludge).

For the light intensity study, the accumulator reactors were operated in the same manner as the above case B (i.e. starting with [Acet] = 30 CmM) and four increasing specific light intensities were tested: 0.96 ± 0.04 W/g X, 4.1 ± 0.04 W/g X, 10.2 ± 0.4 W/g X, 18.5 ± 1.3 W/g X.

To evaluate the culture capability of using a real fermented waste with varied VFAs, sludge from the SBR was centrifuged at 9000 g and the supernatant was removed, thus eliminating interferences from residual acetate. The sludge volume was readjusted with fresh medium lacking carbon and NH4 (NH4 limitation prevents growth and should favor polymer accumulation). The accumulator reactors were operated at a light intensity of 19 W/g X and fermented cheese whey (FCW) was pulse fed to the culture. Table 1 indicates the composition of the FCW.

Results

Results from the effect of acetate concentration study indicate that the culture presents higher acetate uptake rate when operated with a lower initial acetate concentration (Fig 1).

![Figure 1. Specific acetate uptake rate in relation to the acetate concentration at the beginning of the accumulation tests. Error bars calculated from duplicate tests.](image-url)

In fact, the rate increased 48% from 0.93 ± 0.01 to 1.38 ± 0.12 Cmol Acet/Cmol X d when the initial acetate concentration was decreased by 60%. It is possible that higher acetate concentrations can be inhibitory to the culture, decreasing its
metabolism, suggesting that future accumulator reactors operation should occur at lower acetate concentrations. As for the effect of light intensity, results indicate that the specific acetate uptake and PHB production entered a plateau around the maximum tested specific light intensity of 18.5 ± 1.3 W/g X. At this intensity, the culture presented PHA production rates of 2.21 ± 0.07 Cmol PHA/Cmol X d, which is almost 3 times more than previously registered in non-optimized accumulation tests. This enabled PHA contents to increase from 15 to 30% PHA/VSS in less than 4h. These results indicate that the highest specific PHA production rates can be obtained when operating the accumulator reactor at low acetate concentration and specific light intensities around 20 W/g X. Also, in this work it was shown for the first time the culture capability of using a real waste, fermented cheese whey, to produce PHA with an HV content of 12%. Results indicate the expected culture preference for acetate consumption, with this substrate being completely consumed at each pulse, while the other compounds tended to accumulate. The total specific substrate uptake rate was in average 1.0 Cmol substrate/Cmol X d, with acetate contributing for more than 80% of this rate (Table 2). The PHA production rate was 0.52 Cmol PHA/Cmol X d (12% of HV monomers production) which resulted in a PHA content variation from 10 to 20%. These differences in relation to previous tests with synthetic acetate feeding are likely related to the influence of the other VFAs or other compounds present in the FCW.

Conclusions
These results prospect the utilization of cheap VFA-rich fermented wastes as substrates for PHA production with PMCs and open up the possibility for direct sunlight illumination since the light intensities used in this work (20 W/g X) can be naturally obtained in sunny regions. All combined, results indicate that phototrophic mixed cultures can lead to a more cost-effective and environmentally sustainable PHA production process.

Table 1. Composition of the fermented cheese whey used in the accumulation tests.

<table>
<thead>
<tr>
<th>FCW composition</th>
<th>Lactic Acid</th>
<th>Acetic Acid</th>
<th>Propionic Acid</th>
<th>Butyric Acid</th>
<th>Valeric Acid</th>
<th>Ethanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>% C</td>
<td>16</td>
<td>45</td>
<td>14</td>
<td>23</td>
<td>6</td>
<td>5</td>
</tr>
<tr>
<td>Concentration (Cmmol/L)</td>
<td>52</td>
<td>146</td>
<td>45</td>
<td>74</td>
<td>19</td>
<td>16</td>
</tr>
</tbody>
</table>

Table 2. Average uptake rates of the different compounds during accumulation tests calculated for the six pulses of FCW. The standard deviation for each uptake rate is shown in italic. Uptake rates are in Cmmol/L d.

<table>
<thead>
<tr>
<th></th>
<th>Lactic Acid</th>
<th>Acetic Acid</th>
<th>Propionic Acid</th>
<th>Ethanol</th>
<th>Butyric Acid</th>
<th>Valeric Acid</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>FCW</td>
<td>0.6</td>
<td>36</td>
<td>2.6</td>
<td>1.4</td>
<td>1.2</td>
<td>0.0</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>(0.5)</td>
<td>(2)</td>
<td>(0.4)</td>
<td>(0.6)</td>
<td>(0.8)</td>
<td>(0.0)</td>
<td>(2)</td>
</tr>
</tbody>
</table>

Acknowledgements
The authors would like to acknowledge the Fundação para a Ciência e Tecnologia (Portugal) for funding through SFRH/BPD/101642/2014. UCIBIO acknowledges financing by national funds from FCT/MEC (UID/Multi/04378/2013) and co-financed by ERDF under PT2020 Partnership Agreement (POCI-01-0145-FEDER-007728). NoAW project has received funding from the European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation programme (grant agreement 688338).

References
Impact of textile wastewater composition on the performance and properties of an aerobic granular sludge-sequencing batch reactor system during operation after granule storage

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In this study, two sequencing batch reactors (SBRs) were inoculated with aerobic granular sludge (AGS) previously used to treat simulated textile wastewater containing the azo dye Acid Red 14 and stored for 18 days at 4°C. The effect of silver nanoparticles (AgNPs, 10 mg/L) was assessed during AGS reactivation, its supply being interrupted after 35 days of operation. The impact of changes in wastewater composition on AGS properties and treatment performance was studied, including a three-fold increase in the concentration of dye, and the addition of nitrate salts, namely Ca(NO₃)₂•4H₂O (60 and 120 mg NO₃/L) and KNO₃ (120 mg NO₃/L). AgNPs and Ca(NO₃)₂•4H₂O seemed to have promoted granulation and biomass accumulation. Color and organic matter removal yields of around 80% were obtained, irrespective of the presence of AgNPs. The addition of Ca(NO₃)₂•4H₂O or KNO₃ did not reduce the color removal yields, but decolorization was delayed in the first SBR cycles.

Introduction

The textile industry is one of the main sources of water pollution problems worldwide due to the production of large volumes of wastewater characterized by extreme variations in chemical oxygen demand (COD), pH, color and salinity [1]. Textile dyes, azo dyes being the most commonly used [2], are often able to persist in natural water bodies during large periods of time, hindering light penetration and photosynthetic activity [1]. Recently, the textile industry has found great interest in engineered nanoparticles (ENPs). Silver nanoparticles (AgNPs), in particular, have proved to be a viable option for antimicrobial finishing of textile materials, and are currently the most commercialized ENPs [3, 4]. As the use of AgNPs is growing and their presence in textile wastewaters is expected to increase accordingly, concerns about their potential impact on the biological treatment of textile wastewaters have been emerging.

Aerobic granular sludge (AGS) was developed to overcome the drawbacks of conventional activated sludge systems, such as poor sludge settling ability, and is mainly obtained in sequencing batch reactors (SBRs). The AGS-SBR technology has been recently scaled-up to treat domestic and food industry wastewater [5]. Information regarding the use of AGS-SBR technology for textile wastewater treatment is still scarce, but its potential as an effective alternative to the flocculent anaerobic/aerobic SBR technology for color and COD removal has been reported [6]. Additionally, as one of the major appeals of the AGS-SBR technology is its ability to treat high-strength wastewaters containing nitrogen, phosphorus and toxic substances, AGS has a high potential to efficiently treat textile wastewater when exposed to AgNPs [7]. The aim of this study was to analyze the impact of changes in dye-laden textile wastewater composition on AGS properties and treatment performance, including the presence of AgNPs (10 mg/L), a three-fold increase in dye concentration (dye shock-load) and the supply of different nitrate salts, namely Ca(NO₃)₂•4H₂O (60 and 120 mg NO₃/L) and KNO₃ (120 mg NO₃/L), to promote denitrification.

Materials and methods

The experimental system consisted of two 1.5-L anaerobic-aerobic SBRs, SBR1 and SBR2, inoculated with AGS previously used to treat simulated textile wastewater and then stored at 4°C during 18 days. SBR1 and SBR2 were fed with a simulated textile wastewater containing the azo dye Acid Red 14 (AR14). A starch-based sizing agent (hydrolyzed Emsize E1) was used as carbon source, and supplemented with pH buffering phosphates and nutrients [8]. The operation comprised seven experimental periods, in which different changes were introduced in the wastewater composition: I (days 0–35) – SBR1 was supplemented with AgNPs (<100 nm particle size) at 10 mg/L at the onset of the reaction phase; II (days 36–118) – AgNPs supply to SBR1 was interrupted from day 36 on; III (days 119–134) – a three-fold increase in AR14 concentration to 120 mg/L was imposed in SBR1 and SBR2; IV (days 135–146) – AR14 concentration was reset at 40 mg/L; V (days 147–153) – Ca(NO₃)₂•4H₂O was introduced in the feed of SBR1 at 60 mg NO₃/L; VI (days 154–205) – Ca(NO₃)₂•4H₂O concentration in the feed of SBR1 was increased to 120 mg NO₃/L; VII (days 206–224) – Ca(NO₃)₂•4H₂O supplied to SBR1 was replaced by KNO₃ at 120 mg NO₃/L.

The SBRs were run in parallel at room temperature with 6-h cycles composed by 30 min of static fill, 1.5 h of anaerobic reaction (mechanical mixing, 70 rpm), 3.5 h of aeration (2vvm), 5 min of settling, 1 min of drain and an idle stage. The SBRs were operated with a volume exchange ratio (VER) of 50%, a volumetric organic loading rate (OLR) of 2.00 kgO₂/(m³⋅d) as COD, and a hydraulic retention time (HRT) of 12 h.

AR14 degradation was assessed spectrophotometrically (UV-VIS) and organic matter removal was followed by quantifying soluble COD in the mixed liquor, according to standard procedures [9]. Biomass concentration in the SBRs was assessed by determining the mixed liquor Total Suspended Solids (TSS) and Volatile Suspended Solids (VSS). The particle size distribution in the mixed liquor was determined through sieve analysis, using two sieves with mesh diameters of 0.2 mm and 0.65 mm. Sludge settling
ability was evaluated through the measurement of sludge volume index (SVI) values after 5 and 30 minutes (SVI5 and SVI30, respectively) [9]. Biomass morphology was analyzed using a transmission light microscope (BA200, Motic) fitted with a digital camera and respective software (Moticam 2, Moticam).

Results and Conclusions
As shown in Figure 1, aerobic granules were formed in the two SBRs during the first 36 days of operation. The sieving results obtained between days 36 and 118 suggest that AgNPs may have had a lasting effect on sludge behavior, since a greater amount of granules was observed in SBR1 when compared to SBR2 (average percentage of small+large granules of 37% and 21% in SBR1 and SBR2, respectively), even after the supply of AgNPs to SBR1 was interrupted.

In Figure 1, results registered between days 163 and 204 suggest that Ca(NO3)2·4H2O promoted the development of aerobic granules, since the granule fraction increased along this period in SBR1 and was significantly higher than in SBR2. Regarding the AGS settling ability, SVI results during period I and periods II, III and IV (data not shown) suggest that AgNPs had both a short- and a long-term enhancing effect on the settling ability of AGS in SBR1. Furthermore, a significant decrease in SVI values (SVI5 and SVI30) was observed in SBR1 during period VI, which suggests that Ca(NO3)2·4H2O also increased the settling ability of AGS.

Acknowledgements
This work was financed by Fundação para a Ciência e a Tecnologia (FCT, Portugal), projects PTDC/AAG-TEC/4501/2014 and UID/BIO/04565/2013. Funding from Programa Operacional Regional de Lisboa 2020 (Projects N. 007317 and RNEM-022125) is also acknowledged. R.D.G. Franca acknowledges FCT for a doctoral research grant (SFRH/BD/95415/2013).

References
Effect of engineered silver nanoparticles on the performance of aerobic granular sludge regarding the potential for abatement of textile wastewater toxicity

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The present work aimed at examining the influence of silver nanoparticles (Ag NPs) on the performance of aerobic granular sludge (AGS) sequencing batch reactors (SBR) with respect to the potential detoxification of a simulated textile wastewater (TWW). Two types of TWW, with and without Ag NPs, both containing the azo dye Acid Red 14 (AR14), were fed in static conditions into two SBRs. Samples were collected from both SBRs at different cycle stages and assessed in terms of potential toxicity using yeast-based assays. Based on toxicity data over yeast growth, no relevant differences were found due to the presence of the Ag NPs during the SBR operation under the conditions tested. Measurement of possible changes in the transcript levels of two toxicity-indicator yeast genes brought about by the samples is in course. Data from the two types of bioassays at molecular and phenotypic levels will be integrated and discussed with respect to potential detoxification of the dye-laden TWW.

Introduction

The textile industry is an important source of water pollution, in terms of discharged volumes, high organic loads and color content – often from azo dyes – of the released textile wastewater (TWW). Bioreduction of the azo dyes can be promoted under anaerobic conditions leading to decolorization and the formation of aromatic amines that are frequently resistant to further biodegradation. However, these amines and possibly other azo dye degradation products can be more toxic than the parent dye molecule [1,2]. The aerobic granular sludge (AGS) technology proved to be an auspicious bioprocess for TWW treatment due to, among other important attributes, the presence of anoxic/anaerobic and aerobic zones within the granules, potentially contributing to azo dye and aromatic amine biodegradation, and the improvement of TWW detoxification [1]. Furthermore, there are emerging concerns regarding the rising utilization of nanoparticles (NPs) in the textile industry and their consequent occurrence in TWW. In particular, silver nanoparticles (Ag NPs) can be toxic to microorganisms due to the bioavailability of the soluble metal fraction (ionic Ag) as well as through the largely size-dependent interaction of NPs with cell surfaces and cellular internalization [3]. For the reasons referred above, it is critical to assess the toxicity of textile effluents containing mixtures of the dyes, the metabolites formed from dye degradation and Ag NPs, in order to evaluate the efficacy of the TWW treatment process and to be able to predict risks associated to the release of the partially treated TWW into natural water courses [2]. Therefore, the present work aimed at examining the influence of Ag NPs on the performance of AGS sequencing batch reactors (SBRs) with respect to the potential detoxification of a simulated TWW. For that, synthetic TWW containing the azo dye Acid Red 14 (AR14), with or without Ag NPs, were fed in static conditions into two SBRs. The potential toxicity of the TWW during long-term operation of the SBRs was assessed by using two bioassays with the microbial eukaryotic model Saccharomyces cerevisiae [1]. The microplate susceptibility assay measures the inhibitory effects of the samples on yeast growth, as indication of their potential cytotoxicity [1]. The gene expression assay measures changes in the transcript levels of two stress-responsive yeast genes, namely GRE2 (induced by general stress) and RAD54 (related with specific response to DNA damage) that may be caused by the samples [1]. These relatively cost-effective, small-scale and alternative-to-animal bioassays are intended to provide a preliminary toxicity screening of the TWW samples before more complex ecotoxicity assays are eventually performed.

Methods

Two 1.5-L SBRs (H/D=2.5) were inoculated with conventional activated sludge, harvested from a domestic wastewater treatment plant (Chelas, Lisboa). A fresh simulated TWW containing a pre-hyrdolysed starch-based sizing agent (Emsize E1) as carbon source, phosphorus and nitrogen salts, other nutrients and AR14 at 40 mg L⁻¹ [4], was supplied to the SBRs at a hydraulic retention time of 12 h and an organic loading rate of 2.0 kg m⁻³ d⁻¹, as chemical oxygen demand (COD). SBR1 was supplied with an Ag NPs suspension (<100 nm particle size) to an initial concentration of 10 mg L⁻¹ at the onset of the reaction phase. SBR2 was used as an Ag NPs-free control. The SBRs operated in 6-h cycles with five discrete sequential stages: fill (30-min static anaerobic feeding with exchange ratio of 50%), reaction (5-h reaction with a mixed anaerobic stage (1.5 h) followed by an aerated stage (3.5 h)), settle (5 min), drain (1 min) and idle. Samples were collected for toxicity assessment at different cycle stages, namely, from the feed solution (WWfeed), from the mixed liquor at the end of the anaerobic reaction stage (WWanaer) and from the supernatant at the end of the settling stage, i.e., from the effluent of treatment cycle (WWefflue). A sample of feed solution without dye or Ag NPs was used as control (WWcontrol). All samples were filter-sterilized (0.2 µm) before the toxicity assays.

To assess the potential toxicity of the collected samples, S. cerevisiae BY4741 Δcwp1Δcwp2, with double deletion mutations (genes encoding two cell wall mannoproteins) known to confer increased cell permeability and sensitivity to several genotoxic xenobiotics [5], was used as test organism. A microplate susceptibility assay was used to assess the...
inhibitory effects of the samples on yeast growth as described elsewhere [1]. Briefly, a standardized population grown to mid-exponential phase (optical density at 640nm (OD) ~ 0.35-0.4) was used to inoculate 2 mL of mixtures (1870 µL of each sample or control solution and 67 µL concentrated YPD growth medium) to attain an OD~0.01. The obtained suspensions were distributed (five replicates for each sample; 150 µL/well) into sterile microplate wells, which were incubated for 22 h at 30°C and 1200 rpm. Yeast growth was assessed by measuring the culture OD using a microplate reader. The potential toxicity was expressed as the growth inhibition ratio ODx/ODxWWcontrol, where ODx and ODWWcontrol represent the OD values attained with the sample mixture and the WWcontrol, respectively. Preliminary tests indicated that the used Ag NPs at concentrations up to 20 mg L\(^{-1}\) do not negatively affect yeast growth. Assessment of changes in the transcript levels of the genes GRE2 and RAD54 by quantitative reverse transcriptase real-time PCR, using as template total RNA extracted (hot-phenol method) from the standardized yeast cells upon 2 h of exposure to the samples collected from the SBRs or the WWcontrol is in course; this assay is carried out as described elsewhere [1].

Results and Discussion

The inhibitory effects on yeast growth of the samples WWanaer and WWefflue on days 51, 65, 78, 93, 101 and 113 of SBR1 and SBR2 operation are shown in Figure 1.

The sample WWfeed, with 20 mg L\(^{-1}\) of AR14, caused no significant inhibition on yeast growth (relative to WWcontrol) as reported before [1]. On the contrary, almost all WWanaer and WWefflue samples from both SBRs presented a growth inhibition ratio lower than 0.4 or close to this value, except for the sample collected on operational day 51 at the end of the anaerobic stage (WWanaer) in SBR2, with a value around 0.7 (Figure 1). Samples from SBR1 (with Ag NPs) showed a slight decrease in toxicity after the aeration stage (WWefflue) when compared to the end of the anaerobic stage (WWanaer) (except for the sample from day 113), whereas for the Ag NPs-free SBR2 a generalized increase in toxicity after aeration (WWefflue) was observed (except for the day 101 sample) (Figure 1). From operation day 51 to day 93, the samples from the Ag NPs-free SBR2 showed a slightly lower level of potential cytotoxicity when compared to samples from SBR1, but after 93 days of operation, no significant differences were found between the two SBRs. Based on the obtained data on toxicity over yeast growth, no significant differences in the potential toxicity of the TWW effluent at the end of the treatment cycles were found due to the presence of Ag NPs, during the AGS SBR operation under the conditions tested in the present work, particularly from day 78 on.

![Figure 1: Toxicity assessment based on the inhibitory effects on yeast growth of the samples WWanaer and WWefflue collected in operation days 51, 65, 78, 93, 101 and 113 of SBR1 (with Ag NPs) and Ag NPS-free SBR2, and of the simulated wastewater at the onset of the reaction cycle (WWfeed). Data correspond to growth-inhibition ratio values relative to WWcontrol. Error bars represent the standard deviation of the mean value from a total of fifteen determinations for each sample (five technical replicates tested with three independent yeast cultures).](image)

Measurement of possible changes in the transcript levels of the toxicity-responsive yeast genes GRE2 and RAD54 by the samples WWfeed, WWanaer and WWefflue collected from the two SBRs is in course. Changes in gene expression are anticipated to reflect more sensitive and rapid responses than reductions of activity at higher levels of biological organization, such as those leading to growth inhibition [1,6]. Data from the two types of yeast-based bioassays, at molecular and phenotypic levels, will be integrated aiming at obtaining a clearer picture of the impact of Ag NPs in the performance of the AGS SBR regarding potential detoxification of the simulated TWW, in parallel with decolorization and COD removal.

Acknowledgements

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References

From fruit pulp waste to biogas: The assessment of substrate shifts on the performance of a two-stage anaerobic digestion system

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A two-stage anaerobic digestion system was operated treating different fruit pulp wastes (peach, raspberry and white guava) in a sequential operation in order to assess the effect of substrate shifts and operational conditions on the system’s performance. Substrate shifts did not cause long-term instability issues and no evident association was found between operational condition changes and the effluent’s composition and acidification degrees in the first stage. However, substrate composition seemed to affect the gas production and its composition in the first stage whereas it had no effect on the gas production and composition in the second stage. The biogas produced in the second stage was rich in methane (>75.9%) and high methane yields (>0.30 Lsv/gCOD) and productivities (up to 2.13 L/(L.d)) were achieved throughout the operation. This process was shown to be robust in treating seasonal wastes from the fruit juice industry, suggesting that it could be applied in full scale.

Introduction

Anaerobic digestion (AD) has been recognised as an efficient strategy to treat food wastes, such as, fruit pulp waste [1]. AD is typically conducted in a single reactor system, where each microbial group is not subjected to their optimum conditions. This can cause instability throughout the operation. In order to provide the optimal conditions for each group of microorganisms, a two-stage AD system can be used in order to separate the hydrolytic/acidogenic stages from the methanogenic stage [2]. With this separation, the biogas produced is mainly composed of carbon dioxide (CO₂) and hydrogen (H₂) in the first stage whereas methane (CH₄) is the main gas produced in the second stage. Substrate composition can affect biogas production and composition, and due to the seasonality of the waste feedstocks it is of great importance to study the robustness of systems treating seasonal wastes with different compositions in a continuous operation. A recent study [1] showed that the two-stage system is a suitable process to treat seasonal wastes, however information concerning the impact of the shifts on the biogas in each phase is lacking. The main objective of the present study was to study the effect of substrate shifts and operational conditions on the overall system’s performance and more importantly on the biogas composition.

Methods

A two-phase AD system (each reactor with a working volume of 5 L) was used to treat peach, raspberry and white guava pulp wastes in a sequential operation under different operational conditions (pH, hydraulic retention time (HRT)/organic loading rate (OLR)). The acidogenic effluent obtained was subsequently used as substrate for the methanogenic operation. Both reactors were operated at 30°C and the pH was automatically controlled between 4.5 – 5.5 (depending on the condition) in the acidogenic reactor and at 7.5 in the methanogenic reactor by the automatic addition of NaOH 5M. The acidogenic reactor was inoculated with floccular sludge, while the methanogenic reactor was inoculated with a mixture of granular and floccular sludge. Different conditions were studied for both reactors: in the acidogenic stage, two HRT levels (1 or 2 days corresponding an OLR between 11.9 and 25.7 gCOD/(Ld)) and three pH levels (4.5, 5.0 and 5.5) were tested; in the methanogenic stage four HRT levels were tested (2, 2.5, 5 and 8.6, corresponding an OLR between 1.9 and 7.4 gCOD/(Ld)). The reactors were monitored on a daily basis in order to assess the system performance and the microbial community present in each reactor was analysed by Fluorescence in situ hybridisation (FISH).

Results

Independently of the waste composition and of the operational conditions, the acidogenic effluents composition were similar and stabilised over a short period of time after each substrate shift, indicating that the microbial community was robust and diverse enough to convert the organic matter in fermentation products (FP) for long periods without the need to stop the operation, which represents an important advantage in full scale operations. For all conditions, ethanol was the main FP present in the acidogenic effluent, followed by butyric, acetic and valeric acid. High sugar (>94%) and protein (>65%) removals were obtained and the acidification degrees obtained throughout the whole operation varied between 53.7% and 76.4%. All of these parameters were not affected by the change of operational conditions. Regarding to the biogas production, and at the same pH and HRT/OLR, raspberry pulp waste fermentation resulted in the highest volume of gas produced whereas peach pulp waste was associated with the lowest volume of gas produced (Figure 1). Different gas production between different fruit pulp wastes seems to be a result of the sugar content, in which higher sugar concentration was associated with higher gas production. Furthermore, substantial variations on gas production were detected within each condition, which was probably due to the influent degradation. With a high degradation degree (lower sugar content), less gas was produced, which can further indicate that sugar content had a direct impact on the gas production. Moreover, the composition of the produced gas, namely in hydrogen content (0-24%), was also different between wastes.

The FP produced in the acidogenic stage were converted into biogas rich in CH₄ (75.6%) in the methanogenic stage. The chemical oxygen demand (COD) removal efficiencies were similar under the same conditions and independent of the influent used (Table 1). After the change of OLR or substrate, an increase of FP concentration in the methanogenic effluent
was observed. Regardless, the system recovered rapidly achieving a low and stable FP concentration. The results suggest that the microbial community in the granules was diverse enough to metabolise different ratios of FP and remained equally active after the substrate or HRT/OLR changes. The microbial community was predominantly composed of archaea enriched in *Methanoseta*, *Methanosarcina* and Methanomicrobiales (in a smaller amount). Still, there was no apparent relation between OLR/HRT changes and the relative composition of the archaecal community since the community seemed to have remained constant throughout time (data not shown). CH$_4$ productivities increased with HRT decrease/OLR increase, which also resulted in the increase in gas production (Table 1). The CH$_4$ yields obtained in the present study were close to the theoretical value of 0.35 L$_{CH4}$/gCOD and seemed not to be affected by the HRT/OLR changes (Table 1).

![Figure 1. Acidogenic gas composition in each condition tested in the acidogenic reactor after reactor stabilisation.](image)

Overall, the results obtained in this study showed a good performance in each reactor. Bouallagui’s study [3] obtained a lower acidification degree (38.9 – 44.4%) when treating fruit and vegetable wastes. Carvalheira’s study [1] obtained similar acidification degrees (60.9 – 89.1%) when treating fruit pulp wastes and also observed that the substrate shift did not affect the overall acidogenic performance. Differences in H$_2$ production observed in this study could be a consequence of the different substrate composition, since the potential for H$_2$ production of a certain organic waste is greatly dependent on its carbohydrate content [4]. The CH$_4$ productivities obtained were similar to the other studies [1,3] and the CH$_4$ content and yield obtained in this study is amongst the highest observed in two-stage AD systems treating similar substrates. Achieving a good performance in both reactors at the same HRT (2 days) is a great accomplishment since this enables the use of same volume reactors without accumulation from the first stage. This eliminates the need to use a bigger reactor for the second stage, reducing the costs of application in the industry.

### Conclusions

The two-stage AD system was efficient in the treatment of seasonal wastes of the fruit juice industry, being able to promptly adapt after changing operational conditions or substrates avoiding operation failure. Further, high acidification degrees (53.7% – 76.4%) and COD removal efficiencies (82.1 – 93.2%) were obtained. The gas production in the acidogenic reactor seemed to be affected by the sugar content, higher sugar concentration led to higher gas production. Moreover, the composition of the produced gas, namely in hydrogen content (0-24%), was also different between wastes. On the other hand, biogas production and composition were not affected by substrate shifts in the methanogenic reactor. Stable biogas production with high CH$_4$ content (>76%) was achieved and the CH$_4$ productivity (up to 2.13 L$_{CH4}$/L.d) increased with OLR increase. The full-scale industrial implementation of a similar system would be able to treat the diversity of wastes produced while providing an extra energy source for the company.

### Table 1. Methanogenic reactor’s performance in different operational conditions.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>OLR (gCOD/(L.d))</th>
<th>COD removal (%)</th>
<th>Biogas production (L/d)</th>
<th>CH$<em>4$ yield (L$</em>{CH4}$/gCOD)</th>
<th>CH$<em>4$ productivity (L$</em>{CH4}$/L.d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peach influent</td>
<td>1.9 ± 0.1</td>
<td>92.8 ± 1.5</td>
<td>3.6 ± 0.8</td>
<td>0.30 ± 0.03</td>
<td>0.54 ± 0.02</td>
</tr>
<tr>
<td>Raspberry influent</td>
<td>1.9 ± 0.1</td>
<td>93.2 ± 2.6</td>
<td>3.7 ± 0.8</td>
<td>0.30 ± 0.03</td>
<td>0.56 ± 0.06</td>
</tr>
<tr>
<td>White Guava influent</td>
<td>3.7 ± 0.1</td>
<td>92.5 ± 2.9</td>
<td>6.9 ± 1.0</td>
<td>0.32 ± 0.01</td>
<td>1.08 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>7.4 ± 0.6</td>
<td>82.1 ± 3.9</td>
<td>12.8 ± 1.7</td>
<td>0.32 ± 0.05</td>
<td>1.93 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>6.8 ± 0.7</td>
<td>85.7 ± 0.5</td>
<td>12.6 ± 1.8</td>
<td>0.37 ± 0.03</td>
<td>2.13 ± 0.13</td>
</tr>
</tbody>
</table>

### Acknowledgements

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### References


NMR kinetic and cytotoxicity studies of sesquiterpene lactones

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Sesquiterpene lactones (STL) are natural compounds that exert important biological activities by binding covalently to cysteine (CYS), 1, residues in target proteins. The aim of this study was to develop a method for studying the reaction between CYS and parthenolide (PT), 2, a natural STL with promising antineoplastic activity, and to study PT’s cytotoxic activity against a tumor cell line. PT’s derivative, dimethylaminoparthenolide (DMAPT), 3, is in cancer clinical trials. PT was mixed with CYS at an equimolar ratio and the reaction was followed over time by NMR spectroscopy. PT reacted very quickly with a half-life time lower than 5 min, following a second order kinetics and giving rise to parthenolide-cysteine adduct (PT-CYS), 4. The in vitro cytotoxicity of 2 and 3 against CaSki cells were compared. 2 was more active than 3, with IC50 of 5 and 12 μM, respectively. Further studies will clarify whether this difference in cytotoxicity is related to different CYS-binding potentials.

Introduction
STLs are a large group of secondary plant metabolites with more than 3000 known structures and a wide variety of biological activities, including antineoplastic activity. These compounds have been studied for the treatment of several diseases and some are already in clinical trials [1].

The antineoplastic activity of STLs has been attributed to Michael-type addition reaction of their α-methylene-γ-lactone moiety with biological nucleophiles, especially with CYS residues in proteins. However STLs may contain other groups responsible for this reaction [2]. One important mechanism of STLs antineoplastic effect is the inhibition of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), by binding to Cys38 on its p65 subunit [3]. NF-κB is active in numerous cancers, including those induced by Human papillomaviruses (HPV), like cervical cancer, where it promotes resistance to chemotherapy [3]. STLs could conceivably be included in combination therapies for advanced cervical cancers, to overcome chemoresistance mediated by NF-κB.

Kinetic studies of the reaction between STLs and CYS have allowed a better understanding of the mechanisms underlying their pharmacological activity. Conjugating such studies with in vitro tests may allow the study of structure-activity relationships. Although several methods have been employed, only one resorted to NMR and none addressed the reaction of PT with CYS [4,7].

In this study, the reaction of PT with CYS was monitored using NMR spectroscopy. Kinetic parameters and the structure of the products were determined. The cytotoxic activity of PT was then studied in an HPV-transformed cancer cell line, (CaSki cells), and compared with that of its water-soluble derivative, DMAPT.

Methods
Preparation of reactants solutions, PT was dissolved in 200 μL of CD$_3$OD and D$_2$O and CYS was dissolved in D$_2$O with pH adjusted to 7.3, so that when mixed they reached an equimolar concentration of 5 mM.

NMR spectroscopy, 1H NMR spectra were recorded on a BRUKER AVANCE III HD 600 MHz, 14.1 Tesla, δ in ppm using 3-(Trimethylsilyl) propionic acid D4 sodium salt as internal standard (TSP = 0.0).

Reaction monitoring by 1H NMR. PT was mixed with CYS in equimolar amounts into an NMR tube. The reaction took place at 27 °C during the first 27.5 hours and then at 20°C until the end of the experiment. NMR signals in spectra recorded at sequential times during the reaction were integrated and reaction diagrams obtained by plotting the area of the decreasing signals of PT (H13α/β) vs CYS-α/β, by the rapid disappearance of H13α/β PT reacted with CYS.

Cell viability evaluation. CaSki cells were grown in RPMI 1640 medium supplemented with 10% FBS, 10 μg·mL$^{-1}$ penicillin and 10 μg·mL$^{-1}$ streptomycin at 37°C in a humidified atmosphere containing 95% air/5% CO$_2$. Seeding was made in 96 well plates at cell densities of 3.2x10$^4$ cells. mL$^{-1}$ and 1.6 x10$^5$ cells. mL$^{-1}$, for incubation at 24h and 72h, respectively. PT and DMAPT stock solutions were prepared in DMSO and PBS so that its dilutions had 0.2% of DMSO. After 24h of cell adherence, incubation with different concentrations of the compounds were made, and after 24h and 72h, cell viability was evaluated using an MTT assay.

Results and discussion
PT reacted with CYS via its α-methylene-γ-lactone moiety as observed by the rapid disappearance of H-13α/β PT peaks (δ 6.302 and δ 5.858 ppm, respectively) over time, in Figure 1. At five minutes, the first time point analyzed by NMR after addition of CYS, unreacted PT decreased to 45 %, indicating that the half-life time is lower than five minutes. Integration of these peak’s areas and its relationship with time, permitted to study the reaction’s kinetics (Figure 2). Linearization of this plot indicated that it follows a second order kinetics, since the plot 1/A$^{31}$ vs time yields a linear trend line with R$^2$ close to 0.99.

These results are in agreement with previous studies where a quick reaction of another STL, helenanolide, with CYS was
described, and the half-live was five minutes [4], as well as the reaction monitorization of PT with serum albumin by UHPLC/ESI-QqTOF MS, which presents a half-live time of 37 minutes [5]. Both studies resulted in second order kinetics. The analysis of the reaction product’s 1H NMR spectra together with 13C NMR allowed to determine the structure formed - PT-CYS, that is represented by compound 3. The study of PT cytotoxicity supports its possible application in treatment of HPV-derived cancers since it gave a half inhibitory concentration (IC50) values of 10 µM and 5 µM after 24h and 72h of incubation, respectively. Its derivative present in clinical trials, DMAPT, was also evaluated and was quite active with IC50 values of 18 µM and 12µM after 24h and 72h of incubation, respectively. Further studies could be made, namely the study of the reactivity of DMAPT with CYS, and of others STL and its derivatives, being able to create structure-activity studies and to give rise to a screening tool of potential antineoplastic compounds.

Table 1. IC50 values of PT and DMAPT against CaSki.

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Compound</th>
<th>PT</th>
<th>DMAPT</th>
<th>PT</th>
<th>DMAPT</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 h</td>
<td>IC50 (µM)</td>
<td>10</td>
<td>18</td>
<td>5</td>
<td>12</td>
</tr>
<tr>
<td>72h</td>
<td>IC50 (µM)</td>
<td>10</td>
<td>18</td>
<td>5</td>
<td>12</td>
</tr>
</tbody>
</table>

Figure 1. HNMR spectra (600 MHz, CD3OD/D2O) obtained during the reaction of PT with equimolar quantity of CYS.

Figure 2. Time course of the reaction between PT and CYS at an equimolar concentration (1:1, 5 mM). (a) The area of H13-PT peak versus time; (b) linearization of the time course according to second order law.

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References
Optimizing locked nucleic acid/2′-O-methyl-RNA fluorescence in situ hybridization (LNA/2′OMe-FISH) for bacterial detection

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Introduction
Fluorescence in situ hybridization (FISH) is one of the most well-established molecular biology techniques used for the rapid and direct detection, localization and quantification of microorganisms in many fields of microbiology (e.g. 1-3). Recently, the combination of the FISH method with nucleic acid mimics, such as peptide nucleic acid (PNA), locked nucleic acid (LNA) and 2′-O-methyl RNA (2′OMe), has shown to have advantages compared to DNA-FISH [3]. For instance, introducing LNA monomers at every third position of 2′OMe probes is a common approach used to improve FISH experiments in terms of affinity and sensitivity (e.g. 2). This also allows for a thorough control of the thermodynamic parameters, facilitating multiplex approaches (detection of multiple targets simultaneously). Despite the obvious advantages of LNA/2′OMe molecules in terms of improving the accuracy, stability, robustness and simplicity of the FISH, there is an absence of studies that have analyzed the impact of denaturant and salt concentration on the LNA/2′OMe-FISH efficiency. This information is very important to find the more suitable hybridization conditions for bacteria detection either in an individual or in a multiplex assay, and to move towards a tailored design of hybridization experiments.

Methods
The optimization of the hybridization conditions may be time-consuming due to the large number of factors that affect the FISH efficiency. As such, the effect and the interplay of hybridization temperature, NaCl and denaturant (formamide, ethylene carbonate and urea) on LNA/2′OMe-FISH was studied using an universal LNA/2′OMe probe for the Eubacteria domain (5′mT*G*I4*C*mC*G*T*mC*mC*I4*C*mG*mT*IA*mG*mG*I4*AI4*I4*3′; “I” - LNA; “m” - 2′OMe; * - phosphonothioates backbone) based on Amann et al. (1990) [4], through Response Surface Methodology (RSM). RSM, a mathematical and statistical tool, was applied to model the data obtained from 3 Gram-negative (E. coli CECT 515, C. freundii SGSC 5345 and P. aeruginosa PA01) and 2 Gram-positive bacteria (E. faecalis CECT 184 and S. epidermidis RP61A). Hence, to evaluate the effect of the three factors on the fluorescence intensity of LNA/2′OMe-FISH method, the hybridizations were performed in suspension based on Azevedo et al. (2015) [2], followed by signal quantification using flow cytometry. The standard central composite designs (CCD) were set up for each bacterium, using the statistical software Design Expert® 11 (Stat-Ease Inc., Minneapolis, USA) to estimate the coefficients of the model. In designs, the hybridization temperature (x1), denaturant concentration (x2) and salt concentration (x3) were considered the independent variables and the fluorescence intensity was the response. To find the optimum hybridization conditions for all five species in the study, the obtained fluorescence values were fitted to a quadratic model. Each model was analyzed using analysis of variance (ANOVA) to test the significance and adequacy of the model. Finally, the optimum conditions within the experimental range that maximize the fluorescence intensity were estimated using the optimization function of the Design Expert® 11. The value estimated for the optimum conditions was, then verified for each bacterium on a confirmation experiment, in triplicate.

Results and Conclusion
In this work, different species were selected to include bacteria with different characteristics, including different cell wall structures and compositions. Furthermore, three denaturant agents were included, because even though the formamide is more frequently used in FISH methodology, there are also studies using less hazardous compounds as denaturant agents, including urea [e.g. 2] and ethylene carbonate [e.g. 5]. Most of the quadratic models obtained for each bacterium were highly significant (p<0.05), confirming the adequacy of the model fits. Furthermore, the coefficients of determination, R2 (0.70-0.90) confirmed a good fit between predicted values and the
experimental data points. Hence, using the successful modelling of the hybridization temperature, NaCl and denaturant concentration, we have been able to obtain the optimum hybridization conditions that lead to the maximum intensity fluorescence for all the five bacteria (Table 1). These optimum ranges presented in Table 1 will provide guidelines on compromise conditions for each variable. Surprisingly, observing Table 1, the optimal NaCl concentration, ranging from ~2M to ~5M, is higher than those used in conventional LNA/2′OMe-FISH protocols (0.9 M) (e.g. 2). Optimal values were not related with the type of denaturant. In fact, NaCl is highly important in the hybridization to stabilize the repulsive interactions of LNA/2′OMe-rRNA duplexes. These electrostatic repulsions can be reduced by increasing the NaCl concentration that will stabilize the negative charges of the duplex, allowing the binding of LNA/2′OMe probes to the rRNA target. Regarding the denaturants, the use of urea provided values of fluorescence more homogenous, with *S. epidermidis* values being in lines with the fluorescence values obtained for the other species. Urea is a chaotropic agent that has been studied for its effect on permeabilization of cells [6] and destabilization of proteins and nucleic acids [7], which might enable a higher accessibility of the probe to the target. Moreover, another main observation is related with the fact that, when urea was applied in LNA/2′OMe-FISH, the ranges of the optimal urea concentration are overlapped (e.g. 1 M to 4 M of urea for *E. coli* and *E. faecalis*; 0.6 M to 2 M of urea for *P. aeruginosa, C. freundii* and *S. epidermidis*) (Table 1), which simplifies the design of any multiplex approach.

In conclusion, urea and high salt concentrations seem to be an adequate choice to balance fluorescence signal among species and to reach an universal hybridization solution for multiplex assays. However, according to the properties of the target bacteria some minor adjustments should be performed in optimal hybridization conditions to improve the efficiency of the hybridization. This study gives general recommendations for, at least, the starting point on optimization experiments of LNA/2′OMe-FISH method would include approximately 2 M of urea, 4 M of NaCl and 62 °C of hybridization temperature.

Table 1. Optimal ranges of hybridization temperature, NaCl and denaturant concentration predicted through the RSM models for each bacterium. The predicted and experimental fluorescence values are also shown. Ranges have been stabilised assuming a fluorescence intensity of at least 85% of the maximum value.

<table>
<thead>
<tr>
<th>Denaturant</th>
<th>Bacteria</th>
<th>Temperature (°C)</th>
<th>[NaCl] (M)</th>
<th>[Denaturant] (M; % v/v)</th>
<th>Predicted fluorescence (a.u.)</th>
<th>Obtained fluorescence (a.u.)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formamide</td>
<td><em>E. coli</em></td>
<td>51.41-76.08</td>
<td>0.03-1.99</td>
<td>1.18-4.40</td>
<td>296.13-348.87</td>
<td>369.14</td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em></td>
<td>56.40-74.19</td>
<td>2.11-4.92</td>
<td>4.27-12.80</td>
<td>344.46-405.25</td>
<td>225.30</td>
</tr>
<tr>
<td></td>
<td><em>C. freundii</em></td>
<td>50.00-79.00</td>
<td>2.25-5.00</td>
<td>9.00-17.69</td>
<td>113.76-133.83</td>
<td>141.26</td>
</tr>
<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>50.00-69.29</td>
<td>2.66-5.00</td>
<td>22.00-32.81</td>
<td>287.84-338.63</td>
<td>131.15</td>
</tr>
<tr>
<td></td>
<td><em>S. epidermidis</em></td>
<td>62.00-82.00</td>
<td>2.00-5.00</td>
<td>3.00-13.00</td>
<td>47.51-55.89</td>
<td>296.62</td>
</tr>
<tr>
<td>Ethylene carbonate</td>
<td><em>E. coli</em></td>
<td>55.53-77.88</td>
<td>2.00-5.00</td>
<td>3.13-12.42</td>
<td>278.02-327.08</td>
<td>346.03</td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em></td>
<td>53.77-78.09</td>
<td>2.03-3.42</td>
<td>4.02-12.71</td>
<td>201.12-236.61</td>
<td>291.57</td>
</tr>
<tr>
<td></td>
<td><em>C. freundii</em></td>
<td>57.58-79.00</td>
<td>2.00-4.89</td>
<td>3.76-13.00</td>
<td>266.56-313.59</td>
<td>353.14</td>
</tr>
<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>46.00-63.00</td>
<td>2.00-5.00</td>
<td>1.00-4.00</td>
<td>170.34-200.40</td>
<td>126.34</td>
</tr>
<tr>
<td></td>
<td><em>S. epidermidis</em></td>
<td>62.00-82.00</td>
<td>2.90-5.00</td>
<td>0.00-2.00</td>
<td>106.68-125.50</td>
<td>254.37</td>
</tr>
<tr>
<td>Urea</td>
<td><em>E. coli</em></td>
<td>50.13-60.04</td>
<td>2.41-5.01</td>
<td>1.41-4.09</td>
<td>200.40-235.76</td>
<td>253.87</td>
</tr>
<tr>
<td></td>
<td><em>P. aeruginosa</em></td>
<td>47.12-58.12</td>
<td>2.45-5.01</td>
<td>0.67-2.37</td>
<td>395.90-465.75</td>
<td>324.35</td>
</tr>
<tr>
<td></td>
<td><em>C. freundii</em></td>
<td>50.21-63.29</td>
<td>2.42-4.57</td>
<td>0.63-2.08</td>
<td>183.51-215.89</td>
<td>225.82</td>
</tr>
<tr>
<td></td>
<td><em>E. faecalis</em></td>
<td>50.13-79.87</td>
<td>2.59-5.01</td>
<td>1.41-4.09</td>
<td>345.03-405.92</td>
<td>205.58</td>
</tr>
<tr>
<td></td>
<td><em>S. epidermidis</em></td>
<td>64.16-82.91</td>
<td>3.37-5.01</td>
<td>0.61-1.84</td>
<td>188.04-221.22</td>
<td>314.17</td>
</tr>
</tbody>
</table>

*The obtained fluorescence was evaluated using the optimum hybridization temperature, denaturant and salt concentration predicted through the RSM models for each bacterium.

Acknowledgements

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References

Aqueous solutions of tensioactive ionic liquids: alternative solvents in the chlorophyll extraction from green macroalgae

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Recently, the interest in the products obtained from natural sources has grown in relation to synthetic products. Thus, industry and academia have given particular attention to products derived from natural sources. Some of these interesting compounds can be found in abundance in green algae. Chlorophylls appear as such an example. However, the conventional extraction methodologies are based on organic solvents, which present a low selectivity and are often recognized by negatively environmental impacts. In this work, a set of experimental operational conditions were studied in order to develop an efficient method to extract efficiently chlorophyll from fresh Ulva rigida. Aqueous solutions of tensioactive ionic liquids and surfactants were tested, and the extraction conditions evaluated for the best solvents selected. After, studies on environmental and economic impacts were carried out and compared with the conventional methodology.

Introduction
Chlorophyll is a photosynthetic pigment present in natural sources like plants and algae. Apart from its key role in photosynthesis, chlorophyll and their derivatives are compounds with important characteristics and bioactivities, namely as antioxidant [1] and anticarcinogenic [2] agents. Moreover, these natural porphyrins are also able to form complexes with some carcinogenic compounds [3]. To obtain chlorophyll, volatile and non-selective organic solvents are traditionally used, however these can have negative effects, not only from the operational point of view, but also in terms of their negative environmental impact. In this context, aqueous solutions of surface-active compounds such as surfactants and ionic liquids have been recognized as alternative solvents in the extraction of hydrophobic molecules like chlorophyll [4].

Objectives
This work aims to extract chlorophylls from the fresh samples of Ulva rigida using aqueous solutions of tensioactive ionic liquids and common surfactants. Moreover, and after the proper optimization of all operational parameters, the alternative method developed will be compared with a conventional one using organic solvents. To compare both alternative and conventional methods, the extraction yields, economic and environmental impacts will be assessed.

Methods
Ulva rigida was kindly provided by ALGAplus, Ltda, a company specialized in the production of marine macroalgae, located in Ilhavo, Portugal. The macroalgae were washed and stored in freezer at -20°C for further use. The frozen macroalgae samples were grounded in liquid nitrogen and homogenized in different solvents with a solid-liquid ratio of 1:100 (w/v). The extraction was performed in a shaker IKA™ Traryzer Digital at 80 rpm, for 30 minutes at room temperature and protected from light exposure. Then, the solutions were centrifuged at 5000 rpm for 30 minutes at 20 °C to allow the recovery of the supernatant. The absorption spectra were determined in each extract in the interval between 300-700 nm in a UV-Vis microplate reader (Synergy HT microplate reader -BioTek) and the chlorophyll concentration was calculated. The same method was used to optimize the operational conditions, i.e. solid-liquid ratio [from 0.008 to 0.2 (w/v)], concentration of solvent in water (from 50 to 500 mM), and time of extraction (from 5 to 120 minutes).

Results
An initial screening was performed, in which thirteen different aqueous solvents and ethanol (solvent control) were considered. A common surfactant and a phosphonium-based IL stood out as the best solvents to extract chlorophylls. These two solvents were chosen for further operational conditions optimization. The behaviour of both solvents was similar, reaching a highest extraction yield for an intermediate solid-liquid ratio. After the optimization of the best conditions of concentration of tensioactive compound in water and time of extraction, an increase in the extraction yield of 31% was obtained when compared with the ethanol performance (conventional solvent). In the end, an efficient process to extract chlorophylls from fresh macroalgae cells was envisioned and its economic and environmental impact analysed.

Conclusion
An efficient and simple method to extract chlorophyll from Ulva rigida was developed. Several different aqueous solutions of common surfactants and tensioactive ionic liquids were evaluated aiming at to maximize the chlorophyll extraction. Besides, operational conditions were optimized namely solid-liquid ratio, concentration of tensioactive in water and time of extraction. Two solvents were found as the best solvents, and for those, the operational conditions of the solid-liquid extraction were optimized meaning an increase in the extraction yield in 31% in comparison with the conventional solvent.

Acknowledgements
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the PT2020 Partnership Agreement. The authors thank FCT for the doctoral grant SFRH/BD/122220/2016 of M. Martins. S.P.M. Ventura acknowledges FCT for the IF contract ref: IF/00402/2015.

References
The potential of bacterial cellulose as hemostatic material

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Plants hold the most common cellulose biosynthesis pathway, although it can be also synthesized by bacteria, algae and fungi. Vegetable cellulose is almost an unlimited organic polymer resource on earth. However, bacterial cellulose (BC) produced by Komagataeibacter represents an alternative source with great potential for some specific applications, given its very special technical properties. In particular, the purity and ability to absorb a very high amount of water makes BC a unique source of cellulose [1]. Beside its good mechanical stability and high crystallinity, BC is also a biocompatible, hydrophilic and non-toxic material that has great mechanical properties (Young’s modulus around 15-35 GPa and tensile strength of 200-300 MPa), high degree of polymerization and high water content - up to 200 times its dry weight [2]. Overall, BC is an astonishing material with a huge potential to be applied in several fields such as in food and cosmetic industries and also in biomedical field where biodegradation is a desirable requirement for many applications. The modification of BC in order to increase and improve its biodegradability has been attempted. BC may be chemically modified through its hydroxyl groups. Thus, an improvement on biodegradation may be achieved through the oxidation of BC making it reabsorbable by the organism. Besides this, after oxidation BC displays other characteristics such as hemostatic features being a suitable raw material for some applications as medical devices [3]. In this work, the oxidation of BC membranes was achieved using tetramethylpiperidine-1-oxyl (TEMPO) radical through electrochemical oxidation methods. TEMPO has been chosen as the primary method to convert polysaccharides into the corresponding polyuronic acids through the selective oxidation of the primary hydroxyl groups, i.e., only the primary hydroxyl group at C6 is oxidized to carboxyl groups. Besides this, this approach is a suitable alternative to the chemical co-oxidants like NaClO-NaBr, water-acetonitrile-NaClO-NaClO2 and is considered as cleaner since it is possible the anodic regeneration of the oxidizing species instead of the primary oxidant [4].

This project thus aims to develop a hemostatic and resorbable material based on BC. After oxidation, using a total current of 400 and 700C, BC membranes were deeply characterized through different techniques such as: FT-IR, SEM and 13C-NMR. The carboxyl content after oxidation was also determined, and the in vitro degradability in ultra-pure water was evaluated after 3, 7, 14 and 63 days. Finally, the hemostatic behavior was investigated through whole blood coagulation tests.

FT-IR spectra of the oxidized cellulose showed an increase of the absorption band around 1628 cm\(^{-1}\) attributed to the carboxylic acid vibration, in comparison with that obtained for non-oxidized cellulose [5]. The bands were also more intense on membranes with a higher degree of oxidation. On the other hand, through SEM analysis it was possible to assess the morphology of BC network with and without oxidation. The obtained imagens revealed that the morphology of the membranes was not changed by the oxidation which is in accordance to the literature [6]. It was also performed 13C-NMR analysis to evaluate the specific oxidation of BC membranes on C6. The obtained results showed the usual six signals of BC ascribed to each C atom. The signal around 62 ppm corresponding to C6 membrane was deeply characterized and the selective oxidation of C6 was confirmed by 13C-NMR. SEM was used to assess the surface morphology of BC membranes and the in vitro degradation was investigated in ultra-pure water. The hemostatic behavior was evaluated using whole blood coagulation tests.
Acknowledgements

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References

Bacterial cellulose as a stabilizer for oil-in-water emulsions

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Cellulose is the most abundant biopolymer on earth, being plant sources the most significant. This material has been showing increasing applications in many fields: biomedicine, food, cosmetics, electronics, composite materials, among others. Cellulose from bacterial sources is a prominent alternative to the already marketed cellulosics, being more pure, crystalline, having nanoscale fibres and excellent mechanical properties. One of the many uses of cellulose, in its various forms, is the stabilization of heterogeneous systems – more specifically, the stabilization of oil-in-water emulsions. In this work, bacterial cellulose’s potential as a stabilizer for Pickering emulsions was assessed. A model system of an isohexadecane-in-water emulsion was studied in detail. A dry bacterial cellulose formulation was able to lower the interfacial tension between the immiscible phases. Emulsions prepared with this formulation showed good stability over time and desired rheological behavior.

Emulsions are obtained from at least two immiscible liquids, when they are mixed together and one of the phases arranges in small droplets (internal phase) dispersed in the other continuous outer phase. In an oil-in-water (o/w) emulsion, the oil is the dispersed/internal phase, and the water is the continuous phase. This sort of mixture is unstable without the addition of an emulsifying agent of some kind. Conventionally, to produce and stabilize an emulsion, surfactant molecules are added in order to lower the interfacial tension (IFT) between the immiscible liquid phases – in other words, to reduce the energy of the liquid-liquid interface. Surfactants arrange in the oil-water interface because they are amphiphilic molecules [1, 2]. Particle-stabilized emulsions - known as Pickering emulsions - have gained increasing interest, including for food applications. Particles suitable for Pickering stabilization of emulsions strongly and irreversibly adsorb at the oil-water interface, depending on their relative affinity for both phases. Organic solid particles, or more precisely biopolymers (polysaccharides, aromatic macromolecules and polypeptides), have been shown to be capable of stabilizing emulsions: they exhibit surface activity at liquid–liquid interfaces [1]. The solid particle coating at the droplets surface acts as a mechanical barrier, preventing coagulation and coalescence phenomena [3, 4, 5].

Besides the stabilization attained by adsorption to the interface of two distinct fluids, solid particles have yet another mechanism of stabilization: structuring the continuous phase. When the interfacial area of a biphasic system is completely covered, and an excess amount of particles remains in the continuous phase, they interact with each other and can form a three-dimensional structure. The viscosity of the continuous phase increases, providing higher support to the disperse phase droplets, reducing coalescence and creaming. Thickening of the emulsions and foams slows down the processes that lead to phase separation, improving long-time storage, an interesting outcome for some applications [1, 3, 4].

Solid cellulosic particles, in the form of nano- or micro- fibres or crystals, also show capability to form Pickering emulsions. Despite the overall hydrophilic nature of cellulose, the less significant hydrophobic domains within the crystal structure greatly contribute for the stabilization of Pickering oil-in-water emulsions, by adsorbing to the interface [6].

Hydrocolloidal microcrystalline cellulose (MCC), from plant sources, is already widely used in food industry to regulate the stability, texture, rheology and organoleptic properties of many food and cosmetic formulations [7]. Bacterial cellulose (BC) is a sophisticated material produced biotechnologically by different microorganisms, but most efficiently by acetic acid bacteria from the genera *Gluconacetobacter* [8, 9]. While chemically identical to plant cellulose, BC is chemically pure. Each BC nanofiber is a bundle of cellulose nanofibrils. Due to their nano-size, these aggregates of extended cellulose chains have a rather large surface area. The unique properties of BC account for extraordinary physico-chemical and mechanical behavior [7]. This biomaterial has also been used for the stabilization of o/w emulsions [6, 10], but in its hydrated form. For the sake of storage, economy and practicality, additives for industries are preferentially provided in a dry or powder form. After drying, most cellulose products do not re-hydrate properly. Co-drying with water soluble polysaccharides helps dispersion of the dried fibres or crystals, while maintaining the rheologic and structuring properties. Thus, it is very common to find i.e. carboxymethyl cellulose (CMC) in commercial MCC formulations, for the most diversified applications.

The main objective of this study was to assess the stabilizing properties of BC in o/w emulsions, for use as a novel hydrocolloid. For this, an equimassic formulation of BC and 90 kDa CMC (BC:CMC) was prepared and spray dried. Isohexadecane-in-water emulsions (10:90) were prepared in the presence of 0.1%, 0.25% and 0.5% of the BC:CMC formulation, previously dispersed in the water phase. Samples were mixed with a homogenizer for 4 minutes at 20 000 rpm, and stored at room temperature. Visual and microscopic aspect of the emulsions was registered over time. Samples were also visualized in Cryo-SEM the day after preparation. Rheological tests were performed to assess the emulsion’s viscosity profile, storage and loss moduli.

Interfacial tension between the immiscible phases was measured both with the pendant drop method and with the Du Noüy ring method. BC:CMC dispersions density was assumed the same as of the distilled water (1.004 g/mL, determined at room temperature, approximately 22 °C). Isohexadecane’s density at the same temperature was 0.783 g/mL.
Microscopic analyses showed large oil droplets, but stable over time. Despite a visible creaming in the emulsions with lower BC:CMC concentration (0.1% and 0.25%), there was no evident separation of the oil phase. This reaffirms that the particle adsorption to the oil-water interface is strong, and that the amount of particles was sufficient to cover all the interfacial area. The emulsions showed a viscoelastic and shear-thinning behavior, with a yield stress between 0.01 s\(^{-1}\) and 0.1 s\(^{-1}\). An increase in BC:CMC concentration results in higher emulsion viscosity, along with progressively less significant creaming phenomenon. The storage and loss moduli also increase with BC:CMC concentration, and the elastic behavior becomes more prominent.

IFT measurements (Table 1) showed that the presence of the BC:CMC formulation actually diminishes the interfacial energy between the immiscible phases. At a concentration of 0.5%, the dry BC:CMC formulation was able to effectively stabilize the o/w emulsions against coalescence or creaming for up to 60 days without the need to add any other emulsifying agents, thus confirming the potential of BC as a stabilizing agent for Pickering emulsions.

<table>
<thead>
<tr>
<th>Method</th>
<th>H_2O IFT (mN/m)</th>
<th>0.1% BC:CMC IFT (mN/m)</th>
<th>0.25% BC:CMC IFT (mN/m)</th>
<th>0.5% BC:CMC IFT (mN/m)</th>
<th>0.5% CMC IFT (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Du Noüy Ring</td>
<td>45.9 ± 1.3</td>
<td>44.8 ± 1.6</td>
<td>40.6 ± 1.2</td>
<td>36.6 ± 1.7</td>
<td>-</td>
</tr>
<tr>
<td>Pendant Drop</td>
<td>48.5 ± 1.7</td>
<td>45.9 ± 1.1</td>
<td>42.1 ± 0.8</td>
<td>35.2 ± 1.2</td>
<td>48.9 ± 0.2</td>
</tr>
</tbody>
</table>

Acknowledgements
This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO04469 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

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References
Development of a water-soluble dextrin-amphotericin B conjugate for the treatment of Leishmaniasis

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Leishmaniasis has been classified as one of the most neglected tropical diseases that affects around 2 million people worldwide (with an incidence of about 0.5 million cases annually) causing morbidity and mortality [1]. This disease, which is endemic in poor countries, is also found in Europe (transmitted as a zoonosis). In fact, there are 12 million people currently infected globally and 350 million people in 88 countries all over the world living at risk of developing one of the many forms of the disease [1, 2]. Protozoan parasites of the genus Leishmania are responsible for this life-threatening disease. These parasites can maintain their life cycle through transmission between two forms: the extracellular motile promastigote found in insects (sandfly) and the intracellular non-motile amastigote inside the macrophages of mammalian hosts [3]. Similarly to other intracellular pathogens, such as Mycobacterium tuberculosis, this life-threatening parasite can cause a range of diseases, especially in immunocompromised patients [1, 3].

More specifically, some Leishmania species (e.g. L. major, L. mexicana, L. amazonensis and L. braziliensis) promote cutaneous, mucocutaneous or diffuse cutaneous leishmaniasis, characterized by localized symptoms. Other Leishmania species that are more aggressive (e.g. L. donovani, L. chagasi, L. infantum), target internal organs causing visceral leishmaniasis. This form of the disease is responsible for approximately 70,000 deaths per year and is associated to different symptoms: fever, weight loss, splenomegaly, hepatomegaly and anemia [2, 3]. Despite the well-established knowledge and the recent advance in our understanding of leishmanial biology, some aspects of this disease remain enigmatic and because of that the current control or treatment strategies are rather inadequate [3, 4]. This is either due to limited availability of effective parenteral drug formulations or constant appearance of new fungal infections, which are resistant to the available drugs on the market. Besides, as there are currently no effective vaccines to prevent human leishmaniasis, the management/cure of the disease relies on chemotheraphy, where the drugs are of high toxicity, low efficacy and difficult to administer [2, 5]. Amphotericin B (AMB) - a highly toxic water-insoluble compound - is a polyene antibiotic used as standard drug for fungal infections. Currently it is recommended as a second-line treatment for visceral and mucocutaneous leishmaniasis. Nevertheless, this therapy is limited since AMB is difficult to solubilize, promote side effects (nausea, fever and chills) and is toxic, mainly to the kidneys, central nervous system and liver. Considering that, strategies such as the use of combination therapy, modification of the AMB molecule, modification of the physical state of AMB and use of drug delivery system (liposomal formulations, lipid complexes, colloidal dispersions, among others) have been the cornerstones to improve the therapeutic efficacy and to reduce the toxicity of AMB, even at high doses. However, there are some lipid products (e.g. AmBisome) or micellar formulations (e.g. sodium deoxycholate AMB - Fungizone) that, despite being on the market, exhibit major clinical limitations [5, 6].

The use of proteins, polypeptides, polysaccharides and synthetic polymers to achieve water-soluble polymer-drug conjugates has attracted considerable attention in recent years since it may enable drug targeting while reducing drug toxicity [6]. Different polymeric drug carriers have been widely studied for this purpose, including polysaccharides [5, 7, 8], which possess high water-solubility, low toxicity, a high degree of biodegradability and biocompatibility [9].

In this work, we hypothesize that dextrin may be an interesting polysaccharide for the development of a drug delivery system as it is a biocompatible and nonimmunogenic material, degradable in vivo by amylases, making it a potential asset for use in the biomaterials field [10]. Considering that, the purpose of this work was to achieve and characterize a new water-soluble dextrin-amphotericin B (DEX-AMB) conjugate and test its efficacy against Leishmania infection. The conjugate, which was obtained by mixing dextrin with AMB, was characterized in terms of size/morphology by cryo-SEM. Furthermore, an HPLC-MS detection method was optimized and used to determine the AMB concentration and the encapsulation efficiency (EE) in the conjugate. Leishmanicidal activity of the DEX-AMB was assessed in vitro in axenic cultures of Leishmania amazonensis by resazurin and infected bone marrow-derived macrophages stained with different fluorescent probes using high-content microscopy (IN Cell Analyzer 2000). The cytotoxic effects of the conjugate were assessed on bone marrow-derived macrophages by resazurin. In terms of size/morphology characterization, cryo-SEM analysis has shown that this conjugate has the ability to form spherical particles in aqueous solution. Those particles are within the nanometric size range, with a hydrodynamic diameter of around 100 - 200 nm. This analysis also prove that the conjugate is soluble in water, since no signs of drug precipitation were observed.

As previously stated, the development of an HPLC-MS quantification method was very important in this work, since it
has been found that the commonly used methods, such as spectrophotometry or HPLC-UV, are not reliable. In this work, a discussion on the limitations of the commonly used methods for estimation of AMB will be provided and a new method is proposed.

In vitro assays were performed to evaluate possible cytotoxic effects against bone marrow-derived macrophages and to assess capacity of the DEX-AMB to reduce the parasite infection. Comparatively to the non-encapsulated AMB, the conjugate material showed less cytotoxicity at the higher tested doses. In the L. amazonensis-infected bone marrow-derived macrophages, all the tested doses of our conjugate promoted a slightly higher inhibition of the infection comparatively to the non-encapsulated drug.

Acknowledgements
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References

In conclusion, this work has shown that DEX-AMB is a promising drug delivery system for the treatment of Leishmaniasis. This is proved by the capacity of our polymer-drug conjugate to generate similar effects to the ones obtained with non-encapsulated AMB against Leishmania-infected macrophages and Leishmania axenic cultures, with the advantage of being significantly less cytotoxic.
Density and sludge volume index estimation in mature aerobic granular sludge by quantitative image analysis and chemometric tools

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Quantitative image analysis (QIA) has already been applied for activated sludge dysfunctions and anaerobic granular sludge processes elucidation and its application to aerobic granular sludge characterization is considered of major interest. In this work, a sequencing batch reactor (SBR) was operated using mature aerobic granular sludge for pharmacologically active compounds (PAC) removal. The physicochemical parameters (total and volatile suspended solids, sludge volume index and density), prior to the introduction of PAC, were determined by analytical methods. The mature aerobic granular and suspended sludge morphological and structural parameters were established by QIA. The objective of this study is to characterize this mature granular sludge by QIA, and establish relationships for physicochemical parameters (aggregates density and SVI30) with QIA data using chemometric tools. Indeed, relevant relationships could be found for both density (R² of 0.81) and SVI30 (R² of 0.87).

Introduction
Conventional activated sludge (CAS) systems are prone to be affected by dysfunctions (pinpoint flocs formation, filamentous and viscous bulking, foaming, etc.) leading to a decrease in treatment efficiency. However, the traditional methods to monitor these phenomena are usually time-consuming. In this sense, quantitative image analysis (QIA) has already been applied to monitor and predict these abnormalities in CAS [1]. On the other hand, the aerobic granular sludge (AGS) systems are a promising technology to replace CAS due to the smaller footprint and the possibility to remove organic matter and nutrients (N and P) simultaneously. Furthermore, its use for pharmacologically active compounds (PAC) removal, within a sequencing batch reactor (SBR), is gaining attention. However, the granular aggregates can suffer from instability phenomena, which affects the performance of the biological process. Recently, it has been recognized that the stability of the AGS process is dependent on a good balance between the suspended and granular sludge [2]. Again, QIA techniques have been successfully applied to monitor the anaerobic granulation process performance [3]. Therefore, the application of QIA can be a useful tool to characterize the biomass structure and even to predict and diagnose changes in AGS by the presence of certain compounds like PAC. This research presents the first study, to the authors’ knowledge, on the application of QIA to the suspended and granular fractions of a mature AGS system directed to estimate its density and sludge volume index (SVI1). Due to the large amount of data obtained by QIA, chemometric tools were applied to pursue and validate the obtained correlations.

Objectives
The main objective of this study is to characterize mature aerobic granular sludge in an SBR, prior to the introduction of PAC, by QIA and establish relevant relationships for aggregates density and SVI30 using chemometric tools.

Methods
A 5 L SBR was fed with a synthetic medium [4] for 6 hours cycles encompassing 120 min of feeding in plug-flow, 232 min of aeration, 3 min of settling and 5 min of effluent withdrawal, presenting a hydraulic retention time of 12h. The SBR was inoculated with AGS from a municipal WWTP (Portugal). A stabilization period of 66 days was allowed before monitoring took place. Then, the characterization of the mature and stable AGS was performed once per week for a period of two months. Total and volatile suspended solids (TSS and VSS) and SVI at 30 minutes (SVI30) were determined according to standard methods [5], whereas the density was determined with Blue dextran [4]. For the biomass morphological and structural characterization, the suspended and granular sludge fractions were separated with a 500 µm mesh sieves. The suspended biomass images were further acquired with an Olympus BX51 microscope (Olympus, Shinjuku, Japan) with a total magnification of 40x, and for the granular biomass an Olympus SZ 40 stereomicroscope (Olympus, Shinjuku, Japan) was used with a total magnification of 15x. The image processing and analysis programs, for the characterization of the suspended (flocs and filaments) and granular sludge were developed in-house in Matlab 7.3 (The Mathworks, Inc., Natick, USA), adapting a previous version developed by Amaral [6]. The total filaments length (TL) and the flocs size values were obtained by QIA. The flocs were then divided into three size classes in equivalent diameter (Deq): F1 (<25 µm in Deq), F2 (25-250 µm), and F3 (>250 µm). Regarding the granular biomass also three classes were used: G1 (<0.25 mm in Deq); G2 (0.25-2.5 mm); and G3 (>2.5 mm). The performed overlapping allowed accounting for flocculent biomass that could be trapped in the sieve, though presenting a diameter smaller than 500 µm. Finally, a multiple linear regression (MLR) was performed in order to establish relevant relationships concerning the studied parameters: aggregates density and SVI30.

Results
Table 1 presents the main physicochemical parameters determined in the monitored operational period. During the monitoring period TSS were always above 4.6 g L⁻¹, presenting the largest value in the last monitoring sample. Throughout this entire period, the VSS/TSS ratio presented a value around 0.9. The SVI30 values were always below 65 mL g⁻¹.

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indicating a good sludge settleability, with particular emphasis towards the monitoring period end (16.3 mL g\(^{-1}\)). Taking into account the granules volume, the obtained density, presented an average value of 18.1 g vss L\(^{-1}\) biomass, which can be considered relatively low according to [4] (below 50 g vss L\(^{-1}\) biomass), though it raised at the end of period. In addition, the main mature biomass structural parameters were determined by QIA in the monitored operational period (Table 2).

Table 1. Main physicochemical parameters values

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Min</th>
<th>Avg</th>
<th>Max</th>
<th>STD</th>
<th>Final</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS (g L(^{-1}))</td>
<td>4.6</td>
<td>6.3</td>
<td>13.4</td>
<td>2.7</td>
<td>13.4</td>
</tr>
<tr>
<td>VSS/TSS</td>
<td>0.87</td>
<td>0.90</td>
<td>0.91</td>
<td>0.01</td>
<td>0.90</td>
</tr>
<tr>
<td>SVI(_{30}) (mL g(^{-1}))</td>
<td>16.3</td>
<td>41.4</td>
<td>64.6</td>
<td>14.7</td>
<td>16.3</td>
</tr>
<tr>
<td>Density (gVSS L(^{-1}) biomass)</td>
<td>9.4</td>
<td>18.1</td>
<td>30.2</td>
<td>7.5</td>
<td>30.2</td>
</tr>
<tr>
<td>Gran. vol. %</td>
<td>22.6</td>
<td>41.7</td>
<td>70.5</td>
<td>16.1</td>
<td>40.1</td>
</tr>
</tbody>
</table>

The overall granules area percentage was, throughout the monitoring period, always above 85% and reaching 97.8% in the end. All but one, of the samples presented G3 (larger granules) area % above 50%, reaching 73.2% at the end. This configures a highly granular biomass stratification. Such granules were also found to possess good morphological qualities. Given the TL/TSS values always under 7 m mg\(^{-1}\) [7], and reaching 0.2 m mg\(^{-1}\) at the end, no filamentous bulking occurred. Furthermore, the SVI\(_{30}\) values under 65 mL g\(^{-1}\) indicate good granules settling properties, whereas the F1 area percentage below 2.5% excluded the existence of a pinpoint flocs problem [1].

Next, a multiple linear regression (MLR) was performed in order to establish the relationships concerning the studied parameters:

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References


Figure 1. Relationship between the parameters predicted by MLR (pred) and the experimental measurements for: (a) Density and (b) SVI\(_{30}\).

Conclusion

The use of QIA, coupled with chemometric analysis allowed to characterize mature AGS and establish relevant relationships for the aggregates density and SVI\(_{30}\) with QIA data using chemometric tools. Thus, it is expected that the use of such techniques and correlations can be useful in further experiments regarding the AGS use for PAC removal, to predict possible changes in the biomass morphology due to the presence of those compounds.
Aqueous biphasic systems (ABS) are commonly used as biocompatible liquid-liquid separation strategies – a main result of their high water content. ABS composed of ionic liquids (ILs) were introduced in 2003 [1] as promising substitutes of the well-studied polymer-based systems. Due to the wide variety of ILs chemical structures, IL-based ABS allow to overcome the narrow hydrophilic-hydrophobic range of the more traditional systems [2].

ILs have been applied as designer solvents, mainly because of their ability to combine highly diverse cations and anions [3]. Furthermore, the use of ILs mixtures further enhances this chemical flexibility [4], which can be advantageous on the development of efficient separation platforms using IL-based ABS. Based on this possibility, herein we show the results obtained with ABS composed of mixtures of ILs, while envisaging the broadening of their phases’ polarities and ability to undergo liquid-liquid demixing.

Novel ABS (pseudo-quaternary) phase diagrams were determined for systems formed with the inorganic salt K$_2$CO$_3$, water and mixtures of 1-butyl-3-methylimidazolium trifluoromethanesulfonate ([C$_4$C$_1$im][CF$_3$SO$_3$]) and 1-butyl-3-methylimidazolium chloride ([C$_4$C$_1$im][Cl]) ILs at different mole fractions. The liquid-liquid phase behavior of these new systems was compared to that of the ABS composed of each of the ILs. The partition coefficients and extraction efficiencies of the investigated ABS were evaluated for a series of bioactive compounds, namely alkaloids (caffeine), amino acids (L-tryptophan and L-tryptophan), and antioxidants (gallic acid and vanillic acid). The composition of the coexisting phases was quantified at the mixture composition used for the extraction experiments.

According to the obtained results, the most hydrophobic IL ([C$_4$C$_1$im][CF$_3$SO$_3$]) is preferentially salted-out by the salt over [C$_4$C$_1$im][Cl]. Furthermore, it was found a selective partitioning of biocompounds to the IL-rich phase, which is mainly dependent on the IL ratio present in each mixture. The partition coefficients of all biocompounds closely correlate with the distribution profiles of the IL ions between the coexisting phases, confirming that the presence of a given IL and its nature allows the tailoring of the phases’ polarities and extraction performance.

In conclusion, ABS composed of mixtures of ILs can act as separation systems of tailored polarity, an essential feature regarding the extraction and purification of value-added compounds obtained from biotechnological processes.

Acknowledgements

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References

Dextrin: a platform for the development of drug delivery systems

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Dextrin is a biocompatible polysaccharide that can be suited for the conjugation with bioactive agents while enabling controlled release at the target site. LLKKK18, an LL37 human cathelicidin analog, has been engineered to enhance antimicrobial properties and decrease toxicity. In this work, chemically modified dextrin bearing a carboxylic group has been produced to allow the conjugation with LLKKK18 for the treatment of osteomyelitis. FT-IR spectra confirmed dextrin modification while maximum degradability was achieved after approximately 2h. A batch of 30% mol modification was used to conjugate with LLKKK18 and incubated in vitro with Staphylococcus aureus. Conjugates incubated with α-amylase were more effective in killing bacteria when compared to conjugates with intact bonds, emphasizing the protective effect of dextrin and indicating a controlled peptide release.

Dextrin is a glucose polymer, generally regarded as safe (GRAS), obtained from partial hydrolysis of starch. It is a biocompatible material, non-immunogenic and degradable in vivo by α-amylases. Regarding the biomedical field, the polymer has been shown to envelope a protein thus masking its bioactivity, while enabling controlled restoration of activity at the target site by triggered polymer degradation [1]. Moreover, in previous work, we have demonstrated the conjugation of the antimicrobial LLKKK18 peptide with a carboxyl group created in the dextrin backbone [2].

Antimicrobial peptides (AMPs) are part of the innate immune system with potential as novel therapeutic agents due to its high spectrum of antimicrobial activity and low propensity for bacteria to developing resistance [3]. The bactericidal effect of LL37, the only known human cathelicidin, has been reported [4]. LLKKK18, an LL37 analog, has been engineered to enhance antimicrobial properties and decrease toxicity, being three-fold more effective in the killing of mycobacteria than LL37. Despite these advantages, exogenous administration is limited by enzymatic degradation, ultimately leading to an unsuccessful local delivery of AMPs.

Osteomyelitis is an inflammation of the bone triggered by an infection, which results in inflammatory destruction and tissue necrosis. The antimicrobial effect of LLKKK18 associated to a proper nanocarrier, emerges as an innovative and promising solution to treat this pathological condition.

In this work we hypothesized that the modified polymer would be slowly degraded by amylases, releasing the peptide in a controlled fashion avoiding its early degradation, thus improving antimicrobial effectiveness. The activity of the conjugates obtained was assessed in vitro, using a relevant strain mostly responsible for osteomyelitis, Staphylococcus aureus.

Dextrin was succinoylated using a modified version of a previously described method [5], based on the introduction of a carboxyl group in the dextrin backbone via an ester linkage, in a 4-dimethylaminopyridine (DMAP)-catalyzed reaction. The ratio of reagents used was optimized to yield a modified dextrin that: (a) would have enough functional groups to bind LLKKK18, and (b) would be able to delay degradation by amylases in order to provide a sustained release of the peptide. Modification % of several batches was confirmed by FT-IR and quantified by titration with sodium hydroxide. Succinoylated dextrin (DexSuc) spectrum showed a transmittance peak around 1729 cm\(^{-1}\) that is typical for the ester group. DexSuc degradation by dinitrosalicylic colorimetric method took around 2h, and increasing % modification slowed the degradation rate. LLKKK18 was then conjugated with dextrin [5], based on the binding of the peptide free amines to the free carboxyl groups in the succinoylated dextrin backbone, which results in the formation of an amide (as shown in the Graphical Abstract).

In vitro studies with S. aureus showed that incubation of conjugates with α-amylase increased the microbialic activity as compared to intact conjugates, emphasizing the protective effect of dextrin and indicating a controlled peptide release. In fact, conjugates with α-amylase and intact conjugates corresponding to a peptide concentration of 1.17 µg/mL, were able to reduce viability to 0% and 32.8%, respectively. These results are promising and will soon be further investigated in a rat model of osteomyelitis.

Acknowledgements

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References
Application of enzyme technology in the improvement of wastewater treatment systems

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Urban wastewater treatment is commonly based on biological processes, and therefore its success depends on the uptake of contaminants by the microorganisms present in the treatment units. Many of the conventional treatment systems are inefficient and the final effluent does not meet the emission limit values required for the discharge to the environment (D91/271/EEC) or its further reuse, showing low rate of degradation of organic matter (presence of recalcitrant components). Therefore, it is crucial to develop technological innovations to make the treatment of wastewater an efficient and effective process. In this sense, the use of enzymatic biocatalysts could lead to a significant improvement in the performance of wastewater treatment plants. In this work, batch tests to assess the effect of enzymes dosage to an activated sludge system are proposed. Although, initially, no effect is observed on the removal of organic matter, a reduction in the sewage sludge production is detected.

Biological reactors are able to bring about the degradation of the target pollutants primarily due to their enzymes. Therefore enzymes, both intracellular and extracellular, are being explored as biochemical means of wastewater treatment. In general, enzymes are highly specific and extremely efficient catalysts [1]. They can selectively degrade a target pollutant without affecting the other components in the effluent. Therefore, enzymatic treatment is suitable for effluents that contain relatively large amounts of the recalcitrant target pollutants in comparison to others. More importantly, they can operate under mild reaction conditions, especially temperature and pH. In this respect, enzymes outperform the regular catalysts (transition elements like Cu, Ni etc.). From the environmental perspective, enzymes are more acceptable due to their biodegradability [2].

The potential advantages of enzymatic treatment as compared with conventional treatment include: application to biorefractory compounds; operation at high and low contaminant concentrations; operation over a wide range of pH, temperature and salinity; absence of shock loading effects; absence of delays associated with the acclimatization of biomass; reduction in sludge volume and the ease and simplicity of controlling the process [3].

The use of enzymes for the food, chemical, pharmaceutical industries, among others, is widespread. They have been also applied in environmental processes, mainly soil and bioremediation with satisfactory results [4]. However, the application of enzyme technology in the urban wastewater treatment sector is still in an emerging stage. The main factor limiting the use of enzymes in this sector could be the cost of the commercial enzyme preparations currently available on the market. So the development of processes leading to the production of these enzymes “in situ” in a feasible way is crucial for its deployment.

In this study, batch tests have been employed for the assessment of the application enzymes to a biological reactor addressed to the treatment of urban wastewater.

Figure 1. Experimental set: Oxitops ® and batch reactors employed in this study.

The enzymes and dosage employed in these tests are:

- Subtilisin (0.05%; 0.1 % and 0.5%), group of serine proteases that – like all serine proteases – initiate the nucleophilic attack on the peptide (amide) bond through a serine residue at the active site.
- Papain (0.05%; 0.1 % and 0.5%), enzyme with both exo- and endo-peptidase activity. Papain breaks peptide bonds.
- Cellulase (0.05%; 0.1 % and 0.5%), catalyzes cellulolysis, the decomposition of cellulose and of some related polysaccharides.
Lipase (0.05%; 0.1 % and 0.5%), catalyzes the hydrolysis of fats (lipids).

Combination of protease and lipase (P-L), to study synergistic effects.

The reactors are initially fed with real wastewater from the Experimental Centre of CENTA in Seville (Spain). All the reactors contain the same volume of activated sludge at the beginning of the trials.

In all the batch trials, a control reactor with the denaturalized enzyme is prepared. The effect on the organic matter removal is calculated through the difference between the COD (mg/l) at the beginning of the test and at the end (up to 5 days). The sludge production is estimated through the 30 Minute Settling Test (V30) and the determination of suspended solids. All the analytical determinations are based on Standard Methods [5].

According to the results in the batch trials, the addition of enzymes on the bioreactors does not have a noticeable effect on the degradation of organic matter. Concretely, the COD in the final effluent is practically the same in all the reactors at the end of the trial. On the contrary, a smaller sludge production is observed in those reactors with enzymes.

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References
Harvesting of Arthrospira maxima by coagulation/flocculation

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Biodiesel production from microalgae still faces big challenges to achieve a commercial and profitable production, in particular due to harvesting, one of the most energy intensive steps of the whole process. On the other hand, use of complex chemicals may pose additional problems to the later water disposal. Thus, in this study, the effectiveness of coagulation-flocculation methods induced only by pH variation and by the addition of flocculants were evaluated for Arthrospira maxima. Results show that Arthrospira maxima can be effectively harvested by increasing the pH over 10 with NaOH or by adding calcium chloride as flocculant with a concentration of about 0.2-0.3 g/L and a 1:30 volume ratio of CaCl2/microalgae culture. Other chemicals, such as NH₃, Al₂(SO₄)₃, Na₂PO₄, HCl and MgSO₄ have also been tested but without positive results.

Introduction
Microalgae are potential feedstock for biofuels production, as they do not require much land area for cultivation, possess a high growth rate and contain higher lipid content than traditional food crops [1].

Generally, a microalgae biomass production system includes microalgae cultivation in an environment that favors accumulation of target metabolites and recovery of the microalgal biomass for downstream processing. However, due to the small size (5-50 μm) and low biomass concentration (0.5-5 g/L) of microalgal cells, harvesting microalgal biomass from growth medium is still a challenge, which accounts for more than 30 % of the total production cost from microalgae to biodiesel [2]. Therefore, it is necessary to develop effective and economic technologies for microalgae harvesting, bearing in mind that the least addition of chemicals should be done, so that water can be further reused or disposed of easily.

As microalgae usually have negative surface charge (about -7.5-40 mV) they have a strong affinity to cationic additives such as metal coagulants. Consequently, coagulation (e.g. with Al or Mg salts) is regarded as an effective technology for removing them from the culture medium. However, it has the disadvantage to contaminate biomass with the metal ions. Other flocculants that do not cause metal ions contamination and that do not change the algae cell metabolic components have also been proposed, such as ammonia [3]. Hence, this study aims to evaluate the effectiveness of coagulation-flocculation methods for Arthrospira maxima, induced only by pH variation and by the addition of flocculants.

Material and Methods
Arthrospira maxima is an aquatic blue green alga that, in spite of having relatively low lipid content, presents a fast growth rate. It is usually cultured for its high protein and nutrient content, and can tolerate high levels of bicarbonate, carbonate, salinity and pH, what prevents its contamination and suggests it can be cultivated using wastewater, instead of pure fresh culture mediums. However, in this case, the use of Arthrospira maxima for feeding purposes may not be admissible [4-5]. Therefore, its use as a raw material for biofuel production can be of interest.

A culture of Arthrospira maxima SAG 84.79 from the German SAG (Sammlung von Algenkulturen Göttingen) collection was used in this work.

Different chemicals have been referred to in literature, as potential coagulation/flocculation inducers. Therefore, the effects of NH₃, Al₂(SO₄)₃, Na₂PO₄, CaCl₂ and MgSO₄ were tested, using several different concentrations. To quantitatively analyze the coagulation/flocculation effect of a pH variation, HCl and NaOH were used to decrease and increase the pH, respectively. For the pH decrease, the pH was adjusted to 9, 8, 7 and 6 in 250 ml beakers containing the microalgae cultures (with an initial pH of 10) and samples were taken each 15 min to measure the absorbance at 680 nm until a maximum 4 h of resting time. This way the biomass removal efficient was measured. The same procedure was followed for the increase of pH with NaOH to 11 and 12.

To analyze coagulation and flocculation effectiveness of different flocculants, after adding the chemical compound, the microalgae culture was stirred 1 min at 75 rpm and then 15 min at 25 rpm in a jar test apparatus. After this time, samples were taken for absorbance measurements (at 680 nm) at different time intervals during a resting time that last for about 4 h.

Results and Conclusions
As summarized in Table 1, the coagulation/flocculation tests undertaken have shown that, for the range of conditions tested, addition of NaOH or CaCl₂ could promote coagulation or flocculation of the microalgal cells. This way, there is no need for addition of further chemicals that contaminate the wastewater, preventing its reuse. These results have been observed and are demonstrated in the photos in Figure 1, where the effects of NaOH or CaCl₂ addition are substantially different from those obtained after the addition of any of the other chemicals tested. Results show that Arthrospira platensis can be effectively harvested by increasing the pH over 10 with caustic soda or by adding calcium chloride as flocculant with a concentration of about 0.2-0.3 g/L or a 1:30 volume ratio of CaCl₂/microalgae culture, respectively. These chemicals are not expensive, are easy to use and do not pose any substantial contamination risks of the recovered microalgae biomass.
Figure 1. Microalgae coagulation/flocculation tests with a) hydrochloric acid, HCl, b) caustic soda, NaOH, c) ammonia, NH₃, d) aluminium sulphate, Al(SO₄)₃, e) sodium phosphate, Na₃PO₄, f) calcium chloride, CaCl₂, g) magnesium sulphate, MgSO₄.

Table 1. Summary of microalgae coagulation/flocculation tests

<table>
<thead>
<tr>
<th>Coagulants/ Flocculants</th>
<th>Method</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>pH decrease from 10 to 9, 8, 7 and 6.</td>
<td>No coagulation-flocculation occurred.</td>
</tr>
<tr>
<td>NaOH</td>
<td>pH increase from 10 to 11 and 12.</td>
<td>Coagulation after 15 min. Coagulated cells remained suspended in the growth medium.</td>
</tr>
<tr>
<td>NH₃</td>
<td>50 ml of ammonia solution (25 ml of NH₃ + 25 ml of deionized water) in 250 ml of microalgae culture.</td>
<td>No coagulation-flocculation occurred. Biomass degraded by ammonia.</td>
</tr>
<tr>
<td>Al(SO₄)₃</td>
<td>Tested concentrations from 0.5 to 4.5 g/L of 10 ml solution in 250 ml of microalgae culture.</td>
<td>No coagulation-flocculation occurred. Microalgae biomass contaminated by aluminum.</td>
</tr>
<tr>
<td>Na₃PO₄</td>
<td>Tested concentrations from 0.5 to 1.5 g/L of 10 ml solution in 250 ml of microalgae culture.</td>
<td>No coagulation-flocculation occurred.</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>Tested concentrations from 0.2 to 2.0 g/L of 10 ml solution in 300 ml of microalgae culture.</td>
<td>Flocculation after 15 min. Microalgae flocculated in the bottom of the recipient.</td>
</tr>
<tr>
<td>MgSO₄</td>
<td>Tested concentrations from 1.0 to 2.0 g/L of 10 ml solution in 300 ml of microalgae culture.</td>
<td>No coagulation-flocculation occurred.</td>
</tr>
</tbody>
</table>

Acknowledgements
To FCT, for funding project IF/01093/2014/CP1249/CT0003, research grants IF/01093/2014 and SFRH/BPD/112003/2015, and Center for Innovation in Engineering and Industrial Technology - CIETI, UID/EQU/00305/2013. Financial support of POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy - LEPABE, UID/EQU/00511/2013) funded by FEDER through COMPETE2020-POCI and by national funds through FCT.

References
Toxicity of ammonium-based zwitterions to aquatic organisms

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Our goal was to assess the toxicity of four ammonium-based zwitterions (ZIs) with different alkyl side chain lengths to a marine organism (bacterium Vibrio fischeri) and a freshwater organism (microalga Raphidocelis subcapitata). None of the tested ZIs was hazardous to these species. For both organisms, the increase of the ZI alkyl chain resulted in higher toxicity, which is likely related to the ZIs hydrophobicity. The toxicity was higher for V. fischeri than for R. subcapitata. For the algae, the toxicity varied widely among the ZIs, with a maximum variation of 17-fold. Concerning the bacteria, the toxicity varied slightly, with a maximum variation of 3-fold. This, allied to the fact that ZI’s were more toxic to the marine than to the freshwater species, raises concern about the effects of ZIs to marine organisms and highlights the importance on screening their toxicity. Moreover, the results suggest that the toxicity tests should not be performed exclusively with freshwater species.

Acknowledgements

Thanks are due for the financial support to CESAM (UID/AMB/50017 - POCI-01-0145-FEDER-007638), to FCT/MCTES through national funds (PIDDAC), and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020. This work was also developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and
when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors also acknowledge the support by the Portuguese Foundation for Science and Technology (FCT) through the project PTDC/ATP-EAM/5331/2014. SPM Ventura acknowledges FCT for the contract IF/00402/2015.

References
Unraveling the ecotoxicity of deep eutectic solvents using the mixture toxicity theory

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DES are mixtures as tested in aqueous test media

Their toxicity cannot be predicted based on that of HBA and HBD

Antagonism Synergism

This study aims to assess the ecotoxicological profile of deep eutectic solvents (DES) based on three quaternary ammonium salts as hydrogen bond acceptors (HBA) combined with two hydrogen bond donors (HBD), through the Microtox® Acute Toxicity Test. Concentration addition (CA) and independent action (IA) with deviations were fitted to the experimental data. DES toxicity followed the same trend as the salts, highlighting the role of HBAs on DES toxicity. Antagonism was observed for HBA1-based DES and synergism for HBA3-based DES. As demonstrated here for the first time, the application of the mixture toxicity models represents a breakthrough in the problematic of assessing DES toxicity, given the potentially countless number of DES that can be created with the same starting materials. The graphical representation of the response curves will allow the prediction of DES toxicity (varying ratios of the same HBA:HBD) without having to test them all experimentally.

Introduction and objectives

The interest on deep eutectic solvents (DES) has been increasing. However, the ecotoxicological profile of DES, particularly non cholinium-based DES, is scarcely known. Despite previous studies showing that DES components dissociate in significant amounts of water, none assessed DES toxicity using the classical and adequate models for mixture toxicity prediction - concentration addition (CA) and independent action (IA). These models, with deviations describing synergism/antagonism (S/A), dose-ratio (DR) and dose-level (DL) effects are commonly used to predict the toxicity of a mixture based on the toxicities of its individual components [1].

Thus, the aim of this study was to evaluate the ecotoxicological profile of DES based on quaternary ammonium compounds as hydrogen bond acceptors (HBA) combined with two hydrogen-bond donors (HBD), to assess whether the CA and IA models can be used to predict their toxicity. The quaternary ammonium salts HBA1, HBA2 and HBA3, with increasing alkyl side chain lengths, and two different alcohols (HBD1 and HBD2) were used in the preparation of DES. The marine bacteria Vibrio fischeri was used as the biological model, and the inhibition of its luminescence was used as the target endpoint through the Microtox® Acute Toxicity Test.

Methods

Briefly, the bacteria V. fischeri were exposed to a range of diluted aqueous solutions of each starting material and DES (different molar ratios between starting materials were considered) and their luminescence was measured after 30 minutes of exposure. These data were used to estimate the concentrations eliciting 50% luminescence inhibition (EC50) using the least-squares method to fit the data to the logistic equation in STATISTICA 8.0. The experimental responses to all tested DES and to each corresponding starting material were used to fit mixtures response surface modelling for each DES. The joint action within each DES was assessed by fitting the experimental data into the reference mixture models of CA and IA and their deviation functions, allowing the assessment of S/A, DL and DR dependent effects, as described in Jonker et al. [2]. Contour plots showing the interactive effects between HBA and HBD within each DES towards V. fischeri were created to facilitate interpretation. Several statistic parameters were determined to allow comparison of the model-deviation fit [2].

Results

Neither any of the starting materials, nor DES, could be considered hazardous towards the V. fischeri, as their EC50 values are above 100 g L−1. Among the starting materials, HBA3 was the most toxic (EC50= 0.5456 g L−1). The toxicity of HBA increased in the following order: HBA1 < HBA2 < HBA3, i.e., increased toxicity with longer alkyl side chains. This variation agrees with the side-chain effect as defined for ionic liquids [3, 4], which could be attributed to the increased lipophilicity caused by the elongation of the alkyl chain [5], with consequent higher reactivity with the biological membranes and embedded proteins [6]. It was found valid for DES with both HBD, highlighting the central role of HBA in these DES toxicity.

The toxicity of the tested DES, assumed as binary mixtures HBD: HBA, was well described by CA and IA (r² ≥0.87). The IA model with specific deviations adjusted better to the experimental data in 5 out of 6 DES investigated (Table 1). Antagonism was observed for HBA1-based DES, and synergism for HBA3-based DES. These interactive effects between HBA and HBD are illustrated in the contour plots depicted in the graphical abstract. Indeed, antagonism was found for the DES HBD1:HBA1, evident through the convex shape of the isobologram (graphical abstract, A); synergism was found for the DES HBD2:HBA3, evident through the concave shape of the isobologram (graphical abstract, B). The interactions found between DES components shows that DES toxicity cannot be predicted based solely on the toxicity of the starting materials. Moreover, this approach highlighted the role of both the HBD and HBA on DES toxicity, corroborating results of a previous study assessing DES toxicity to a bacterium [7].

The knowledge of the type of interaction between the DES components (HBA and HBD) is of great importance for a better appraisal of the toxicological profile of DES. Synergism
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is of particular concern, as it means that the toxicity of the mixture (DES aqueous solution) is greater than the toxicity of each of its components separately, thus potentially more environmentally hazardous. In these cases, the toxicity of DES solutions would be underestimated if predicted based only on the toxicity of their components. In the present study, synergism was found for HBA3-based DES and also for HBD2-HBA2. The toxicity of HBA3-based DES raises special concern, not only because of the strong synergism between their components but also because of their high overall toxicity compared to the other DES. However, it should be kept in mind that the response of different organisms to the same DES may vary [7]. In other words, the fact that synergism (or antagonism) occurred between the components of a DES for a certain species does not mean that the same deviation will occur for other species. For this reason, from an environmentally precautionary perspective, studies covering several functional levels are essential to gain comprehensive knowledge about the effects of these solvents on the aquatic ecosystems, thus improving the knowledge of their ecotoxicological profile and ultimately, leading to a more realistic environmental risk assessment of such chemicals.

Conclusion

Considering the tailor-made character of DES, the number of DES that can be created with the same starting materials is potentially countless, this making their toxicity assessment a daunting task. The appropriateness of mixture toxicity models to describe DES toxicity, as demonstrated for the first time in the present study, configures a breakthrough in this problematic. By feasibly modelling response surfaces of each DES (covering all theoretical combinations, i.e. molar ratios between a given HBA and HBD) based on the experimental assessment of rationally selected ratios, one gains a broad overview of DES toxicity profiles, properly appraising interactive effects (synergism or antagonism). Such predictive tools can be valuable to retrieve theoretical ECx values that may hold as environmental benchmarks useful in the environmental risk assessment of new DES. Additionally, these tools provide the possibility of a priori selecting, among DES that fit the desired properties regarding functionality for a given application, those bearing lower environmental hazardous potential.

Table 1. Summary of the mixture toxicity model that best describes the toxicity of the DES: a – model that best describes the mixture; b – deviation function from the reference model; c – inhibition effect; d - coefficient of determination describing the adjustment of the model to the experimental data.

<table>
<thead>
<tr>
<th></th>
<th>HBA1</th>
<th>HBA2</th>
<th>HBA3</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBD1</td>
<td>Concentration Addition</td>
<td>a Independent Action</td>
<td>a Independent Action</td>
</tr>
<tr>
<td>b Dose level</td>
<td>b Synergism/Antagonism</td>
<td>b Dose/ratio</td>
<td></td>
</tr>
<tr>
<td>c antagonism</td>
<td>c synergism</td>
<td>c synergism</td>
<td></td>
</tr>
<tr>
<td>d $r^2 = 0.970$</td>
<td>d $r^2 = 0.958$</td>
<td>d $r^2 = 0.909$</td>
<td></td>
</tr>
<tr>
<td>HBD2</td>
<td>a Independent Action</td>
<td>a Independent Action</td>
<td>a Independent Action</td>
</tr>
<tr>
<td>b Synergism/Antagonism</td>
<td>b Dose/ratio</td>
<td>b Dose/ratio</td>
<td></td>
</tr>
<tr>
<td>c antagonism</td>
<td>c synergism</td>
<td>c synergism</td>
<td></td>
</tr>
<tr>
<td>d $r^2 = 0.981$</td>
<td>d $r^2 = 0.926$</td>
<td>d $r^2 = 0.867$</td>
<td></td>
</tr>
</tbody>
</table>

Acknowledgements

Thanks are due for the financial support to CESAM (UID/AMB/50017 - POCI-01-0145-FEDER-007638), to FCT/MCTES through national funds (PIDDAC), and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020. This work was also developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors also acknowledge the support by the Portuguese Foundation for Science and Technology (FCT) through the project PTDC/ATM-CHEM/1699/2014, the doctoral grant of I.P.E. Macário (SFRH/BD/123850/2016) and post-doctoral grants of A.M.M. Gonçalves and J.L. Pereira (SFRH/BPD/97210/2013, SFRH/BPD/101971/2014), respectively. S.P.M. Ventura acknowledges FCT for the contract IF/00402/2015.

References


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The contribution of carbomer in pluronic-based thermoreversible gels of carbamazepine.

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Introduction

The intranasal administration of drugs may have advantages over oral administration like avoiding the degradation of the drug in the gastrointestinal tract and providing a direct delivery pathway to the central nervous system [1]. Several excipients can be envisaged when formulating for this route with the objective of increasing the retention time in the nasal cavity. Pluronic (poloxamer) and Carbopol (carbomer) are polymers that have been used to obtain thermoreversible gels for intranasal delivery of drugs like carbamazepine in non-clinical pharmacokinetic studies [2].

Objectives

To evaluate the influence of Carbopol in thermoreversible formulation’s viscosity, drug precipitation and in-vitro release of carbamazepine.

Methods

Four thermoreversible gels were prepared with Pluronic F-127 at 15% (m/m), one without Carbopol (P-CBZTranscutol), one with Carbopol 974P at 0.169% without pH neutralization (PC0.169%+CBZTranscutol), as published [2], and two with Carbopol 974P at 0.080% (PC0.080%+CBZTranscutol) or 0.040% (m/m) (PC0.040%+CBZTranscutol) followed by pH neutralization (Table 1). Viscosity was measured using a cone-plate rheometer Brookfield DV3TRVC with the help of software Rheocalc T. All measurements were performed using cone CPA-40Z and the viscosity was determined varying the temperature or the shear rate. In vitro release of carbamazepine was tested in horizontal Ussing Chambers using a hydrophilic polyethersulfone membrane with a pore size of 0.2 µm and an exposed surface area of 0.64 cm². Samples were collected from the receiving chamber over the time until 180 minutes, with volume reposition, and drug concentration was determined using a plate spectrophotometer at 385 nm. One aqueous solution of carbamazepine at concentration of 0.83 mg/ml (with 18.75% transcutol) was used as positive control (CBZTranscutol). A representative formulation without carbamazepine was used as negative control to attest method selectivity. Physical instability of formulations was evaluated by the formation of visible crystals.

Results

At 32 °C the formulations with higher concentrations of carbopol, PC0.169%+CBZTranscutol and PC0.080%+CBZTranscutol showed pseudoplastic behavior with viscosity in the range of 100 to 200 mPa.s and the formulations PC0.040%+CBZTranscutol and P+CBZTranscutol were Newtonian fluids with viscosity of about 76 and 66 mPa.s, respectively. Formulations with carbopol, at 0% and 0.04%, showed higher rate of drug release than the other two although retarded drug released compared with the positive control (Figure 1). PC0.040%+CBZTranscutol and P+CBZTranscutol had higher drug strength but showed physical stability of only 1 hour and and PC0.080%+CBZTranscutol of only 2 hours at room temperature. PC0.169%+CBZTranscutol has several days stability when stored at 4 °C.

Conclusion

Carbopol at 0.04% can prevent short-term drug precipitation, allowing higher drug strength, without inhibiting drug release, and without changing the viscosity of the poloxamer formulation. Although this formulation strategy may be useful for the development of novel thermoreversible gels for drugs with carbamazepine-like characteristics for future pharmacokinetic studies in animal models, it is not useful for clinical transposition due to the short physical stability.
<table>
<thead>
<tr>
<th>Formulation</th>
<th>Polymer composition of the gel before solvent addition (% m/m)</th>
<th>Solvent used</th>
<th>Tg-Solv (°C)</th>
<th>CBZ (mg/ml)</th>
<th>NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pluronic F-127 + CBZ-Etanol [2]</td>
<td>15.23 0.169 + -</td>
<td>95:5 (v/v)</td>
<td>1 -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pluronic + CBZ-Transcutol</td>
<td>15.00 0.080 - +</td>
<td>90:10 (m/m)</td>
<td>1 -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pluronic + CBZ-Transcutol</td>
<td>15.00 0.040 - +</td>
<td>90:10 (m/m)</td>
<td>1 -</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pluronic + CBZ-Transcutol</td>
<td>15.00</td>
<td>95:5 (m/m)</td>
<td>2 -</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CBZ-carbamazepine; NaOH- Sodium hydroxide;

Acknowledgements

This work was partially supported by “Programa Operacional do Centro, Centro 2020” through the funding of the ICON project (Interdisciplinary Challenges On Neurodegeneration; CENTRO-01-0145-FEDER-000013)” and by FEDER funds through the POCI - COMPETE 2020 - Operational Programme Competitiveness and Internationalisation in Axis I - Strengthening research, technological development and innovation (Project POCI-01-0145-FEDER-007491) and National Funds by FCT - Foundation for Science and Technology (Project UID/Multi/00709/2013)

References

Bioethanol is the biofuel produced at the largest scale. However, since it is mainly obtained from agricultural crops that interfere with the food chain, its sustainability has been questioned [1]. In this regard, the production of bioethanol from lignocellulosic biomass (LCB) is a promising alternative [2]. Despite all the advantages, its large-scale production is not yet implemented since it requires a high capital investment [3]. The pulp and paper industry contributes significantly to the Portuguese economy [4]. Kraft pulping allows for the removal of lignin from wood and is the most widely used pulping process. Its use as a pretreatment of forest residues before their conversion into bioethanol LCB would be advantageous [5]. It can be integrated in a LCB biorefinery following the circular economy concept. The aim of this work was the study of the production of bioethanol from unbleached Kraft pulps of *Eucalyptus globulus*.

Worldwide dependence on fossil fuels is responsible for devastating effects, including climate change, reduced air quality, and global warming [1]. These environmental problems, coupled with the non-renewable nature of fossil fuels, led researchers to envisage a viable solution, and, thus, biofuels emerged as a sustainable source of energy [2]. Currently, bioethanol is the biofuel produced on the largest scale. However, this is mainly obtained from agricultural crops, which sparked some controversy regarding the sustainability of this alternative since the raw materials used play a role in the food chain [1]. The second generation bioethanol produced from agricultural, forestry, industrial or municipal residues started to be considered as a sustainable alternative. Many of these residues are rich in lignocellulosic biomass (LCB), which is composed of variable amounts of cellulose, hemicellulose, and lignin, depending on the source [6]. Cellulose and hemicelluloses represent about two-thirds of the total dry weight of biomass, and are a source of sugars as glucose and xylose that can act as substrates for the production of ethanol. This is a promising alternative as it does not potentiate the competition between food and fuel feedstocks. In addition, it is an abundant, and cheap raw material throughout the world [7]. Despite all these advantages, the large-scale of bioethanol production from LCB is not yet implemented in the market, since it requires a high capital investment, besides research and technology development, still representing an economic risk [3].

The pulp and paper industry contributes significantly to the socio-economic development at national level. In 2016 Portugal was considered the third largest pulp producer in Europe [4], [8]. Kraft pulping is the most widely used process for removing the lignin, and hemicellulose, from cellulose. For this reason, Kraft process can be considered as a possible pretreatment of lignocellulosic biomass for bioethanol production. Since Kraft pulping process has many industrial units already implemented, it will facilitate the integration of bioethanol production in a biorefinery based on pulp and paper pulping plants. This process will allow the conversion of wood residues or pulps of low quality, rejected by the pulping industry. In this way, a diversification of products of pulp and paper industry is created, allowing the rise of its profitability, since those are mostly value-added products [5].

The main objective of this work was to study the feasibility of the production of bioethanol from wood chips of *Eucalyptus globulus*. First the wood chips were submitted to the Kraft process and converted into unbleached pulp. Then, the pulp was subjected to an enzymatic hydrolysis to convert the polysaccharides to monomers in order to be fermented by the yeast *Saccharomyces cerevisiae* to produce bioethanol.

An experimental design of fermentation assays was performed in Erlenmeyer flasks (500 mL) using the enzymatic hydrolysate obtained from unbleached Kraft pulp in order to study the influence of the agitation rate (180 and 250 rpm), working volume (200 and 300 mL) and filtering area of Erlenmeyer flasks in the bioethanol fermentation production. After optimization of operating conditions, fermentation assays were carried in bioreactor with automatic control of pH, agitation, and temperature. Concluding, this work intended to contribute to the integration of a biorefinery in the pulp and paper industry. In addition, it supports the concept of the circular economy since the hydrolysate used in the assays was obtained from unbleached Kraft pulps. In the future, the feasibility of using the low-quality raw material and the agroforestry residues will be studied.

**Acknowledgements**

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References


Effect of glycerol on the anaerobic co-digestion of the organic fraction of municipal solid wastes

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The Anaerobic co-Digestion (AcoD) of the Organic Fraction of Municipal Solid Wastes (OFMSW) with other substrates is one of the most efficient processes to overcome the Carbon limitation of OFMSW. Anaerobic Digestion (AD) and AcoD of a hydrolysed OFMSW (hOFMSW) was tested under three different organic load rates (OLR): AD with hOFMSW = 2.92 g COD/L*d; AcoD with PG = 20% and 45% increases of OLR when compared to the assay with hOFMSW alone. In AcoD assays, it was possible to achieve, on average, an increase of 20% in biogas daily production when compared to hOFMSW alone. Although CO₂ and H₂S have increased with the addition of PG, biogas and methane yields showed no significant variation from the AD assay to the AcoD assays. An increase of 20% OLR with PG has stabilised the biogas daily production, while an increase of 45% OLR resulted in an unstable process with H₂S reaching the highest concentration (higher than 1500 ppmv).

Introduction
Anaerobic Digestion (AD) is a well-established biological process, suitable to convert the Organic Fraction of Municipal Solid Wastes (OFMSW) into biogas; this is a renewable biofuel useful to be converted into electricity and heat. OFMSW is a Nitrogen-rich but a Carbon-defective substrate, due to the high concentration of proteins; therefore, it is characterised by a low C:N ratio that can cause ammonia accumulation and consequently AD inhibition. The Anaerobic co-Digestion (AcoD) of OFMSW with other C-rich substrates is one of the most efficient processes to overcome these nutrient imbalances [1]. Glycerol has recently gained attention as a possible co-substrate for AcoD due to two major aspects: (i) availability and (ii) destination problems, which are both related to its excessive amount worldwide due to biodiesel production. Crude glycerol can be used, after proper purification, in food and pharmaceutical industries, but the amount currently available in some countries surpasses the industry and market capacities to use this by-product. Glycerol is a C-based substrate characterized by a high chemical oxygen demand (COD) and high biodegradability; therefore, it has a potential to be used in AcoD.

This work is the first part of a larger research that aims to assess the use of both pure and crude glycerol in AcoD with OFMSW. In this first part, only the effect of pure glycerol (PG) on biogas production and its contribution to AcoD stability are reported.

Materials and methods
In this work, a hydrolysed fraction of OFMSW (hOFMSW) was used. hOFMSW was collected in the hydrolysis tank of an AD plant located in Lisboa surroundings. This AD plant processes the organic wastes collected in restaurants, canteens and malls, PG (99.96% purity) (Fisher Scientific) was used. The AD and AcoD assays were performed in a lab-scale stirred tank bioreactor (working volume: 2.1 L), equipped with controlling systems for temperature, redox potential and pH. The assays were done under thermophilic conditions (50 ± 1°C). Initially, the bioreactor was feed with hOFMSW alone (Organic Load Rate; OLR = 2.92 g COD/L*d) with a hydraulic retention time of 19.5 days (AD assay); two AcoD assays were performed with hOFMSW and PG under OLR increases of 20% and 45%. Biogas volume was measured through acid-water displacement and biogas composition (CH₄, CO₂, and H₂S) by gas chromatography according to ASTM D 1946.

Results and discussion
During the AD and AcoD assays, slight increases in C:N ratio were observed from 10.4:1 in AD assay with hOFMSW alone to 10.6:1 (20% increase OLR) and to 11.4:1 (45% increase OLR) in AcoD assays. The C:N ratio increase did not promote any significant enhancement in total and soluble COD removal percentages during AcoD assays when compared with the AD assay of standalone hOFMSW (Figure 1). A COD increase in the influent does not promote necessarily an increase in COD removal percentages [2]. On the other hand, increases of 31% and 26% of the removal percentages of Total Solids (TS) and Volatile Solids (VS), respectively, were observed in AcoD assay in which an increase of 20% OLR was tested, when compared with the other two assays (Figure 2). This result suggests that supplying a C-rich and easily biodegradable substrate, such as PG, promotes the degradation activity of the microbial population, enhancing the degradation of hOFMSW, as suggested by [3]. pH values showed a slight increase from AD to AcoD assays (8.0 to 8.2) and redox potential presented a slight improvement in the presence of PG with a decrease towards the optimal value for AD (∼350 mV) [4], confirming the good performance of microbial population.

The daily production of biogas during both AD and AcoD assays is reported in Figure 3. The variation in daily production of biogas is mainly due to the feeding process used in this work, which was characterised by time intervals of 48 h. A more stable production occurred in AcoD with an increase of 20% OLR, and an unstable production was detected in the AcoD assay with 45% increase of OLR, indicating the beginning of a possible inhibition; this inhibition was already reported in literature for higher values of OLR when using glycerol [5]. The highest daily production of biogas (4.7 L/d) was registered for AcoD assay of hOFMSW + PG with an increase of 20% OLR. The AcoD assay of hOFMSW + PG with an increase of 45% OLR provided a similar average daily production of biogas.
(4.3 L/d). Globally, an increase of 20% in the daily production of biogas was registered for both AcoD assays when compared with hOFMSW alone.

![Figure 1](image1.png)

Figure 1. Removal percentages of total and soluble COD.

![Figure 2](image2.png)

Figure 2. Removal percentages of TS and VS.

![Figure 3](image3.png)

Figure 3. Daily production of biogas in AD and AcoD assays.

The biogas composition (Table 2) showed that by using PG the concentrations of CH₄ slightly decreased and CO₂ increased. However, as reported in Figure 4, the values of methane yield showed almost no variation between AD and AcoD assays. In this sense, although the quality of biogas slightly decreased with the use of PG, biogas and CH₄ yields were compensated by the increase of biogas volume.

![Figure 4](image4.png)

Figure 4. Biogas and methane yields in AD and AcoD assays.

The slight decrease of CH₄ when PG is used, is in line with another work available in literature, in which a slight decrease in the quality of biogas was registered with the increase of COD through the use of glycerol [3]. Table 2 also shows the increase of H₂S with the increase of PG in the influent of the bioreactor. The highest H₂S concentration was registered for the increase of OLR in 45%, pointing out for an unstable process that shows a possible inhibition in higher values of OLR with glycerol.

**Conclusions**

PG seemed to improve the anaerobic microbial activity, promoting a stable process with the addition of PG under an increase of 20% OLR when compared to the OLR used for hOFMSW alone. The beginning of an unstable process was registered with the addition of PG for an OLR 45% higher than with hOFMSW alone. The biogas quality in AcoD assays showed a slight decrease in comparison to hOFMSW alone, registering increases in CO₂ and H₂S with the use of PG. An increase of 20% of biogas volume was obtained with the addition of PG. Biogas and methane yields remained almost the same in the three assays. AcoD of hOFMSW with glycerol can be an interesting option to achieve an optimization in biogas production and a more anaerobic stable process, provided the OLR applied are adequately controlled.

Table 2. Biogas composition in AD and AcoD assays.

<table>
<thead>
<tr>
<th>AD and AcoD assays</th>
<th>CH₄ (% v/v)</th>
<th>CO₂ (% v/v)</th>
<th>H₂S (ppmv)</th>
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</thead>
<tbody>
<tr>
<td>hOFMSW (OLR = 2.92 g COD/L.d)</td>
<td>70.5</td>
<td>28.6</td>
<td>768</td>
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<tr>
<td>hOFMSW + PG (increase of 20% OLR; OLR = 3.50 g COD/L.d)</td>
<td>68.2</td>
<td>30.7</td>
<td>838</td>
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<tr>
<td>hOFMSW + PG (increase of 45% OLR; OLR = 4.23 g COD/L.d)</td>
<td>66.6</td>
<td>32.7</td>
<td>1505</td>
</tr>
</tbody>
</table>

**Acknowledgements**

Authors acknowledge LAQV/REQUIMTE for funding this work, as well as the Foundation for Science and Technology for funding LAQV/REQUIMTE through Portuguese funds (UID/QUI/50006/2013) and co-funds by the ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007265).

**References**

Valorise saline wastewaters through the production of polyhydroxyalkanoates (PHA) biopolymers by mixed microbial consortia (MMC)

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PHAs (Polyhydroxyalkanoates) are microbial polyesters that can be used to produce bioplastics. A cost-effective and eco-friendly approach to produce PHAs is based on Mixed Microbial Consortia (MMC) processes and the use of waste or industry by-products as feedstocks. Within the scope of MultiBiorefinery project, a process to valorise industrial saline wastewaters through the production of PHA biopolymers by MMC is on development. A stable PHA-producing halotolerant MMC-based process was effectively established from a salt non-acclimatised inoculum, applying a successful start-up strategy and using a synthetic Volatile Fatty Acids (VFAs) mixture with 20 gNaCl.L⁻¹. The following step consists in validating the developed star-up strategy to real industrial wastages, salted cheese whey and fish canning residues.

Introduction
Up to 300M tons of plastic are produced every year, half of which is for single use and more than 8M tons/year are dumped into the oceans [1]. This represents a tremendous ecological burden in terms of energy consumption, greenhouse emissions, plastics waste accumulation, and pollution. Microbial polyhydroxyalkanoates (PHAs) have appeared as a solution since they can be used to produce bioplastics, with comparable characteristics to conventional plastics and have a wide range of properties and numerous potential applications [2]. PHAs produced by pure microbial cultures are already commercially available, but their establishment as an alternative to traditional plastics has been constrained by the associated high production cost. On the other hand, production by mixed microbial consortia (MMC) is a recognised alternative for a cost-effective PHA production. As it eliminates costs associated with maintaining aseptic conditions and allows the use of waste or industry by-products as feedstocks [3], MMC PHA production generally comprises three stages: (1) acidogenic fermentation stage – organic carbon is converted into organic acids; (2) culture selection stage – ecological selection principles are applied permanently in order to enrich an MMC in PHA-accumulators; and (3) PHA production stage – the selected MMC is fed with organics acids from stage (1), aiming to achieve culture’s maximum PHA production capacity.

The work presented here is within the scope of Multibiorefinery project and aims to develop eco-design approaches to valorise saline wastewaters from industries through the production of PHA biopolymers by MMC. Specifically, this study assesses an adequate start-up strategy for selecting a halotolerant PHA-producing MMC and develop an innovative MMC-based process for PHA production under saline condition.

Methods
The selection reactor was carried out in a 2 L working volume SBR operated under feast and famine regime and additional uncoupling of carbon (during feast, for storage) and nitrogen (during famine, for growth) availabilities [4]. The SBR was inoculated with concentrated activated sludge harvested from the aeration tank of a municipal wastewater treatment plant (Mutela, Portugal). The culture was fed with a synthetic VFAs mixture, containing (COD.%): 66 acetic acid, 37 butyric acid, 21 propionic acid, 8 valeric acid, and with pH adjusted to 7. Commercial allylthiourea (ATU) was added to inhibit the nitrification activity. Additionally, nitrogen was added in a NHCl solution. The SBR cycle length was 12 h, comprising seven discrete phases: (1) 10 min carbon source addition; (2) aerobicosis I (carbon availability phase); (3) 1 min nitrogen solution addition; (4) aerobicosis II (nitrogen availability and famine phases); (5) 2 min biomass purge; (6) 15 min settling; and (7) 2 min supernatant/effluent withdrawal. Phases (2) and (4) duration were adjusted in order to follow the duration of carbon consumption. Some operational parameters were constant during the entire operation: cycle time length, 12 h; HRT, 1 d; SRT, 4 d; salinity, 20 gNaCl.L⁻¹; carbon to phosphorus ratio, (C/P) 100/1 Cmol.Pmol⁻¹; while other parameters were changed, namely the carbon to nitrogen ratio (C/N, Cmol.Nmol⁻¹) and the OLR (Cmmol VFA L⁻¹ d⁻¹). The bioreactor was mechanically mixed at 300 rpm and aerated through a bubble diffusor located at the bottom of the vessel, with the air flow rate controlled by a mass flow-controller (Aalborg, Denmark). The pH was controlled between 7 and 8 through the automatic addition of 1 M of NaOH and HCl. Pumps (fill and withdraw), aeration, and mixing were automatically controlled by a software program developed within the research group, which also enabled online pH and dissolved oxygen (DO) concentration data acquisition, allowing the online monitoring of the biological process. Additionally, DO data was interpreted via respirometry for estimation of stoichiometric and kinetic parameters of the microbial community [5].

Results
Different selection reactor start-up strategies were intended to be tested: the gradual increase on salinity, to allow the possibly slow culture’s adaptation to salt presence; and the abrupt increase on salinity, testing the ability of the culture fast adaptation and/or fast halotolerant organisms’ proliferation over halosensitive organisms. The abrupt increase on salinity strategy was firstly tested applying a salinity of 20 gNaCl.L⁻¹, with the premise that by being effective would annul the need to test the salinity gradual increase strategy. A low OLR (1.1 gCOD.L⁻¹.d⁻¹) and C/N ratio (10/0.2 Cmol.Nmol⁻¹) were adopted, on one hand to allow a short feast, and on the other hand to avoid nitrogen to leak from one cycle
to the following cycle. The OLR was gradually increased to 1.6 gCOD L⁻¹ d⁻¹ and C/N ratios to 10/1 Cmol:Nmol⁻¹ (Figure 1).

![OLR and C/N ratios](image)

Figure 1: Feast to famine ratio (A, F/f), and storage yield, estimated through respirometry (B, YPHA/S) and active biomass concentration (B, X) over time. OLR is organic loading rate (gCOD L⁻¹ d⁻¹) and N is the amount on nitrogen (Nmol) in relation to carbon (10 Cmol).

The adopted start-up strategy allowed to fast select a halotolerant PHA-producing MMC. Indeed, in 15 days of operation, a stable storing culture was achieved and maintained in the selection reactor, as it may be observed by the attained stable high storage yields (Figure 1B) and low F/f ratios (Figure 1A). It is generally recognised that, with a F/f ratio lower than 0.2-0.3, an efficient enrichment in PHA storing organisms is obtained [6]. By manipulating the C/N ratio and the OLR, the biomass growth was improved (Figure 1B).

In the end of the feast, the final selected consortia contained ca. 30±4% in weight of PHA, composed of (wt.%): 91% HB, 7% HV and 2% HMV.

Further work is being developed using artificially salted cheese whey and fermented brine wastewater as feedstock and salted acclimatised inoculum collected from a sea salted lake (Albufeira, Setúbal).

**Conclusions**

A strategy for starting-up the selection SBR was established, which allowed to achieve a stable PHA-producing halotolerant MMC in a relative fast time. This start-up strategy is based on applying an abrupt salinity increase (20 gNaCl L⁻¹) to a salt non-acclimatised inoculum and a relative low initial OLR and high C/N ratio, and run the SBR under feast and famine regime, along with uncoupling carbon and nitrogen availabilities. This strategy was established with a saline synthetic VFAs mixture. Since VFAs from cheese whey and fish canning residues are now available, the following step will allow to validate the developed strategy with a real feedstock.

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**References**

Bio-electro-Fenton hybrid process as a plausible methodology for the degradation of ionic liquids

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In the present study, the degradation of the ionic liquid 1-butyl-1-methylpyrrolidinium chloride has been carried out through a system combining an electro-Fenton pre-treatment and a biological post-treatment. The effect of the key parameters was evaluated, since the effect of the iron dosage (0.1-0.2 mM), the applied current (50-1000 mA) and the ionic liquid initial concentration (0.075-2.5 mM), using boron doped diamond (BDD) and sub-stoichiometric titanium oxide anodes. A total organic carbon decay of 98% was obtained with a dosage of iron 0.1 mM, 1000 mA current and a concentration of ionic liquid 0.15 mM. Before coupling the biological process, the average oxidation state and carbon oxidation state were calculated to evaluate the biodegradability of the solution. Based on the results, after an electro-Fenton treatment of 30 min, the biological process was attached. The efficiency of the system was followed by the total organic carbon and released carboxylic acids measurements.

Introduction

In recent years, there is a growing interest in a new kind of compounds, known as ionic liquids, since they are a possible alternative to traditional solvents. Ionic liquids are salts composed by organic cations and inorganic or organic anions. A negligible vapor pressure, a low melting point and a high thermal and chemical stability characterize them [1]. However, with deeper studies, it has been discovered that they are not as “green” as they were considered, since their high solubility, potential toxicity and low biodegradability makes them potential pollutants [2].

Electrochemical advanced oxidation processes (EAOPs) have gained more and more interest as alternative to conventional wastewater treatments. The main advantages of EAOPs are outstanding oxidation capacity, versatility, eco-friendly character and easy-handling operation [3]. They are based in the in situ generation of highly oxidizing agents such as hydroxyl radicals (HO·). The most popular process is electro-Fenton (EF), in which H₂O₂ is produced by the reduction of dissolved O₂ (Eq. 1). Electrogenerated H₂O₂ then reacts with the externally added iron (II) (catalyst) to produce the HO• (Fenton’s reaction, Eq. 2). Fe²⁺ ion is then regenerated in the cathode (Eq. 3) [4].

\[ O_2 + 2H^+ + 2e^- \rightarrow H_2O_2 \]  \hspace{1cm} (1)

\[ H_2O_2 + Fe^{2+} \rightarrow Fe^{3+} + HO^- + HO^+ \]  \hspace{1cm} (2)

\[ Fe^{3+} + e^- \rightarrow Fe^{2+} \]  \hspace{1cm} (3)

Nevertheless, despite the advantages of EAOPs, these processes require a high energy consumption on longer electrolysis time with the consequent cost-intensive operations. As a promising alternative, the development of a hybrid system combining EAOPs with a biological treatment has been newly proposed [5]. Therefore, the aim of this study was to evaluate the degradation of the ionic liquid 1-butyl-1-methylpyrrolidinium chloride ([bmpyr]Cl) by coupling the EF process with a biological treatment.

Materials and methods

Chemicals. Ionic liquid [bmpyr]Cl and sodium sulfate were purchased from Sigma-Aldrich. Sulfuric acid and iron (II) sulfate heptahydrate were supplied by Acros Organics. All reagents were of analytical grade.

EF assays. EF experiments were carried out in a cylindrical glass cell with an operational volume of 230 mL at room temperature. The initial concentration of 1-butyl-1-methylpyrrolidinium chloride was 0.15 mM. Carbon felt (Mersen, France) was used as cathode and it was placed on the inner wall of the cell. The anode was a boron-doped diamond (BDD) (Condias GmbH, Germany) or a sub-stoichiometric titanium oxide (TiO₂) thin-film (Saint Gobain C.R.E.E., France), centered in the cell. Both electrodes were connected to a direct current power supply (HAMEG 7042-5) and 50 mM of electrolyte was added to the solution to be treated. The different tests were carried out at room temperature, pH 3 and 0.1 mM Fe²⁺ was used as catalyst.

Biological treatment. A consortium of microorganisms has been used in the biological experiments. Bacteria and fungi were grown at 150 rpm and 30 °C in a TSB and YMS medium [5], respectively. Before the tests, biomass was washed, centrifugated and resuspended in the solution with the ionic liquid. The pH was adjusted at 7 and minerals salts were added to the solution (5 mM NaH₂PO₄, 1.5 mM MgSO₄, 0.5 mM CaCl₂, 1.4 mM NH₄Cl) [5]. The experiments were carried out at 150 rpm and 30°C.

Analytical methods. The ionic liquid removal was monitored by a Dionex ICS-1000 Basic Ion Chromatography system equipped with a D56 conductivity detector and an IonPAC CS12A (250 x 4 mm) column. The production of carboxylic acids was followed by ion-exclusion HPLC (Agilent 1100) equipped with a Rezex™ ROA-Organic Acid H⁺ column (300 x 7.8 mm) at 60°C. Chemical oxygen demand (COD) was calculated using a Lange cuvette test (LCK1414) in a Hach Lange DR 2800. Total organic carbon (TOC) measurements were performed by thermal catalytic oxidation using a Shimadzu VCSH TOC analyzer. Specific energy
consumption (ECTOC) was determined per unit TOC mass at time \( t \) (h) using the following Eq. 4, where \( E_{\text{cell}} \) is the average potential difference between the anode and the cathode, \( V_\text{s} \) is the solution volume (L), \( I \) is the applied current intensity (A) and \( \Delta \text{TOC} \) is the corresponding TOC abatement (mg/L).

\[
\text{ECTOC} \ (\text{kWh/g TOC}) = \frac{E_{\text{cell}} \ I \ t}{V_\text{s} \ \Delta \text{TOC}} \tag{4}
\]

Results and discussion
Initially, the effect of the key parameters (iron dosage, applied current intensity andionic liquid concentration) on EF process was evaluated. In addition, different anode materials (BDD and TiO\(_2\)) were tested in EF process.

Effect of iron dosage. The concentration of Fe\(^{2+}\) used as catalyst is one of the key parameters in EF process. To evaluate this factor, two concentration of Fe\(^{2+}\) were tested (0.1-0.2 mM). The experiments were carried out at 50 mA and the BDD and TiO\(_2\) anodes were tested. The degradation kinetics was similar with both concentrations, whereas the TOC decay is slightly higher with 0.1 mM Fe\(^{2+}\), reaching over 90% (BDD) and 57% (TiO\(_2\)) within 240 min.

Effect of the applied current. The effect of the current on the mineralization efficiency was evaluated in the range of 50-1000 mA for the BDD anode. As can be seen in the Figure 2, greater removal percentages of TOC were reached with higher currents.

On the other hand, for TiO\(_2\) anode, experiments were conducted for 50-200 mA. The same trend was observed with this anode, attaining a TOC decay of 75% within 240 min at 200 mA. Regarding the [bmpyr]Cl removal, the degradation rate, with both anodes, is raised by increasing applied current intensity.

Effect of the initial concentration of [bmpyr]Cl. The initial concentration of the pollutant was investigated in the range of 0.075-2.5 mM. The experiments were carried out with the BDD anode, Na\(_2\)SO\(_4\) 50 mM and Fe\(^{2+}\) 0.1 mM at 200 mA. After 240 min electrolysis, the TOC decay was similar with all the concentrations (over 90%), except 2.5 mM (66%). The values of E\(_{\text{TOC}}\) were calculated, varying between 2.080 and 0.147 kWh/g TOC at 240 min.

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References
Introduction

Kefir is a fermented milk beverage with unique sensory properties and high nutritional and therapeutic values. Currently, there is an increase in its consumption and manufacture in many countries, however, in Portugal, only recently this beverage has started to be produced industrially. Therefore, a detailed study of kefir production should be done to help expanding the implementation of kefir industrial process in Portugal. In this work, several fermentation conditions were studied and compared, concerning pH values, lactose, lactic and acetic acids, and ethanol concentrations: initial kefir grains mass concentration (3 %, 6 % and 9 % (m/v)), initial milk volume (100 mL and 200 mL), temperature (28 ºC and room temperature) and agitation rate (0, 60 and 180 rpm). The best conditions for kefir production were achieved with 9 % (m/v) of kefir grains, 200 mL of milk volume, 28 ºC and without agitation, allowing the production of kefir in approximately 8 h.

Results

An increase on the initial kefir grains concentration resulted in a decrease on the time required for kefir production: with 3% inoculum kefir production was accomplished between 22 and 23 h, with 6% inoculum around 9 h, and with 9% inoculum in approximately 8 h. No differences were observed on the appearance of the beverages produced under these conditions, thus an inoculum of 9% was chosen for the following assays, since resulted in a faster kefir fermentation. No acetic acid or other organic acids besides lactic acid were produced during the fermentation assays performed.

When the milk volume was reduced to 100 mL, kefir production was accomplished between 5 and 6 h. However, the differences observed were probably a result of the sampling process, since the ratio lactose/biomass was similar to the assay with 200 mL. No differences were also observed on the appearance of the beverages produced under these conditions, meaning that a higher head space did not provide worse results in this fermentation.

An increase on the temperature resulted in a faster kefir production process: with room temperature (21-25 ºC) kefir production was accomplished in around 15 h, and with controlled temperature of 28 ºC in approximately 8 h. No differences were observed on the appearance of the beverages produced under these conditions thus controlled temperature of 28 ºC was chosen.

The agitated assays provided a faster kefir production process than the static assays since the imposition of an agitation speed of 60 rpm resulted in a production time of 6 h and those with 180 rpm provided less than 6 h. However, the beverages obtained in the agitated process had different organoleptic properties since presented visual differences when compared

Objectives

The aim of this work was the study of the effect of operational conditions in kefir production, in order to optimize the process, namely initial kefir grains mass concentration, initial milk volume, temperature and agitation rate.

Methods

In this work, kefir grains were inoculated in commercial UHT whole fat cow milk and incubated for about 24 h in batch mode. Different fermentation conditions were studied and compared (Table 1): initial kefir grains mass concentration (3 %, 6 % and 9 % (m/v)), initial milk volume (100 and 200 mL), temperature (28 ºC and room temperature) and agitation rate (0, 60 and 180 rpm). Samples were taken during the time to determine pH and to later analyze lactose, lactic and acetic acids, and ethanol concentrations by HPLC. The best conditions determined for kefir production were also evaluated using a raw cow milk sample provided by a dairy industry.

References


with beverages obtained in the static process: kefir produced with 60 rpm consisted of a heterogeneous mixture with suspended particles and in the assay with 180 rpm precipitation occurred. Since both situations were undesired in organoleptic terms, despite the occurrence of a faster fermentation with agitation, the static process was preferred. From all the conditions tested, the best results were achieved with 9 % (m/v) of kefir grains, 200 mL of milk volume, 28 °C and without agitation, allowing production of kefir in approximately 8 hours. At this time of fermentation, when using the milk sample provided by the dairy industry, pH value attained was 4.44, and lactose, lactic acid and ethanol concentrations were 42.9 g/L, 8.74 g/L and 2.13 g/L, respectively. All values were in agreement with those expected in a typical kefir, as described in the literature [1,2].

**Conclusion**
The best conditions for kefir production were achieved with 9 % (m/v) of kefir grains, 200 mL of milk volume, 28 °C and without agitation, allowing the production of kefir in approximately 8 h, with both milk samples. Sensorial analysis would also be important to perform to better understand the influence of the different conditions tested in the final product.

<table>
<thead>
<tr>
<th>Assay</th>
<th>Initial Kefir Grains Mass Concentration (%(m/v))</th>
<th>Initial Milk Volume (mL)</th>
<th>Temperature (°C)</th>
<th>Agitation rate (rpm)</th>
<th>Cow milk Sample</th>
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<tbody>
<tr>
<td>1</td>
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<tr>
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**References**
IgG purification with alginate-protein fibril composites

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Significant advances of therapeutics action have been observed using biopharmaceuticals, namely with antibodies such as Immunoglobulin G (IgG). However, the purification of antibodies for therapeutic applications requires the use of highly selective and robust technologies, which are highly expensive. In this work, alginate fibril composites were prepared, characterized and applied for the IgG purification from human serum. Several operating conditions were optimized: pH, adsorption time and serum concentration, aiming at improving the IgG recovery and purity. After the adsorption step, the IgG desorption and recovery were addressed. IgG with a purity of 80% and a recovery yield of 41% was obtained. The reuse of the composite materials was also evaluated and no loss in their adsorption performance was observed. This work demonstrates the potential of seaweed polysaccharide-based materials for the purification of high value (bio)molecules.

Antibodies (or immunoglobulins) bind to antigens with high affinity and specificity [1]. Recovered antibodies can be used in research, diagnosis, and therapeutic applications. However, the purification of IgG is rather difficult since the original media in which they are naturally present or produced are highly complex, with a large number of other biomolecules [2]. Their separation and purification from natural sources for further use requires the use of highly selective and robust technologies [3]. As a result, the main drawback associated to their regular use is their current high cost. When considering antibodies as alternative biopharmaceuticals, the problem is exacerbated with therapies that need high doses or the regular administration of these compounds. Therefore, the development of a cost-effective and scalable strategy for the purification of IgG is a crucial demand for IgG industrial production, with high impact in economics and human healthcare.

Alginate is a broadly used hydrophilic biopolymer, extracted from certain species of brown seaweeds (e.g., Phaeophyceae) [4]. It has been described as a promising biopolymer for several applications because of its added advantages: biodegradability, biocompatibility, non-toxicity and relatively low-cost. Moreover, alginate has been used in the development of alginate beads for a wide range of applications, such as drug delivery, heavy metal sorption, protein and drug encapsulation, adsorption of protein or dyes, etc. [5]. In this work, alginate-protein fibril composites-based hybrid cryogel beads were prepared with silk fibers (Alg-SSF) or egg white protein fibers (Alg-EWF). After their complete characterization, these materials were applied for the separation and purification of IgG from real matrices of human serum. The cryogel composites beads were chemically, structurally and morphologically characterized by Fourier transform infrared spectroscopy (FTIR), thermogravimetric analysis (TGA), powder X-ray diffraction (XRD), and scanning electronic microscopy (SEM).

For the selective extraction of IgG from human serum samples, several operating conditions were optimized aiming at improving the IgG recovery yield and purity. Different cryogel beads (Alg, Alg-SSF and Alg-EWF), pH, adsorption time and serum concentration were investigated. The purity and stability of the IgG after the recovery step were also evaluated and compared with standard/commercial IgG by size exclusion high performance liquid chromatography (SE-HPLC), sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), circular dichroism (CD) and FTIR spectroscopy. The IgG adsorption mechanism was evaluated by determining the respective adsorption isotherms. The equilibrium data were best described by the Langmuir model [6].

The highest adsorption of IgG was obtained with Alg-SSF at pH 5 and a desorption process consisting of two steps: purity of 80% and a recovery yield of 41%. The recovery and reusability of the cryogel beads were evaluated in the separation and purification of IgG from human serum samples and no loss in their IgG adsorption capacity was observed. Alginate-protein fibril composites-based hybrid cryogel beads can be considered as bio-based alternative platforms for the purification and recovery of IgG from human serum and should be explored in the future for the purification of other value-added compounds.

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References
Integrated biocatalytic processes by using thermoreversible aqueous biphasic systems

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Thermoreversible aqueous biphasic systems (ABS) composed of ammonium-based zwitterions (ZIs) and polymers are here disclosed to act as integrated bioreaction-separation processes. The biocatalytic reaction involving laccase occurs in homogeneous media, after which small changes in temperature induce the formation of two phases and the complete separation of the enzyme from the products in a single-step. These systems also allow the recovery and reuse of the enzyme, along with the ZI-rich phase, contributing towards the development of sustainable biocatalytic processes.

Introduction/Objectives
Several industries use enzymes in catalytic processes, mainly due to their high activity, specificity and selectivity, and minimization of raw materials consumption and by-products production [1]. Despite these advantages, the maintenance of the enzymes structural stability during the biochemical reaction and their reusability is yet a huge challenge [1]. Reversible aqueous biphasic systems (ABS) appear as an interesting alternative in catalytic processes since several processing steps can be combined into a single operation. Furthermore, ABS are composed of two immiscible aqueous-rich phases, valuable when dealing with biologically active compounds [2]. Based on the need of finding integrated reaction-separation processes for biocatalysis, in this work, we investigated and designed novel zwitterion-polymer-based ABS with a thermal switchable behavior, at temperatures amenable to deal with proteins. These systems were tested as integrated reaction-separation platforms, where a homogeneous catalytic reaction followed by the enzyme separation from the product, by a change in temperature, is performed in one-step. To this end, laccase (EC 1.10.3.2) was used as the biocatalyst and 2,2’-azinobis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) as the substrate.

Methods
1. Determination of the binodal curves of thermoreversible ABS. The binodal curves were determined through the cloud point titration method, previously described by us [2] at 25, 35, and 45 °C (± 1 °C) and atmospheric pressure. Aqueous solutions of polymer and aqueous solutions of each zwitterion (ZI) were prepared and used. The polymer used was polyethylene glycol (PEG) with different molecular weights, namely 1540, 2000, 4000 and 6000 g/mol. The water-soluble ammonium-based ZIs used were N,N,N-tripropyl-3-sulfonyl-1-propaneammonium (N35C3S), N,N,N-tripropyl-3-sulfonyl-1-propaneammonium (N35C3S), N,N,N-trimethyl-3-sulfonyl-1-propaneammonium (N11C3S). Their molecular structures are depicted in Figure 1.

2. Biocatalytic oxidation of ABTS by laccase. The following mixtures compositions were used to carry out the enzymatic catalysis: 39 wt% of N35C3S + 13 wt% of PEG 6000 and 20 wt% of N11C3S + 20 wt% of PEG 6000. The oxidation reaction occurred in aqueous PEG/ZI monophasic systems. In each system, a small amount of the solution containing laccase and a small amount of the solution containing ABTS were added to the phase-forming components to reach a total weight of 1 g. The oxidative reactions were carried out at 45 °C and 25 °C in N35C3S- and N11C3S-based ABS, respectively. Each mixture was carefully mixed during 1 min. Then, the reaction systems were cooled down to 25 °C or heated up to 45 °C in N35C3S- and N11C3S-based ABS, respectively. A careful separation of both phases was then performed. The quantification of oxidized ABTS was carried by UV-spectroscopy, using a UV-Vis spectrophotometer, at a wavelength of 420 nm, in each phase. Laccase was quantified by its activity. To investigate the recovery and stability of laccase, five consecutive cycles of oxidative reaction of ABTS were performed. Each cycle (reaction + separation) was carried out in a maximum of 1 h. The upper phase was removed (substrate-rich phase), and a fresh substrate solution was added and a new cycle started. The summary of the process is depicted in Figure 2.

![Figure 1: Chemical structures and acronyms of the ZIs used.](image)

![Figure 2: Flowchart of the integrated reaction-separation process developed.](image)

Results
1. Determination of the binodal curves of thermoreversible ABS. The temperature-reversible behavior of the studied systems is depicted in Figure 3. ZI-polymer-based ABS present two distinct behaviors with temperature, which depend on the ZIs alkyl chains length: (i) an increase of the immiscibility region...
with an increase in temperature for N$_{111}$C$_{3}$S-based ABS; and (ii) a decrease of the biphasic region with a decrease in temperature for the N$_{555}$C$_{3}$S-based systems. ABS composed of ZIs with smaller alkyl chains (N$_{111}$C$_{3}$S) display a lower critical solution temperature (LCST)-type behavior when mixed with polymers, following the same behavior as polymer-salt ABS. On the other hand, the ABS formed with the most hydrophobic ZI investigated - N$_{555}$C$_{3}$S - present an upper critical solution temperature (USCT)-type behavior, similar to those of polymer-polymer ABS.

![Figure 3](image-url)  
**Figure 3.** Temperature effect in the phase diagrams of ternary systems composed of ZI + PEG 6000 + H$_2$O at 25 °C ( ), 35 °C ( ) and 45 °C ( ). (A) N$_{111}$C$_{3}$S- and (B) N$_{555}$C$_{3}$S-based ABS. There is no formation of ABS comprising PEG 6000 and N$_{555}$C$_{3}$S at 45°C.

2. Biocatalytic oxidation of ABTS by laccase. The reaction step occurs at a homogeneous medium, followed by the enzyme and products separation in liquid-liquid systems promoted by small changes in temperature (Figure 2). For the two studied thermoreversible ABS, it was observed that laccase successfully catalyzed the oxidation of ABTS (colorless) to a highly stable green-colored ABTS$^+$ radical. After that, a temperature change (from 25 to 40 °C or vice-versa) was applied, inducing the separation of the system into two phases, allowing the selective separation of the oxidized ABTS and laccase, as shown in Figure 2. Figure 4 shows the extraction efficiencies (EE%) of the studied systems for both laccase and oxidized ABTS, which correspond to the percentage recovery of each compound to a given phase. Remarkably, laccase and the reaction product partition to opposite phases, with extraction efficiencies higher than 81% achieved in a single-step. For the two studied ABS, laccase preferentially partitions to the ZI-rich phase and the reaction product to the PEG-rich phase, allowing thus the selective separation of the enzyme and product. This approach avoids the need of vigorous stirring to improve mass transfer, as typically carried out in heterogeneous reactions, thus contributing to the maintenance of the enzyme activity. Reaction and separation steps can be carried out sequentially by taking advantage of their temperature switchable behavior. Furthermore, the thermoreversible nature of ZI-PEG-ABS occurs at temperatures amenable for keeping the enzymes stability and activity (25-45 °C).

![Figure 4](image-url)  
**Figure 4.** EE% of laccase (yellow bars) and EE% of the green-colored ABTS$^+$ radical (green bars) in ABS formed by PEG 6000 and N$_{555}$C$_{3}$S or N$_{111}$C$_{3}$S.

In addition to the complete separation of the enzyme and the reaction product, it was further confirmed that laccase maintains its biocatalytic activity after the separation step. This maintenance in activity is of crucial relevance when envisaging the development of processes in which the enzyme can be recovered and reused. Remarkably, it is possible to recover laccase and reuse it without losses in the catalytic activity, for at least 5 consecutive cycles. This approach not only allows the recovery and reuse of the enzyme, but also allows the recovery and reuse of the ZI-phase required to form ABS, contributing towards the development of sustainable biocatalytic processes.

**Conclusions**

Temperature-driven reversible ABS composed of ZIs and polymers are efficient and sustainable integrated reaction-separation platforms in biocatalytic processes. These systems allow the complete separation of the enzyme and the products in one-step, which are enriched in opposite phases, followed by the recovery and reuse of both the ZI-rich phase and enzyme [3].

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**References**

Activation of laccase in the presence of natural deep eutectic solvents

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Introduction

Sustainable and novel green solvents for advances in biocatalysis have provided alternative options to perform enzymatic reactions in partly or fully nonaqueous environments [1]. Since the shift to green chemistry puts high stress on the biocatalytic process, deep eutectic solvents (DES) have proved to be a good alternative to traditional organic solvents [1]. DES consist of two or more compounds (usually solids) in different stoichiometric ratios that can self-associate often through strong hydrogen bond interactions, reducing significantly their melting point when compared to the initial compounds [2]. DES display advantages in comparison to the traditional solvents such as high biodegradability, low toxicity, low cost and simple preparation. Traditionally, biocatalysis have been performed in aqueous media or in organic solvents, when is desired to enhance the solubility of hydrophobic substrate. However, the presence of organic solvents could promote enzyme denaturation and/or deactivation.

Laccases are multicopper oxidases (EC 1.10.3.2) catalysing diverse oxidative reactions. This type of enzyme only uses molecular oxygen and substrate to initiate their catalysis, i.e., the electrons are removed from the reducing substrate molecules and transferred to oxygen to produce water [3]. Laccase has received great attention from academic and industrial community due to its ability (simple requirements, apparent stability and lack of inhibition) to degrade a diversity of substrates with industrial interest [3]. More specifically, laccase oxidizes an extensive variety of organic and inorganic compounds (see graphical abstract) being applied in bioremediation of phenolic compounds, dyes removal, pulp and paper processing, phenolic compounds removal in wine, fruit juice and beer, biosensors, medical care and synthesis of fine chemicals [3]. However, since some these laccase substrates cannot be completely dissolved in an aqueous medium, the use of organic solvents are required [4]. Thus, the use of DES in enzymatic reactions with laccase are potential and safe alternatives to organic solvents due to their potential advantages as mentioned above.

Based on this, the present work aims to evaluate DES aqueous solutions as potential co-solvents for biocatalytic reactions with laccase. Several DES constituted by choline chloride (ChCl), choline dihydrogen phosphate (ChDHP), choline dihydrogen citrate (ChDHc), betaine (Bet) as hydrogen-bond acceptor and ethylene glycol (EG), glycerol (Gly), erythritol (Ery) and xylitol (Xyl) as hydrogen-bond donor at different ratios were evaluated.

Methods

The synthesis of DES was carried out by adding the hydrogen-bond acceptor (Bet, ChCl, ChDHc or ChDHP) to the hydrogen-bond donor (Ery, Gly, Xly or EG) at three molar ratios of 1:2, 1:1 or 2:1. Laccase stability was evaluated in a phosphate buffer (50 mM, pH 7.0) aqueous solution containing DES at concentrations of 10, 25 and 50 wt%. The mixture was incubated at room temperature and then a sample was taken for laccase activity assay.

The activity of laccase was assayed according to the method previously described by Ander and Messner [5]. One unit (U) of laccase activity is defined as the amount of enzyme able to oxidize 1 μmol of ABTS per minute. The conversion of ABTS was monitored at 420 nm (ε = 36,000 M⁻¹cm⁻¹). The laccase activities are presented in U L⁻¹. Laccase thermal stability was evaluated by incubating the enzyme in the absence and presence of DES at -80°C and 60°C. Samples were collected at various intervals of time, and the residual activity was measured as described previously.

Results

To assess the effects on enzyme activity, cholines/polyols DES solutions showed that the laccase activity was positively and negatively affected. For ChCl/polyols DES (50 wt%), a high decrease of 60-80% of laccase activity was obtained when compared to the control. On the other hand, ChDHP and ChDHc DES presented remarkable results, since the laccase activity increase significantly. In the presence of these DES solutions, the positive effect was more pronounced for the polyols with more hydroxyl groups, i.e. Ery (four OH groups) and Xyl (six OH groups). For example, comparing the effect of polyols for ChDHP/polyols, ChDHP:Xyl (1:2) presented the best results with an increase in the laccase activity from 100% (control) to 170% at two concentrations of 10 and 25 wt%.

Due to the protector effect betaine for proteins against denaturation and aggregation [6], betaine-based DES was also studied in the activity of laccase. The results were promisor,
however, the increase in laccase activity was low when compared to ChDHP DES, with an increase in activity from 100% (control) to 120% in the presence of Bet/Xyl (all molar ration and concentrations).

Based on previous results, ChDHP/Xyl DES solutions were selected to evaluate the laccase storage thermal stability at 60 and -80°C in the absence and presence of ChDHP/Xyl (1:2) 10%; ChDHP/Xyl (1:2) 25% and ChDHP/Xyl (2:1) 25% for prolonged period of time. According to results, when the enzyme was incubated at -80°C, the activity increases 1.33 times (using a blank control – without DES) up to 9 days of incubation.

On the other hand, no positive effect of the DES on laccase activity was detected at 60ºC, being after 2 days no laccase activity detected.

**Conclusion**

This study demonstrates that deep eutectic solvents are promising alternatives to traditional co-solvents for biocatalysis using laccases. These results could open a novel perspective for applying these environmentally benign solvents to a broad range of important oxidative biotransformations with laccase.

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**References**

Support ed ionic liquids for the removal of cyclophosphamide from aqueous solutions

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The incidence of cancer is increasing and with it also the consumption of anti-cancer drugs. As with other drugs, these compounds are not completely metabolized and are excreted through the urine, ultimately reaching waste water treatment plants (WWTPs). Anti-cancer drugs are emerging pollutants, reinforcing the need for developing effective treatment or removal strategies. This work aims to contribute to the development of an efficient material for the removal of anti-cancer drugs, namely cyclophosphamide (CP), from urine. This strategy envisages the removal of anti-cancer drugs at the entrance point to avoid the contamination of water streams. To this end, six supported ionic liquids (SILs) were synthesized and characterized. A preliminary study was carried out to address the efficiency of these materials for the removal of CP from aqueous solutions. Kinetic and adsorption isotherms were determined for the best materials, and future studies will be carried out with urine samples.

Nowadays, cancer is one of the most relevant causes of death worldwide. It is expected that the incidence of this disease will increase in the following years and, therefore, the use of anticancer drugs (cytostatics) will continue to rise. Oral therapy is being used as an alternative and/or complement to traditional chemotherapy, and therefore most of the cytostatic drugs are consumed by outbound patients [1]. Among these, cyclophosphamide (CP) is one of the most used cytostatic drugs to treat cancer – Figure 1.

CP as well as other cytostatics are not completely metabolized by humans, being excreted by urine (ca. 25% of the consumed drugs) [2]. These compounds ultimately reach waste water treatment plants (WWTPs) that are unable to efficiently remove or treat them [3]. Thus, the development of materials that could be used as adsorbents of cytostatic drugs from the urine of oncologic patients, i.e. at their entrance point and before they reach WWTPs, is highly relevant to avoid the entrance of such pollutants into the environment.

The main objective of this work is to prepare and identify low-cost materials functionalized with ionic liquids capable of efficiently removing cytostatics from urine samples. Supported ILs (SILs) were already described as efficient materials to remove other pharmaceutical compounds, such as nonsteroidal anti-inflammatory drugs (sodium diclofenac, ibuprofen, naproxen, and ketoprofen) from aqueous samples [4], but were never investigated for the removal of cytostatic drugs. In this work, experiments were carried out with CP, as a main representative of cytostatic drugs. Six SILs (SilPrMImCl, SilPrNMeBzCl, SilPrNEtCl, SilPrNC(C3)Cl, SilPrNMeBuCl, SilPrNBu2Cl) were synthesized and characterized by SEM, 13C solid NMR, elemental analysis and surface charge (pzc) assays. Preliminary adsorption studies (at a fixed drug concentration, material weight and solution volume) of CP from aqueous solutions were carried out to identify the most efficient SILs. After identifying the best SILs (SilPrNBu2Cl, SilPrNEtCl and SilPrN(C3)2Cl), kinetic and adsorption isotherms were determined. CP was quantified by DAD-HPLC in all experiments.

The experimental values obtained were adjusted to two kinetic models, in which the pseudo-second order model was found as the best model to describe the experimental data. The experimental values were also adjusted by two equilibrium isotherm models: the Langmuir model [5] and the Freundlich model [6]. The experimental results were better described by the Langmuir model, where the maximum concentrations of adsorbate in equilibrium in the solid phase (qₑₛ) were found to be 0.0161, 0.0173 and 0.0163 mg·g⁻¹ for SilPrNEtCl, SilPrNBu2Cl, and SilPrN(C3)2Cl, respectively. The results obtained show that these materials can effectively remove the studied drug from aqueous solutions. Further investigations on these materials performance, to remove cytostatic drugs from urine samples, are ongoing.

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References
Valorization of spent coffee grounds with supercritical fluids

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Spent coffee grounds (SCGs) are a potential sustainable source of high value added compounds. Extractions with pure supercritical CO2 (scCO2) and scCO2+co-solvents (10% ethanol, 10% i-propanol and 5% ethyl lactate) of SCGs were used for valorization of the waste biomass. Conditions for highest yields (30 MPa and 323 K for extractions with co-solvents and 40 MPa and 313 K - for those with pure scCO2) were identified and applied for comparison. The results, in terms of global yield and elemental composition, were compared with those achieved by pure scCO2 and by conventional Soxhlet extraction with n-hexane. The highest yield of 12.3% was obtained with scCO2+5% ethyl lactate, yields of (10.1 to 12)% - with the rest of the extractions, and 10.4% - by the Soxhlet extraction. Quantitative identification of diterpenes in the extracts was carried out by H-NMR. Elemental analysis provided useful information for the application of SCGs products, obtained with the tested extraction technologies.

Introduction

The selection of potential feedstock sources of high value added bioproducts with a wide spectrum of applications like biofuels, foods additives, pharmaceutical, cosmetics and other health and well-being related compounds should take into account the abundance and composition of original biomass. The spent coffee grounds (SCGs) are biowastes, obtained in significant amounts after the consumption of non-soluble coffee and from the production of soluble coffee. Coffee is one of the world’s most popular beverages and the second largest operated commodity after petroleum [1]. The importance of SCGs can be appreciated taking into consideration that coffee production in the world for 2015/2016 was 8.6 million tones according to ICO [2] (9.2 million tones, according to USDA [3]). The oil from SCGs consists mainly of triglycerides and small amounts of diglycerides, free fatty acids, together with terpenes, sterols and tocopherols [1], thus representing an important source of raw materials for a variety of industries.

Materials and Methods

The SCGs were obtained from a local coffee shop. The conventional Soxhlet extraction with n-hexane was performed for 4 h. The solvent was removed by reduced pressure evaporation and the average yield of coffee oil thus obtained was 10.4%.

The supercritical extraction (SCE) was carried out in a laboratory apparatus “Applied Separations” with some modifications, namely an addition of a second high pressure pump for the co-solvents used. The pressure of 30 MPa and temperature of 323 K and the solvent to co-solvent ratio were chosen to provide a basis for adequate comparison of the global oil yields obtained.

The 1H-NMR spectra of the crude oil extracts were recorded on a Bruker Avance 400 MHz NMR spectrometer (Bruker Inc., Bremen, Germany), working at 400.13 MHz for 1H-NMR equipped with a 5 mm PABBO BB-1H probe with 90° proton pulse length of 11.8 μs and an interval time between acquisitions of 30 s, using standard Bruker routines. A CHNO elemental analysis of the samples from the initial, residue and oils obtained from SGC were also performed.

Results and Conclusion

Figure 1 shows the extraction kinetic curves in terms of SCGs oil yield, obtained with scCO2+ different co-solvents, and with pure scCO2 at the optimum conditions of 40 MPa and 313 K. The highest yield was obtained with 5% ethyl lactate (ELactate). The qualitative and quantitative patterns of the other two extraction curves obtained with isobutanol (IsBut) and ethanol (EtOH) are very similar as final values of the cumulative yields achieved for the SCE without a co-solvent applied, not only the yield is lower but it also takes longer time to reach the plateau of the extraction.

1H-NMR analysis showed that the oils are largely dominated by triacylglycerols (TAGs) with minor amounts of 1,2 diacylglycerols. The main esters are of saturated fatty acids (44.4 - 45.9%), diunsaturated fatty acids (40.3 - 41.5%) and monounsaturated fatty acids (13.9 - 15.0%). Diterpenoids were also quantified in the extracts namely, cafestol (2.23 - 3.29%), 16-O-Methyl-Cafestol (2.21 - 3.61%) and kahweol (0.97 - 1.33%).
Table 1 shows the data for the CHNO elemental analysis of the dry SCGs, before extraction, the SCGs after extraction and the extracts, obtained from the SGC. The results obtained show elemental compositions similar to those published by other authors. The higher C and H contents and the lower N and O contents in the extracts are in agreement with the analysis of ¹H-NMR where the predominant compounds identified are acylglycerols. All depleted solid matrices contain more nitrogen and less carbon which make them more suitable for certain applications, like composting.

The eventual presence of nitrogen in the extracts needs more precise estimation, since nitrogen compounds in fuels should be limited from an air pollution point of view, but they might be useful as antioxidants. In any case, Table 1 results show once again that circular production in sustainable biorefineries should separate and use all useful components. In this instance, elemental analysis is a relatively inexpensive tool which in combination with other analytical methods can provide useful information for the development of extraction technologies.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Carbon</th>
<th>Hydrogen</th>
<th>Nitrogen</th>
<th>Oxygen</th>
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<tbody>
<tr>
<td>Dry Spent Coffee Grounds, before extraction</td>
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<td>7.04</td>
<td>2.10</td>
<td>39.84</td>
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<tr>
<td>Average for the SCG, after the extractions</td>
<td>46.54</td>
<td>6.22</td>
<td>2.28</td>
<td>44.96</td>
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<tr>
<td>Oil from hexane extraction</td>
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<td>11.74</td>
<td>&lt; 0.5</td>
<td>11.26</td>
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<tr>
<td>Oil from SCO₂₋₄₀/313 (conditions?)</td>
<td>77.03</td>
<td>12.14</td>
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<td>10.83</td>
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<tr>
<td>Oil from SCO₂₋₃₀/323, 10% Ethanol</td>
<td>76.63</td>
<td>11.49</td>
<td>&lt; 0.5</td>
<td>11.88</td>
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<tr>
<td>Oil from SCO₂₋₃₀/323, 10% Isobutanol</td>
<td>76.51</td>
<td>11.38</td>
<td>&lt; 0.5</td>
<td>12.11</td>
</tr>
<tr>
<td>Oil from SCO₂₋₃₀/323, 5% Ethyl lactate</td>
<td>76.79</td>
<td>11.52</td>
<td>&lt; 0.5</td>
<td>11.69</td>
</tr>
</tbody>
</table>

*Oxygen is calculated by difference to 100 % (for the extracts – without nitrogen). Less than 0.5 % nitrogen could not be determined quantitatively by the used method. The code used for the SC extractions (e.g., 40/313), shows pressure (MPa)/temperature (K).

Acknowledgements
The authors acknowledge the funding received from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 778168 and to National Science Fund, Ministry of Education and Science of Bulgaria under Contract Grant DH 07/12 J. Coelho, and M. Robalo are thankful for the financial support from Fundação para a Ciência e a Tecnologia, Portugal, under projects UID/QUI/00100/2013.

References
Introduction

LF is a globular single-chain glycoprotein of the transferrin family folded into two globular lobules, which is present in several fluids such as milk, saliva, tears and nasal secretion. As one of the components of the immune system of the body, LF has great biological properties such as antibacterial, antiviral, immunomodulatory and iron binding capacity [1]. Curcumin, a yellowish polyphenol from Turmeric spice (Curcuma Longa), has shown to have multiple health benefits due to its anti-inflammatory and antioxidant potential. However, this nutraceutical is sparingly soluble in aqueous solutions and also presents low bioavailability [2]. Protein nanohydrogels are characterized by their three-dimensional and hydrophilic nano-sized networks coupled with their large surface area, and an interior network for incorporation of nutraceuticals, enabling: (i) their encapsulation and controlled release; (ii) their improved solubility and bioavailability; (iii) their target deliver in the associated tissues and/or protecting them against degradation and undesirable chemical reactions; and (iv) their stability in the GI tract [3].

This study is focused on the bovine LF nanohydrogel behavior as an encapsulating agent of curcumin, as well as on the release profiles of the chosen nutraceutical model when incorporated into food simulant models.

Materials and methods

Nanohydrogels preparation was based on a methodology described by other authors, with some modifications [4]. Briefly, a weighted amount of LF was dissolved in distilled water at 25 °C and stirred at 500 rpm for 1 h until reach a 0.2 % (w/v) concentration. The solution pH was adjusted to 7.0, with 0.1 mol L⁻¹ of sodium hydroxide. LF aqueous solution was submitted to a thermal treatment in which it was heated at 75°C for 0, 5, 10, 15 and 20 min in a water bath (closed system), to promote the formation of a monodisperse nanohydrogel solution. All the samples were made in triplicate and kept at room temperature (25 °C) for at least 30 min until further characterization.

Initially, it was prepared a range of concentrations between 5 and 80 µg/mL from a curcumin stock solution, previously dissolved in pure ethanol. A given volume from each of these solutions was added to the LF solution and, after 20 min of gentle stirring, LF-curcumin mixtures were finally heated at 75 °C in a water bath (closed system) for 10 min. The unbound curcumin was removed from the nanohydrogel solutions by centrifuging at 12 000g for 20 min. The pellet composed by undissolved curcumin was thoroughly dissolved in pure ethanol and further quantified spectroscopically at 425 nm [5]. In order to determine the highest association efficiency (AE) for this system, the amount of free curcumin was estimated using a calibration curve previously made with the same conditions of the free curcumin solutions. Finally, the obtained values were used as the main variables in a standard AE equation.

The physicochemical characterization was carried out starting with DLS to access hydrodynamic diameter, polydispersity index (PDI) and ζ-potential of the LF-curcumin nanoparticles. Therefore, to analyze protein-nutraceutical interactions, fluorescence measurements such as ANS (1-anilinonaphthalene-8-sulfonic acid) probe and FRET occurrence analysis were performed. To evaluate the effect of curcumin association on LF secondary structure, a CD measurement was accomplished. In order to perceive the system binding types as well as to confirm LF-curcumin association, FTIR measurements were realized. TEM was also used to evaluate the morphology of the nanohydrogels and to confirm the nanoparticles formation. A release kinetics assay was performed with 2 different food simulants at room temperature (25 °C). Nanohydrogels solutions were placed in dialysis membranes with 10 kDa cut-off, which in turn those emerged into glass release reactors. Firstly, it was traced the curcumin release profile from nanohydrogels in a lipophilic food simulant (ethanol at 50%), followed by the hydrophilic food simulant (ethanol at 10%) one.

In order to evaluate LF-curcumin nanohydrogel, the storage stability over time was performed at 4 °C and 25 °C during 35 days, separately. The stability of nanohydrogels was accessed by measuring its size, PDI and ζ-potential.

Results

LF nanohydrogel system can associate curcumin with an efficiency of 90 ± 1.09 % and loading capacity of 2.6 ± 0.02 %. For curcumin at 80 µg/mL (as shown in Table 1). Above this concentration, the system can also associate curcumin but, it...
proved to be unstable, revealing a curcumin precipitate after 3 days of storage at 4 °C. Nanohydrogels have sizes around 89.4 ± 2.2 nm with 0.197 ± 0.019 PdI and a ζ-potential of 23.4 ± 2.05 mV. To evaluate potential hydrophobic interactions between LF and curcumin, an ANS probe extrinsic fluorescence measurement (with excitation wavelength at 370 nm) was carried out with a range of curcumin concentrations between 10 and 80 µg/mL. The results reveal that there is a curcumin-ANS competition for LF hydrophobic sites. While at the lower concentration ANS still fluoresces, at the following concentrations, the fluorescence intensity signal presents an abrupt loss, suggesting that curcumin is occupying the majority of LF hydrophobic sites. Regarding FRET analysis, results have shown that, in this system, there is energy transfer from LF tryptophan and tyrosine residues (donor fluorophores in the excited state) to curcumin chromophores (acceptor ligands in the ground state), since it can be visualized by the spectral overlap between LF fluorescence and curcumin absorbance spectra. This confirms the occurrence of FRET phenomenon, which can provide accurate structural information leading to determine protein-ligand binding distances [6].

Concerning protein secondary structure, the CD spectra shows no significant differences between native LF, LF nanohydrogels and LF-curcumin nanohydrogels. These results suggest that curcumin association does not affect LF secondary structure at a significant level. As shown in Figure 1, the release rates of curcumin from LF nanohydrogels proved to be higher in the lipophilic food simulant rather than the hydrophilic one. It was also observed that, in the case of the hydrophilic food simulants, LF nanohydrogels nets can retain curcumin for more than over 9 days, since only ~1.6 µg of curcumin was released during this period, reaching stabilization after ~10 hours of release. On the other hand, ~16 µg of curcumin were released from LF nanohydrogels to the ethanol 50 % medium. During 35 days, nanohydrogels showed a constant stability in terms of size, PdI and ζ-potential, at 4 °C. Solutions kept their orange-ish aspect, showing no signs of precipitate and possible contaminations. On the opposite way, after 14 days, solutions stored at 25 °C presented loss in color and it was also observed the presence of curcumin precipitates. DLS measurements showed significant changes in terms of size and PdI values, evidencing the nanohydrogels instability under such conditions.

![Figure 1. Release kinetics profile of curcumin from LF nanohydrogels in hydrophilic medium ethanol 10 % (○) and lipophilic medium ethanol 50 % (●).](image)

**Conclusions**

LF nanohydrogel has the ability to associate lipophilic nutraceuticals, such as curcumin, with remarkable association efficiency and loading capacity values. This system can also be stable over time when submitted to storage conditions (4 °C during 35 days), making it a valuable candidate to serve as vehicle for nutraceuticals controlled release. FRET occurrence brings out the possibility to obtain valuable structural information in what LF-curcumin binding concerns. Curcumin release rates indicate that its retention in LF nanohydrogel nets can be higher when in contact with a hydrophilic food matrix. This information suggests which matrix character would be more appropriate to incorporate LF-curcumin nanohydrogel.

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**Table 2. Effect of curcumin concentration on association efficiency and loading capacity of LF nanohydrogels, where data are presented as mean ± 95 % confidence interval. Different letters indicate statistically significant differences between values (p < 0.05).**

<table>
<thead>
<tr>
<th>Curcumin Concentration (µg/mL)</th>
<th>Association Efficiency (%)</th>
<th>Loading Capacity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10 µg/mL</td>
<td>78.9 ± 2.0 % a</td>
<td>0.3 ± 0.01% a</td>
</tr>
<tr>
<td>20 µg/mL</td>
<td>80.8 ± 4.6% a</td>
<td>0.6 ± 0.04% b</td>
</tr>
<tr>
<td>40 µg/mL</td>
<td>84.4 ± 2.1% ab</td>
<td>1.2 ± 0.06% c</td>
</tr>
<tr>
<td>60 µg/mL</td>
<td>85.2 ± 1.7% ab</td>
<td>2.1 ± 0.05% d</td>
</tr>
<tr>
<td>80 µg/mL</td>
<td>90.0 ± 1.1% b</td>
<td>2.6 ± 0.02% e</td>
</tr>
</tbody>
</table>

**Acknowledgements**

Oscar L. Ramos acknowledge his Post-Doctoral grant (SFRH/BPD/80766/2011) to the Fundação para a Ciência e Tecnologia (FCT, Portugal). This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UID/BIO/04469 unit and COMPETE 2020 (POCI-01-0145-FEDER-006684) and BioTecNorte operation (NORTE-01-0145-FEDER-000004) funded by the European Regional Development Fund under the scope of Norte2020 - Programa Operacional Regional do Norte.

João F. Araújo acknowledge the Escola de Ciências and Centro de Engenharia Biológica from Universidade do Minho (Braga, Portugal) for their support, under the scope of the Biophysics and Bionanosystems Master program.

**References**

The contamination of the aquatic environment by various pollutants, such as endocrine disruptors (bisphenol A, BPA), has been a major concern in human health and environment [1]. BPA is an ubiquitous compound present in consumer products, such as plastic bottles, food packaging, water pipes, thermal paper, dental devices, toys, computers, helmets, sunglasses, among others, ending in WWTPs. Within the endocrine disruptors class, BPA is associated with metabolic malfunction including obesity and diabetes, cardiovascular diseases, breast and ovarian cancer, and infertility [2]. The levels of BPA in effluents of WWTPs vary from non-detectable levels to 370 μg L⁻¹, being this highest level detected in Japan [3]. BPA has also been identified in potable water samples with average and maximum values of 14 ng L⁻¹ and 1.3 μg L⁻¹, respectively [4].

Several techniques have been proposed to remove BPA from WWTPs, such as oxidation, membrane filtration and solid phase adsorption. However, these processes lack in efficiency, and if degradation is carried out instead of removal approaches, the production of other contaminants/pollutants may occur.

The science of materials has experienced tremendous advances leading to the development of effective adsorbents specifically designed for the treatment of water [5]. Among these materials, supported ionic liquid phases (SILPs) may be used as an alternative removal/adsorption approach [6]. The first application of SILPs refers to the extraction of aromatic compounds (benzene, toluene, ethylbenzene, and xylenes) from paints by a solid phase microextraction technique (MEFS) [7].

Since this pioneering work, a significant interest has been verified in the development of extraction, separation and pretreatment processes using SILPs. SILPs have been studied in a wide range of areas, such as in the removal of herbicides, organic acids, amines and aldehydes, and in the extraction of bioactive compounds from natural plants [8]. The main advantage of SILPs is associated to their tailoring ability, which is directly connected to ILs and their wide chemical diversity.

Given the major concerns associated to endocrine disruptors, the main objective of this work is to identify an effective SILP with high adsorption capacity for BPA. Several SILPs were synthesized, characterized by SEM, Elemental Analysis, Potential Zeta and ¹³C NMR, and evaluated in terms of removal performance for BPA by determining the respective kinetic and adsorption isotherms. The regeneration of the material was also investigated to infer their reusability after saturation.

Under the best conditions, two SILPs with a removal efficiency higher than 80% were identified (BPA concentration of 0.6 mM, volume of BPA aqueous solution of 10 mL and material weight of 50 mg). 95% of desorption of BPA was also accomplished, allowing the materials reuse. Although further studies are still ongoing, SILPs may be considered as effective materials for the adsorption and removal of endocrine disruptors from the aquatic environment.

Acknowledgements
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References
CHEMPOR 2018


Several studies have been published regarding pretreatments for the optimization of the production process of biofuels from sugarcane. Pretreatments performed with dilute acids and alkaline substances are considered the most efficient in rupturing the rigid structure of the lignocellulosic material. Research on the hybrid has been intensified due to the possibility of increasing the energy matrix. Due to its high fiber content and low sucrose content, energy-cane proved itself as a superior material for biofuels. Furthermore, the hybrid is more adaptable to climate variations and has a higher yield per hectare production. In addition, studies estimate that the production of ethanol from energy-cane has the potential to produce approximately twice as much ethanol as sugarcane. In this context, the present study evaluates the efficiency of the pretreatment stage of energy-cane using dilute sulfuric and phosphoric acids to remove hemicellulose compared to the results of sugarcane bagasse.

Introduction and Objectives

In Brazil, sugarcane, produced on a large scale, has as main feature the high sugar content and low fiber content [2], which is better adapted to sugar production, because it contains a high content of sucrose in the structure. In addition, the species requires a large area of planting, which is detrimental to food production [3]. Sugarcane remains as a biomass proper to produce biofuels because of its high productivity. However, its contribution to the energy matrix can be increased with the production of energy cane, a hybrid of sugarcane from *Saccharum spontaneum* [2][4][5].

In Brazil, GranBio has developed a variety of sugarcane to produce second generation ethanol (E2G) and biochemicals. Cana Vertix®, as it was called, is being obtained from the genetic cross-breeding of commercial hybrids with ancestral types of sugarcane. Table 1 shows the main characteristics of sugarcane and energy cane.

Table 1. Comparison of characteristics of sugarcane and energy cane [6].

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Sugarcane</th>
<th>Energy Cane</th>
</tr>
</thead>
<tbody>
<tr>
<td>Productivity</td>
<td>85 t/ha</td>
<td>200 t/ha</td>
</tr>
<tr>
<td>Fertility requirement</td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td>Resistance to pest and diseases</td>
<td>Low</td>
<td>High</td>
</tr>
<tr>
<td>Number of crops</td>
<td>4 – 5</td>
<td>12 – 15</td>
</tr>
<tr>
<td>Multiplication rate</td>
<td>1:10</td>
<td>1:30</td>
</tr>
<tr>
<td>Time to development</td>
<td>8 to 12 years</td>
<td>3 to 5 years</td>
</tr>
</tbody>
</table>

Considering the characteristics of energy cane, the Brazilian soil and climate conditions, the production of ethanol from this hybrid can be viable due to the low cost of cultivation and the use of degraded agricultural areas. The production of E2G consists of several steps which are generally executed in the following order: chemical or physical pretreatment, hydrolysis to produce fermentable sugars, fermentation of these sugars and, distillation for separation of product. All stages are interconnected, requiring the study of each stage to improve them [7]. The chemical pretreatment realized with diluted acids consists in increasing the accessibility of the enzymes used in the subsequent processes, such as hydrolysis and fermentation, by breaking the rigid structure of the hemicellulose, transforming it into xylose, glucose, arabinose and galactose - simple sugars - and a residue consisting of cellulose and lignin. This is due to the amorphous structure of hemicellulose, making it less resistant to the attack of chemical agents [8][10].

Hence, the objective of this work is to evaluate the viability of producing E2G from energy cane, analyzing its composition and the effects of acid pretreatment on the removal of hemicellulose, comparing with the results for sugarcane.

Experimental Methods

For the pretreatment, the biomass was stirred in the Fisatom rotary evaporator with *H*₃*PO*₄, *H*₂*SO*₄ and the acids blend, at a concentration of 1.45% (v/v). The samples were pretreated for 45 minutes at a temperature of 120°C. The determination of the composition of the lignocellulosic fraction, before and after the pretreatment, was obtained using the High-Performance Liquid Chromatography (HPLC). The characterization was performed through Fourier Transform Infrared Spectroscopy (FTIR) in order to analyze the structural changes undergone after pretreatment.

Results and Discussion

Through the chemical characterization was possible to obtain significant results in relation to the chemical composition of the sample, comparing them with those found in the literature, as shown in Figure 1.

Figure 2 shows the results obtained in the infrared spectroscopy analysis, being possible to observe the bands referring to the predominant functional groups. The wavenumber from 3640 to 3200 cm⁻¹ is attributed to hydroxyl (OH), predominant functional groups in lignin and cellulose. In the absorption range from 3000 to 2840 cm⁻¹, the wavenumber corresponds to *CH*₂ and *CH*₃, which are attributed to cellulose. The absorption bands from 1870 to 1540 cm⁻¹ correspond to the carbonyl properties (C=O) attributed to hemicellulose. The wavenumber between 1420 and 1330 cm⁻¹ are attributed to lignin and carbohydrates and from 1150 to 1085 cm⁻¹ is attributed to the bond (C-O-C) functional groups present in the lignin [11].

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Fig. 1. Chemical characterization of biomass on dry basis.

Fig. 2. Absorption spectrum of (a) energy cane and (b) sugarcane bagasse both without extractives and after pretreatment.

Fig. 3. Chemical characterization of biomass after acid pretreatment.

The results of the characterization after the acid pretreatment, Fig. 3, demonstrate that the pretreatment performed with sulfuric acid and the combined acids were efficient in the removal of hemicellulose, however, the combined pretreatment presented a lower percentage of cellulose, which may indicate its degradation.

Conclusions

Based on the chemical characterization, concludes that the pretreatment with sulfuric acid is the most appropriate for this stage of the second-generation ethanol production, due to the results in the removal of hemicellulose and to the fact that the cellulose concentrations were maintained. Concerning the advantages of using energy cane, considering Brazil’s climate and soil conditions and the characteristics of energy cane, its planting for the production of biofuels may be more feasible than sugarcane, because the organic composition is similar. The next stage of this work will be the realization of different alkaline pretreatment to define a route of greater efficiency in ethanol production.

References

Extraction of laccase from Trametes versicolor growth media using aqueous biphasic systems

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Laccases are copper-containing oxidases that are produced by plants, bacteria, insects, and especially by white-rot fungus, such as Trametes versicolor. Due to that, this fungus has been widely used in wood delignification [1], bioremediation [2], decolorization of dyes [2] and other applications, since laccase oxidizes various aromatic substrates such as polyphenols, aromatic amines, and methoxy-substituted phenols [3]. Nevertheless, the purification of laccase from the fermentation medium is still considered an expensive and laborious step, requiring techniques such as precipitation, flocculation, immobilization, gel filtration, chromatography, among others [4]. Moreover, some of the applied techniques also require time consuming sample pretreatment. Therefore, there is a crucial need on the development of cost-effective techniques to recover and purify laccase from fermentation media, as well as to find effective preservation solvents. To attain this aim, aqueous biphasic systems (ABS) can be used. ABS are considered a valid technique for the extraction, concentration and purification of proteins mainly due to their high-water content [5].

In this work we studied ABS as effective platforms for the extraction, concentration and purification of laccase from T. versicolor fermented medium, followed by the use of the ABS coexisting phases as preservation media.

Nowadays, laccase from microorganisms such as Aspergillus oryzae (Novozym® 51003) that is also a filamentous fungus is preserved in a mixture containing water (66%), Propylene glycol (PPG) (25%), Sucrose/Glucose (4%) and Glycine (2%). That way, ABS composed of ILs or amino acids and polymers, such as polypropylene glycol 400 (PPG 400) and polyethylene glycol (PEG) 400 and 600 were investigated, based on previous preservation mixtures. The first step consisted in the screening of the best components that could form ABS in presence of a commercial laccase solution, allowing the purification of the oxidase when dealing with the original medium. After that, the best systems were characterized by the determination of the respective phase diagrams at several temperatures, comprising the respective tie-lines and tie-lines lengths. Thenceforth, with the previous variables determined, the theoretical biomolecule concentration factor was calculated through the lever rule. The tested systems for the laccase concentration allowed to calculate two parameters: top or bottom phase yield (Y) and the top or bottom concentration factor (CF), depending of the enzyme partition. The Y variable represents the fraction of enzymatic activity recovered in a specified phase in relation to the total activity introduced into the system. The CF denotes the T. versicolor fermented media enrichment when compared to the ABS total volume [6]. The higher these parameters, the successful the enzyme concentration process.

As previously referred, after the screening of the best ABS components, the systems were applied in the partition of a commercial laccase, where the activity of the enzyme was evaluated and the best systems (ABS formed by betaine + PEG600 and glycine + PPG400) were identified. The previous systems were then tested as new platforms for extraction of laccase directly from the T. versicolor growth media. The ABS composed by PEG and betaine allowed a top phase yield (YP) of 0.31, about half compared to previous laccase concentration studies [6]. Furthermore, these constituents do not allow to form an ABS with a small top phase (betaine rich phase), and thus concentrate the enzyme (laccase partitioned to betaine rich phase). On the other hand, the glycine and PPG400 ABS allowed to form an ABS with a reduced bottom phase (glycine rich phase) recovering and concentrating the enzyme activity. This system was very promising, since the bottom phase yield (YBP) was 0.7, average value using different mixture points. Moreover, the bottom CF was 18.7, double the registered values in the literature [6]. Then, the enzyme stability was determined over a period of time. For this, the enzyme rich phase (bottom phase) was frozen (<80°C) and the activity was measured for 2 weeks, registering an enzyme activity loss of 20%. Although other preservation alternatives should be explored to preserve the laccase activity, this preliminary study shows that properly designed ABS can act as simultaneous extraction, concentration and preservation media.

Acknowledgements

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the PT2020 Partnership Agreement. A.P.M. Tavares acknowledges FCT for the Investigator Programme IF/01634/2015. M.G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ERC-2013-StG-3377.

References
**Exploring glutathione as an adjuvant of anti-biofilm strategies against Pseudomonas aeruginosa**

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**Pseudomonas aeruginosa** biofilms are involved in several infectious diseases and their eradication remains a challenge. Disrupting the biofilm matrix is the most attractive way to facilitate biofilm-cells eradication and glutathione has been exhibiting great potential as a biofilm disrupter agent. Therefore, this study aimed to explore anti-biofilm strategies based on the combination of glutathione with two in-use antibiotics. Biofilms were formed and subjected to the action of combinatorial arrangements of glutathione with ciprofloxacin or tobramycin. Preliminary results showed that PAO1 biofilms were eradicated using glutathione-ciprofloxacin, in contrast to biofilms formed by a clinical isolate (PAI). Better outcomes were obtained using glutathione-tobramycin since biomass reduction occurred with lower dosages. Also, a different action than the usually described for glutathione is suggested. In conclusion, glutathione-tobramycin could be a potential anti-biofilm strategy.

**P. aeruginosa** is a gram-negative, ubiquitous environmental bacterium frequently found in diverse environments such as water, soil and plants in the form of biofilms. Biofilms are defined as highly structured, surface-attached communities of cells encased within a self-produced extracellular polymeric matrix forming a shell around a microbial community conferring to the microorganisms a protective environment [1]. *P. aeruginosa* biofilms have been linked to several human infectious diseases, such as nosocomial infections and cystic fibrosis, and to medical equipment [2–4]. The eradication of these biofilms poses several challenges because the antimicrobial resistance of biofilms is multifactorial resulting from the combination of different mechanisms, including restricted penetration of antimicrobials through the exopolysaccharide matrix, slow growth of bacteria within biofilms and cell-to-cell communication systems [5]. Attempting to eradicate *P. aeruginosa* biofilms, several treatments have been used but, unfortunately, *P. aeruginosa* infections still persist. Therefore, new strategies to eradicate *P. aeruginosa* biofilms are required. Biofilm matrix disruption has been considered an attractive approach because it can expose biofilm-cells to the action of antimicrobial agents. Typically, *P. aeruginosa* biofilm matrix contains a lot of compounds including significant amounts of pyocyanin, a blue reox-active phenazine that confers structural integrity to the biofilm, and extracellular DNA (eDNA) [6,7]. Glutathione is an human antioxidant that reacts with pyocyanin and eDNA intercalation being thus proposed as a potential matrix disruptor agent [7–9]. We hypothesized that combined with antibiotics, glutathione could weaken biofilms affecting the biofilm integrity and so augmenting the efficacy of the antimicrobial agents using reduced concentrations. Therefore, this study aimed to determine the anti-biofilm potential of glutathione in the disruption of biofilm matrix and to develop anti-biofilm strategies based on a possible synergistic effect resulting from combining glutathione with two different antibiotics, ciprofloxacin and tobramycin.

In this study, two *P. aeruginosa* strains were used, a laboratory strain PAO1, antibiotic sensitive and non-pyocyanin producer, and a clinical isolate PAI, antibiotic resistant and pyocyanin producer in order to reflect some of the *P. aeruginosa* biofilms diversity found in the hospital and industrial settings. Biofilms were formed on a 96-well microtitre plate for 24, 48 and 72 h to obtain different biofilm ages (immature and mature biofilms). Briefly, 200 µL per well of 1x10^7 CFU/mL overnight bacterial suspensions prepared in TSB were transferred into a 96-well microtitre plate and incubated aerobically at 37 °C under agitation (120 rpm). After biofilm formation, the content of the wells was discarded and biofilms washed to remove weakly attached cells and further treated with the combinatorial strategies. Checkerboard arrangements of glutathione, ranging from 1 to 8 mM, with ciprofloxacin, ranging from 1 to 32 µg/mL, or tobramycin ranging from 1 to 64 µg/mL were performed to determine the most effective anti-biofilm strategy. All these dual-arrangements were in contact with biofilms for 24 h. Biofilm biomass (determined by crystal violet assay [10]) and viable cells counting were determined before and after the application of the antimicrobial strategies to evaluate their anti-biofilm efficacy.

The preliminary results showed that the efficacy of the anti-biofilm approach of glutathione-ciprofloxacin reduced as the biofilm became more mature, since 24 h-old-biofilms were more susceptible than 48 h- and 72 h-old-biofilms. To augment anti-biofilm efficacy, increased concentrations of ciprofloxacin were needed and, even so, eradication did not occur for the two *P. aeruginosa* biofilms. The antibiotic susceptibility of *P. aeruginosa* strains seemed to have a great impact on the efficacy of this anti-biofilm strategy since all PAO1 biofilms suffered biomass reduction or were eradicated using glutathione-ciprofloxacin, in contrast to PAI biofilms in which no effect was observed. This result may be explained by the ciprofloxacin tolerance of PAI.

Interestingly, it was verified that glutathione-ciprofloxacin combination seemed to have action over PAO1 biofilms where there is no pyocyanin within the matrix. This finding led us to speculate that glutathione may not only act over pyocyanin and eDNA intercalation.

The combination glutathione-tobramycin exhibited improved preliminary results; although the same loss of efficacy was observed as biofilms became more mature. PAO1 biofilms were eradicated using low concentrations of glutathione and tobramycin, 1.5 mM and 16 µg/mL, respectively. The increased susceptibility of PAO1 biofilms to this last combination, reinforced our assumption that glutathione may play another role in biofilm matrix disruption. Young 24 h-old PAI biofilms were load reduced using low dosages of glutathione and tobramycin, 1.5 mM and 32 µg/mL, respectively. However, mature PAI biofilms with 48 h and 72 h of growth were again more tolerant to glutathione and tobramycin combination and increased concentrations of glutathione and tobramycin, 2 mM
and 64 µg/mL, respectively were needed to obtain a significant load reduction of biofilm cells and biomass. Moreover, it was also observed that glutathione, ciprofloxacin and tobramycin did not exhibit increased or equal efficacy against *P. aeruginosa* biofilms when applied alone highlighting the beneficial result of co-applying different kind of agents to eradicate biofilms.

In conclusion, the combination of glutathione with tobramycin could be a potential anti-biofilm strategy to be applied in clinical biofilms. This finding was quite relevant to continue exploring other biotechnological solutions based on the synergistic effect between antimicrobials and glutathione to eradicate *P. aeruginosa* biofilms.

**Acknowledgements**

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**References**


Novel glycine betaine ionic liquids analogues as components of aqueous biphasic systems with improved performance to separate amino acids

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Given the biotechnology advances observed in recent years in terms of upstream, the development of effective downstream processes becomes mandatory to decrease the associated costs of biotechnological-based products. Although a large interest has been devoted to ionic-liquid-based aqueous biphasic systems (IL-based ABS) as tailored separation platforms, imidazolium-based ILs have been the preferred choice as phase-forming agents. To overcome some toxicity and biodegradability issues associated to imidazolium-based ILs, novel ABS composed of ILs analogues of glycine-betaine (AGB-ILs) are here proposed and investigated. The ABS phase diagrams were determined for mixtures composed of water, AGB-ILs and Na2SO4 at 298 K. Their performance as extraction strategies was evaluated with five amino acids. In all studied systems, amino acids preferentially migrate to the IL-rich phase, with amino acid one-step extraction efficiencies ranging between 65% and 100%.

The need of finding new cost-effective purification techniques for value-added biocompounds, while maintaining their biological activity, is an actual challenge [1]. In the last decade, a large interest has been devoted to ionic-liquid-based aqueous biphasic systems (IL-based ABS) as tailored separation platforms [2]. ILs are salts with melting temperatures below 100°C, negligible volatility at atmospheric conditions, nonflammability, high thermal and chemical stabilities, and a strong solvation ability for a wide range of compounds. In addition, ILs are also labelled as “designer solvents” due to the possibility of combining a large number of ions with significantly different chemical structures, allowing their design for a particular application [2]. IL-based ABS have been considered as a biocompatible and effective alternative to common separation methods, mainly due to their high water content, remarkable extraction efficiencies, and high selectivity. ILs-based ABS have been successfully applied in the separation of several biomolecules, like amino acids and proteins/enzymes [2]. Among all the ILs-based ABS investigated, imidazolium-based ILs have been the preferred choice as phase-forming components. Nevertheless, imidazolium-based ILs have some associated toxicity that can compromise the biological activity of molecules, and low biodegradability, thus motivating the research on more benign ILs to be applied in ABS. ILs derived from natural sources can be seen as a promising alternative, namely ILs derived from analogues of glycine-betaine (AGB), which could be explored in the creation of ABS. Glycine betaine is composed of a quaternary zwitterionic tri(methyl)ammonium containing an acetate group, and these organic osmoles accumulate in a wide variety of plant species in response to environmental stress [3]. Hydrophobic (non-water miscible) ILs analogues of AGB have been recently proposed, and applied in the extraction of pesticides and metal ions [4]. However, based on their natural-derived source and if coupled with anions that turn them miscible with water, these ILs can be used in the creation of ABS aiming the separation and recovery of biologically active compounds. Considering this possibility and advantages connected to AGB-ILs, this work is focused on the development of more biocompatible IL-based ABS and on the evaluation of their performance to extract amino acids. With this objective, five AGB-ILs were synthesized, characterized in terms of ecotoxicity towards the marine bacterium Vibrio fischeri, and applied in the development of novel ABS formed with water and Na2SO4. For comparison purposes three commercial ILs were also used. The respective ABS ternary phase diagrams, as well as the tie-lines and tie-line lengths, were determined at 298 K and atmospheric pressure. Their extraction performance was evaluated with five amino acids, namely L-tryptophan, L-phenylalanine, D-phenylalanine, L-tyrosine and L-dopa. The five AGB-ILs investigated display a lower toxicity to V. fischeri than those commercially available and normally used in the preparation of ABS. The amino acids preferentially migrate to the IL-rich phase in all studied systems, where amino acid extraction efficiencies ranging between 65-100% were achieved in a single-step using AGB-ILs. These values are higher to those obtained by conventional polymer-based ABS [5]. In addition, the five AGB-ILs display a higher ability to form ABS and extract amino acids when compared to ABS composed of more traditional and commercial ILs. These results confirm the efficiency of IL-based ABS as extraction strategies, as well as the potential of AGB-ILs to substitute the largely investigated imidazolium-based compounds. Besides the negligible ecotoxicity of AGB-ILs, the extraction efficiencies obtained are competitive compared to those using commercial and more toxic ILs, while using lower amounts of salt and IL. Thus, AGB-ILs can form novel ABS that can be used as alternative separation platforms for amino acids and may be used for the separation of other value-added compounds of biotechnological interest.

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References
Ionic-liquid-based aqueous biphasic systems as enhanced extraction platforms for bovine serum albumin


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Aqueous biphasic systems (ABS) have been frequently applied in the development of novel ABS formed with ionic-liquid-based ABS, allowing remarkable extraction efficiencies and high selectivity compared to traditional polymer-based ABS. Ionic-liquid-based ABS have been successfully applied in the purification of different biological materials, such as cells, nucleic acids, lipids, amino acids, proteins, antibodies and enzymes [2]. Infact, the coexisting phases in these more conventional polymer-based ABS display a restricted polarity difference, hampering enhanced extraction efficiencies and high selectivity to be achieved in a single-step [1]. Several attempts to improve the performance of polymer-based ABS have been carried out, either by introducing additives or by carrying out the functionalization of polymers [3]. Ionic-liquid-based ABS were later proposed as an alternative of polymer-based ABS, offering several advantages over conventional ABS [4]. IL-based ABS are less viscous than typical ABS and usually display faster phase separation rates, and also lead to higher extraction efficiencies. ILs are salts that present melting temperatures below 100 °C and are normally constituted by an organic cation and an organic or inorganic anion [5]. Most ILs present a negligible volatility at atmospheric conditions, high thermal and chemical stabilities, a strong solvation capability for a large variety of compounds, and a capacity of conjugating ions according to the intended application, being designated by “designer solvents”. IL-based ABS have been applied in the extraction of a wide diversity of compounds, including proteins [6].

Proteins are biomolecules with applications in many fields. Depending on the environment in which they are inserted and changes in the medium composition, temperature and pH, proteins can suffer changes in their function and activity [7]. Thus, it is crucial to find methods of extraction and purification for proteins capable of maintaining their native structure and function/activity. In this work, novel IL-based ABS were investigated, and their extraction performance for bovine serum albumin (BSA) as a model protein was evaluated. Imidazolium-, phosphonium- and ammonium-based ILs combined with the anions acetate, arginate and good buffer MES were synthesized, characterized, and applied in the development of novel ABS formed with K2HPO4/KH2PO4 buffer at pH 7. The respective ABS ternary phase diagrams, as well as the tie-lines and tie-line lengths, were determined at 25°C. The effect of the IL anion and cation was studied in the formation of ABS and extraction performance. In all studied systems, BSA preferentially migrates to the IL-rich phase, with extraction efficiencies of 100% obtained in a single-step. Depending on the IL applied, the BSA recovery yield ranges between 42% and 100%. In conclusion, the new ABS here proposed may be used as an alternative platform for the extraction and purification of biomolecules, such as proteins, and if properly designed they do not lead to proteins losses.

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References


Removal of dyes using surfactant ionic liquids for in situ biodegradation with laccase

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Increased contamination of water is a serious threat to human health and ecosystem. In this sense, effective approaches for the treatment of industrial aqueous wastes and aqueous effluents, aiming at eliminating their detrimental effects and reduce costs, should be investigated and adopted. Many of the conventional effluent treatments are not completely effective, and some are complex and costly Therefore, the use of surfactants that could incorporate appropriate enzymes for the degradation of persistent pollutants can be considered as a potential alternative. However, attention must be given to these surfactants environmental risks and to their release into aqueous streams [1]. Ionic liquids (ILs), as eco-friendly solvent media and with unique and tunable physical properties, play very important roles in many enzymatic reactions, such as oxidative biotransformation of pollutants [2]. At critical micellar concentration (CMC), the surfactant molecules associate with their hydrophobic chains to form micelles with a generally hydrophilic exterior exposed to water and a hydrophobic interior. Some enzymes are more active in micellar systems than in reference solutions, the kinetics of the reactions catalyzed by these enzymes is improved and the yields of the reaction increased [3].

Laccase is one of the few enzymes that have the ability (simple requirements, apparent stability and lack of inhibition) to degrade a diversity of substrates with industrial interest [4]. More specifically, laccase oxidizes an extensive variety of organic and inorganic compounds being applied in bioremediation of phenolic compounds such dyes removal. In this work, biocompatible surfactant-based ILs with concentrations of IL above the CMC (micellar form) were used to encapsulate oxidative enzyme and colored pollutants (indigo carmine (IC), remazol brilliant blue R (RBRB) and rhodamine 6G within the micelles for in situ enzymatic biodegradation.

Methods

The catalytic performance of commercial laccase in two ILs families, namely 1-alkyl-trimethylammonium bromide and 1-alkyl-3-methylimidazolium chloride (cationic surfactants), was assessed towards the oxidation of 2,2-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). Decyltrimethylammonium bromide ([C10TMA]Br) and 1-Decyl-3-methylimidazolium chloride ([C10 mim]Cl) were the ILs where the laccase presented more activity above the CMC and were therefore used for the degradation of the dyes. The dye degradation conditions were optimized, namely pH and reaction time. The presence of the enzyme and the dyes inside or outside the micelles was evaluated by optical microscopy.

Results

The degradation of the IC, RBBR and Rhodamine 6G dyes by the laccase was tested in a medium with and without surfactant-based ILs. For the dye degradation tests, [C$_{10}$TMA]Br and [C$_{10}$ mim]Cl were used. For IC dye, the percentage of color removal (>90%) was higher and faster in the presence of ILs when compared to the control. Moreover, at the end of one day, almost complete degradation (>99%) of IC was achieved. With the C$_{10}$ TMABr, about 82% of IC was degraded in 30 min, whereas without IL a very low dye degradation of 10% was obtained. For the RBBR and Rhodamine 6G dyes, the results show that the degradation was more quickly in the absence of the ILs.

The presence of enzyme and dyes inside or outside the micelles was evaluated by microscopic analysis. It was observed that the enzyme and the dye are in or out of the micelle, depending on the IL used. In the case of [C$_{10}$ TMA]Br, it was observed that the enzyme and the dye were both inside the micelle formed by this LI. These results are important and relevant since the toxicity of industrial effluents is essentially associated with dyes and their decomposition intermediates [5].

Conclusion

It can be concluded that ILs improve the enzymatic reactions by laccase (the enzyme showed higher catalytic activity in the presence of micellar systems in the medium) for dye degradation. This work presented an environmentally friendly technology for the removal of colored pollutants which can be applied in industrial aqueous effluents. However, although the results suggest the potential application of these surfactant LI as a favorable approach, more studies will still be needed to achieve more efficient results in pollutant degradation tests and a better understanding of the impact of the use of these compounds.
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References
Monoclonal antibodies (mAbs) have been playing an important role in the pharmaceutical industry; they can be used in the treatment of several diseases, being sometimes the only available therapy for a particular disorder [1]. Up to date, their production for therapeutic purposes has passed through continuous developments, and their upstream processing is actually well-developed by biological processes. However, this fact shifts the spotlights towards the downstream processing, being now considered the bottleneck in the antibodies production [1]. Currently, Protein A affinity chromatography is the gold standard for the capture and purification of mAbs in the pharmaceutical industry, although it increases the global production costs up to 80% [1]. Ionic-liquid-based aqueous biphasic systems (IL-based ABS) appear as a cost-effective and efficient extraction technique, which was already successfully applied in the extraction and purification of numerous (bio)molecules [2], and thus can be foreseen as promising alternatives for mAbs downstream processing. However, the toxicity features of some ILs should not be discarded. Glycine-betaine analogous ILs (AGB-ILs) allow to overcome this issue, since they are biocompatible and non-toxic ILs, and are produced from natural sources [3]. In this work, novel AGB-ILs were synthesized and characterized, and the phase diagrams of ABS composed of AGB-ILs and phosphate buffer at pH 7 were determined at room temperature. Then, their extraction and purification performance for anti-human interleukin-8 (anti-IL-8) mAbs directly from Chinese Hamster Ovary (CHO) cell culture supernatants was evaluated. According to the obtained results, AGB-ILs not only successfully allow the extraction of anti-IL-8, but also improve the purity factor of mAbs up to 3.5 over the initial feed, in a single step. Therefore, this novel technology emerges as a potential alternative and biocompatible technique for the downstream processing of mAbs directly from the complex medium.

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References
Dinoflagellates: unique microalgae for sustained supply of bioactive compounds

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An interest in bioactive compounds produced by dinoflagellates has boomed in recent years. Such compounds exhibit a wide chemical diversity and complexity, and possess remarkable bioactivities – thus holding a strong potential for application as pharmaceutical leads. However, their production to sufficient extent remains an issue, thus hampering preclinical testing and eventual commercial exploitation. Such strategies as de novo chemical synthesis or genetic engineering are too laborious or even unfeasible at present. The most reasonable approach is dinoflagellate culturing in photobioreactor – yet unique designs and improved operating conditions are a must; their being fastidious microorganism and extremely sensitive to shear stress pose indeed serious challenges. Advances so far have encompassed culture medium formulation and light supply in situ; improvement of classical designs and proposal of novel configurations for dinoflagellate growth are under way.

Dinoflagellate bioactive compounds (potential application)

The long-lasting interest in bioactive molecules (namely biotoxins) produced by dinoflagellates (a group of microalgae) has risen in recent years. Dinoflagellates are able to produce bioactive compounds with a wide chemical diversity – namely such complex molecules as macrolides, cyclic polyethers, spirilides and purine alkaloids[1]. Their unique functional groups, and associated toxicological and biological features justify the broad range of bioactivities of such metabolites – with a remarkable potential to be explored as pharmaceutical leads. Such bioactivities include: anti-inflammatory, antiviral, anti-infective, cytotoxic, analgesic, immuno-suppressive, anticoagulant and anti-infective[2].

Several scientific reports and patents, encompassing applications of dinoflagellates as per their bioxin-related applications have emerged in recent years – and are a clue for the enormous interest in R&D efforts encompassing this group of microalgae.

Approaches to Biotoxin Supply (Constraints)

Despite their biotechnological potential in the pharmaceutical industry, unavailability of dinoflagellate-generated material has raised systematic difficulties – namely with regard to elucidation of the mechanism of action. Clinical trials and eventual development of new commercial products have indeed been hindered by unavailability of suitable amounts of dinoflagellate derived-compounds.

The portfolio of compounds commercially available is scarce – an associated with only a few dinoflagellates (e.g. Proorocentrum concavum, Gambierdiscus toxicus, Karenia brevis); the corresponding prices range, however, from 1000 up to 500,000 €/mg, depending on purity and source. Furthermore, said substances are often discontinued, while the effective purity and quantity claimed by the manufacturers is often inaccurate[2].

The limited availability of natural sources, along with major difficulties for de novo synthesis, or else genetic or metabolic engineering[2] have left bioreactor engineering approaches as sole strategy; however, difficulties in growing them at lab scale have hampered further experimentation downstream.

In fact, the route of chemical synthesis is too much complex, and in some cases requires more than 100 steps – being thus economically unfeasible at present. Genetic and metabolic engineering are also too complex, due to the peculiar DNA of dinoflagellates - while their complex and specific metabolism have greatly constrained applicability of genetic and metabolic tools already available for other organisms[3].

On the other hand, mass production of such metabolites in bioreactor has proven extremely difficult in conventional photobioreactors[4]. Dinoflagellates at large grow slowly, and are very shear-sensitive. They also obey strict circadian cycles, in which a vertical migration pattern is established according to daylight and nutrient level. Therefore, conventional photobioreactors will hardly provide suitable conditions to achieve high biomass productivities and biotoxin concentrations. Remember they entail simplistic modes of light supply (e.g. continuous external illumination) — which, combined with the typical uniform levels of nutrients, may break down natural rhythms, and cause deviations from the original metabolic behavior. Continuous supply of CO2 is also required for photosynthesis, owing to its low solubility in water – which calls for turbulence to minimize resistance to mass transfer. However, dinoflagellates have been found to be extremely sensitive to turbulence, meaning that shear stress readily produces cell damage[2].

Culture of Dinoflagellates and Biotoxin Production

Researchers have been investing a lot of effort towards development of dinoflagellate controlled cultures – by resorting to medium, light and stirring engineering.

Nutritional requirements. Attempts to improve nutritional requirements of dinoflagellates has been done by a genetic algorithm (GA). GA-based stochastic search is able to explore a large experimental space, and has been successfully applied in medium formulation of dinoflagellates Protoceratium reticulatum and Karlodinium veneficum. The new developed media allowed 40% and 190% enhancement of biotoxin titer, and 60% and 120% final cell concentration relative to the basal L1 medium, respectively [5,6].
Light provision. Both natural and artificial light have been reported to support growth of dinoflagellates of interest ([7]). Artificial light provides better control of light spectrum, irradiance (or photosynthetic photon flux density) and photoperiod in closed photobioreactors. Several culture studies have employed conventional cool fluorescent lamp, yet light-emitting diodes (LEDs) are gaining importance in dinoflagellate cultures. So far, *Karlodinium veneficum* and *Alexandrium tamarense* dinoflagellates were the only species of interest to be cultured with success with LED technology at pilot-scale [8,9].

Patterns of agitation/aeration. If aeration is provided and carefully regulated, improvement in biomass productivities results – as well as enhancement of rate of biotoxin synthesis. Hu et al. [10] has worked with a two-step batch culture method – first favoring growth in static conditions, and applying aeration in subsequent steps in order to improve saxitoxin yields by *Alexandrium tamarense*.

Bioreactor culture. Based on different bioreactor technologies employed, with volumes ranging from 2 to 700 L, a number of cultivation strategies have been tested aiming to optimize growth conditions and biotoxin levels [11]. Configurations tested encompass carboys, chemostats and stirred-tanks, airlift, bubble column, tubular reactor and flat-plate photobioreactors. The technology Twin-Layer PBR was also implemented at laboratory scale to characterize growth of *Symbiodinium voratum* and production of pigment thereby in an immobilized support; said novel configuration approach appears suitable for enhanced biotoxin production.

Final considerations. Dinoflagellates have proven a rich biotechnological source of compounds with interesting biological activities, potentially useful for pharmacological purposes. Despite such a recognized potential value, scarcity of those compounds for preclinical testing (and later for commercial exploitation) remains a major issue. Major efforts and resources have been directed to improved modes of culturing dinoflagellates in photobioreactors, for enhanced biotoxin concentrations. Nutritional profile and operational conditions, such as light and aeration/agitation patterns, have to take into account that dinoflagellates obey specific circadian rhythms, and are extremely sensitive to turbulence when cultivated in bioreactor. Therefore, the feasibility of dinoflagellate mass cultivation will require challenges on conventional bioreactor designs in attempts to circumvent their fastidious growth and shear-sensitivity. It is hoped that an improved strategy will allow production of sufficient amounts of biotoxins, for biochemical investigational and clinical trials and the like a posteriori.

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References

Nucleic-acid-based products have been identified as promising biopharmaceuticals and as relevant biomolecules in biological assays. However, the understanding of the biological role of RNAs or their use as biotherapeutic agents require pure and intact RNA with adequate integrity, stability and biological activity, in the last case also to meet the demands of regulatory agencies. In addition, nucleic acids are highly susceptible to nuclease cleavage, and although RNA is more prone to degradation than DNA, most studies aiming at finding adequate preservation media focused on improving the stability of DNA. Based on this lacuna, we evaluated the potential of self-buffering ILs for extending the shelf life of a recombinant small RNA (sRNA) fraction derived from Escherichia coli, containing the therapeutically relevant microRNA-29. Distinct GB-ILs comprising the cholinium cation and anions derived from biological Good’s buffers, namely MES, TES, Tricine and HEPES, were prepared, and their aqueous solutions appraised as preservation media to improve the stability of recombinant RNA. It is shown that the thermal stability of RNA is highly enhanced by GB-ILs, with an increase of 14 °C in the biopolymer melting temperature - the highest increase observed up to date with ILs. Most GB-ILs investigated improve the stability of RNA at least up to 30-days, both at 25 and at 4 °C, without requiring the typical samples freezing. Molecular dynamics simulations demonstrated that the number of IL cations surrounding the RNA chain is similar, yet with differences found for the IL anions, which are responsible for the observed improved stability. No cytotoxicity of RNA and ILs at 20 % (w/w) solutions was observed onto two distinct human cell lines, reinforcing their potential to act as preservation media when foreseeing biopharmaceutical applications. Finally, high-integrity RNA was successfully recovered from the ILs aqueous solutions, and the IL recycled and reused. In summary, aqueous solutions of [Ch][MES] and [Ch][HEPES] as remarkable stabilizing media of recombinant sRNAs at room temperature, without requiring the typically carried out samples freezing, thus reinforcing their effectiveness in RNA bioprocessing.

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References
Nucleic acids are fundamental in many biological processes and in the progression of diseases, being therefore important targets in fundamental and applied research, but also for therapeutic purposes\(^1\). However, the current methodologies for the purification of nucleic acids, particularly RNA, are costly, laborious, time-consuming, require the use of organic solvents or imply the structural modification of RNAs\(^2\). Indeed, the search on chromatographic ligand for RNA purification endowed of superior selectivity, robustness and reproducibility is still in high demand, and for which ionic liquids could be investigated as alternative/effective ligands. Ionic liquids are a group of organic salts, with great versatility to manipulate their cation/anion design, and presenting particular physicochemical characteristics (non-volatility, non-flammability, among others)\(^3\). These compounds have been applied in liquid chromatography in distinct ways\(^4\), including as ligands in the stationary phases; moreover, it has been shown that they stabilize the conformational structures of nucleic acids, being therefore projected as proper solvents and preservation media. Therefore, the main aim of this work consisted on the development of new and effective macroporous-based matrices functionalized with ionic liquids for preparative liquid chromatography, envisaging the separation of nucleic acids. Initially, different ILs were covalently attached onto silica (SILs), which was used as the stationary phase for the screening and identification of the more suitable ILs for nucleic acids purification. This approach revealed to be a reproducible and more economical method as a pre-evaluation stage before using chromatographic columns. In general, the obtained SILs allow different interactions with nucleic acids, mainly hydrophobic and ionic interactions. Then, macroporous-based matrices were modified with the most promising ILs, in which the obtained results showed that these columns are able to selectively isolate ribosomal RNA from small RNAs and DNA in a single purification step, thus representing a step forward in nucleic acids isolation methodologies. These IL-based matrices present high robustness, high reproducibility and high dynamic binding capacity, even after regeneration steps. Overall, the successful separation of nucleic acids by IL-macroporous based chromatography may open news perspectives in nucleic acids purification methodologies, with a wide applicability for nucleic acids structural and functional studies and to prepare these samples for therapeutic approaches.

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References
Isolation and preliminary characterization of a new bacteriophage against *Sphaerotilus natans*

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Activated sludge process, the most commonly used system for biological wastewater treatment, is driven by a community of microorganisms that play a key role in the conversion of the organic matter and removal of nutrients from wastewater. In this artificial ecosystem, filamentous and floc-forming bacteria co-exist in a dynamic equilibrium. However, filamentous bacteria overgrowth lead to a decrease of performance of the plant by causing two well-known phenomena: bulking and/or foaming. *Sphaerotilus natans* is a filamentous bacterium that can cause or contribute to the malfunction of these systems by leading to bulking occurrence. Bacteriophages are regarded as possible novel treatment against the filamentous overgrowth. In this work, the isolation and preliminary characterization of a new lytic bacteriophage against *S. natans* are described. Results clearly indicate the potential effect of this bacteriophage for future wastewater treatment system management.

**Introduction**

Bacteriophages (phages) are viruses that specifically infect bacteria. They are the most abundant entities in the biosphere, being found in every environment where their bacterial hosts are present [1]. Phages are parasites that invade the bacterial cells and reproduce themselves by using the bacterial machinery. These have an easy, rapid and relatively inexpensive production. *S. natans* is a rod-shaped Gram-negative microorganism [2]. This species is aerobic and growth at 28 °C [3].

In this work, one lytic phage for *S. natans* was isolated, using sewage water from an urban WWTP. The phage was tested against other species found in WWTP to conclude about its lytic spectra. Stability of the phage solution at different temperatures and pH was determined. Morphologic analysis by Transmission Electron Microscopy (TEM) of the *S. natans* phage was also performed.

**Materials and Methods**

A culture of *S. natans* (DSM 6575) from the Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ) collection was used to isolate the phages. For the host range screening of the isolated phage, strains of *Sphaerotilus montanus*, *Gordonia amarae*, *Rhodococcus rhodochrous*, *Staphylococcus epidermidis* and *Escherichia coli* were used.

Sewage water from a municipal WWTP was used to isolate phages of *S. natans*. Spot assays were performed against bacterial lawn to check the presence of phages. Plaque picking was repeated until single-plaque morphology was observed. Thermal stability tests were carried out at −20 °C, 4 °C (as control), room temperature (25 °C), 28 °C, 40 °C and 50 °C for 24 h. Similarly, the effect of pH was also evaluated using a universal pH buffer with pH adjusted to 3, 4, 5, 6, 7 (as control), 9, 10, 11 and 12 at room temperature for 24 h.

Transmission Electron Microscopy (TEM) was used to morphologically characterize the *S. natans* phage.

**Results**

New phage against filamentous bacteria found in activated sludge. This work allowed to isolate one *S. natans* phage. Plaque morphology of the *S. natans* phage was characterized as clear and uniform small plaques on the host strain.

**Host range screening.** *S. natans* phage did not show a lytic effect against any of the other bacteria tested.

**pH and temperature stability.** The results demonstrate that *S. natans* phage was stable after 24 h, at 4 °C, 21 °C, 28 °C and 40 °C, showing a concentration about 7 orders-of-magnitude. At -20 °C and 50 °C, *S. natans* phage concentration decreased about 1 and 1.5 orders-of-magnitude, respectively, in comparison with the control (4 °C).

*S. natans* phage was stable in the pH range 5.0–11.0. In the pH range 3.0, 4.0 and at pH 12.0, the *S. natans* phage was completely inactivated.

**TEM of *S. natans* phage.** According to the morphological evaluation [4], the isolated phage must be included in the *Caudovirales* order. *S. natans* phage has a short non-contractile tail and an icosahedral head and consequently was considered to belong to the *Podoviridae* family.

**Conclusions**

One new *S. natans* phage was isolated and its lytic spectrum and stability at different temperatures and pH values determined. The morphologic analyse by TEM was also carried on. The *S. natans* phage shows clear and uniform small plaques on the host strain. *S. natans* phage was stable after 24 h, at 4 °C, 21 °C, 28 °C and 40 °C, and in the pH range 5.0–11.0. The TEM analysis indicates that *S. natans* phage belongs to the *Podoviridae* family. From the results obtained it can be hypothesized that this phage has a potential effect on the removal of the excess of filamentous bacteria and might be used for future wastewater treatment system management.

Further work is required to determine if *S. natans* phage can control *S. natans* overgrowth phenomena in wastewater treatment plants, by using lab-scale reactors.
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References
Bioengineered in vitro 3D hydrogel-based cancer model using chemically modified biopolymers

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The development of hydrogel-based in vitro models using nature-derived bioactive materials such as gelatin and hyaluronic acid, is a promising approach for the discovery of new anti-tumor therapies in a faster and expeditious way. Herein we describe the chemical modification of gelatin and hyaluronic acid and subsequently engineering a photocrosslinking hydrogel composed of gelatin-methacryloyl and hyaluronic acid modified with thiol groups (GelMA-HASH), which intends to mimic the extracellular matrix of tumor microenvironment. In vitro multicellular 3D models, composed of cancer cells and fibroblasts, were also produced to mimic the cellular heterogeneity present in this disease. Each macromer was characterized by 1H NMR and the biological functionality and viscoelastic properties of the 3D models produced by GelMA-HASH hydrogels were evaluated. Globally, the formulated hydrogel was considered to be a promising scaffold for bioengineering of 3D in vitro prostate cancer models and is envisioned to be used as a drug screening platform in the future.

Introduction

The tumor microenvironment (TME) is comprised for different cell types including cancer cells and non-cancer cells, which are embedded in a tumor-specific extracellular matrix (ECM) [1]. For the past decades researchers have aimed to capture the complexity of human prostate cancer by developing various types of disease models that could help in understanding fundamental tumor biology and development, as well as aid in the discovery of more effective treatments. The in vitro 2D cancer models, widely used in cancer research, although they have helped to understand some aspects related to the progression of the disease, they fail in fully mimicking some typical features of cancer tissues. In this sense, in vitro 3D models have been gaining some particular attention, since they represent more accurately the biological behavior and the tumor microenvironment, which is essential for understanding the mechanisms underlying disease progression, contributing significantly to future advances in anti-cancer [2]. In order to produce the most biomimetic 3D in vitro cancer models possible, they need to provide the appropriate ECM components in a 3D configuration found in vivo. In addition, the co-culture of cancer cells with non-malignant stromal cells related with TME, such as fibroblasts is required. To date various techniques have emerged to allow the establishment of tumor-mimicking 3D cell cultures in controlled in vitro environments. Particularly, 3D disease models can be assembled by using scaffold-free models, which comprise the aggregation of the cells so as to form spheroids (microtumors), and scaffold-based models, where the cells are embedded in a matrix which is intended to mimic the extracellular matrix in vivo. To recreate this matrix, hydrogels have been the most used materials due to their properties, with gelatin and hyaluronic acid being the most reported biomaterials in disease models. HA and collagen are major native ECM components in various tissues. However, when used as single component biomaterials to generate 3D models they demonstrate several drawbacks. In this research work, to improve the physical and biological properties of it natural polymers, HASH and GelMA macromers were synthetized, and HASH-GelMA hybrid hydrogels were fabricated. This two macromers can be crosslinked one with each other by photopolymerization, an irreversible crosslinked network through covalent crosslinking, producing a stable structure to support a biomimetic disease model.

Objective

The main aim of this research work is the development of a 3D biomimetic prostate cancer model, using functionalized polymers, that allows an accurately preclinical validation of new anti-cancer therapies.

Methods

First, two polymers, GelMA and HASH, were synthesized as previously described [3,4]. The occurrence of the modification was confirmed by analysis of the 1H NMR spectra and the degree of modification of GelMA and HASH was obtained by performing the fluorodehyde assay and by integrating 1H NMR characteristic peaks, respectively. Thereafter, a GelMA-HASH hydrogel was synthesized by combining the two polymers with a photoinitiator aliquot and subjecting the mixture to U.V. light at 365 nm. Different formulations of this hydrogel were then formulated, varying the concentration of each of the macromers, and the biological and viscoelastic properties were analyzed. To assess the biological functionality of the hydrogel, prostate cancer cells (PC-3) and Fibroblasts (HF) were co-cultured in the different hydrogel formulations and Live/Dead assay was performed at 7 days of culture. In addition, spheroids comprising prostate cancer cells and stromal cells (fibroblasts) were assembled by seeding both cells in ultra-low attachment plates. The necrotic core formation was assessed by using the Live/Dead assay at specified timepoints.

Results

GelMA and HASH preparation and characterization

Gelatin-methacryloyl (GelMA) was synthesized through the reaction of the amino and hydroxyl groups in gelatin molecules with methacrylic anhydride. The 1H NMR spectra of gelatin (Gel) and GelMA macromers are shown in figure 1, (a), and allowed the confirmation of the substitution. To estimate the degree of functionalization of the gelatin the fluorodehyde assay was performed (figure 1, (b)) and the degree of functionalization (DoF) obtained for synthetized GelMA was 89.53%.

With regard to the HASH macromer, thiol groups were introduced on hyaluronic acid using a standard carbodiimide-mediated reaction of the HA carboxylic groups with the amine group of cysteamine. The representative 1H NMR spectra of HA
and HASH and the signal assignment are shown in Figure 1, (c).
Integration of the $^1$H-NMR signals indicated that the degree of modification of HA–SH was about 30.3%.

Figure 1. Characterization of the degree of modification of GelMA and HASH.

Synthesis and Characterization of GelMA-HASH hydrogel

To synthesize the GelMA-HASH hydrogel, the two polymers were mixed with an aliquot of Irgacure 2959 and the blend was subjected to UV light at 365 nm, thereby forming a hydrogel chemically bound by covalent bonds. With the aim of achieving the best formulation for the cell culture, hydrogels were prepared with varying concentrations of GelMA and HASH, and viscoelastic properties and the biological functionality were analysed (Figure 2 and Figure 3, respectively).

Figure 2. Storage modulus ($G'$) of 10% GelMA-based hydrogel and of 5% GelMA based hydrogels.

The viscoelastic properties of the gels were probed through the storage modulus ($G'$), which was continuously monitored during photopolymerization, allowing a qualitative comparison of the stiffness between the different hydrogels formulations. As shown in Figure 2, it was possible to conclude that higher concentration of HASH into gelatin-based hydrogels produced softer hydrogels. Regarding the biological functionality, it was possible to verify that the hydrogel is biocompatible and therefore suitable for cell culture.

Figure 3. Live/dead staining PC-3:HF co-cultures in GelMA and GelMA-HASH hydrogels at day 7. Scale bars=200 µm.

Related to scaffold-free models, spheroid models can represent key factors such as necrotic core formation and the recapitulation of cellular interactions between different populations, that are crucial to production predictive disease models. With the objective of produce microtumors with characteristic necrotic core similar to that obtained in human solid tumors, 3D co-culture spheroids with PC-3 and HF cells, were produced at different cellular densities. To assess if a dense mass of necrotic cells was formed, Live/Dead assay at days 3 and 7 of culture was performed (Figure 4). It was possible to observe the formation of necrotic core at day 7 in the co-cultures with the highest cell density, mimicking the hypoxic core of tumor in vivo.

Figure 4. Fluorescence microscopy micrographs of PC-3:HF spheroids Live/Dead staining. Green channel: live cells (Calcein-AM labelled), Red channel: dead cells (PI labelled).

Conclusion

3D in vitro models provide an environment in which cells interact directly with involving adjacent cell in a three-dimensional space, expanding, contracting, and assembling into microtissues with morphological, phenotypical and genetic characteristics similar to solid human tumors. In this way, with the aim of produce a 3D prostate cancer model, we formulated a biocompatible and tunable GelMA-HASH hydrogel which intended mimic the in vivo features of ECM. By using this strategy, 3D co-culture microtumours comprised for prostate cancer cells and stromal cells, were obtained with success and this model is expected to be used in the future as a platform to screen for new prostate cancer therapies.

Acknowledgements

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In the 21st century, nanotechnology has been emerging as a very promising field, with numerous applications in distinct areas, such as textiles, food agriculture, environment, electronics and in the biomedical field. Among the different categories of nanomaterials, zeolites are a very important class of inorganic materials that have been used in a wide range of applications. In the current work, we used zeolite NaY as host to incorporate silver ions and the antineoplastic drug 5-Fluorouracil (5-FU) for microbial infections and non-melanoma skin cancer treatment, respectively. Both situations present a major threat to public health: microbial infections due to their increasing resistance to antibiotics and cancer because of the difficulty of an efficient treatment especially when the disease has progressed considerably. So, since zeolites have a range of structural and physical properties that make them suitable for therapeutic delivery systems, they could be interesting candidates for this purpose. Our goal is to create an efficient and biocompatible dual topical delivery system with zeolites and, this way, combine the antimicrobial and the antineoplastic activity in the same formulation.

Introduction
Zeolites are microporous crystalline nanomaterials that contain alkaline metal ions (that can be readily exchanged by other cations in solution) and water molecules within their structure and are composed by silicon, aluminum and oxygen. Their 3D framework of SiO4 and AlO4 tetrahedra results in a uniform network of nano-channels and pores. Water molecules can be easily removed upon heating, resulting in a high surface area and an accessible pore volume that allows diffusion of the molecules to the interior of the zeolite particle [1-3]. The number of areas utilizing zeolites has grown tremendously and several studies have reported its use in the industrial sector, as detergent water softeners, as food additives, as adsorbents for wastewater treatment [4-5], as catalysts in industrial processes and in the biomedical field, such as magnetic resonance imaging, wound treatment, as drug delivery systems (DDS) and as antimicrobial agent (by incorporating cations with antimicrobial action) [6-8].

In this work, we explored and combined these last two applications loading zeolite NaY with silver ions by ion exchange followed by loading with 5-Fluorouracil (5-FU), originating a dual biocompatible nanomaterial system, (Ag/5-FU)Y as DDS for topical delivery.

Methods
1. Preparation of DDS. A solution of 0.01 M of silver(I) nitrate (Sigma-Aldrich) was added to the zeolite NaY (Zeolyst International) and the resulting suspension was maintained under constant stirring at 300 rpm for 24 h at room temperature. Suspension was then filtered and washed with deionized water and dried overnight at 60 °C to obtain the AgY. The resulting sample was calcined at 350 °C for 4 h. Loading of the drug 5-FU into the zeolitic structure was achieved by adding 200 mg of AgY to a solution of 5-FU (0.023 mM) in acetone (80 % acetone/20 % water; v/v). The suspension was kept under stirring at room temperature for 48 h. The resulting mixture was filtered and washed and the DDS ((Ag/5-FU)Y) was dried at 60 °C for 12 h to evaporate the solvent. Na(5-FU)Y was prepared as described in [2].

2. Evaluation of antimicrobial activity. To evaluate the antimicrobial activity of the prepared samples, several bacterial strains which are known to be capable of triggering several infections (including skin infections) and therefore compromise human health, were selected as predictive models. 5 µL drops of each inoculum (bacterial cultures at OD = 0.4–0.6) were placed on top of agar plates containing the culture medium supplemented with zeolite nanomaterials. Assays were performed in the presence of 0.2, 0.5, 1 and 2 µg/mL of each zeolite sample. Minimum inhibitory concentration (MIC), defined as the lowest concentrations that prevent bacterial growth, were determined for each pair sample/bacterial strain tested.

3. Cell viability assays. Cell viability studies were performed using a human skin cancer cell line, A375. The cell line was routinely cultured in DMEM, supplemented with 10 % (v/v) Fetal Bovine Serum and 1 % (v/v) penicillin–streptomycin and maintained in a 5 % CO2 humidified atmosphere. Cells were subcultured every 2 to 3 days (approximately when they reached 80% confluence) to ensure their proper growth and health. To evaluate the effect of zeolite-mediated cytotoxicity and cell proliferation in vitro, Sulforhodamine B (SRB) colorimetric assay was performed.

Results
We observed that bacterial growth was unaffected by the presence of parent zeolite, NaY, regardless of the concentration tested, meaning that the zeolite itself revealed no antibacterial effects. However, AgY exhibited antibacterial activity against all the bacterial strains tested and no viable bacterial cells were detected in the presence of 1 mg/mL of AgY, proving that the introduction of silver ions in the zeolitic structure gives the antibacterial properties. The DDS (Ag/5-FU)Y also exhibited inhibitory effects on bacterial growth, showing that the zeolite maintains its antibacterial capacity in the presence of both...
agents, so the presence of 5-FU does not interfere with the release and action of silver ions. Interestingly, the MIC values obtained with (Ag/5-FU)Y were lower than the values for AgY, meaning that the hybrid system exhibited enhanced antibacterial properties which may be caused by the combined action of 5-FU and silver ions (Table 1).

Table 1. MIC values for NaY, AgY and (Ag/5-FU)Y against each of the tested indicator bacterial strains

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>MIC (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NaY</td>
</tr>
<tr>
<td><em>P. acnes</em></td>
<td>&gt;2</td>
</tr>
<tr>
<td>MRSA</td>
<td>&gt;2</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>&gt;2</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>&gt;2</td>
</tr>
<tr>
<td><em>P. aeruginosa</em></td>
<td>&gt;2</td>
</tr>
</tbody>
</table>

*P. acnes* - Propionibacterium acnes, MRSA - Methicillin-resistant Staphylococcus aureus, *S. aureus* - Staphylococcus aureus, *E. coli* - Escherichia coli and *P. aeruginosa* - Pseudomonas aeruginosa

Regarding the cancer cell viability tests (Figure 1), NaY did not interfere with the cell viability in the tested range of sample concentrations and throughout the entire period of exposure of the cells to the samples (up to 72 h), indicating its suitability as a drug delivery system for this type of cancer cells. (Ag/5-FU)Y caused a very accentuated decrease in cell viability. Similarly, AgY, which does not contain the antineoplastic drug 5-FU, also showed the same behavior, indicating that silver ions are highly cytotoxic to A375 cells. This way, both agents incorporated in the zeolite structure, silver ions and 5-FU, have an active role on the decrease of cell viability.

Figure 1. Effect of NaY (blue), AgY (green), Na(5-FU)Y (purple) and (Ag/5-FU)Y (red) on A375 human cells viability.

**Conclusions**

(Ag/5-FU)Y showed good antimicrobial properties, and in some cases with lower MIC values than the sample with only silver ions, suggesting that 5-FU itself has some antibacterial effect. Moreover, the DDS proved to be highly cytotoxic to A375 cells, an effect that is not only the result of 5-FU action but is also due to the presence of Ag⁺ ions. These results highlight the great potential of the dual nanomaterial system and, with further work, it could be eventually incorporated into a biocompatible cream to be used for topical delivery, and this way combine the advantageous effects of the antimicrobial and antineoplastic activity in the same formulation.

**Acknowledgements**

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Flavonoids exhibit useful antioxidant activities and pharmacological properties, such as the capability of inhibiting the enzyme xanthine oxidase involved in inflammatory-related processes [1,2]. Among them, rutin has been demonstrated to possess beneficial features for preventing various diseases. However, this compound shows poor solubility in water, thus implying scarce bioavailability, and low thermal stability [3]. Rutin has been previously oligomerized in a laccase-catalyzed reaction leading to products with enhanced aqueous solubility and superoxide scavenging activity [4]. As seen in our latest research [5], laccase activity played an important role in rutin oligomerization in a reaction medium containing 50% of ethanol as co-solvent to enhance initial rutin solubility.

The main purpose of this work was to probe possible and characterize the oligomerization of rutin in complete absence of organic solvents and to provide information for the optimization of this process. To fulfill this objective, we studied the influence of both laccase activity and temperature on the oligomerization of rutin when no co-solvents are used, and initial substrate forms a suspension of non-dissolved rutin in water. Results from this work will lead to a future design of an enzymatic reactor that will allow the re-use of laccase in more than one cycle of this process. To fulfill this objective, we studied the influence of both laccase activity and temperature on the oligomerization of rutin when no co-solvents are used, and initial substrate forms a suspension of non-dissolved rutin in water. Results from this work will lead to a future design of an enzymatic reactor that will allow the re-use of laccase in more than one cycle of production.

Three enzymatic activities (laccase from Trametes versicolor) were tested in this work: 1000, 500 and 100 U/L. All reactions were conducted in a CH-4127 Adolf Kühner AG incubator under controlled stirring (225 rpm) and temperature (25-35-45°C). Laccase activity and rutin concentration in the reaction medium were monitored during 24 h of reaction. Final lyophilized products were analyzed in terms of antioxidative capacity. Methods for monitoring and analysis were as described in [5].

As can be seen in Table 1, adequate rutin conversions were obtained for reactions involving 1000 and 500 U/L, with an increase of ~10% when rising the temperature from 25 to 45°C. As shown in Figure 1.a the use of higher enzymatic activities led to products with worse properties when compared to their respective controls. However, differences between oligorutin obtained using 500 and 1000 U/L were negligible. Xanthine oxidase inhibitory activity of oligomers remained mostly unaltered and were comparable to the monomer (Figure 1.b).

Table 1. Rutin conversion reached at 24 h under different temperatures and laccase activities.

<table>
<thead>
<tr>
<th>Enzymatic activity (U/L)</th>
<th>25°C</th>
<th>35°C</th>
<th>45°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1000</td>
<td>88.5 ± 1.5</td>
<td>87.4 ± 9.0</td>
<td>97.8 ± 1.7</td>
</tr>
<tr>
<td>500</td>
<td>83.1 ± 3.2</td>
<td>93.8 ± 2.0</td>
<td>95.8 ± 1.0</td>
</tr>
<tr>
<td>100</td>
<td>29.1 ± 5.2</td>
<td>50.3 ± 4.4</td>
<td>49.5 ± 11.9</td>
</tr>
</tbody>
</table>

Figure 1. Effect of temperature and laccase activity on a) antioxidant activity and b) xanthine oxidase inhibitory activity (half maximal inhibitory concentration); ■25°C, □35°C, ◆45°C.

An increase in temperature resulted in a higher rutin conversion during the first hours of reaction (Figure 2.a and 2.b) but accompanied by a greater activity loss than in reactions that took place at 25°C (Figure 2.c and 2.d). At short times, the effect of the laccase activity used upon rutin conversion was more dependent on temperature for a lower initial laccase activity. The oligomerization of rutin in a reaction medium free of organic solvents was performed under different...
temperatures and starting enzymatic activities. Despite rutin low aqueous solubility, adequate oligomer production yields were reached with 500 and 1000 U/L at 24 h of reaction, obtaining products with higher aqueous solubility, similar xanthine oxidase inhibitory capacity and reduced antioxidant activity, no matter the temperature used. As a conclusion of this work, it would be possible to operate an enzymatic reactor using 1000 U/L laccase activity at 25°C with the objective of re-using the same enzyme for further production cycles, but reaction time could be shortened to 6 h, thus allowing a rutin conversion of ~80% with no initial loss in enzymatic activity in the first oligomerization reaction. Further experimental work will be performed regarding laccase re-use for rutin oligomerization.

![Figure 2](image_url)

**Figure 2.** Rutin concentration and enzymatic activity during the reaction time expressed in relation to their initial values for the reaction catalyzed by 1000 (a, b) and 500 U/L laccase (c, d) at different temperatures; ●: 25°C, ●: 35°C, ●: 45°C.

**Acknowledgements**

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**References**

Effect of processing on the antioxidant activity of different varieties of peppers (Capsicum annuum)

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Vegetables have bioactive components (polyphenols, phytosterols, carotenoids, glucosinolates) that lead to important health benefits. However, there are vegetables that to be preserved or ingested, need to undergo heat treatments which can cause changes in physicochemical properties, composition and antioxidant activity [1,2]. In this work, the antioxidant activity of different types of peppers (green, red, yellow and orange), raw and submitted to processing (hot water and microwave bleaching, stewed, grilling and frozen) was evaluated and expressed in IC50. After extraction with a solution of ethanol-water 80 %, the extracts were analyzed for its ability to inhibit radicals of 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antioxidant activity of the extracts depends on the peppers variety with IC50 values from 32.8±3.6 to 47.7±2.7 mg dry matter/µmol DPPH in raw yellow and orange peppers, respectively. Regarding the type of processing, some variations in these IC50 results were found.

Introduction
The interest in compounds with antioxidant properties is due to the fact that they have important functions in food preservation and health protection.

Fruits and vegetables are sources of natural antioxidants such as vitamins, carotenoids, flavonoids and other polyphenols [1,2]. According to epidemiological studies that have been carried out in the last years, frequent consumption of these products contributes to a significant reduction in the risk of coronary diseases and cancer development. In addition, the World Cancer Research Fund and the American Institute for Cancer Research have warned since 1997 about the benefits of a diet rich in fruits and vegetables recommending a daily intake of at least 400 - 800 g of these products.

However, there are vegetables that, in order to be preserved or ingested, need to undergo heat treatments, which can cause changes in physicochemical properties, composition and antioxidant activity [1,2].

Pepper fruits (Capsicum annuum) are used as vegetable foods as well as a spice, mainly in Mediterranean diet. It can be found in the markets a lot of very different cultivars showing a wide range of morphological and organoleptic characteristics, including color, size, shape and taste that can determine use of these vegetables.

Peppers are an important source of nutrients in the human diet and an excellent way to obtain provitamin A compounds, like carotenoids, as well as vitamins C and E, with well-known antioxidants properties. However, it is known that levels of these compounds are influenced by growing conditions and can be modified or lost after processing [3].

Objective
From the evaluation of the antioxidant activity of vegetables frequently grown in Portugal, it will be possible to obtain information that may contribute to the valorization of some of these products as a possible source of antioxidants with health benefits to the consumers.

In this way, the aim of the present work was to determine and compare the antioxidant activity of different varieties of peppers and discuss the effects of different cooking and preservation techniques namely bleaching, stewed, grilling and frozen on the antioxidant properties of the processed products.

Methods
Sample preparation. After washed and seeds removed, fresh peppers were cut and divided into portions to be analyzed in raw state and after applying the different processing operations: bleaching in hot water and microwave (mw), stewed, grilling and frozen for 17 days. Samples of 2.0 g were subjected to extraction with 10 mL of a mixture of ethanol-water 80 % (v/v) for 15 minutes.

Water content. All samples were oven dried at 100 °C until constant weight. The water content of the peppers was calculated, in percentage, for the different samples.

Bleaching in hot water. The sample was immersed in 200 mL of hot water during 30 seconds and, then, cooled in cold water.

Bleaching mw. The sample was placed in 10 mL of water and submitted to microwave during 2 minutes in an oven and next cooled with cold water.

Stewed technique. Pieces of peppers were seasoned with salt and olive oil and cooked as usually are consumed.

Grilled technique. Pieces of peppers were grilled after only seasoned with salt.

Frozen process. The samples of the different pepper varieties were frozen at -20 °C for 17 days.

Antioxidant Activity. The antioxidant capacity was evaluated from the scavenging activity of the extracts of pepper samples for the 2,2 diphenyl-1-picrylhydrazyl (DPPH) radicals, measuring the absorbance decrease at 517 nm. In the assays 0.1 mM DPPH solutions were prepared daily and used to obtain the corresponding calibration curves for the determination of the DPPH radical concentration in the reaction mixture and IC50 calculation.

Results and Conclusions
The kinetics of inhibition of the DPPH radicals by solutions of equal concentration of the raw peppers extracts was followed for fifteen minutes as it is presented in Figure 1.
The results of this assay showed for all pepper extracts a similar behavior for the kinetics inhibition with the absorbance of the reaction mixtures remained constant after about 5 minutes of reaction.

Considering the effect of the different techniques applied to the four varieties of peppers, it was generally verified that hot water bleaching did not significantly modify the antioxidant activity of peppers.

On the other hand, when microwave bleaching was applied the results showed that there was a tendency to decrease the antioxidant activity of the extracts with the exception of yellow peppers for which an increase of 22% was observed.

Applying the techniques of stewed and grilling, there is a clear effect of increasing the antioxidant capacity, especially in the case of red pepper extract which activity increased about 45% when compared with raw state.

Finally, it was observed that using freezing as a preservation method during 17 days of storage, all the peppers extracts showed a higher antioxidant activity.

In this study, orange pepper extract revealed the highest antioxidant activity in the raw state and after processing only exceeded by the others varieties of peppers when the grilled technique was applied.

Origin and variety of peppers, conditions of processing (temperature, time of application) and facility/difficulty to carry out the extractions are important factors which effects must be considered in the antioxidant capacity of these vegetables.

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To Jessica Mota, student from Escola Profissional de Setúbal, for the collaboration in the experimental work.

References
Bacterial degradation of the veterinary antibiotic florfenicol

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Florfenicol (FF) is an antibiotic largely used in aquaculture and its presence in their aeous streams can affect the treatment process. Bacteria can play an important role in the cleanup of contaminated sites due to their ability to degrade an impressive variety of pollutants, using them as carbon and energy source. This study aimed to investigate the degradation of FF by a single bacterial strain, Labrys portucalensis F11. Biodegradation of FF was assessed in batch mode in the presence of a conventional carbon source and the bacterium was capable to remove ca. 80% of the FF supplied. When acetate was periodically added, although the cell growth was improved, no effect on the uptake of FF was observed but its defluorination was greatly enhanced.

Introduction

Florfenicol (FF) is a synthetic phenicol antibiotic, widely used in veterinary medicine. It is one of the few approved antibiotics for use in aquaculture during both production and processing operations, mainly to prevent and treat bacterial diseases [1,2]. Therefore, this antibiotic is inevitably present in the effluents from fish farms and eventually directly discharged in surface waters. The accumulation of pharmaceuticals in natural environment due to its extensive use is causing an increasing concern due to potential adverse effects on human health and aquatic ecosystems.

Up to now, removal of FF has been mainly reported using physical-chemical processes [3–5] but its removal by biological processes is scarce. Through biodegradation microorganisms are able to transform or mineralize organic contaminants into less harmful, non-hazardous substances, which are then integrated into natural biogeochemical cycles [6]. Bioremediation has been considered the strategy of choice to reduce the risk of hazardous chemicals. Therefore, microorganisms able to deal with FF can contribute for the development and enhancement of bioremediation processes towards more efficient biological removal processes. The main aim of this study was to investigate FF degradation by a previously isolated bacterial strain, Labrys portucalensis F11, capable to degrade other pharmaceuticals. The effect of the periodic feeding with a conventional carbon source on the FF degradation by this bacterium was also investigated.

Methods

Florfenicol degradation. The degradation of FF was tested at a concentration of 10 µM with the addition of a supplementary carbon source, sodium acetate, at 5.9 mM. Assays with a periodic feeding with the same concentration of sodium acetate were also established. Cells of L. portucalensis F11 previously grown on NA plates were inoculated to an OD600 of ca. 0.1 into 250 mL flasks containing 75 mL of mineral medium supplemented with FF. Experiments were performed in triplicate under sterile conditions. Control assays without inoculation and controls inoculated with autoclaved (i.e. non-viable) L. portucalensis F11 cells were performed to evaluate abiotic degradation and adsorption. A control for cell growth was established with the same concentration of acetate but without FF addition. All suspensions were incubated for 28 days, at 25 °C, with constant shaking (120 rpm) and protected from light, to avoid FF photodegradation. Samples were collected twice a week to determine FF removal, fluoride release and cell density.

Analytical methods. FF removal was evaluated by HPLC-DAD [7]. The concentration of fluoride ion was measured with a fluoride electrode as previously described by Amorim et al. [8]. Growth was monitored spectrophotometrically by measuring the optical density at 600 nm.

Results

The ability of L. portucalensis F11 to degrade FF in the presence of an additional carbon source was evaluated. When acetate was added only at the beginning of the experiment, at the end of 28 days, strain F11 was able to remove about 80% of the FF initially supplied. Considering the reaction stoichiometry, the fluoride release corresponded to 40% of the total amount of consumed substrate. The growth pattern observed for cultures supplied solely with acetate was similar to the one for the cultures supplied simultaneously with acetate and FF. This result revealed that despite FF antibiotic properties, there was no evidence of toxic effects of FF on L. portucalensis F11 cells, whose growth was not affected at the supplied concentration. Moreover, FF degradation started from the beginning of the experiment, showing that the bacterium was able to degrade the pollutant without the need of any pre-induction. Periodic feeding (once and twice a week) with the same concentration of acetate was carried out to investigate if a higher cell mass would improve the biodegradation efficiency of the target compound. In the same period of time, approx. 80% of the initial FF was consumed. Although the presence of a conventional carbon source that could be easily metabolised by strain F11 improved cell growth, FF degradation was not enhanced. Nevertheless, a positive effect of acetate supplementation was observed for FF defluorination. The release of fluoride when acetate was supplied once and twice a week corresponded to 73 and 80% of the total amount of consumed substrate, respectively. The higher availability of a
second carbon source to supply energy to the cells may be a critical factor for biodegradation. The release of the halogen substituent is a critical step in the biodegradation of halogenated compounds. The positive effect of acetate periodic feeding on the dehalogenation indicates that the presence of the additional carbon source could have contributed to circumvent the enzymatic rate-limiting step of FF metabolism.

Conclusion
In this study, FF biodegradation by *L. portucalensis* F11 was observed in the presence of an additional carbon source. Periodic feeding with the additional carbon source did not improve the removal of FF but it had a positive effect on the defluorination of the target compound. An improvement of about 24 and 30% in the fluoride release extent in the cultures fed once and twice a week respectively, with acetate was observed as compared to the culture fed only at the beginning.

Acknowledgements
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References
Changes in the biochemical composition of selected Tetraselmis species, cultured semi-continuously under distinct renewal rates

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Several strains of Tetraselmis suecica (UW 605 and AROSA) and of Tetraselmis sp. that were characterized as lipogenic (RG 63, RG 75 and RG 74) and non-lipogenic (RG 44 and RG 46) through the flotation method were cultured semi-continuously using two different daily renewal rates, 10 % and 30 % of the volume of the cultures, in attempts to ascertain the changes in production rates of proteins, carbohydrates and lipids. As expected, an increase in renewal rate produced an increase in protein content of the biomass but a decrease in carbohydrate content in all strains. Lipid content decreased for RG 63 and RG 74 strains (lipogenic) with a renewal rate of 30 % and increased for RG 44 and RG 46 strains (non-lipogenic). Our results prove the influence of cultivation mode upon the biochemical composition of microalgae.

Introduction
Microalgae are efficient biological systems in terms of capture of solar energy to synthesize organic compounds. Their biomass contains several lipids, proteins and carbohydrates, with potential application in several sectors. In attempts to increase the market value of microalgae while reducing production costs, it is important to ascertain the full potential of their biomass constituents, and to develop a strategy for extraction [1]; furthermore the biomass biochemical composition can be manipulated by changing growth conditions [2] – so preferential synthesis of selected compounds is in principle possible [3].

Semi-continuous cultivation has been described as suitable for large-scale application [4,5]. Dilutions are made every 24 h; and no special equipment is required, while the resulting biomass exhibits a stable biochemical composition [5]. Periodic withdrawal of product and addition of substrates reduces the stagnant time for collection of biomass products and photobioreactor cleaning. Additionally, it allows higher productivities to be achieved as compared to batch cultures.

In the present work, results are presented on the biochemical variability generated through manipulation of renewal rates in semi-continuous cultures of several Tetraselmis species.

Experimental Methodologies
Two Tetraselmis suecica strains (UW 605 and AROSA) and five strains of Tetraselmis sp. isolated by Prof. Ralph Lewin and classified as lipogenic (RG 63, RG 75 and RG 74) and non-lipogenic (RG 46 and RG 44) according to the flotation characteristics of stationary-phase cells, were cultured in 80 mL test tubes, with filtered sea water (35 % salinity), sterilized and enriched with nutrients and vitamins, according to Fabregas et al. [6] with a final concentration of nitrogen in the culture medium of 8 mM. They were submitted to 12 h light: 12 h dark photoperiod, with a light intensity of 152 μmol photons m⁻² s⁻¹. Cells were inoculated at a density of 0.5 x 10⁶ cell. mL⁻¹, and subjected to aeration of 250 mL/min supplemented with CO₂, in order to keep the pH below 8 at a temperature of 20 °C. As soon as cultures reached the early stationary phase of growth, the semi-continuous growth regime was initiated with daily renewal of 10 % or 30 % of the culture medium, in the first hour of the light period. Renewal of the cultures was carried out with sterilized sea water enriched with nutrients at the same initial concentration. Cultures reached steady-state after 5-8 days, under the semi-continuous regime. And were maintained at steady state for 21 days. Cell density was monitored daily using a Neubauer haemacytometer. Biomass from early morning harvested cultures was collected by centrifugation. Fresh biomass was used for all biochemical analyses – lipids, proteins and carbohydrates. All analyses were carried out in triplicate. Carbohydrates were measured by the phenol-sulphuric acid method [7], lipids by the charring method [8] and proteins by the Folin-phenol method [9].

Results
A one-way analysis of variance (ANOVA); data normality previously tested with the Shapiro-Wilk test, P < 0.05 was applied to the presented results. The cell density achieved in the 80 ml culture units at early stationary phase was higher for Tetraselmis suecica AROSA and Tetraselmis sp. RG 74, with values of 2.44 x 10⁷ ± 5.69 x 10⁶ cell. mL⁻¹ and 2.08 x 10⁷ ± 1.04 x 10⁶ cell. mL⁻¹, respectively. From inspection of Figure 1, it is possible to see that steady-state cell density decreased from the stationary phase to the renewal rate 30 % for all strains (statistically significant difference for AROSA, RG 75 and RG 44 (p < 0.0001, p < 0.05 and p < 0.001 respectively), which is in accordance with previous researches [2] that reported steady-state cell density decreases with increase in the renewal rate. The only exceptions are RG 74 and RG 46. The differences between renewal rates found in these two strains
are not statistically significant. In the strains 605 and RG 63, a subtle increase in steady-state cell density was achieved (not statistically significant) for the renewal rate 10 % which is possible to happen due to the presence of a limiting nutrient or the accumulation of a self-limiting metabolite.

The increase in renewal rate of the culture medium favored production of proteins by the cells (Figure 2), as expected in view of results by Otero and Fábregas [10]. The observed differences between renewal rates are statistically significant, except for *Tetraselmis* sp. RG 74 strain. Maximal protein content was obtained by *Tetraselmis* sp. RG 46 strain, with 54 pg cell⁻¹.

Unlike to what happens with protein synthesis, the renewal rate of 30 % did not favor production of carbohydrates by the cells, as also found previously [10] – (Figure 3). The observed differences are statistically significant. Maximal carbohydrate content was obtained by *Tetraselmis* sp. RG 46 strain, with 2646 pg cell⁻¹.

Regarding lipid cell content obtained, the strains who showed a statistically significant difference between renewal rates (p < 0.0001) were RG 63 (lipogenic), RG 74 (lipogenic), RG 44 (non-lipogenic) and RG 46 (non-lipogenic). However in RG 63 and RG 74 strains, lipid cell content was slightly lower with a renewal rate of 30 % - the authors Fábregas *et al.* [2] also reported a decrease in lipids percentage as a result of the increase in nitrogen availability caused by increasing renewal rates with *Phaeodactylum tricornutum* microalga.

With RG 44 and RG 46 strains, the increase in the renewal rate favor lipids production from 12.9 to 17.2 pg. cell⁻¹ and 9.1 to 15.1 pg. cell⁻¹, respectively, which is in accordance with authors Otero and Fábregas [10], who showed that in a semi-continuous growth regime, an increase in the renewal rate increased lipid percent in *Tetraselmis suecica*.

**Conclusion**

Our results suggest that semi-continuous culturing provides an interesting experimental system for nutritional studies, since different biochemical compositions can be generated with the same microalgal species. Changes in operational variables has indeed proven a useful tool for manipulation of biochemical composition of microalgal cells.

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**References**

Effect of polysaccharides in neuronal ROS production

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Introduction
Over time, constant daily changes, especially with regard to eating habits, sleep quality and stress level, affect the way the body processes its metabolic activity. Among the various reactions that occur in aerobic beings is the production of free radicals, such as reactive oxygen species (ROS). This type of cellular damage is involved in many diseases such as cancers, neural disorders and premature aging.

The aim of this study was to evaluate the effect of polysaccharides extracted from the mushroom Tricholoma equestre, in the formation of neuronal ROS, using the fluorescent indicator H2DCFDA. The experiments were performed in brain slices, using a polysaccharide mixture at the concentrations of 0.1 g.L⁻¹, 0.5 g.L⁻¹ and 1 g.L⁻¹. Significant changes were observed only for the 1 g.L⁻¹ concentration, which indicated a decrease of about 5% in ROS production. The observed inhibition is in agreement with the idea that the used polysaccharides have an antioxidant action.

Material and Methods
For the preparation of the polysaccharides solutions, the solid fraction of the polysaccharides extracted from the Tricholoma equestre mushroom was added to the ACSF solution.

The solid extract was previously ground with the aid of a mortar in order to reduce particle size. This solid mixture, after being homogenized, was used to prepare solutions with three different concentrations: 0.1 g.L⁻¹, 0.5 g.L⁻¹ and 1 g.L⁻¹.

The neuronal studies, were made in hippocampal slices from the brain of Wistar pregnant rats (8 to 12 weeks old, with 16 to 18 days of gestation), that were sacrificed by cervical dislocation.

The slices, with a thickness of 400 µm, were kept in an artificial cerebrospinal fluid (ACSF) solution with the following composition (in mM): NaCl 124.0; KCl 3.5; NaHCO₃ 24.0; NaH₂PO₄ 1.25; MgCl₂ 2.0; CaCl₂ 2.0 and D-Glucose 10.0), pH 7.4, oxygenated with 95% O₂ and 5% CO₂. For ROS detection the slices were incubated for 1 h, in an ACSF medium containing 20 µM of the fluorescent ROS indicator H₂DCFDA. After the incubation, the slices were perfused at a rate of 1.5 to 1.8 mL/min with ACSF at 30-32 °C, in an experimental chamber mounted in a fluorescence microscope (Zeiss Axioskop) equipped with a water immersion objective (40x, N.A. 0.75, 1.6 mm working distance). The optical signals were recorded at the mossy fiber synapses from hippocampal CA3 area, using an halogen lamp (12V, 100 W), excitation and emission filters of 480 nm and >500 nm, respectively, and a photodiode (Hamamatsu, 1 mm²). The data were processed using a 16 bit data acquisition system and the Signal Express analysis software (National Instruments).

Results and Discussion
The effect of the mixture of polysaccharides (0.1 g.L⁻¹, 0.5 g.L⁻¹ and 1 g.L⁻¹) was evaluated in slices with the fluorescent ROS indicator H₂DCFDA. Figure 1 shows that during the first 10 min in the ACSF medium, which correspond to the baseline, the measured ROS signals, plotted at 1 min intervals, remained stable. When the slices became in contact with the 1 g.L⁻¹ polysaccharide solution, for 30 min, the ROS signals decreased by about 5% (n=2). Afterwards the ACSF solution was
reperfused leading to the recovery of the signals and their subsequent increase to a level above the baseline. These results suggest that in the presence of the polysaccharide solution there is a lower production of ROS, which was expected since the polysaccharides capture ROS due to its antioxidant properties.

![Figure 1](image_url)

Figure 1. Neuronal ROS signals for the solution containing 1 g.L\(^{-1}\) of the polysaccharides mixture. The bar represents the time during which this solution was perfused. The average of the first 10 responses was used to normalize all data points which are represented as the mean±SEM (n=2).

The ROS signals detected for the lower concentrations of the polysaccharides mixture, 0.1 g.L\(^{-1}\) and 0.5 g.L\(^{-1}\), showed no clear changes during the 30 min application period, suggesting that the concentrations used were too low to affect significantly ROS production.

**Conclusions**

The decay of the ROS signals for the 1 g.L\(^{-1}\) polysaccharide solution indicates that this solution has a negative effect on the formation of these reactive species and that the polysaccharides lower the amount of these radicals. However, for the concentrations of 0.1 g.L\(^{-1}\) and 0.5 g.L\(^{-1}\) of the polysaccharide mixture, no significant ROS changes were observed.

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**References**

O mundo da CUF sempre foi composto de mudança. Constantes foram sempre as ligações a Portugal, às pessoas e ao futuro. Hoje, os desafios internacionais levam-nos a assumir uma nova marca: Bondalti. Reforçando a nossa herança e uma forma de estar onde a eficiência e a criação de valor se conjugam com a sustentabilidade e os valores humanos.

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Poster Session

BIOREFINERY AND SUSTAINABILITY
Direct transformation of cellulose to ethylene glycol using Ru-W bimetallic catalysts supported on glucose-derived carbon materials

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The catalytic conversion of lignocellulosic biomass to valuable chemicals has attracted global interest. In this work, Ru-W bimetallic catalysts supported on glucose-based carbon materials were synthesized to perform the hydrolytic hydrogenation of cellulose to ethylene glycol (EG) in a single step. The catalysts were examined for the one-pot reaction and have shown to be efficient for the direct production of EG from cellulose. Conversions of cellulose of 100% were achieved in just 2 h of reaction and yields of ethylene glycol up to 35% were obtained after 5 h. The prepared materials are promising alternatives to carbon nanotubes and other materials as catalyst supports for the production of EG directly from cellulose in a more economic, sustainable, faster and easier way.

To help with issues of global environmental problems and diminishing fossil fuel reserves, lignocellulosic biomass is attracting attention as raw-material for the production of green chemicals [1,2]. Lignocellulosic biomass is mainly composed of cellulose, hemicelluloses and lignin, cellulose being the main component (40-50 %). One of the most interesting routes for cellulose valorization is its one-pot hydrolytic hydrogenation into valuable chemicals [3]. Among them, ethylene glycol (EG) is one of the most highlighted target molecules, since it can be used as antifreeze or coolant liquid in vehicles, or as precursor and coating agent in the plastics and food industries such as for polyester fibers and polyethylene terephthalate (PET) [2,4]. However, the major challenge to directly convert cellulose to EG is the establishment of a highly efficient and selective catalytic system. Recently, many research groups have reported several efficient systems. For example, in 2008 the direct conversion of cellulose to EG was performed using a Ni-W/CAC catalyst with 61 % yield of EG after 30 min at 245 ºC and 60 bar of H2 [5]. Later, the EG yield reached up to 76.1 % using Ni-W/SBA-15 catalysts under the same conditions [6]. Since then, many other bi-functional catalysts have been studied and it has been established that the one-pot conversion of cellulose to EG proceeds through hydrolysis, retro-aldol condensation and hydrogenation [1,2,7]. Besides the presence of one or more active sites, understanding the properties of the support materials is fundamental to design the strategy of modifying the surface and to study the interaction between the supports and the metals in the developed catalytic systems. The main goal of this work was the development of an efficient and sustainable process for the direct transformation of cellulose into EG using Ru-W bimetallic catalysts supported on glucose-derived carbon materials. The effect of the preparation method on the final properties of the catalysts was evaluated for cellulose direct conversion to EG.

Two different carbon supports were prepared by hydrothermal treatment: i) a glucose-derived carbon support (CG) and ii) a glucose-derived carbon/carbon nanotube hybrid (CG-CNT). Tungsten monometallic catalysts were then prepared by incipient wetness impregnation of the carbon supports. W was added in three different stages of the preparation process of the supports, resulting in the following catalysts: i) W/CG and W/CG-CNT from W addition after carbonization of the carbon supports; ii) W/CGorg and W/CG-CNTorg from W addition prior to the thermal treatment and iii) W/CGsys and W/CG-CNTsys from W introduction prior to the hydrothermal treatment. Then, Ru-W bimetallic catalysts were prepared via incipient wetness impregnation of the Ru precursor on the already prepared W monometallic catalysts (previously heat treated and reduced at 700 ºC). After impregnation, the Ru-W catalysts were heat treated and reduced at 250 ºC and denoted as Ru-W/CG; Ru-W/CG-CNT, Ru-W/CGorg, Ru-W/CG-CNTorg, Ru-W/CGsys and Ru-W/CG-CNTsys. The amount of the metal precursors was calculated to achieve a metal loading of 0.4 wt.% Ru and 30 wt.% W. Supports and catalysts were characterized by nitrogen adsorption at -196 ºC, thermogravimetric analysis, scanning electron microscopy, temperature programmed techniques and X-ray photoelectron spectroscopy. The conversion of cellulose into ethylene glycol was tested by one-pot reaction. In standard tests, 300 mL of water, 750 mg of ball-milled cellulose (4 h at 20 s-1) and 300 mg of catalyst were introduced to a 1000 mL Parr reactor. After heating to 205 ºC under N2, the reaction was initiated by switching to H2 (50 bar). The conversion of cellulose was determined by TOC and the products distribution by HPLC.

The textural properties of the supports and the metal catalysts were determined by N2 adsorption isotherms. The CG support presented a type I isotherm, characteristic of microporous materials. The volume of N2 adsorbed at low relative pressures decreased by adding CNT, resulting in a material with lower microporosity. Also, CG-CNT samples exhibited a hysteresis loop, which indicates the presence of mesopores. The catalysts presented lower surface areas than their corresponding supports (Table 1) according to the nominal loading of Ru and W. However, samples Ru-W/CGsys and Ru-W/CG-CNTsys showed higher volumes of N2 adsorbed at low relative pressures than the catalysts prepared by incipient impregnation method (Ru-W/CG, Ru-W/CG-CNT, Ru-W/CGorg and Ru-W/CG-CNTorg). Such result could be explained by a lower tungsten content of these materials since after the hydrothermal treatment they are washed to remove unreacted products and part of W can be leached. This observation is confirmed by thermogravimetric analyses (Table 1) that showed a lower percentage of ashes for these two catalysts.

The prepared Ru-W bimetallic catalysts supported on glucose-derived carbon materials were tested for the one-pot conversion of cellulose to EG and, in general, the catalysts were highly...
efficient, with 100 % cellulose conversion after just 2 h of reaction. When the catalysts were prepared by W deposition in organic or carbonized glucose, the presence of CNT did not favor the production of EG (Figure 1). However, when W was added during the hydrothermal process, the presence of CNT resulted in an increased EG yield from 18.9 to 29.7 % after 5 h. Furthermore, and independently of the presence of CNT, although no significant difference was observed on the porous properties of the materials prepared by W deposition in organic or carbonized glucose, CG supported catalysts led to higher yields of EG than CGorg supported catalysts (Figure 1).

To conclude, the catalyst prepared on the carbonized glucose (Ru-W/CG) was the most efficient for the production of EG, with a yield close to 35 % after 5 h of reaction, which is the same that was previously obtained using Ru/CNT [7,8]. These results indicate that carbon nanotubes can be successfully replaced by cheaper supports derived from biomass.

![Figure 1. Yields of EG obtained after 5 h.](image)

Table 1. Characterization results of the supports and catalysts.

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<th>V\text{\text{\textsuperscript{p}}} (cm\textsuperscript{3} g\textsuperscript{-1})</th>
<th>Volatile matter (%)</th>
<th>C\text{\text{\textsubscript{load}}} (%)</th>
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References
Tunable hydrophobic eutectic solvents based on terpenes and monocarboxylic acids


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Introduction

Nowadays, developments in engineering and technology are strongly influenced by the concepts of green chemistry and sustainability. Within this framework, there is a demand for new eco-friendly solvents able to dissolve a large spectrum of solutes. Currently, one of the most important focus of research for novel solvents are eutectic mixtures, and particularly the so-called deep eutectic solvents (DES). Most of the DES proposed so far were prepared through the combination of materials from renewable resources with nontoxic and biodegradable compounds such as carboxylic acids, polyols, and sugars; being the vast majority hydrophilic. To the best of our knowledge, only a limited number of works reported hydrophobic eutectic mixtures. However, in these studies, the solid-liquid equilibria (SLE) phase diagrams were not characterized despite the relevant information that they can provide on the range of composition and temperature for operating these systems. Moreover, a complete physicochemical characterization of their properties is also poor.

Due to their very low solubility in water and relatively low price terpenes appeared as good candidates to prepare sustainable and cheap hydrophobic solvents. Menthol and thymol are monoterpenoids used in various industrial processes and commercial products, and the use of their eutectic mixtures has been investigated.

Thus, the main goal of this work was to prepare and characterize eutectic mixtures composed by the terpenes L(−)-menthol or thymol and the monocarboxylic acids: caprylic, capric, lauric, myristic, palmitic and stearic acids. Solid-liquid phase diagrams of these mixtures were measured in the whole composition range, through differential scanning calorimetry and modelled by the Perturbed Chain-Statistical Associating Fluid Theory (PC-SAFT) equation of state. Moreover, the densities, viscosities, mutual water solubilities and solvatocromatic parameters for these systems were measured at compositions close to the eutectic point.

Recently some works claim that hydrophobic deep eutectic solvents could be prepared based on menthol and monocarboxylic acids. Despite of some promising potential applications these systems were poorly understood and this work addresses this issue. Here the characterization of eutectic solvents composed by the terpenes thymol or L(−)-menthol and monocarboxylic acids is studied aiming the design of these solvents. Their SLE phase diagrams were measured by differential scanning calorimetry in the whole composition range, showing that a broader composition range, and not only fixed stoichiometric proportions can be used as solvents at low temperatures. Moreover, the densities, viscosities, solvatocromatic parameters and mutual solubilities with water were measured at compositions close to the eutectic point. The mutual solubilities with water attest the hydrophobic character of the mixtures investigated. The experimental SLE phase diagrams were described using the PC-SAFT EoS.

Results and Conclusions

The SLE phase diagrams of the investigated mixtures were measured in the whole composition range using DSC, and showed a broader composition range in the liquid state, at room temperature than previously admitted. Generally the systems exhibited small deviations from ideality and a eutectic point close to that predicted assuming ideality. Therefore, although often labeled as DES, these systems do not present negative deviations large enough to induce a significant melting point depression. However, it must be stressed that room temperature solvents can be obtained for many of these mixtures on a wide composition range and not fixed to any particular stoichiometric relationship between the hydrogen bond donor and acceptor, even at the eutectic point, what reinforces the tunable character of the liquid phase region of these mixtures. The experimental solid-liquid phase diagrams were successfully described using the PC-SAFT EoS, which provided reliable estimates of the eutectic points and of the solvents densities. This EoS also showed that liquid phases are quasi-ideal. The eutectic mixtures present densities lower than water and low viscosities (1.3 – 50.6 mPa-s) and in general eutectic mixtures containing thymol were less viscous but more dense than those with L(−)-menthol. A series of solvatocromatic parameters were measured in order to address the polarity of the mixtures investigated. All the parameters are strongly influenced by the terpene used and in some cases vary with the alkyln chain length of the monocarboxylic acid. Mixtures involving thymol present a higher hydrogen-bond acidity character, as well as higher nonspecific interactions. L(−)-menthol presents a higher hydrogen-bond basicity character and a slight increase of this parameter with the increase of the alkyln chain of the monocarboxylic acid. Moreover, the mixtures reported here displays a high capacity to donate (thymol-based mixtures) and accept (L(−)-menthol based mixtures) protons when compared to some organic molecular solvents and very close to water. The polarity dependence on the alkyln chain length of the monocarboxylic acid favors the design of new solvents. The measured mutual solubilities with...
water prove the hydrophobic character of the mixtures investigated. The systems investigated were mixed with water in the presence of dyes. Results are presented in the Graphical Abstract that shows a separation between the organic and aqueous phases. Rhodamine 6G, presenting a non-polar character seems to be completely extracted into the hydrophobic organic phase (pink-dyed phase), terpene + monocarboxylic acid. By the other hand the Brilliant Blue FCF (E133) migrates into the water phase (blue-dyed phase).

Acknowledgements
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Pre-treatment of maize cob waste with hydrogen peroxide for biogas enhancement

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The enhancement of biogas and CH4 yields through anaerobic co-digestion (co-AD) of the pre-hydrolysed Organic Fraction of Municipal Solid Waste (hOFMSW) and Maize Cob Waste (MCW) was tested in a lab-scale thermophilic anaerobic reactor. This enhancement is relevant if the biogas has to be upgraded to biomethane. Before the co-AD, MCW was chemically pre-treated with H2O2 at room temperature and optimized conditions of MCW percentage and reaction time. The co-AD of hOFMSW with MCW, pre-treated with 10% MCW wMCW/alkaline water for 4h of contact time and a wH2O2/wMCW ratio of 0.5, increased the biogas and CH4 production yields by 65% and 48%, respectively, when compared to the results obtained with the standalone hOFMSW. Moreover, during this co-AD assay, the biogas stream provided a Low Heating Value (LHV) and a daily biogas energy content of 4% and 1% higher, respectively, than those obtained with co-AD of hOFMSW with non-pre-treated MCW.

Introduction
The Organic Fraction of Municipal Solid Wastes (OFMSW) and Maize Cob Wastes (MCW) are residues suitable for Anaerobic Digestion (AD). The OFMSW is characterised by a low C:N ratio due to its high protein content. Maize Cob is a carbon rich biomass, with relatively low biodegradability, and its harvesting from crop fields may enhance crop yields. MCW is a lignocellulosic biomass mainly composed of cellulose, hemicellulose and lignin. Only cellulose and hemicellulose are fermentable by bacteria after hydrolysis; thus, a previous pre-treatment makes these compounds more accessible for biological degradation and allows some lignin solubilisation before AD. The pre-treatment conditions have to be accurately chosen, because severe conditions may promote metabolic and physiologic inhibitions of AD bacteria, due to the formation of phenolic compounds or furfural and 5-hydroxymethylfurfurals (5-HMF) [1]. This work aims to assess the Anaerobic co-Digestion (co-AD) efficiency of a hydrolysed sample of OFMSW (hOFMSW) with MCW pre-treated in the presence of hydrogen peroxide (H2O2), at room temperature and optimized conditions of MCW percentage and reaction time. This work contributes to the development of a new approach to valorise MCW, that is currently exploited below its potential, and enhance the methane (CH4) yield in the biogas produced.

Material and Methods
The hOFMSW was collected in the outflow of the hydrolysis tank of an industrial Portuguese AD plant, located in Lisbon area. This waste is produced through the acidogenic bacterial hydrolysis of OFMSW generated in canteens, restaurants and malls. MCW was collected from a local Portuguese farmer located in Coruche (Lisbon surroundings).

In order to remove lignin and solubilise part of the hemicellulose and cellulose into digestible sugars and trying to reduce as much as possible the formation of AD inhibitors, MCW was submitted to chemical pre-treatments at 23 °C in the presence of H2O2, at a wH2O2/wMCW ratio of 0.5, pH of 9.8, with 10% wMCW/alkaline water, and 4 hours reaction time (Pre3). The results obtained with this pre-treatment were compared to the results obtained with pre-treatments Pre1 and Pre2 (Table 1), previously tested by the authors [2]. MCW pre-treated under Pre1, Pre2 and Pre3, as well as non-pre-treated MCW were then submitted to co-AD with hOFMSW in a lab scale Stirred Tank Reactor (STR). The bioreactor was inoculated with a thermophilic anaerobic sludge obtained in the same industrial AD plant as for hOFMSW. The co-AD was performed under thermophilic conditions (50 ± 2°C). The bioreactor was operated with an Organic Load Rate (OLR) of 2.48 g VS/L.d, a Hydraulic Retention Time (HRT) of 21 days and fed through a semi-continuous regime every two days. The AD with hOFMSW alone was tested as a control assay.

Results and Discussion
After pre-treatment Pre2, the solubilization of cellulose, lignin and hemicellulose was always higher than 60% w/w. In Pre1 and Pre3, lignin, hemicellulose and cellulose removals did not exceed 5.0% w/w (Pre3), 5.6% w/w (Pre3) and 11.3% w/w (Pre1), respectively. The increase of MCW concentration from 2% wMCW/alkaline water (Pre1) to 10% wMCW/alkaline water (Pre3), keeping constant the reaction time (4 h), allowed only slight lignin solubilisation, ranging from 0% w/w in Pre1 to 5% w/w in Pre3. During Pre2 and Pre3, glucose and fructose solubilizations were on average 4 times higher than in Pre1. Regarding the inhibitors formation, in all the samples tested neither furfural nor 5-HMF were detected (DL furfural: <0.1 mg/L; DL 5-HMF: <0.5 mg/L). The concentrations of phenolic compounds were always below those considered critical for the AD process, according to the available literature [3]. To note that Pre2 (72 h reaction time) generated concentrations of p-coumaric acid and ferulic acid of 50% and 325% higher, respectively, than those obtained with Pre3. The C:N ratio in the influent increased with co-AD from 9.46 in the AD assay with hOFMSW to 9.66, 12.53, 21.21 and 12.57 in the co-AD assays with hOFMSW + Pre1, hOFMSW + Pre2, hOFMSW + Pre3 and with hOFMSW + MCW, respectively. The C:N ratio observed in the co-AD hOFMSW + Pre3 is quite close to the theoretical optimal value of 25 [4]. The increase of C:N ratio during the co-AD is reflected in the increase of biogas and methane yields, confirming that MCW, if properly pre-treated, can balance the C demand for an enhanced co-AD (Table 2). The CH4 content in biogas streams did not show any significant difference (ANOVA, p<0.05) during the AD of
hOFMSW alone and co-AD of hOFMSW + Pre1 and OFMSW + Pre2, with an average value of 66.5% v/v (Table 2). A slight decrease in CH₄ content was observed in the biogas streams obtained during co-AD of hOFMSW + Pre3 (63.1% v/v) and hOFMSW + MCW (60.1% v/v). During these two latter assays, it was observed the most favourable average biogas productions (4830 mL/d and 5017 mL/d, respectively).

Table 2. CH₄ and CO₂ contents (% v/v) during Anaerobic Digestion and co-Anaerobic Digestion experiments (STP).

<table>
<thead>
<tr>
<th>Assay</th>
<th>CH₄ (%)</th>
<th>CO₂ (%)</th>
<th>Biogas (mL/d)</th>
<th>LHV (MJ/m³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>hOFMSW</td>
<td>66.1</td>
<td>32.4</td>
<td>2389</td>
<td>22.4</td>
</tr>
<tr>
<td>hOFMSW+Pre1</td>
<td>66.5</td>
<td>32.6</td>
<td>3787</td>
<td>22.5</td>
</tr>
<tr>
<td>hOFMSW+Pre2</td>
<td>66.8</td>
<td>32.2</td>
<td>3941</td>
<td>22.6</td>
</tr>
<tr>
<td>hOFMSW+Pre3</td>
<td>63.0</td>
<td>36.9</td>
<td>4830</td>
<td>21.3</td>
</tr>
<tr>
<td>hOFMSW+MCW</td>
<td>60.3</td>
<td>39.6</td>
<td>5017</td>
<td>20.4</td>
</tr>
</tbody>
</table>

The co-digestion of OFMSW + Pre3 increased biogas and methane yields by 65 % and by 48 %, and the co-digestion of hOFMSW with non-pre-treated MCW by 84 % and 57 %, respectively, when compared to the hOFMSW alone (Figure 1).

Figure 1. Biogas and methane yields during Anaerobic Digestion and co-Anaerobic Digestion experiments.

Despite the highest yield and similar biogas volume produced during these two co-digestion assays, the biogas stream obtained during co-AD of OFMSW + MCW has a Low Heating Value (LHV) on average 4 % lower than the LHV obtained in the co-AD of OFMSW + Pre3. Moreover, the Daily Energy Content (LHV×Volume of biogas per day) of the biogas obtained in the co-AD of hOFMSW with non-pre-treated MCW was 1% lower than that obtained with co-AD of hOFMSW + Pre3.

From the viewpoint of process stability, during the co-AD of hOFMSW + MCW, which produced the highest biogas and methane yields, the NH₄-N concentration (2544 mg/L) slightly exceeded the threshold-limit for AD (2500 mg/L) [5], which can cause ammonia accumulation and inhibition of acetotrophic methanogens. During this assay, the concentration of total Volatile Fatty Acids (tVFAs) (2512 mg/L) was closer to the threshold considered critical for AD (4000 mg/L) [6] and higher than for co-AD with hOFMSW + Pre3 (2205 mg/L).

Conclusions
The results showed that the chemical pre-treatment catalyzed by H₂O₂, at pH 9.8, at room temperature and 4 h reaction time (Pre3) is a promising and low energy demanding pre-treatment applicable to MCW to allow its co-AD with hOFMSW. Pre3 is characterized by the best compromise in terms of lignin, cellulose and hemicellulose solubilisation, high concentration of carbohydrates and low production of inhibitors, when compared to Pre1 and Pre2. The co-AD of Pre3 with hOFMSW enhanced biogas and methane yields by 65% and 48%, respectively if compared with AD of standalone hOFMSW, as well as it provided the highest biogas Daily Energy Content when compared with the other co-AD assays performed. Moreover, the co-AD of hOFMSW + MCW, characterised by the highest biogas and methane yields, is affected by some process instability due to N-NH₄ and tVFA accumulation. In summary, a pre-treatment is recommended before submitting MCW to AD and the co-digestion of hOFMSW with pre-treated MCW with H₂O₂ allows a significant enhancement of biogas and methane yields. This is relevant if one intends to further use this enhanced biogas to upgrade it to biomethane.

Table 1. Pre-treatment conditions applied to Maize Cob Waste before co-Anaerobic Digestion with hOFMSW.

<table>
<thead>
<tr>
<th>Pre-Treatments</th>
<th>Optimized Conditions</th>
<th>Catalyst (w/mcwalk)</th>
<th>T (°C)</th>
<th>Reaction Time (h)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre1</td>
<td>0.5 H₂O₂/MCW (w/w)</td>
<td>2 %</td>
<td>23</td>
<td>4</td>
<td>Surra et al. (2017)</td>
</tr>
<tr>
<td>Pre2</td>
<td>0.5 H₂O₂/MCW (w/w)</td>
<td>ratio at pH 9.8</td>
<td>10 %</td>
<td>23</td>
<td>72</td>
</tr>
<tr>
<td>Pre3</td>
<td>0.5 H₂O₂/MCW (w/w)</td>
<td>ratio at pH 9.8</td>
<td>10 %</td>
<td>23</td>
<td>This work</td>
</tr>
</tbody>
</table>

Acknowledgments
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References
Bacterial cellulose production through hydrolysates produced with cellulosic residues

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In the paper industry, significant fraction of fibers that cannot be re-utilized are wasted by the paper companies, which raise economic and environmental concerns [1]. Additionally, aggressive methods to retrieve the cellulose are used for papermaking, such as acid hydrolysis. Therefore, alternative materials were being studied in the last decade[2,3].

An increasing demand of bacterial cellulose (BC) has been noticed in the last years. Bacterial cellulose (BC) is a known polymer produced by Acetobacteraceae family, which consist of a wide group of strictly aerobic, gram negative bacteria[4]. Among several genera of the Acetobacteraceae family, aceticobacter and komagataebacter genus are known have high yields of BC production. The interest in producing bacterial cellulose is due to the advantages that this product offers among the unique characteristics such as high porosity, high water retention capacity, low density, biocompatibility, non-toxicity and biodegradability, which promotes a variety of applications in the food industry as food additive, in paper industry as coating material and in the biomedical industry as regenerative material[2]. However, the production of BC still presents challenges, as high production costs[5]. Therefore, the use of low-value waste is an alternative that may lower the cost of producing BC and at same time, take advantage of using by-products of the papermaking industry[6]. In order to ally, the recycling of lignocellulosic residues and the production of bacterial cellulose through lignocellulosic residues has been studied. Recycled paper sludge from RENOVA and rejected fibers from EUROPAC were kindly supplied for this study. Recycled paper sludge (RPS) is a residue originated from the paper recycling process, more specifically, from the treatment of the liquid effluents generated in that process. It is mostly composed of small fibers with approximately 40% of carbohydrates that cannot be incorporated on recycled paper [7]. Rejected fibers (EUR) is a residue originated from the paper pulp production. Therefore, the goal is to obtain interesting yields of BC from this lignocellulosic residues, which could be difficult due to contaminants present in the residues and the availability of the cellulose in the residue.

To achieve the goals established above, RPS and EUR residues were hydrolyzed enzymatically (with Celliec Ctec 2; Novozymes) in order to obtain the hydrolysate for bacterial cellulose production [7]. Along with enzymatic hydrolysis, acid hydrolysis were also performed in order characterize each residue in terms of cellulose and hemicellulose present in the residues [4]. The concentration of reducing sugars retrieved through the saccharification process were 54 g/L (yield recovery sugars of 69,58 %) and 24 g/L (yield recovery sugars of 42,30%) for RPS and EUR respectively.

Table 1: Characterization of RPS and EUR

<table>
<thead>
<tr>
<th>Sample</th>
<th>RPS</th>
<th>EUR</th>
</tr>
</thead>
<tbody>
<tr>
<td>glucans (%)*</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Xylans (%)*</td>
<td>16,11</td>
<td>17,8</td>
</tr>
<tr>
<td>Reducing sugars (g/L)**</td>
<td>54,24</td>
<td>24,29</td>
</tr>
<tr>
<td>yield recovery sugars (%%)**</td>
<td>69,58</td>
<td>42,30</td>
</tr>
</tbody>
</table>

*Ac*Acid hydrolysis characterization
**E**Enzymatic hydrolysis results

After hydrolysate production, an assay of BC production through static fermentation for 9 days at 30 ºC was performed with the goal of optimizing the yield of BC by testing two different strains (Gluconacetobacter hansenii ATCC 53582 and Glucanacetobacter xylinus ATCC 700178) and nitrogen sources (combination of yeast extract/peptone and corn steep liquor (CSL)) on the hydrolysates previously prepared [8]. For the strain G. hansenii, the highest yield (6 g/L) was obtained with the standard medium (Hestrin & Schramm medium). However, RPS hydrolysate showed to be an interesting alternative carbon source for G. hansenii since the yield of BC with RPS was around 5 g/L. For the G. xylinum strain, EUR hydrolysate (4-5 g/L) is shown as an interesting alternative to HS medium (2-3g/L). Overall, the results suggest that RPS and EUR residues have potential to be alternatives of carbon source for BC production, after a further optimization of the BC production and the enzymatic hydrolysis.
Acknowledgements
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References
Assessment of agroforestry residues potential within the biorefinery context

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AGROFORESTRY RESIDUES
PB PS
TW WW

The interest in converting biomass resources into energy and value-added bioproducts is increasing in modern societies. In this way, the aim of this work is to evaluate the potential of four Portuguese agroforestry residues within the biorefinery context: pine branches and stumps, winery and tomato industrial wastes. Volatiles and other extractable fractions with biological properties, such as repellent/attractant, antioxidant activity and flavonoid content were obtained, being these properties relevant for food, pharmaceutical, and agroforestry industries. The potential for biogas production was assessed and encouraging results were obtained. Stumps potential for bioethanol production was evaluated by their characterization in terms of holocellulose (cellulose and hemicellulose) content, while the potential for bio-oil production was estimated by solvolysis process. All residues are promising for a bio-based economy.

Introduction
In the context of biorefineries and sustainable economy, the potential of several agroforestry residues for the production of value-added bioproducts has been performed in an ongoing multidisciplinary research project [1]. The biorefinery concept is nowadays a recommended strategy to convert biomass resources into energy, biofuel and marketable biochemicals. A number of biomass resources has been explored, including wood, grasses, corn, among others [2,3]. In Portugal, the potential and technologies related to the use of biomass resources have been mainly focused on the production of bioenergy [4]. In this context, it is important to identify and to characterize readily available residues, as well as to assess their potential to produce other unexplored commodities.

Objectives
The aim of this work was (i) to characterize four residues obtained from the Portuguese agroforestry industry: pine branches (PB) and stump (PS), tomato waste (TW) and winery waste (WW), and (ii) to evaluate their potential within the biorefinery context. The production of bio-oil, bioethanol, biogas, and byproducts for food/pharmaceutical/agroforestry industries are proposed in this work.

Methods
PB and PS from P. pinaster were milled (< 2 mm for PB and 0.210 – 0.841 μm for PS). PS samples were air dried and analysed following extraction, and acid hydrolysis. WW was obtained from wine distillation, and frozen until analysis. TW (S. lycopersicum) was obtained on a local store in a semi-rotten state, minced mechanically and frozen. For some analyses, the agro-wastes were freeze-dried (Figure 1).

The characterization was performed according to the nature of each raw material and included the parameters such as: moisture content (thermogravimetric analysis); elemental composition (C, H, O, N); volatile fraction (extracted by solid-phase microextraction, SPME, and analyzed by GC-MS); other extractable fractions (solid-liquid extractions using water); natural pH (in water suspension); total solids (TS) and volatile solids (VS); chemical oxygen demand (COD); total lignin and sugar (HPLC).

Extractable fractions were also characterized by spectrophotometry (UV-Vis) in terms of antioxidant activity (DPPH method) and flavonoid content.

Results
Moisture content was close to 50% for PB and 10 % for PS samples, while both agro-wastes (WW and TW) exhibited higher values, around 95%. The elemental analysis results are included in Figure 2 and revealed ~50% C for all the residues, ~40% O for PB, WW and TW, while PS had the highest O content (47%). Values between 6 and 8% were found for H and low amounts of N were present in all residues (0-3%).

Figure 1. PB and PS after milling, and TW and WW after freeze drying.

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Emitted volatiles from agroforestry residues were analyzed in order to find compounds that can be used as insect control strategies (e.g., bioinsecticides, attractants and/or repellents). PB volatile fraction was mainly composed of terpenes such as α-pinene, β-pinene, β-myrcene, and β-caryophyllene, which is in accordance with compounds already found by other authors for P. pinaster needles and branches. For PS, the α-pinene was the predominant volatile, followed by β-caryophyllene and longifolene. Note that α-pinene has already been reported as an attractant for some insects [5]. For the WW, the volatile fraction included compounds such as benzeneethanol, limonene and ethanol.

Regarding the anaerobic digestion context, the natural pH was measured, and took values close to 4 (PB, WW, TW). The percentage of TS and VS (expressed as %TS) were also analyzed and the results are shown in Figure 3. All of these substrates contain VS higher than 65%. Total COD was also determined, and all residues had values in the range considered as adequate for anaerobic digestion (2500 mg O₂ gVS⁻¹ for agro-wastes and 1300 mg O₂ gVS⁻¹ for PB) [6]. Indeed, experimental biochemical methane potential (BMP) measured to TW, for example, was close to 300 mL CH₄ gVS⁻¹. Therefore, these residues showed potential for biogas production.

To evaluate the suitability of these agroforestry residues for bio-oil/biofuels production, the content in lignin and holocellulose was determined [7]. Acid-soluble and -insoluble lignin was c.a. 3 and 27% for scCO₂ pre-extracted PB, whilst carbohydrate fraction was around 53%. For PS, soluble and insoluble lignin was c.a. 2 and 28%, respectively and neutral sugars took values of 54%, which could theoretically yield 0.30 g of ethanol per gram of dry PS, strongly depending on the pre-treatment applied to obtain cellulose and hemicelluloses from PS. Preliminary saccharification and fermentation tests on pre-treated PS yielded up to 0.20 g of ethanol per gram of PS. Solvolyis process was effective in the production of a bio-oil from PS since mass yields higher than 97% after 2.5 h of reaction at 160°C were achieved. Derived bio-oil constituents (identified by GC/MS) could be distributed by several chemical families, such as alcohols, acids, esters, ketones, phenols and their derivatives.

Acknowledgements

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References

One-pot conversion of furfural to bioproducts over mesoporous bimetallic catalysts

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The renewable platform chemical furfural is produced industrially, and used as solvent or intermediate for synthesizing various bioproducts with different applications in the chemical industry. The present work focusses on the production of the bioproducts 2-(alkoxyethyl)furans, 2-methylfuran, 4-oxopentanal and its acetal in one-pot from Fur. For such an achievement, bimetallic Pd-Ni TUD-1 mesoporous catalysts were used. These multifunctional catalysts allowed the in situ hydrogen supply from formic acid, responsible for the reduction steps involved. Different procedures were applied for the synthesis of the materials, and the influence of the materials' properties on the catalytic performances was studied. Detailed characterization and catalytic studies (with the identification of reaction intermediates) led to insights into the reaction mechanism, the roles of the different types of metal species on the complex reaction mechanism, and the catalyst stability.

Introduction

Plant biomass is an important renewable carbon source for producing bio-based products, which may complement or substitute fossil fuel-derived chemicals. Specifically, furfural (Fur) is obtained from carbohydrates (the largest component of vegetable biomass) through a series of acid-catalyzed hydrolysis and dehydration reactions. Fur valorization is highly desirable for a biobased economy. One of the most important steps of Fur conversion is its reduction to furfuryl alcohol (FA), with applications in the foundry, polymer and drug industries. The conversion of Fur to the bioproducts (bioPs) 2-(alkoxyethyl)furans (AoFs), 2-methylfuran (2MF) and 4-oxopentanal (OP) is complex and involves acid and reduction reactions (Scheme 1). The reduction pathways require the use of a reducing agent. In this work, formic acid (FAc) is used as an attractive H-source with advantages over the use of high pressure H2, such as safe transport and storage, relatively low toxicity and cost, and, on the other hand, FAc is a co-product of many carbohydrate biomass conversion processes and thus its repurposing is highly desirable. The production of bioPs and the in situ hydrogen supply from FAc requires the use of appropriate multifunctional heterogeneous catalysts. Bimetallic mesoporous catalysts of the type Pd-Ni TUD-1 were used in this work; the palladium and nickel species played roles in the reduction and acid reactions, respectively [1].

Objectives

The main objective was to find a suitable multifunctional heterogeneous catalyst to enable both acid and reduction steps involved in the conversion of Fur to bioPs, and to use a cheap, safe H-source able to produce hydrogen in situ, instead of using high-pressure H2.

Experimental

Pd-Ni TUD-1 materials with molar ratios Si/(Ni+Pd) in the range 9-34 were prepared from aqueous suspensions of a palladium precursor and silicates NiTUD(x) (the latter were previously prepared by hydrothermal synthesis), via incipient wetness impregnation with solvent evaporation (iw) or filtration and washing procedures (fw), as described in ref. [1].

Characterization of the materials included powder X-ray diffraction (PXRD) for determination of crystallite sizes, scanning transmission electron microscopy (STEM) for morphology and elemental mappings (Si, Ni and Pd), inductively coupled plasma atomic emission spectroscopy (ICP-AES) for quantification of Si, Ni and Pd; N2 adsorption-desorption isotherms for the textural properties, temperature-programmed reduction with hydrogen (TPR-H2) for reduction properties, and acid properties measurement by FT-IR of adsorbed pyridine.

Catalytic tests were performed in batch mode using Fur as substrate, FAc as hydrogen source, and ethanol (EtOH) or 1-butanol (BuOH). Quantification of the products was based on GC analysis, while the quantification of Fur was based on HPLC analysis. The reaction products were identified by GC-MS.

Results

PXRD suggested that the precursors NiTUD(x) possessed essentially isolated nickel sites. Upon the impregnation of palladium, some aggregation of nickel species occurred, especially for the materials prepared via the iw method [1]. Nitrogen adsorption-desorption at -196 °C indicated that the materials prepared possessed mesoporosity, with mesopore sizes in the range 11-32 nm. TPR-H2 analyses indicated that the palladium species of the Pd-Ni TUD-1 materials are reduced at low temperature range (<120 °C) and are thus reducible under the catalytic reaction conditions; conversely, the nickel species are reduced at a higher temperature than the catalytic reaction temperature and thus no nickel reduction was expected during the catalytic process [1]. The Pd-Ni TUD-1 materials were active for the conversion of Fur using FAc as hydrogen source, and EtOH (at 140 °C) or BuOH (at 170 °C) as reacting solvent (Table 1). The bioPs formed were AoF, 2MF, OP and its acetal (OPea, OPba). Blank/control tests were carried out to assess the role of each metal in the overall catalytic process. The simultaneous presence of FAc and the bimetallic catalyst was necessary to trigger Fur conversion. In general, the total bioPs yield tended to be higher for BuOH than EtOH (Table 1). These results may...
be partly due to differences in $\text{H}_2$ solubility and solvent polarity that may lead to competitive adsorption effects of reactants/intermediates/products, influencing product selectivity. Increasing the temperature of the system Fur/FAc/BuOH from 140 °C to 170 °C led to a considerable increase in the Fur conversion and total bioPs yields.

The best-performing catalyst was prepared via the fw method and possessed Si/(Ni+Pd) ratio of 13, namely, PdNi-TUD-1(13), which led to 83% 2MF yield at 98% Fur conversion, reached in 7 h, using BuOH at 170 °C. This catalytic protocol benefits from the fact that there is no need for high-pressure gas or externally supplied $\text{H}_2$, no need for catalyst pre-reduction steps (typically using $\text{H}_2$ at high temperature), and the use of alcohol media allows improved bioPs selectivity.

Table 1- Reaction of Furfural, in the presence of bimetallic PdNi-TUD-1(13) in alcohol (ROH) media at 24 h [1].

<table>
<thead>
<tr>
<th>ROH</th>
<th>T (°C)</th>
<th>Conv. (%)</th>
<th>Yields of bioPs (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>AoF</td>
</tr>
<tr>
<td>EtOH</td>
<td>140 a</td>
<td>81</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>140 b</td>
<td>86</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>140 c</td>
<td>82</td>
<td>9</td>
</tr>
<tr>
<td>BuOH</td>
<td>140</td>
<td>77</td>
<td>24</td>
</tr>
<tr>
<td>BuOH</td>
<td>170</td>
<td>98</td>
<td>1</td>
</tr>
</tbody>
</table>

a) run 1, b) run 2, c) run 3

Acknowledgements
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References
Deep eutectic solvents (DES) present interesting properties, mostly connected to their solvation ability, and have been subject to much research in the recent past. Currently, the discovery of new eutectic solvents is accomplished by experimentally measuring the eutectic point of random systems, often using choline chloride as a hydrogen bond acceptor.

In this work, new choline chloride-based eutectic systems were experimentally assessed. Their eutectic data, along with other previously reported in the literature, was used to evaluate a method based on COSMO-RS to predict the eutectic temperature of choline-chloride based mixtures. The predictive methodology herein developed allows for the quick scanning of a large matrix of systems in order to identify those more promising to be liquid at any given temperature. To validate the method here proposed, the eutectic temperature of pharmaceutical drug-based mixtures was predicted and, then, assessed experimentally, being shown that the COSMO-RS can be used to help design liquid drug-based formulations.

Introduction

Deep eutectic solvents (usually shortened to DES) are a relatively new class of green solvents that were first proposed by Abbott et al. in 2003[1]. Their liquid state at operation temperature arises from the mixing of two or more solid components (typically hydrogen bond acceptors and hydrogen bond donors) presenting a eutectic temperature at or below the operation temperature. Due to being cheap and non-toxic [2], choline chloride is the most recurrent substance used in DES research.

A particular area of DES research is concerned with pharmaceutical compounds[3]. More specifically, the liquefaction of pharmaceutical drugs by the addition of a second component could solve problems such as poor solubility and bioavailability or polymorph instability. This, in turn, would solve non-optimal dosage problems, thus reducing waste, and would increase shelf life in the cases where crystalline morphology changes are an issue. As such, this work has a particular emphasis on binary systems consisting of choline chloride and a pharmaceutical drug.

The present work aims at evaluating the ability of COSMO-RS to predict the eutectic temperature of choline chloride-based eutectic mixtures. For that purpose, a database of eutectic points was collected from the literature and supplemented by novel data measured in this work. The choline chloride was modelled in the COSMO-RS framework as either a single ion pair, or two separated ions, being the two approaches compared. The modelling procedure was validated in the design of novel, pharmaceutical-based DES, predicting the eutectic temperature of mixtures not previously studied, which were then measured to test the quality of the predictions.

Experimental

In order to develop a suitable method to predict eutectic temperatures, a database of eutectic points of several choline chloride-based mixtures was assembled from literature data, further complemented by other novel systems studied in this work.

Before use, each compound was dried under vacuum (0.1 Pa and 298.15 K) and constant stirring for at least 72 h. The water content of all compounds was determined and found to be lower than 600 ppm in every compound.

All mixtures were prepared by weighting, inside a dry-argon glove-box and at room temperature, the amounts of each pure component. Whenever possible, all samples were heated under stirring until complete melting and then recrystallized. The solid-liquid phase diagrams were measured using two different methods, depending on the physical state of the final mixture. For the mixtures with a paste-like consistency, a visual method with an oil bath was employed. For the remaining mixtures, the recrystallized solid was crushed in a mortar inside a glove-box and the powder was filled into a glass capillary. The solid-liquid phase diagrams were measured using a melting point device, with a temperature resolution of 0.1 K.

Model

To describe a eutectic type solid-liquid phase diagram, the equilibrium curve for each component can be calculated, in a reduced, approximated form, by:

$$ \ln(x_i \cdot γ_i) = (ΔmH_i) / (R \cdot (1/T_{mi} - 1/T)) $$

(1)

where $x_i$ is the molar fraction of component $i$, $γ_i$ its activity coefficient, $ΔmH_i$ the melting enthalpy of pure component $i$ and $T_{mi}$ its melting temperature, $R$ is the ideal gas constant and $T$ is the absolute temperature of the system. COSMO-RS, short for Conductor like Screening Model for Real Solvents, is a thermodynamics model, based on quantum mechanics, that predicts the chemical potentials of individual components in liquid mixtures[4,5].

It should be noted that COSMO-RS is not particularly suitable for calculations with ionic species. To attempt to minimize this problem, two distinct approaches are investigated in this work. The first approach considers choline chloride as two individual ions, each molecular file being optimized with TURBOMOLE (model A+B) while in the second approach, choline chloride is...
Results and Discussion

For each system whose eutectic point was taken from the literature or measured in this work, its solid-liquid phase diagram was calculated with COSMO-RS considering the two molecular models adopted for choline chloride. The results for the model AB are shown in the Graphical Abstract.

It is evident that the present version of COSMO-RS is unable to adequately predict the solid liquid phase diagrams for all systems studied. Moreover, COSMO-RS, especially with the model A+B, systematically underestimates the values of the activity coefficients for both components of the mixtures studied, overestimating the non-idealities of the mixtures, leading to eutectic temperatures much lower than those obtained experimentally. For a number of systems, irrespective of the model used to describe choline chloride (A+B or AB), this underestimation of the activity coefficients led to solid-liquid diagrams consisting of two lines that do not intercept each other. Contrasting with this behavior, the AB model, specifically for fatty acids/alkohols, predicted choline chloride to have large positive deviations from ideality. In some cases, immiscibility was also predicted.

The mean absolute error, defined as:

$$\text{MAE} = \frac{1}{N_{\text{systems}}} \sum |T_{\text{model},i} - T_{\text{experimental},i}|$$

obtained for the model AB was 31.4 °C. However, the model displayed abnormal inaccurate predictions for close-ring sugars (glucose, fructose and sucrose), benzoic acid and salicylic acid. In particular, the difficulties herein encountered to describe the choline chloride/sugar systems have already been reported in the literature along with fine-tuning of COSMO-RS parameters to overcome them [7]. If these five systems are disregarded as outliers, the mean deviation of the AB model is decreased to just 11.6 °C. This reasoning is illustrated in the Graphical Abstract.

Contrary to the AB model, from the thirty-four binary systems studied in this work, model A+B was only able to predict a eutectic point for eighteen of them. In the remaining sixteen systems, this model overly underestimated the activity coefficients, leading to gaps in the diagrams that are impossible to close. Moreover, the eutectic temperatures predicted are, in general, too low when compared to those obtained experimentally, with a mean error of 112.4 °C (as per equation 2). As such, the AB model greatly surpasses the predictive ability of the A+B model and should be the one chosen to predict new novel eutectic solvents.

Conclusion

In this work, a methodology to predict new choline chloride based eutectic solvents was developed, based on COSMO-RS. Two different choline chloride models were investigated, the A+B model, where choline chloride is treated as two different ions, and the AB model, where choline chloride is treated as an ion pair. Both models were used to predict eutectic temperatures of several binary systems. Model A+B was shown to be very inaccurate, severely underestimating the activity coefficients of the system’s components, leading to gaps in the diagrams of many of them. On the other hand, model AB was quite accurate for most systems, except those whose composition included close-ring sugars, salicylic acid or benzoic acid.

The methodology herein developed can be used in future work to predict new DES without the need of any experimental data. These new deep eutectic solvents can then be used for their interesting properties, like good solvation properties. This methodology is particularly valuable for pharmaceutical-based eutectic solvents.
Sequential extraction of phycocyanin and chlorophyll from Anabaena cylindrica

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Cyanobacteria are attracting the attention worldwide as a reliable and sustainable feedstock for the production of biofuels, food colorants and biochemically active compounds. Anabaena cylindrica is a cyanobacterium with a significantly widespread occurrence in Portuguese freshwater habitats, with phycocyanin and chlorophyll in abundance. These compounds have a huge market value, namely phycocyanin with an expected market around $60 million by 2019. The present work focuses on the development of a sequential extraction methodology to obtain phycocyanin and chlorophyll from fresh samples of Anabaena cylindrica. Extracting several compounds from the same sample constitutes a great opportunity since they can later be applied in industry at lower costs and regarding less waste of biomass. The proposed process consists on a more sustainable method allowing the extraction of around 90% and 55% of phycocyanin and chlorophyll, present in the sample, respectively.

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References

Analyzing sugar-based NADES: A study based on experimental measurements of solid-liquid phase diagrams and their modeling using COSMO-RS

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Natural based-DES are described as “green solvents” due to their low-toxicity, non-flammability, water-compatibility and negligible vapor pressure. Given the recent interest for NADES and the number of articles reporting applications using sugars as DES precursors, data on the solid-liquid equilibria(SLE) of their mixtures is surprisingly scarce, as well as their physical properties, despite the information it provides on the range of compositions and temperatures to operate these systems. In this work, we studied the SLE of binary and ternary mixtures involving [Ch]Cl+sugars. The experimental phase diagrams were described by two excess Gibbs energy(\(g^E\)) models, namely NRTL and a modified Redlich-Kister expansion, after used to develop tools for predicting the behavior of other sugar-based DES. Densities, viscosities, solvatochromic parameters and water impact were evaluated at the eutectic composition, in order to address the tailoring of their properties using water.

Introduction

Nowadays, the search for “eco-friendly” solvents to be used in chemical processes is focused on the incorporation of bio-based compounds in new formulations. In this context, Deep Eutectic Solvents (DES) based on natural compounds are of increasing interest.\(^1,2\) Natural Deep Eutectic Solvents (NADES) are mainly composed by biomolecules such as urea, choline chloride ([Ch]Cl) and organic acids but, some authors have also proposed sugar-based NADES, due to their easy availability, low cost, non-toxicity, and biodegradability, that could lead to cheap and environmentally friendly solvents. Given the recent interest in this class of NADES\(^3,4\) and the number of publications reporting applications for them experimental data on the solid-liquid equilibria (SLE) and the characterization their physical properties becomes vital but, it is extremely scarce, despite the information it provides on the range of compositions and temperatures to operate these systems.\(^5,6\) Given the lack of experimental data, the thermodynamic modeling of such systems is extremely limited hindering the application of NADES to real processes.

Objectives

This work aims to measure the SLE phase diagrams of ternary mixtures of sugars and [Ch]Cl + sugar binary mixtures, evaluating the non-ideality of these systems and of their eutectic mixtures. The experimental phase diagrams for the binary systems composed by [Ch]Cl and sugars were described by two excess Gibbs energy (\(g^E\)) models, namely NRTL and a modified Redlich-Kister expansion (m-RK). The predictive ability of COSMO-RS was tested, and a few parameters tuned with the knowledge gathered from the new binary experimental data and \(g^E\) modeling. The model was then used to predict new hypothetical NADES that were experimentally validated. Thermophysical properties like densities, viscosities and solvatochromic parameters for the eutectic compositions of [Ch]Cl-based mixtures were also measured. The impact of water upon these properties was also investigated.

Methods

The melting points determination was carried out applying two different methods: 1) For mixtures with a paste-like appearance, a visual method using an oil bath was applied. 2) For the remaining mixtures, a melting point device model M-565 by Bucchi (100-240 V, 50-60 Hz, 150 W) with a temperature resolution of 0.1 K was used. Density and viscosity measurements of the mixtures [Ch]Cl + sugars at the eutectic composition, and of the [Ch]Cl + sugars + water mixtures were carried out at atmospheric pressure, in the temperature range (293.15 to 373.15) K using an automated SVM 3000 Anton Paar rotational Stabinger viscometer-densimeter. The Kamlent Taft solvatochromic parameters \(\pi^*\), \(\beta\) and \(\alpha\) were measured at the eutectic composition for the mixtures [Ch]Cl + sugars, and also for the [Ch]Cl + sugars + water mixtures at 323.15 K. \(\pi^*\) and \(\beta\) were obtained using the longest wavelength absorption band, determined by UV-Vis spectroscopy (BioTek Synergy HT microplate reader) at 323.15 K. \(\alpha\) was measured by 13C nuclear magnetic resonance (NMR) spectra, using a Bruker Avance 300 apparatus at 75 MHz.

Results

The results obtained in this work showed that all systems present a SLE phase diagram characterized by a single eutectic point.\(^7\) Mixtures of sugars were found to present an ideal behavior (Figure 1), while their binary mixtures with [Ch]Cl present negative deviations from the ideal behavior, in the sugar solubility curve. This non-ideal behavior was described using NRTL or m-RK expansion, but due to the marked asymmetry of these systems only m-RK was able to correctly describe the SLE behavior of those mixtures (Figure 2). Targeting liquid mixtures at room temperature, and to avoid an extensive number of experimental measurements, the tuned COSMO-RS was applied to estimate the eutectic points of possible novel ternary DES containing [Ch]Cl and two sugars. Results were checked by measuring experimentally two ternary systems: [Ch]Cl + mannose + xylose (T1) and [Ch]Cl + fructose...
+ glucose (T2), showing that COSMO-RS can be a useful tool for screening and designing new NADES (Figure 3). The amount of water required for the binary [Ch]Cl + sugar mixtures to be liquid at room temperature was found to vary between 3 and 9 wt%. The densities and viscosities of both the dried and watered samples were measured in order to evaluate the potential of these systems to be used as solvents in industrial applications. The mixtures investigated were further characterized by the measurement of their solvatochromic parameters. While choline chloride plays the major role on the dipolarity/polarizability and acidity of the mixture, the parameter β is strongly influenced by both, the HBD and HBA used.

Figure 1. Melting temperatures of ternary system sucrose + glucose + fructose, at constant glucose/fructose molar ratio, as a function of sucrose mole fraction (x1): A) 0.10 glucose + 0.90 fructose; B) 0.25 glucose + 0.75 fructose; C) 0.50 glucose + 0.50 fructose; D) 0.75 glucose + 0.25 fructose; and E) 0.90 glucose + 0.10 fructose. The last figure represents the 3D phase diagram of the mixture. Symbols represent the experimental data while the solid lines depict the ideality curve.

Figure 2. Solid-liquid phase diagrams for the binary mixtures composed of [Ch]Cl and: A) arabinose, B) sucrose. Symbols represent the experimental data, (---) NRTL modeling, and (—) mRK modeling.

Figure 3. Melting temperatures of ternary mixtures T1 (upper row) and T2 (lower row) as a function of [Ch]Cl molar fraction (x1) at different ratios of component 2 and 3: A) and E) 1:2; B) and F) 1:1; C) and G) 2:1. Figures D) and H) represent the 3D phase diagrams of T1 and T2, respectively. Symbols represent the experimental data while the solid lines depict the COSMO-RS modelling.

Conclusions
• Mixtures of sugars present only slight deviations from ideal behavior, while the binary mixtures of choline chloride with sugars present eutectic points with much lower temperatures than the sugar + sugar systems, and important negative deviations from the ideal behavior, especially in the sugar solubility curve.
• The predictions quality was checked experimentally, proving that COSMO-RS can be a useful tool for the design of deep eutectic solvents.
• The density of systems containing water is lower (reduction within the range 0.82 – 2.22%) than the density of the dry mixtures, while the addition of water reduces by approximately half the viscosities of the dry mixtures.
• While choline chloride plays the major role on the dipolarity/polarizability and acidity of the mixture, the parameter β is strongly influenced by both, the HBD and HBA used.
• The addition of water leads to a higher number of potential hydrogen bonds between the solutes and water, decreasing their densities and viscosities, and turning the final mixtures into systems with enhanced transport properties for industrial applications.

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References
The work consists of experimental analyzes and computer simulations in animal fat derived biodiesel industry in which was used antifreeze additive VISCOPLEX, donated by Evonik Industries, S50 diesel donated by Suape Distributor BR and also commercial soybean oil to try to improve the cold flow properties of biodiesel studied. A portion of this work was the realization of physicochemical analyzes on biodiesel from animal fat to study the formation of a precipitate of unknown nature formed at low temperatures. The other portion was to determine the cloud point by computer simulation and compared with the experimentally obtained. To perform the computational model using chromatographic analyzes to determine the mass composition of biodiesel studied was required as well as the properties of thermophysical esters of saturated and unsaturated fatty acids, in particular about the melting enthalpy and melt temperature.

**Materials and Methods**

**Animal Tallow Biodiesel.** The samples of animal tallow biodiesel were transferred by JBS Biodiesel to study the formation of the precipitate formed at low temperatures.

**Soybean Biodiesel.** The soybean oil was purchased commercially for use in transesterification reaction by methyl route. The oil/alcohol molar ratio was 1:6, with KOH being used as the catalyst in the ratio of 1% to the oil mass. The reaction occurred in a batch reactor with a capacity of three liters, equipped with a temperature controlled thermal bath. After the reaction, the mixture was transferred to a settling funnel, in order to separate the phases. After 3 hours two very distinct phases could be observed: a phase rich in methyl esters, less dense and lighter, and a phase richer in glycerin, dense and darker. Diesel. The S50 diesel was supplied by Suape Distributor BR.

**Antifreeze Additive.** The Viscoplex additive was supplied by Evonik Industries. It is a solution of acrylic polymer oil composed of solvent naphtha (petroleum), heavy aromatics, kerosene, 1,2,4-trimethyl benzene, mesitylene, 1,3,5-trimethyl benzene and naphthalene.

**Preparation of the samples for analysis.** The analyzes were divided into three stages. The first was the complementation of the characterization of some parameters of biodiesel of animal fat fundamental for interpretation of the formation of precipitate formed at low temperatures. For the second stage of the analyzes, the biodiesel samples were placed in a 500 ml amber flask and then cooled to phase separation, where the formation of two phases, one solid and one liquid, was observed. The analyzes were divided into three stages. The first was the complementation of the characterization of some parameters of biodiesel from animal fat, fundamental for interpretation of the formation of precipitate formed at low temperatures. The fraction of non-solidifying biodiesel (supernatant) was removed and placed in another amber flask for further refrigeration and subsequent phase separation. This procedure was carried out three times with the same sample to obtain three biodiesel supernatants of different compositions, which were given the names of 1o supernatant, 2o supernatant and 3o supernatant. To these supernatants and biodiesel mixtures were made with diesel, soybean biodiesel and Viscockplex additive in percentages of 0.25; 0.50; 1.0; 1.5; 2.0; 5.0; 10.0; 20.0 in mass, in order to analyze the decrease of the cloud point and pour, and thus to improve the properties at low temperatures. In the third stage of the work, chromatographic tests were carried out in order to obtain the composition of tallow biodiesel esters and their respective supernatants to certify if the formed precipitate had the same composition of the biodiesel and calculate the solid-liquid equilibrium to obtain the cloud point through MATLAB software. To calculate the solid-liquid equilibrium using the MATLAB software, one of the important criteria is the characterization of the ester content in each sample. The larger the chain size and/or the saturated character of the biodiesel molecules, the higher the cloud point, pour [7].
For simulation of the cloud point through the MATLAB software, it’s necessary the melting temperature, the heat of fusion and the molar masses of each ester present in the composition of the biodiesel. Although some $\Delta H_{m}$ values are not available in the literature, the $\Delta H_{m}$ value of fatty acids can be used as an alternative because of the proximity. In the chromatography, to obtain the percentage of each ester in the mixture was used the method of normalization of the area with response factor [8].

Determination of flow properties. The Mini Pour / Cloud Point tester Model MPC-101 was used to determine the cloud point of animal fat biodiesel as well as its blends, including their supernatants in the proportions previously cited. The test was performed according to ASTM D6749-02 (2012).

Results

Figure 1. (a) Result of the analysis of the cloud point of the tallow biodiesel and the mixtures made with different mass percentages. (b) Result of the analysis of the pour point of the biodiesel of tallow and the mixtures made with different mass percentages.

The cloud points and pour of the pure tallow biodiesel and its supernatants were determined so that they could be compared with the diesel later, biodiesel and additive mixtures. Based on Figures 1 (a), it can be observed that the Viscoplex additive in a mass percentage of 20% presented greater influence on the cloud point in pure tallow biodiesel. For the pour point, it can be seen in Figure 1 (b) that both the diesel and the Viscoplex additive showed the same reduction of the point. For the supernatant cases: the Viscoplex additive and the diesel were observed to reduce the cloud point of the 1st supernatant equally. At the pour point, diesel at 20% showed a greater reduction in the point. For the pour point of the 2nd supernatant, the 20% diesel showed a greater reduction of the point. For the 3rd supernatant at 20% in mass fraction, the diesel and the Viscoplex additive showed the same reduction in the cloud point. Regarding the pour point, the mass fraction at 20%, the biodiesel and diesel blend was more fluid, compared to the biodiesel blend and Viscoplex.

Cloud points simulation. From the data obtained in the literature and the chromatographic analyzes, the calculation of the cloud point was carried out by MATLAB software. The results were very satisfactory, considering that the deviations were small in relation to the experimental values. The values in Table 1 can be evaluated. The deviations can be justified by the approximation of the values for the heat of melting of methyl esters.

Conclusion

By means of the physico-chemical analyzes for the studied biodiesel, it was verified that this fuel comprised within specifications and that the precipitate formed at low temperatures was not due to the presence of soaps or impurities. By analyzing the calorific value for both biodiesel and its respective precipitate and supernatant separately, it was concluded that the precipitate formed was biodiesel of the liquid phase with the consecutive cooling of the sample, it’s perceived that the precipitate was nothing more than the saturated fatty acids that at lower temperatures reached their cloud point and began to form crystals, thus causing precipitation. It was also observed that the addition of diesel to the biodiesel obtained good results when dealing with the reduction of the cloud point, while the additive was more efficient in reducing pour point.

Acknowledgements

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References

Microwave drying and/or extraction of bioactive compounds from industrial by-products

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The microwave technology has also been proved to be a feasible approach for tightly bound polysaccharides extraction under pressurized water conditions [1], including the extraction of polysaccharides from spent coffee grounds [3,4]. Depending on temperature and time of extraction, different amounts (Figure 1A) and ratios of arabinogalactan/galactomannan polysaccharides (Figure 1B) can be obtained.

Current circular economy concern has led industries to search for approaches able to reduce waste while increasing value of by-products. However, due to the typically high moisture content of the later, application of dehydration processes is almost mandatory [1]. The microwave technology can be used to dehydrate broccoli by-products while preserving a polysaccharide-rich residual product. This technology, based on hydrodiffusion and gravity, also allows for the simultaneous recovery of diffused water which, in the case of broccoli, contain up to 356 µg/mL glucosinolates [2].

Figure 1. Microwave assisted extraction (MAE) of spent coffee grounds (SCG): A) Yield of total soluble solids as function of temperature and time; B) Effect of MAE temperature for maximization of extraction of galactomannans (GM) vs. arabinogalactans (AG).

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References
IProPBio - Integrated Process and Product Design for Sustainable Biorefineries

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Bio-based economy involves in Europe 22 million people and turns over roughly 2.4 billion €. The full realization of its huge potential, however, requires expert knowledge and synergy of different competencies. The overall goal of IProPBio is to exchange complementary theoretical and experimental knowledge of research staff while looking for innovative answers in the field. IProPBio addresses key engineering, thermodynamic, energy, environmental, safety, production and process challenges in the design, optimization and operation of sustainable biorefinery. IProPBio will significantly impact: a) the competitiveness of EU bioeconomy; b) participants’ potential and new carrier perspectives; c) exchange and transfer of high-quality multidisciplinary knowledge, advanced expertise, research and innovation between academic and non-academic participants in EU member states and third countries through the dissemination of the results achieved to target groups and the general public.

Introduction
IProPBio-Integrated Process and Product Design for Sustainable Biorefineries is a project which has received funding from the European Union’s Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement N°778168. The IProPBio project spans over 4 years (2018-2021) and 10 countries. IProPBio addresses key engineering, thermodynamic, energy, environmental, safety, production and process challenges in the design, optimization and operation of sustainable biorefinery systems for multiproduct portfolios. Biorefinery (BioRef) is a novel industrial concept, recognized as the most promising route for employment of the full potential of the biomass industry to utilize biowastes and industrial crops. The primary objective of biorefinery systems is to maximize the conversion of biomass and waste biomasses into high value products, to reduce the environmental pollution, caused by traditional oil-based chemical industries and to stimulate regional and local development. In the EU, BioRefs social and economic benefits are well recognized. It has been estimated that this sector has the potential to create € 32.3 trillion revenues and 1 million new jobs in Europe by 2020. Hence, the topic of our Action is timely and in complete accord with the EU and MSCA RISE priorities.

Overview of the project
A complex BioRef employs multiple alternative feedstocks, unit operations and synthetic routes, using physicochemical, chemical and biological processes to obtain, separate and purify the bioproducts. In comparable terms, the process system engineering of a sustainable BioRef is more complex than the oil refinery counterpart, due to the significant differences between petroleum and biomass feedstocks. Some of the current challenges to the development of sustainable BioRef systems with multiproduct portfolios are summarized hereunder: a) Design flexible product-tailored processes for a variety of biomass feedstocks and/or their mixtures, integrating chemical and biochemical routes into sustainable biorefining of given feed stocks; b) Relate biomass extraction and separation processes with the properties of the desired products and the sustainable utilization of the depleted matrices; c) Identify, design and use inter-changeable processes, capable of being realized according to market requirements with the same industrial equipment (reactors, separators, etc.); d) Integrate production processes (e.g., extraction, pyrolysis and gasification) to develop closed loop production with no or minimum waste, and ecocompatible impact on environment; e) Ensure a constant flow of biomass feedstocks with significantly different properties for a not interrupted production plant of a competitive size, considering also the low chemical and microbiological stability of feeds, intermediates and products; f) Identify, evaluate and design a competitive portfolio of synthetic biochemicals, based on available feedstocks and given production infrastructure. The IProPBio project faces the above formulated and other relevant challenges. With this aim, the ProPBio Consortium incorporates entities from the academic and non-academic sector from EU countries (Denmark - coordinator, Bulgaria, Greece, Italy, Portugal, Spain, UK) and participants from Third Countries (3 entities from the academic sector). The research groups from organizations located in the Third Countries (TCs)
are not eligible for funding and include one from Mexico, one from the USA and one from Brazil. The EU no-academic partner, PANAX-The “Panax – Homeopathy, Phytotherapy and Aromatherapy Laboratory” has extensive expertise and competencies in herbal and essential oil properties, on their therapeutic effects, on the methods and the conditions for their isolation and on their commercialization and marketing. The company has over 30 years of experience on the development and enhancement of processes for production and isolation of essential oils by purely physical mass-transfer operations, like fractional distillation. To develop an advanced process systems engineering approach for integrated process and product design (PPD) of sustainable and competitive “smart” BioRefs, with maximized profits and minimized wastes through intensive exchange of complementary theoretical knowledge and experimental expertise of the partners, six specific objectives have been identified and converted into work packages (WPs), presented in Figure 1. The specified objectives (SOs) can be summarized as:

1. Selection of alternative feed stocks to generate a variety of high added value bio-products. The selection of alternative feedstocks for the “smart” BioRef” is a key factor of its integrated PPD, which influences the processes and equipment needed for their transformation, the products obtainable and their functional properties, as well as its impact on the environment.

2. Design, validation and implementation of a thermodynamic framework (TMF). The proper design of BioRef systems requires the application of a next generation TMF that overcomes the existing challenges, with the potential to improve the accuracy and provide high predictive ability and reliable description of the thermodynamics and kinetics of the processes involved (in biomass conversion routes and separation and purification technologies) at a wide range of operating conditions.

3. Estimation of properties for integrated process and project design. This specific objective (SOs) addresses another key factor for the realization of IProPBio, as the property values of the selected feedstocks and/or their mixtures are needed in the modeling of the transformation processes (e.g., the TMF), in the formulation of product portfolios and in the evaluation of the pollutants generated.

4. Systematic synthesis of process alternatives. This objective addresses the definition of a novel methodology able to generate all feasible flowsheets related to the purification of the products identified in the objective one.

5. Multi-objective optimization problem. Process integration and intensification of multiproduct portfolio BioRefs involve several numerical challenges. For example, the mass and energy integration in BioRefs is fundamental to reduce waste streams, to minimize energy consumption and to reduce operating costs.

6. Process Integration and Life cycle analysis of different target products. Process integration involves setting targets for best theoretical performance of a process, and solves them for various alternative processes based on constraints imposed by practical considerations.

In brief, the SOs 1, 2 and 3 are targeted in WP1. In this work package: (1) a meticulous research of potential feedstocks will be performed. Key biomass from agricultural and other activities, which are preferably encountered at least in two different countries and the traditional/contemporary methods of biomass production and waste treatment will be charted. SO-4 is explored in WP2. The synthesis of the process alternatives regards the definition of a clear methodology used to define the research space that includes all feasible flowsheets and avoids a random alternative generation or limiting the analysis to configuration already proposed in the literature and readapted to the case examined. SO-5 is executed through WP3. In this part of the work, the appropriate design methods and optimization procedures to quantify the performances of the configurations developed in the WP2 will be defined. BioRefs show a number of challenges related to energy and water integration. Except for thermochemical conversions, they typically operate at relatively low temperatures, which complicate energy integration, involving large amount of water over several stages of the process, from pre-treatment to the synthesis and final product separation. All those problems will be addressed in the WP4.

The ultimate achievement of the successful completion of the IProPBio project include novel research results and advanced knowledge that will lead to considerable societal benefits and could potentially contribute towards practical solutions of existing societal challenges. In view of this, the IProPBio foreground knowledge will be disseminated in an optimum way for impact and re-use.

For more information:
Site of the project: http://ipropbio.sdu.dk/
EU Information: https://cordis.europa.eu/project/rcn/212228_en.html
Twitter: https://twitter.com/IProPBio
E-mail: ipropbio@kbm.sdu.dk

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Simultaneous degradation of hydrocarbons and production of valuable compounds by *Yarrowia lipolytica*

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Yarrowia lipolytica is known as an industrial workhorse because is capable of producing important industrial metabolites and can grow even in media with 10 g·L⁻¹ of hexadecane or hexadecene. In fact, as the hydrocarbons concentration in the petroleum extraction or in the effluents of industries that use petroleum or its derivatives, the ability of *Yarrowia lipolytica* to efficiently degrade hydrocarbons (herein demonstrated with hexadecane and hexadecene) and use them as carbon source to grow and produce valuable compounds was demonstrated. In hexadecane-based medium, *Y. lipolytica* W29 cells were able to accumulate up to 16 % of their cellular dry weight as intracellular lipids. Due to its composition, similar to that of vegetable oils, these microbial lipids can be used as feedstock for biodiesel production. Moreover, the simultaneous production of lipase (2730 U·L⁻¹), which market demand is increasing due to its application in the field of bioenergy, represents an economic advantage. Thus, it is possible to valorize wastes contaminated with hydrocarbons with this bioprocess.

**Introduction**

Hydrocarbons have become one of the most important types of organic pollutants due to spills during the processes of exploration, extraction, refining and transporting. Moreover, the marketing of petroleum products and the inadequate release of hydrocarbons on effluents of various industries that use petroleum or its derivatives have contributed to the increase of pollution by hydrocarbons. A high negative impact on ecosystems is expected by the release of these compounds into the environment [1,2]. Although several physical and chemical processes have been developed to treat hydrocarbon contaminated environments or industrial effluents, some disadvantages are recognized to these techniques. Biological methods are an attractive alternative because are environmentally-friendly, less expensive and less hazardous and can be applied *in situ* (bioremediation) and *ex situ* (in bioreactors) [3]. Several microorganisms possess the ability to secrete enzymes that degrade hydrocarbons [4]. The non-conventional yeast *Yarrowia lipolytica* is an example of microorganism that is able to degrade hydrocarbons [5]. This yeast strain is known as an industrial workhorse because is capable of producing important industrial metabolites and can grow in agro-industrial by-products or wastes [6,7]. In this work, the ability of *Y. lipolytica* W29 to degrade hydrocarbons (hexadecane and hexadecene) and them as carbon source to produce biomass and added-value products (microbial lipids and lipase) was evaluated in batch microplate and flask experiments.

**Methods**

**Yeast Strain.** *Yarrowia lipolytica* W29 (ATCC 20460) was maintained on YPDA medium, composed by (g·L⁻¹): yeast extract 10 g·L⁻¹, peptone 20 g·L⁻¹, glucose 20 g·L⁻¹ and agar 20 g·L⁻¹, at 4 °C to a maximum of two weeks.

**Microplate experiments.** The ability of *Y. lipolytica* W29 to grow in hydrocarbons-based medium was assessed in 96-wells microplates during 48 h. Cells were pre-grown in YPD medium for approximately 19 h and 30 μL of pre-inoculum were transferred to each microplate well with 270 μL of sterilized hydrocarbon medium. The hydrocarbons media were composed by hexadecane or hexadecene (1 g·L⁻¹ – 10 g·L⁻¹) as carbon source, YNB without aminoacids (6.7 g·L⁻¹) and Tween 80 1 % (w/v). A control experiment (YNB 6.7 g·L⁻¹), was also carried out.

**Erlenmeyer flask experiments.** Batch experiments with hexadecane (10 g·L⁻¹), hexadecene (10 g·L⁻¹) and a mixture of both hydrocarbons (5 g·L⁻¹ of each hydrocarbon) as carbon source were carried out in 250-mL and 500-mL Erlenmeyer flasks filled with 100 mL of each culture medium (hydrocarbons, YNB without aminoacids 6.7 g·L⁻¹ and Tween 80 1 % (w/v)).

**Analytical methods.** Biomass concentration was quantified by cell counting in the microscope and converted to cell dry weight by a calibration curve. Lipase activity was measured in the cultures supernatant by enzymatic reaction (absorbance measurement at 410 nm during 10 min at 37 °C), using 1 mM p-nitrophenyl butyrate dissolved in 4 % (v/v) aceton and phosphate buffer 50 mM (pH 7.3) as substrate. One unit of activity was expressed as the quantity of enzyme that produces 1 μmol of *p*-nitrophenol per minute in the assay conditions. Microbial lipids were quantified by the phospho-vanillin colorimetric method, after extraction with methanol and chloroform (1:1, v/v) from lyophilized cells as described by Lopes et al. [8]. The visualization of microbial lipids by fluorescence microscopy was performed after staining with Nile red (0.1 mg/mL in acetone).

**Results**

**Microplate experiments.** The ability of *Y. lipolytica* W29 to grow on hydrocarbons-based medium was evaluated in microplate batch cultures. A considerable yeast growth was obtained in media with hydrocarbons comparatively to the control (without carbon source) (Figure 1). Independently of hydrocarbon used as carbon source, *Y. lipolytica* was able to grow even in media with 10 g·L⁻¹ of hexadecane or hexadecene. In fact, as the hydrocarbons concentration...
increased, highest values of absorbance were attained. These results demonstrated the ability of _Y. lipolytica_ W29 to degrade hexadecane and hexadecene and use them as carbon source to growth.

![Graph A](image1.png)

**Figure 1. Growth of _Y. lipolytica_ W29 on several concentrations of hexadecane (A) and hexadecene (B) obtained in microplate experiments. Values are the average of three independent replicates ± standard deviation.**

Erlenmeyer flask experiments. As no growth inhibition was observed with 10 g·L\(^{-1}\) of each hydrocarbon, yeast growth and metabolites production in 250-mL Erlenmeyer flask experiments were carried out with this concentration. Additionally, an experiment with a mixture of both hydrocarbons was performed. No differences on biomass concentration were observed for hexadecane and hexadecene-based media, but a higher cellular density was attained with the mixture of both hydrocarbons. The highest lipid content and lipase activity were obtained with hexadecane and hexadecene as carbon source, respectively. The effect of oxygen mass transfer was studied increasing the volume of flask and consequently the headspace. This result in an improvement of biomass concentration in both experiments (hexadecane alone or mixture of both hydrocarbons). Also microbial lipids content and lipase activity was considerably augmented with the increase of oxygen transfer, particularly with in experiments with hexadecane as sole carbon source. Microbial lipids were perfectly observed by fluorescence microscopy after staining with Nile red and lipids accumulated in lipid bodies has an intense yellow color (graphical abstract).

**Conclusions**

This work demonstrates the ability of _Y. lipolytica_ W29 to efficiently use hydrocarbons (hexadecane and hexadecene) as sole carbon source to grow and to produce added-value compounds, such as lipase and microbial lipids. Both metabolites have industrial interest: (a) lipase is exploited for several applications, such as additives in food industry, in detergent industry and in biodiesel production; (b) microbial lipids, due to its composition similar to that of common vegetable oils can be used to obtain biodiesel, a renewable fuel. The bioprocess developed herein has an important impact both from an economic and environmental point of view, since simultaneously occurs the degradation of highly pollutant compounds and the production of added-value metabolites.

**Table 1. Values of maximum biomass, microbial lipids content, microbial lipids concentration and maximum lipase activity of _Y. lipolytica_ W29 batch cultures obtained in flask experiments.**

<table>
<thead>
<tr>
<th>Flask</th>
<th>Hydrocarbon</th>
<th>Xmax (g·L(^{-1}))</th>
<th>Microbial lipids (% w/w)</th>
<th>Lipids concentration (g·L(^{-1}))</th>
<th>Lipase (U·L(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>250 mL</td>
<td>Hexadecane</td>
<td>3.5 ± 0.02</td>
<td>8.7 ± 0.7</td>
<td>0.3 ± 0.03</td>
<td>1260 ± 125</td>
</tr>
<tr>
<td></td>
<td>Hexadecene</td>
<td>3.0 ± 0.01</td>
<td>9.9 ± 1.4</td>
<td>0.3 ± 0.04</td>
<td>610 ± 112</td>
</tr>
<tr>
<td></td>
<td>Mixture</td>
<td>5.1 ± 0.04</td>
<td>8.5 ± 0.8</td>
<td>0.4 ± 0.003</td>
<td>567 ± 134</td>
</tr>
<tr>
<td>500 mL</td>
<td>Hexadecane</td>
<td>4.7 ± 0.03</td>
<td>15.6 ± 1.1</td>
<td>0.7 ± 0.02</td>
<td>2730 ± 304</td>
</tr>
<tr>
<td></td>
<td>Mixture</td>
<td>7.3 ± 0.05</td>
<td>6.6 ± 0.9</td>
<td>0.5 ± 0.01</td>
<td>868 ± 270</td>
</tr>
</tbody>
</table>

**Acknowledgements**

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**References**

Valorisation of tomato wastes for energy production through anaerobic digestion

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Introduction
Nowadays, the environmental policies in the European Union (EU) [2], but after harvest one of the biggest concerns that producers face is the lack of treatment/valorisation of several remaining wastes, such as rotten tomato waste (TWR), green tomato waste (TWG), and tomato branches waste (TWB). Several works point out that TWR is a good substrate for AD [3-5], while very little information has been reported about TWG and TWB. As so, it is of the most interest an evaluation of these materials as substrates to AD.

Objectives
The aims of this study are the characterization of rotten tomato waste, green tomato waste and tomato branches waste, and the evaluation of those substrates as feedstock to anaerobic digestion for biogas/biомethane production.

Methods
For the physical and chemical characterization of TWR, TWG and TWB, several parameters were determined. Namely, the total chemical oxygen demand (tCOD) was determined by the close reflux method according to APHA (1998), using potassium dichromate as oxidant. Total solids (TS) were determined by drying the samples at 105 °C until constant mass, according to APHA (1998) and calculated by the mass difference of the of initial and final weight of the sample. Volatile solids (VS) were determined by weighing the sample before and after calcination in a muffle at 550 °C for 2 h. The pH was measured in a suspension at a liquid to solid ratio of 10 L kg⁻¹, using pH meter HANNA HI 2002 edge. Experimental BMP was obtained following the protocol proposed by Holliger and co-workers [6]. For theoretical BMP prediction the models described in Eq. (1) and Eq. (2) were used [7]:

\[
BMP = - 42.849 - 182.814 \times \text{tCOD} + 5.432 \times 10^{-1} \times \text{VS} - 41.544 \times \text{tCOD}^2
\]

(1)

\[
BMP = - 42.849 - 182.814 \times \text{tCOD} + 5.432 \times 10^{-1} \times \text{VS} - 41.544 \times \text{tCOD}^2
\]

(2)

Results
The main physical and chemical parameters of the considered substrates (TWR, TWG and TWB) are shown in Table 1.
rotten tomato waste, as shown in Figure 1. The maximum specific biogas production (SBP\textsubscript{cumul}) obtained was 453.4 NmL gVS\textsuperscript{-1}, using 0.5 S/I ratio.

To predict the biochemical methane potential of these substrates, the models described in Eq. (1) and Eq. (2) were applied, and the results are shown in Figure 2. From these data, it is expected a higher BMP for rotten tomato waste, followed by green tomato waste. The substrate with the lower BMP is tomato branches waste.

The preliminary experimental assays for assessing BMP for rotten tomato waste indicated about 295±4 mLCH\textsubscript{4} gVS\textsuperscript{-1}. The experimental assessment of BMP for TWG and TWB is ongoing, but the models of Eq. (1) and Eq. (2) predict interesting methane quantities for these wastes as well.

**Conclusions**

The physical and chemical properties of the three types of wastes generated in high quantities by the tomato industry showed that these are potentially suitable substrates for anaerobic digestion. The theoretical BMP prediction and the preliminary tests as well have shown that the substrates studied have a interesting energy production potential, fulfilling the aim of a circular economy.

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**References**


Separation of betulinic and oleanolic acids by simulated moving bed

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In this work, a simulated moving bed unit was designed for the separation of betulinic and oleanolic acids. HPLC pulse experiments were conducted to select suitable stationary and mobile phases, and best results were found with an Apollo C18 column with 50/50 (%, v/v) acetonitrile/methanol mixture. Breakthrough experiments of pure components were conducted to determine equilibrium and mass transport parameters. They were then successfully validated through the simulation of a breakthrough assay of a binary mixture of these acids. Rigorous phenomenological simulation results showed that the SMB unit successfully separates the compounds with purities above 99 % (wt. %) from a representative natural extract of *Eucalyptus globulus* bark containing the triterpenic acids under study.

*Eucalyptus globulus* is a predominant species in the Portuguese forest [1] and a vital resource for the pulp and paper industry. The bark, branches and leaves are regarded as residues usually burned for energy production without any further valorization. In what concerns bark residues, these have been extensively studied as a possible source of triterpenic acids (TTAs) such as betulinic and oleanolic acids [2,3]. These compounds are known to possess a wide spectrum of bioactivities, from antioxidant, anti-HIV, anti-inflammatory, anti-tumor to antimicrobial properties [4]. Thus, under the scope of the biorefinery concept, *E. globulus* bark is a potential candidate to extract high-value compounds such as TTAs. However, their separation after extraction is a challenging task as they are isomers (see Figure 1) and occur simultaneously in the same natural matrix.

The simulated moving bed (SMB) chromatography is now a well-established technique [5]. Originally developed for large-scale processes such as purification of petroleum components and separation of fructose/glucose, its applications have been extended to the purification of fine chemicals such as enantiomers [6].

The SMB is a continuous multi-column chromatographic process, consisting of a set of columns connected in sequence, in which the countercurrent movement of the stationary phase is simulated by the periodic shift of inlet and outlet ports. It provides an efficient alternative to batch operations, as the continuous operation in countercurrent of SMB units maximizes the mass transfer driving forces enabling the effective separation of low-resolution compounds. Moreover, this mode of operation reduces significantly mobile phase consumption and stationary phase utilization. Thus, the SMB may be seen as a potential candidate for TTAs separation.

Accordingly, a SMB unit was designed for the separation of betulinic and oleanolic acids. HPLC pulse experiments were conducted to select suitable stationary and mobile phases and best results were found with an Apollo C18 column (250 × 4.6 mm, 5 µm) with 50/50 (%, v/v) acetonitrile/methanol mixture (see Figure 2).

Breakthrough experiments of pure components were conducted to determine equilibrium and mass transport parameters. Afterwards they were successfully validated through the simulation of a breakthrough assay of a binary mixture of these acids. Rigorous phenomenological simulation results, of which the concentration profile in the SMB at cyclic steady state is presented in Figure 3, showed that the SMB unit successfully separates the compounds with purities above 99 wt. % from a representative natural extract of *Eucalyptus globulus* bark containing the TTAs under study.

![Figure 1. Molecular structures of betulinic acid (BA) and oleanolic acid (OA).](image1)

![Figure 2. HPLC chromatogram of betulinic acid (BA) and oleanolic acid (OA) in Apollo C18 column and 50/50 (%, v/v) acetonitrile/methanol.](image2)
Figure 3. Simulation results at the end of the 45th cycle of SMB operation (cyclic steady state already established) for the isolation of betulinic acid (continuous line) from oleanolic acid (dashed line). (E – eluent; F – feed; X – extract; R – raffinate).

Acknowledgements
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References
Introduction
With the increase of the price of oil, the interest of discovering alternative solutions for the production of biofuels grew. This led to an increased interest in the study of microalgae since the fatty acids and carbohydrates contained in the cells could be used to produce biodiesel and bioethanol. In addition, the much smaller area required to produce high amounts of biomass compared to conventional feedstocks, such as soy and sugar cane, made microalgae a very interesting, promising and highly researched topic [1]. However, due to the high costs of harvesting and processing the biomass, the estimated cost of producing biodiesel was very high, so no industrial sized microalgae-based production units have been built [2]. Fortunately, some of the intracellular contents of microalgae, like carotenoids and polyunsaturated fatty acids (PUFA), just to name a few, have a high market value [3]. This led to the creation of the concept of microalgae biorefinery where, similar to a chemical refinery, not only would biodiesel and/or bioethanol be produced from/by microalgae, but also different products with high value could be obtained from the same biomass in order to compensate the previously mentioned high costs [4].

A typical microalgae biorefinery process can be described in five stages (Figure 1):
1. Microalgae Production Stage
2. Harvesting and Dewatering Stage
3. Disruption Stage
4. Extraction Stage
5. Conversion Stage

Figure 1. Microalgae biorefinery stages

Objective
The objective is to develop an optimal biorefinery setup for two distinct microalgae: the first, a genetically modified *Synechocystis* strain that produces and excretes ethanol into the culture medium; and the second one, a Dinophyceae with a high lipid (PUFA) content, with the desired component found inside the microalgae cells.

In order to obtain the optimal process setup, different biorefinery scenarios will be scrutinized, each one with different equipment and final products. An economical analysis of each scenario will be carried out and, based on the Net Present Value (NPV) and Investment Rate of Return (IRR), the 3 best scenarios for each microalgae will be chosen. These chosen scenarios will then undergo a life cycle analysis (LCA) study, in order to choose the most sustainable. In the end, a thorough economic analysis will be performed to the optimal option.

Results
The first step was to design a 10 ha plant for both microalgae strains and perform a thorough mass balance calculation and economic evaluation of the production process. In scenario 1, the production stage was designed to produce 174.6 tons of *Synechocystis* biomass per year with a final biomass concentration of 0.5 g/l, along with 2038.2 m³/year of Ethanol. In scenario 2, the production stage was designed to produce 408.2 tons of *Prorocentrum* biomass per year with a final concentration of 2.4 g/l.

The following step was to select the equipment for biomass harvesting and cell rupture. Although there is numerous equipment that can perform the steps previously mentioned, some are only available at laboratory scale and are still far away from being available for industrial scale. Therefore, only the ones already used in microalgae or similar industries were selected. The chosen equipment can be found in Table 1.

Although the initial objective was to separate the process into two stages (Figure 1), one for ruptured biomass production and a second, the downstream section for ruptured biomass processing, and to study the economics and sustainability separately, it was soon discovered that this was not possible. The motive behind this was that some variables were dependent on values from both stages and therefore could not be analyzed separately. Therefore, it was decided to continue and design first
the downstream process and then combine both stages and perform the economic analysis to the entire process.

Table 1 - Harvesting and cell rupture equipment

<table>
<thead>
<tr>
<th>Harvesting Equipment</th>
<th>Cell Rupture Equipment</th>
</tr>
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<tbody>
<tr>
<td>Membrane</td>
<td>Bead Mill</td>
</tr>
<tr>
<td>Centrifuge</td>
<td>High Pressure Homogenizer</td>
</tr>
<tr>
<td>Dissolved Air Flotation (with flocculant)</td>
<td>Ultrasonication</td>
</tr>
<tr>
<td>Flocculation</td>
<td></td>
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</tbody>
</table>

The downstream section of the biorefinery was designed to fulfill the main objective of a refinery, to obtain the largest possible number of products from the feedstock. Since there are different components in each microalga that can be obtained and used in different products, several methods are required in order to extract all the components. However, while the harvesting and rupture stages are a quite straightforward process, the downstream setup is quite specific to each microalga due to the different target components as well as to the different compositions and therefore has to be hand tailored to the specifications of each microalga.

In the *Synechocystis* scenario, in order to recover the excreted ethanol a distillation column and a pervaporation membrane were selected. In this scenario, although the product of interest is ethanol, the microalga also produces a high value component, a water-soluble protein, phyocyanin, and therefore, the downstream of the biomass in this scenario was designed to extract the highest amount of that component first.

Figure 2. Downstream setup for *Synechocystis* scenario

As can be seen in Figure 2, this process was designed to start with the extraction of water soluble components like proteins and phyocyanin, followed by a solvent extraction of lipids and some carotenoids. For solvent extraction, different methods like conventional solvent extraction or supercritical extraction can be used. The remaining biomass can be treated with acid or enzymes in order to recover the remaining components still present in the biomass. Another option can be to anaerobically digest the remaining biomass, producing fertilizers and biogas.

In the Dinophyceae scenario, the products of interest are fatty acids present in the lipid content of the microalga. Therefore, the main goal of this biorefinery design was to initially extract these lipids from the ruptured biomass and afterwards perform further separation steps to the remaining biomass, in order to remove other possible products from the other microalga components (Figure 3).

Figure 3. Downstream setup for Dinophyceae scenario

As can be seen in Figure 3, a solvent extraction, either conventional or supercritical extraction, can be used to extract the desired lipids. The remaining biomass can then be treated with enzymes or acid in order to obtain the monomers of the remaining components or, similar to the *Synechocystis* scenario, be anaerobically digested.

With the design of the downstream process complete, the following step is to choose the optimal process configuration. Although, both designs were projected to extract all the components, with the highest purity in order to obtain the highest value, they also required more expensive methods of purification. This means that producing the higher value products doesn’t always mean the highest economic return. Therefore, there is room for optimization of the configuration.

Conclusion and future work

As was stated before there are so many process options that it is not possible to select the best ones easily. Therefore, the 3 process designs with the highest NPV and IRR will be selected using an optimization program called GAMS. However, the study of the optimum sustainable option does not only mean economical sustainability but also environmental sustainability. Therefore, the 3 previously chosen options will be analyzed using the LCA method so that the most sustainable option is chosen.

Acknowledgements

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References


Oxidative degradation of vanillin, vanillic acid and acetovanillone

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Introduction and Objectives

Within lignocellulosic biorefineries, lignin conversion to value-added products, such as low molecular weight phenolic compounds, is one of the most straightforward and promising routes for lignin valorization. In this context, oxidation has been the most studied method for lignin depolymerization [1-4]. Lignin oxidation in alkaline medium with oxygen was been the main method studied by LA LSRE-LCM research group and accounts with decades of experience [5-7]. Vanillin (V), vanillic acid (VA) and acetovanillone (VO) are reaction products derived from lignin oxidation. However, if the main goal is to improve lignin oxidation product yields, a deeper knowledge about the oxidative degradation of phenolic compounds is required, since during lignin oxidation, and simultaneously to their formation, they can suffer degradation depending on the oxidation parameters. In the present work, a study concerning the oxidative degradation of V, VA and VO was developed and the effect of temperature (100 - 140 ºC), oxygen partial pressure (2.0 - 5.0 bar) and V, VA and VO initial concentration (1.25 - 5.0 g/L) was evaluated.

Methods

The oxidation experiments were performed in a Büchi AG laboratory autoclave with a capacity of 1 L (model BEP280 type II, Switzerland), showed in the Graphical Abstract. Oxidation reactions were performed in alkaline medium ([NaOH] = 2N, pH ≈ 14) with oxygen.

Quantification of phenolic compounds was made by high performance liquid chromatography coupled to ultraviolet detection (HPLC-UV), as described elsewhere [8].

Results

The study of the effect of initial concentration of V, VA and VO on degradation yields showed that initial concentration do not affect V degradation, since after 100 minutes of reaction concentration of 14%, 15% and 17% of V was degraded when an initial concentration of 1.25, 2.5, and 5.0 g/L were used, respectively (Figure 1). After the same reaction time, VA shows a complete degradation independently of the initial concentration, and VO shows a degradation of 28% when an initial concentration of 2.5 g/L was used.

What concerns the effect of oxygen partial pressure on the phenolic compounds yields the results demonstrate a complete degradation of VA after 40 minutes of reaction for all the oxidation experiments. In the case of V, it was possible to observe that degradation velocity increases with the increase of $pO_2$ (Figure 2). For the reaction with 5 bar of $pO_2$, 40% of initial concentration of V was already degraded after 70 minutes of reaction while for the reactions with 3 and 2 bar of $pO_2$, at the same reaction time, only 28 and 25% of degradation was noticed.

Vanillin (V), vanillic acid (VA) and acetovanillone (VO) are reaction products derived from lignin oxidation. The evaluation of its degradation will enable a better understanding of lignin oxidation reaction, helping to maximize the yields of these target value-added compounds. In this context, the main objective of this work is to study the oxidative degradation of V, VA and VO under the same conditions of lignin oxidation. Oxidation reactions were performed in alkaline medium with oxygen and the effect of temperature (100 - 140 ºC), oxygen partial pressure (2.0 - 5.0 bar) and initial concentration of V, VA and VO (1.25 - 5.0 g/L) was evaluated. The results showed that the different values of initial concentration do not affect V degradation yield; however, the partial pressure of oxygen and the initial temperature increase the degradation of all the phenolic compounds studied.

Figure 1. Concentration of V versus oxidation reaction time for the experiments with an initial concentration of 1.25, 2.5 and 5.0 g/L. Initial reaction conditions: pH=14; $T_t$=120 ºC; $pO_2$=3 bar; $P_{total}$=9.8 bar.

Figure 2. Concentration of V versus oxidation reaction time for the experiments with a $pO_2$ of 2, 3, 5 bar. Initial reaction conditions: NaOH=2N; pH≈14; initial concentration 2.5 g/L; $T_t$ = 140 ºC; $P_{total}$ = 9.8 bar.

The oxidations performed with different initial temperatures showed that temperature enhanced the degradation rate, what is verified for all the compounds. When a temperature of 140 ºC was used, after 1 hour of reaction, a degradation of 24%, 44% and 100% for V, VO and VA, respectively, was observed (Figure 3).
To complete the study and evaluate the existence of interferences or competitiveness between the phenolic compounds an oxidation experiment with a mixture of V, VA and VO was performed (Figure 4). The operating conditions selected were similar to those used in the oxidation experiments of the compounds individually.

It is observed an increase in the degradation velocity of V, VA and VO when a mixture with the three phenolic compounds is used. Considering a reaction time of 100 minutes the degradation reaches 38 and 36% for V and VO, respectively, with respect to the initial concentration. VA is completely degraded at this reaction time, either when is present in the mixture or used individually.

**Conclusion**

The study of V, VA and VO degradation through oxidation in alkaline medium was carried out in order to evaluate the effect of initial concentration, oxygen partial pressure and temperature. The results showed that the initial concentration does not affect V degradation yield, i.e. the degradation is almost the same independently of the initial concentration used. On the other hand oxygen partial pressure increases the degradation of all the phenolic compounds studied. What concerns the effect of temperature results showed that this parameter has a significant impact on the degradation of phenolic compounds, since the increase of the temperature increases its degradation.

**Acknowledgements**

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**References**


The recovery of high-added products from industrial waste has been highly explored, as an important target of the economic and environmental development, aiming to minimize the waste produced and maximize the value of the raw material.

The crustacean processing industry has been producing large amounts of marine crustacean waste worldwide, representing an opportunity to convert them in valuable resources [1]. Crab shell waste represents a valuable raw material for different applications as they contain large amounts of chitin and carotenoids pigments [2].

Carotenoids are compounds of high-added value to the pharma and food industries due to its dye function, natural origin and antioxidant activity [3].

The purpose of this study was to exploit the use of high pressure extractions to recover carotenoid-rich extract from brown crab waste. For all the extractions, three quantitative responses were analyzed: total extraction yield (η_{total}), carotenoids extraction yield (η_{car}), and carotenoids concentration in the extracts (e_{car}).

A first screening was performed to access the influence of extraction process conditions. Within this goal, a design of experiments (DOE) was established for the supercritical CO_{2} extractions with 2 to 6% ethanol as co-solvent. Based on preliminary assays, the parameters range was set as follows: equilibration time (0-30 min), pressure (20-50 MPa), temperature (313-333 K) and solvent:raw material (w/w) ratios of 110 and 135. The extraction time was set at 180 min. According to these results, further high pressure extractions were made to study the relative influence of CO_{2} and ethanol as solvents in the extraction of carotenoid pigments. Within this purpose, 100% sc-CO_{2} and pressurized liquid extractions of CO_{2}:EtOH (50-100% w/w) were investigated. Conventional S-L extractions with ethanol at the same temperature (40°C) were made for comparative purposes. The extract yield was determined from the dried extract obtained and the carotenoid quantification was performed by UV-vis spectrophotometry, using astaxanthin as standard compound.

From the DOE of the sc-CO_{2} extraction assisted with co-solvent, the range of results varied from 0.23 to 0.65% for the extraction yield, 0.03 to 6.01 µg/g for the carotenoids yield and 8.4 to 1023.0 µg/g for the carotenoids content in the extracts. The results for the experiment considered as most representative in terms of the best combined responses of yield and carotenoids selectivity are represented in table 1 (sc-CO_{2}+co-solvent).

From the statistical analysis provided by the DOE (data not shown), it was possible to identify the pressure and temperature as influential parameters in the carotenoids yield (η_{car}) and carotenoids content in the extracts (e_{car}). Higher amounts of carotenoids were extracted for higher pressures and lower temperatures. The remaining parameters showed no significant influence in the studied responses.

According to these results, the further extraction studies were made at the 55 MPa and 313.15K. For the extraction with 100% sc-CO_{2}, the extract yield was below 0.2%, with almost no carotenoids (data not shown). Regarding the pressurized liquid extractions with 50% CO_{2}:EtOH and 100% EtOH, equivalent values of η_{total}, η_{car} and e_{car} were obtained (table 1). These data indicates that, under the studied conditions, ethanol is crucial for the extraction of carotenoids.

Additionally, comparing the PLE results with the conventional S-L extraction (table 1), it is possible to observe that no significant differences were found in the analyzed responses. However, the sc-CO_{2} extraction with ethanol as co-solvent presents lower extraction yields and higher amount of carotenoids in the extract, indicating that this process is more selective for carotenoids extraction than PLE or conventional S-L extraction.

In conclusion, the carotenoids extraction from brown crab residues with sc-CO_{2} and ethanol as co-solvent increases with pressure and decreases with temperature. The supercritical mixture of CO_{2} and ethanol is more selective for the extraction of carotenoids than pressurized liquid mixtures of CO_{2}:EtOH or conventional ethanolic extraction.
<table>
<thead>
<tr>
<th>Extraction</th>
<th>η&lt;sub&gt;total&lt;/sub&gt; (%)</th>
<th>η&lt;sub&gt;car&lt;/sub&gt; (%)</th>
<th>e&lt;sub&gt;car&lt;/sub&gt; (µg carotenoids/g extract)</th>
</tr>
</thead>
<tbody>
<tr>
<td>sc-CO&lt;sub&gt;2&lt;/sub&gt;+co-solvent*</td>
<td>0.63±0.03</td>
<td>5.57±0.61</td>
<td>894.9±144.2</td>
</tr>
<tr>
<td>PLE 50% CO&lt;sub&gt;2&lt;/sub&gt;:EtOH**</td>
<td>1.22±0.23</td>
<td>4.81±0.96</td>
<td>395.0±2.7</td>
</tr>
<tr>
<td>S-L Conventional</td>
<td>1.35±0.11</td>
<td>4.46±1.09</td>
<td>335.0±107.8</td>
</tr>
</tbody>
</table>

*sc-CO<sub>2</sub>+5.5%EtOH w/o equilibrium time (50MPa, 313K)
**Equivalent results for the PLE extraction with 100%EtOH.

Acknowledgements
The authors acknowledge the financial support received from the Portuguese Fundação para a Ciência e Tecnologia (FCT) through the POCI-01-0145-FEDER-016403 project and PEst-OE/EQB/LA0004/2011 grant. iNOVA4Health – ID/Multi/04462/2013, a program financially supported by FCT/Ministério da Educação e Ciência through national funds and co-funded by FEDER under the PT2020 Partnership Agreement is also acknowledged. Ana A. Matias thank FCT for the financial support through the IF StartingGrant – GRAPHYT (IF/00723/2014), respectively. The authors are grateful to Tejo Ribeirinho, Lda for providing the crab shell wastes.

References
The PIV4Algae project aims to: (i) optimise the photosynthetic efficiency of microalgal cultures in the available tubular photobioreactor (PBR) with compound parabolic collectors (CPCs), modifying hardware components; (ii) evaluate the feasibility of wastewater treatment (from different industrial sectors) using microalgal cultures; (iii) analyse the effect of culture variables in the production of high-value (carotenoids, PUFAs and proteins) and strategic (biodiesel) products; (iv) develop a CFD model for the optimisation of outdoor PBRs; (v) construct a new PBR configuration based on CFD simulations to compare its performance with the available PBRs; (vi) demonstrate integrated value chains to deliver proof-of-concept; (vii) assess process sustainability in terms of environment, energy, economy and efficiency; and (viii) engage industry and other stakeholders through dissemination activities.

The major challenge of implementing a microalgal at an industrial scale is the improvement of its economic feasibility. Besides testing new PBR configurations for microalgal cultures, two research paths may be followed to improve the economic viability of microalgal cultures: (i) to use wastewater as culture medium, reducing the requirements on water and nutrients; and (ii) to take credit of all products of commercial interest extracted from microalgae (biorefinery concept). In microalgal cultures, the required amounts of water and nutrients are a significant fraction of the total cost. The use of wastewater as culture medium reduces these requirements and presents itself as an environmental benefit.

The production and release of N- and P-rich effluents is one of the most serious environmental problems posed by several industries, as this can lead to eutrophication. Tackling this problem, the PIV4Algae project aims at the improvement of a bioremediation strategy using microalgal cultures in which the intensification of biomass production and its valorisation will be under focus. The approach relies on the optimisation of the process parameters for the cultivation of microalgae in existing tubular photobioreactors (PBRs). Modifications of the reactors' hardware are to be tested. After determining the best operating conditions, the biomass will be characterised to assess its potential for oil and carotenoid extraction. The insight provided by these experiments and by CFD (Computational Fluid Dynamics) simulations will allow for the design of an improved PBR system, which will be built at a pilot scale. A techno-economic and sustainability assessment will be performed.

Figure 1. Existing pilot-scale photobioreactors for microalgal cultivation.

Modifications in hardware components (tubes and parabolic collectors) will also be studied with this aim. Simultaneously, at lab scale (1-L), microalgal cultivation (Chlorella vulgaris, Haematococcus pluvialis, Nannochloropsis salina, Neochloris oleoabundans and Scenedesmus dimorphus) using real effluents as culture medium will be assessed in terms of biomass productivity, nutrient removal kinetics and efficiency. After determining the best culture conditions in tubular PBRs with CPCs, microalgal cultures will be performed using real wastewaters. Biomass production and efficiency in wastewater treatment will be evaluated [3]. The need for a pre- and/or post-treatment will be analysed. Special attention will be focused on nutrient ratios (C:N and N:P) that strongly influence the microalgal culture performance in nutrient removal. In the case of non-ideal values, nutrients may be added to the culture. For instance, CO2 will be also added in the feed gaseous stream at different concentrations to provide inorganic carbon to the culture and to control the pH. Biomass concentration, nutrient and O2 concentrations in medium, pH, temperature and light supply will be daily monitored. Water quality analysis at the end of the culture time will be performed and compared with the standard limits defined for discharged effluents (EU Directives 91/271/EEC and 1998/15/EC). Biomass will be harvested by centrifugation and biochemical characterization of biomass will be performed to infer its commercial value. Task 4 will focus on high-value products: proteins, PUFAs and carotenoids. Additional experiments will be performed to increase the carotenoids production by promoting microalgal stress with solar radiation [4]. In Task 5, oil extraction from microalgae, biodiesel synthesis and characterisation will be performed. LEPABE has know-how and equipment for biodiesel synthesis
and characterisation using different sources [5-7]. The influence of culture conditions in extractable oil fraction and biodiesel quality will be evaluated. The monitored oil properties will be acid value, water content, iodine value, oxidation stability and composition. Regarding biodiesel quality, the measured parameters will be density, kinematic viscosity, flash point, methyl-ester content and acid value. These parameters will be then compared with the ones defined by EN 14214. In parallel with these tasks and using the obtained experimental data, CFD simulations (Task 2) will be performed and validated [8]. A new PBR design will also be simulated and a pilot unit (100-L) will be constructed and tested in Task 6. A techno-economic analysis and sustainability assessment (GREENSCOPE methodology) of the integration of microalgal cultures for wastewater treatment will be performed and discussed with associated companies. The continuous dissemination activities (Task 1) will engage industries and other stakeholders in following the PIV4Algae technological and operational innovations.

Acknowledgements
This work will be financially supported by the projects POCI-01-0145-FEDER-031736 – PIV4Algae - Process Intensification for microalgal production and Valorisation, and POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013), both financed by the European Regional Development Fund (ERDF), through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by FCT/MCTES through national funds (PIDDAC) as well as by the project "LEPABE-2-ECO-INNOVATION" – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). J.C.M. Pires acknowledges the FCT Investigator 2015 Programme (IF/01341/2015). V.J.P. Vilar acknowledges the FCT Investigator 2013 Programme (IF/00273/2013).

References
Phosphorus adsorption onto biochars from pyrolysis and co-gasification of agricultural biowastes

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Two different biochars were applied in phosphorus (P) removal from aqueous-phase: one biochar resulting from the pyrolysis of vine prune biowaste (PBC) and another one obtained in the co-gasification of rice husk (RH) with corn cob (CC) (50 % w/w RH + 50 % w/w CC) (GBc). Both biochars were characterized and submitted to P removal assays under different initial pH values (5, 6, 7, 8, 9, 10) to assess the pH influence on chars’ P-enrichment. GBc char presented the highest P uptake capacity for all the initial pH values studied, with the best performance of both chars at an initial pH of 9 (uptake efficiency of 25.9 % and uptake capacity of 6.11 mg g⁻¹). PBC also showed its best performance at an initial pH of 9 with a P uptake efficiency of 11.7 % and uptake capacity of 2.62 mg g⁻¹. The higher mineral content of GBc along with its developed porous structure are probably the reasons for the higher P-uptake capacity of this biochar when compared to PBC, for all the initial pH values.

Introduction

Phosphorous (P) is an essential macronutrient to life. It is present in trace concentrations in atmosphere, and it only can be found in high concentrations in geological structures, or weakly dissolved in water bodies or soil water as phosphates. P is also contained in biosphere, being an important component of several vegetal and animal cells.

P is considered by the European Union as a critical raw material both as phosphate rock (extraction stage) and phosphorus (refining stage) [1]. In contrast, eutrophication of natural and artificial water bodies is a global environmental problem that is caused by the discharged of excessive P-loads, from industrial and domestic wastewater and agricultural runoff, into aquatic ecosystems [2]. On the other hand, agricultural wastes have recently gained an increasing interest, due to their potential to be used as raw materials of added-value sorbent products [3]. Vine prunes and biowastes from the production of corn and rice are distributed worldwide being rich in lignin, cellulose, hemicellulose, which makes them interesting materials to be valorised through thermochemical processes into biochars and activated carbons.

High amounts of biowastes are generated throughout the wine life cycle; during grape production, lignocellulosic materials are generated in pre-pruning and pruning of vine yards.

Corn is one of the most important cereals in Europe and worldwide. During its cultivation and processing, large quantities of by-products are generated, such as corn cob; this biowaste does not have a positive impact in soil fertility due to its low biodegradability.

Rice is one of the most produced cereal worldwide, identical to corn. This cereal mainly generates two different biowastes: rice straw and rice husk. The former plays an important role in supplying nutrients to soil, while the latter being generated in rice processing plants can easily be reused.

The main aim of the present work was to enrich biochars with P. Biochars are from both pyrolysis of vine pruning and co-gasification of rice husk with corn cob. This work is part of a larger research in which renewable biofertilizers are intended to be produced.

Materials and Methods

Vine prune biowaste, collected in Douro region (Portugal) was submitted to a pyrolysis process, at 600 °C, for 2 h, in an inert atmosphere of N₂ (flow 150 ml min⁻¹).

Rice husk (RH) and corn cob (CC), both collected in Ribatejo region (Portugal), were submitted to a co-gasification process (50 % w/w RH + 50 % w/w CC) in a fluidized-bed gasifier, at 800 °C. Water vapor was used as gasification agent on a steam/fuel ratio of 1.0. Air was used as oxidizing agent in an equivalent ratio of 0.2.

Under these experimental conditions, two biochars were generated: a pyrolysis biochar (PBC) produced from vine prune biowastes and a co-gasification biochar (GBc) from a blend of RH and CC. Both biochars were sieved to obtain a homogenous granulometric fraction <100 μm.

Both biochars were characterized for: (a) Textural properties – Surface area and pore volume distribution through N₂ adsorption-desorption isotherms, at 77 K, with previous sample degasification under vacuum conditions at 150 °C. (b) Proximate analysis – moisture content (M) (EN 14774-1), volatile matter (VM) (EN 15148), ashes (Ash) (EN 14775) and fixed-C (100%–M–VM–Ash) determined by gravimetric method. (c) pH at the point of zero charge (pHₚzc) – biochars were placed in 0.1 M NaCl solutions with initial pH values (pHᵢ) between 2 and 12, at a solid/liquid ratio (S/L) (biochar mass per solution volume) of 5 g L⁻¹; the solutions were stirred in a rotatable device, at 150 rpm, for 24 h; at the end of agitation time, final pH (pH₉) was measured; pHₚzc corresponds to the point where pHᵢ = pH₉.

P removal assays were performed under the following conditions: P compound = KH₂PO₄ (99.5% purity grade); P initial concentration = 100 mg L⁻¹; S/L ratio = 4 g L⁻¹; Constant stirring with a magnetic stirrer; Contact time = 24 h. Initial pH was varied in the range of 5–10 to test the effect of pH on the P uptake capacity of both biochars.

Discussion and Results

The textural characterization of both biochars (Table 1) showed, as expected for carbonized materials, that PBC and GBc had a not-well developed porous structure, because they were not submitted to an activation process. Nevertheless, GBc presented a better porous structure than PBC, characterised by a higher surface area and total pore volume.

Concerning the proximate analysis (Table 2), PBC was mainly composed of fixed carbon. GBc was mainly composed by ashes, because the gasification process that originated this biochar causes a higher volatilization of organic matter, giving rise to the ash content. Still, GBc showed a high percentage of volatile
matter. Also, the rice husk used in the production of GBc favoured the increase of ashes in this biochar, as it is characterised by a high content of ashes, mainly silicon. pH$_{pec}$ shows the alkaline nature of both biochars.

Table 1. Textural properties of biochars.

<table>
<thead>
<tr>
<th>Samples</th>
<th>S$_{BET}$ (m$^2$ g$^{-1}$)</th>
<th>V$_{total}$ (cm$^3$ g$^{-1}$)</th>
<th>V$_{mic}$ (cm$^3$ g$^{-1}$)</th>
<th>V$_{meso}$ (cm$^3$ g$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBC</td>
<td>15.3</td>
<td>0.01</td>
<td>n.q.</td>
<td>0.01</td>
</tr>
<tr>
<td>GBc</td>
<td>169</td>
<td>0.10</td>
<td>0.06</td>
<td>0.03</td>
</tr>
</tbody>
</table>

S$_{BET}$ – surface area; V$_{total}$ – total pore volume; V$_{mic}$ – micropore volume; V$_{meso}$ – mesopore volume; n.q. – not quantifiable

Table 2. Proximate analysis and pH$_{pec}$ of biochars.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>PBC</th>
<th>GBc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (% w/w ar)</td>
<td>4.03</td>
<td>3.29</td>
</tr>
<tr>
<td>Volatile matter (% w/w db)</td>
<td>18.9</td>
<td>35.7</td>
</tr>
<tr>
<td>Ashes (% w/w db)</td>
<td>6.77</td>
<td>60.6</td>
</tr>
<tr>
<td>Fixed carbon (% w/w db)</td>
<td>70.3</td>
<td>&lt;0.50</td>
</tr>
<tr>
<td>pH$_{pec}$</td>
<td>9.80</td>
<td>9.67</td>
</tr>
</tbody>
</table>

ar: as-received basis; db: dry basis

P removal assays showed that GBc is a better adsorbent than PBC (Figure 1); this happened in all the six pH values studied (Figure 1a) and, consequently, GBc uptake capacity, q$_e$ (Figure 1b), was also the highest. PBC showed the best performance at pH 9 with a P uptake efficiency of 11.7 % and an uptake capacity, q$_e$, of 2.62 mg g$^{-1}$. GBc was also better at pH 9 with a P uptake efficiency of 25.9 % and an uptake capacity, q$_e$, of 6.11 mg g$^{-1}$. For the initial pH values above 8, surface precipitation of phosphates may have played a relatively important role in the removal assay of P, as the final pH values were above 9.0 and increased when the initial pH was increased from 8 to 10. For the initial pH values below 8, biochars surface is positively charged and electrostatic interaction with H$_3$PO$_4^-$ and HPO$_4^{2-}$ ions may have occurred. These two biochars are mainly composed by minerals with Al$^{3+}$, Ca$^{2+}$, Fe$^{3+}$, K$^+$, and Mg$^{2+}$ cations that can form mineral complexes with phosphate ions [4]. The higher ash content of GBc is probably the reason for the higher P-uptake capacity of this biochar compared to PBC, for all the initial pH values. Nevertheless, GBc also presented the highest surface area (Table 1), and this property might have been also important for the higher P sorption capacity.

Further investigations are needed to determine the detailed mechanisms and characteristics of phosphate adsorption onto the produced biochars, as well as the P availability to crops through desorption assays.

Acknowledgements

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References

Remarkable performance of deep eutectic solvents aqueous solutions on lignin solubilization and wood delignification

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The main goal of this work was to develop Deep Eutectic Solvents (DES) for wood fractionation aiming at their exploitation on pulping processes. To achieve this purpose, fundamental studies were performed to better understand the solubility of lignin model compounds and technical lignins in DES and their aqueous solutions. Particularly, aqueous solutions of propionic acid:urea (PA:U) improved lignin solubility by orders of magnitude in comparison to water and conventional lignin solvents (around 400-fold and 500-fold to kraft lignin and organosolv lignin, respectively). It should be highlighted the fundamental role of water, which mainly governs a hydrotropic effect on lignin solubility in DES aqueous solutions. Finally, the high performance of DES aqueous solutions for wood delignification at mild conditions was also demonstrated. These results are therefore quite promising and might suggest a positive impact on the techno-economic feasibility of the process.

Introduction

The Biorefinery concept has emerged as a new approach that involves the integrated fractionation of biomass to produce energy, biofuels, biomaterials and chemicals following a bioeconomy strategy [1]. The pulp and paper industries have been pushing forward the development of biorefinery processes to open up new opportunities for a sustainable valorization of lignocellulosic biomass [2]. In the field of sustainable solvents for biomass fractionation, DES emerge as a new generation of solvents with attractive cost and unique physicochemical properties [3]. The application of these solvents in wood processing has been considered as one of the most promising technologies for pulp production [4]. Although some studies concerning the selective dissolution of lignin in DES have been recently published [5, 6], a deep understanding of the fundamental aspects behind the behavior of DES and the mechanisms driving biomass fractionation in these media is still needed. Therefore, the aim of this work was to assess the potential of DES as new solvents for the delignification process. Aiming at select the best DES to be used as delignification media, the solubility of lignin monomeric model compounds and technical lignins in several DES and their aqueous solutions were investigated in the first place. Afterwards, the high performance of DES aqueous solutions for delignification of E. globulus wood at mild conditions was also demonstrated.

Methods

Starting materials for DES preparation were used as received. The two-component mixtures of DES were placed in a sealed glass vial with a stirring bar and heated in an oil bath, with constant agitation until a transparent liquid was formed [2]. 50 wt % DES aqueous solutions were prepared by diluting the neat DES in deionized water. The water content of the DES precursors was previously checked and the value was taken into account in the preparation of the DES aqueous solution. E. globulus wood (with 20 wt % of lignin) and technical lignins were kindly supplied by The Navigator Company. Solubility assays were performed by adding lignin monomeric model compounds or technical lignins in excess amount to 2.0 ± 0.1 g of each DES aqueous solutions, pure water or pure DES, following the methodology previously reported [4].

Delignification assays were performed in a reaction vessel heated in an oil bath at mild conditions (363.15 K, 8 h, solid/liquid ratio of 1/20 w/w and 300 rpm). After the extraction, the soluble lignin fraction was precipitated from the DES aqueous solution with distilled cold water followed by filtration. The isolated lignin was quantified gravimetrically and their chemical structure characterized by FTIR-ATR. The solid fraction (pulp or partially delignified wood) was washed and dried before the gravimetric analysis.

Results

Aiming at investigating the potential of DES aqueous solutions in delignification processes, the solubility of some lignin monomeric model compounds such as syringaldehyde, and vanillic, syringic and ferulic acids in several DES aqueous solutions at room temperature, were determined. The effect of DES on the solubility enhancement of syringic acid and the respective pH value of DES aqueous solutions are depicted in Figure 1. On this figure de S and So represent the solubility (mg g⁻¹) of syringic acid in DES aqueous solutions and in pure water, respectively. Therefore, the S/So ratio represents the solubility enhancement promoted by DES.

Figure 1. Influence of DES on the solubility enhancement of syringic acid in aqueous solutions with 25 wt % of DES, at 303.15 K. The pH value of the respective DES aqueous solutions is presented in black dots.

These results showed a good performance of carboxylic acids-based DES aqueous solutions to enhance the solubility of syringic acid (around 10-fold). Furthermore, it is perceptible that both components of DES play a significant role in the
solubilization of this compound. A possible synergistic effect between the two DES starting materials is promoted. Particularly, PA:U (2:1) aqueous solution stood as best system for syringic acid dissolution. Taken into account the goal of this work, the solubility of other lignin monomeric model compounds in PA:U (2:1) at different concentrations and temperatures were also studied and the results at 323.15 K are reported in Figure 2 (left graphic), as example.

The influence of PA:U (2:1) concentration on the solubility of each lignin monomeric model compounds are observed in Figure 2 (graphic at left). Aqueous solutions of PA:U (2:1) at concentrations of 50 and 75 wt % seem to allow the best solubility enhancement of these compounds. Furthermore, the shape of these curves, with strong maxima at intermediate compositions between pure water and pure DES, suggests that the solubility of these compounds is driven by a hydrotrropic mechanism. The same behavior was observed for technical lignin solubilization (organosolv and kraft lignins) using PA:U (2:1) aqueous solutions at 313.15 and 323.15 K in Figure 2 (graphic at right).

The organosolv and kraft lignin solubility enhancement depends on both DES concentration and temperature. Concerning the DES concentration, it is possible to observe that aqueous solutions at 50 wt % of PA:U (2:1) achieved an kraft lignin enhancement around 400-fold, while at 75 wt % of PA:U (2:1) an around 500-fold solubility enhancement of organosolv lignin was attained. These results represent a remarkable performance of DES aqueous solution for lignin dissolution to the detriment of the low solubility of technical lignins in pure water.

Taking in account the solubility results, four DES aqueous solutions (PTSA:[Ch]Cl (1:1), LA:[Ch]Cl (10:1) and U:[Ch]Cl (2:1)) were evaluated for the delignification of *E. globulus* wood. The success of the wood delignification was assessed by determining lignin recovery and solid fraction yields. The amount of cellulose in *E. globulus* wood was determined to be 50 wt %. Considering negligible cellulose dissolution in DES [5], wood delignification was conducted by applying the conditions that maximized the lignin recovery yield and allowed to obtain a solid fraction yield near to 50 wt %, simultaneously. The low value of lignin recovery yield and the high value of solid fraction yield observed in Figure 3 (left graphic) using PA:U (2:1), LA:[Ch]Cl (10:1) and U:[Ch]Cl (2:1) aqueous solutions, indicated that wood delignification was inefficient. The successful delignification of *E. globulus* wood was only achieved using PTSA:[Ch]Cl (1:1) aqueous solution (lignin recovery and solid fraction yields of 76.62 ± 2.94 wt % and 49.90 ± 0.95 wt %, respectively). In case of PA:U (2:1) and LA:[Ch]Cl (10:1) aqueous solutions, the addition of catalytic amount of mineral acid is required to attain lignin removal from wood. Although, the PTSA:[Ch]Cl (1:1) aqueous solution was the best system for lignin extraction, some significant structural modifications in the isolated lignin might occur as demonstrated in FT-IR spectra (Figure 3 right graphic).

Conclusions

This work provided a new understanding of lignin solubilization using DES aqueous solutions, and demonstrated the potential of DES for application in new delignification and lignin conversion processes. The next steps will involve a lab-scale process optimization, a deep characterization of the final products (lignin and pulp), and finally the DES recycling process.

Acknowledgements

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References

Phase behavior of binary mixtures of saturated FAMEs and alkanes

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Biodiesel blends with mineral diesel, although improving many characteristics of the fuel and lowering its environmental impact, is detrimental to the low temperature behavior of the diesel. The understanding of the phase behavior of binary mixtures of the main FAMEs present in biodiesel with alkanes in mineral diesel is for great importance to understand, and eventually improve these properties in commercial diesel. This work studied the phase behavior of 6 binary mixtures of a saturated methyl ester with alkanes. These systems show a very complex behavior, with the formation of co-crystals, peritectic reactions, metatectic transitions and the formation of solid solutions. The results here reported show for the first time that in mixtures with equal alkyl chain a co-crystal is formed and the mixtures with an alkane alkyl size two carbon bigger than the FAME lead to the formation of a solid solution increasing the cloud and pour point of the mixture.

Introduction

The energy resources, and their sustainability, are nowadays a key factor of progress. Fossil fuels have well know adverse environmental effects like the emission of CO2 and SO2, their production is limited to a few regions often fraught with political problems so a considerable effort has been developed to replace them for more sustainable and renewable sources of energy. [1] Biofuels are environmentally friendly fuels, which can be produced from renewable feedstocks reducing the dependency on oil imports and CO2 emissions. [2] Among biofuels one of the most used is biodiesel that is “a fuel comprised of monoalkyl esters of long-chain fatty acids made from natural, renewable sources such as new/used vegetable oils or animal fats”. [3] Biodiesel has a lot of advantages, it is renewable, energy efficient, biodegradable, and has a high cetane number. Besides, one of its main advantages is its similarity with conventional diesel, which can be used in conventional diesel engines, without any modification to the engine, or be blended with diesel in any proportion, improving its lubricity, and ignition quality. Biodiesel has a lead role on the alternatives for petrol fuel, but some challenges must be overcome to optimize their production, usability, sustainability and to decrease their environmental impact.[2]

One of the issues with biodiesel is its behavior at low temperatures, since it has higher cloud and pour points than mineral diesel, what could lead to the formation of solid residues that clog fuel filters in vehicles or causing blockage in the fuel lines. This can lead to the fuel starvation in engine operation during operation. [4], [5]

Since biodiesel is seldom used alone but essentially as a blend with mineral diesel it would be very relevant to study the phase behavior of the mixtures of saturated fatty acid methyl esters more abundant in biodiesel (methyl palmitate and stearate) with n-alkanes of similar size. The mixtures of dissimilar size are well established to produce simple eutectic systems with a behavior not far from ideal, and thus of little interest. In this work the phase diagrams of these two fatty acid methyl esters with hexadecane, octadecane and eicosane are studied in detail using DSC, polarized optical microscopy and x-ray diffraction.

Methods

The binary mixtures were prepared gravimetrically (Mettler Toledo AG245, precision of 0.01 mg) with molar fraction between 0.0 to 1.0 in steps of 0.1. Some additional binary mixtures were made when necessary to increase the definition of the phase diagrams. Samples of 0.2 g were prepared by heating and stirring until the complete melting of the components. The thermal profile of each sample was determined by differential scanning calorimetry (DSC, Perkin-Elmer Diamond DSC and NETZSCH 200F3) using nitrogen for purge and liquid nitrogen to cool down the samples. Small quantities of each sample were sealed in an aluminum crucible and heated at a rate of 5 K min⁻¹ to 323.15 K under nitrogen atmosphere. The sample was maintained at this temperature for 5 min and was then cooled at a rate of 5 K min⁻¹ to a temperature of 223.15 K and maintained at this temperature for 10 min. The sample was then heated at a rate of 2 K min⁻¹ until the complete melting of the sample. Taking in account the existence of overlapped peaks which prevents the determination of onset temperature the solid-solid and solid-liquid transactions were determined through peak top temperatures. [6–9]

The solid phases of the mixtures, solid-solid and solid-liquid morphology was evaluated by optical microscopy in an Olympus BX51 optical microscope, equipped with a Linkam T95-PE temperature controller with LTS120, a camera Olympus DP71 with U-TV1x2 and a camera Olympus C-MAX3/U equipped with a Linkam controller (Anton Paar TTK450) and a temperature controller (Anton Paar TCU100 with Eurotherm) to investigate the crystal structure transitions observed in DSC analysis.

Results

For the binary mixtures of algae with an alkyl size more than two carbons bigger than the alkyl size of the methyl ester as the system methyl palmitate (1) + eicosane (2) was obtained a phase diagram with an eutectic point around 295 K and a composition of x₁= 0.8. This system also has two solid-solid transition. The lower temperature transition is an invariant solid-solid transition around 280 K that exist between molar fraction of x₁=0.1 and x₁=0.7. The other solid-solid transition varies with the temperature as function of the concentration characterize by the transition of one solid solution to two different solid-solutions. A similar behavior it is also observed for systems with alkyl size of esters bigger than the alkyl size of the alkane as the binary system methyl stearate (3) + hexadecane (4) that presents a simple eutectic type behavior with an eutectic composition close
to $x_1=0.15$ And an eutectic temperature of 289 K in good agreement with the results for the same system previously published by Lobbia et al. [10] This mixture presents an invariant solid-solid transition at around 278 K, for concentrations above the eutectic.

The binary systems with similar alkyl size as the methyl stearate + octadecane (5) and the methyl palmitate + hexadecane. Also present an eutectic point as the previous systems. The novel feature of this phase diagrams is the formation of an intermediate compound of $x_1=0.5$ and $x_3=0.66$. These compounds decompose is not stable to its melting point decomposing close to $T_1=289$ K and $T_2=300$ K with the formation of a peritectic point. The formation and composition of these compounds is it supported by XRD and by the Tamman plot. In these systems it is also visible solid-solid transitions associated to the alkane. The system methyl palmitate + hexadecane was also visible the existence of a metaeutectic transition above the peritectic line for molar fractions above 0.5.

For the systems with an alkyl size of the alkane two carbons bigger than the methyl ester alkyl size as the systems methyl stearate + eicosane and methyl palmitate + octadecane was obtained a more complex phase diagrams than the previously discussed. From a superficial inspection to its liquidus line an interpretation similar to that for the previous phase diagrams with an eutectic and a peritectic point could be drawn but a significant number of other phase transitions were observed by DSC at lower temperatures that seem to require a more complex phase diagram to fully interpret all observations.

In the region of the left-hand side, close to the pure alkane, solid solution seems to be formed. In this zone of the diagram it is also observed a peritectoid at a mole fraction of 0.2 to both systems and $T_1= 299$ K and $T_2= 308$ K induced by the solid solution. Invariants solid-solid transitions occurs at molar fractions of the ester between $x_1=0.3$ to $x_1=0.5$ and $x_3=0.2$ to $x_3=0.5$ an eutectoid point was also observed at a molar fraction of methyl ester close to 0.5 and $T_1=296$ K and $T_2=306$ K. In the region for concentrations between 0.3 and 0.5 an invariant transition occurs with at higher temperatures a two-phase region formed by the two solid solutions. In the right region of the diagram it is observed melting liquidus line with no other transitions observed in this region of the diagram. The absence of transitions observed in the DSC for concentrations above $x_1= 0.5$ suggests that in this region should exist one solid solution.

**Conclusion**

The systems studied show a very complex behavior. For systems with bigger alkyl size of the ester and the alkyl size two carbon bigger of the alkane than the methyl ester is observed a simple eutectic system with some solid-solid transitions. The other systems with more similar alkyl size the behavior present the formation of co-crystals, peritectic reactions, metatetic transitions and, not previously knowledge, the formation of solid solutions. The results here reported show for the first time that in mixtures with equal alkyl chain a co-crystal is formed and the mixtures with an alkane alkyl size two carbon bigger than the FAME lead to the formation of a solid solution increasing the cloud and pour point of the mixture.

**Acknowledgments**

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**References**

Introduction
Tomato cultivation and processing is a very relevant agro-industrial activity in a Portuguese context that generates a significant amount of residues, which need to be properly treated and disposed of. One way to valorize the tomato residues is through the extraction of high value compounds, such as lycopene, representing this an extra revenue source. However the selection of the most adequate option should consider their potential environmental impacts. Thus, in this work the supercritical fluid extraction of lycopene from tomato residues is analyzed using a Life Cycle Thinking approach, based on the Life Cycle Assessment (LCA) methodology defined in the ISO 14040. The functional unit is 1 g of lycopene produced from tomato residues. The life cycle stages considered include the transportation and drying of residues, and the lycopene extraction. Inventory data was obtained from process simulations using the AspenTech V7.3 complemented with data from the literature and the Ecoinvent V3.4 LCI database. The results show that the energy consumption, in particular electricity, is the main responsible for the environmental impacts, and the nature of the residue and solvent used have a significant impact on the process environmental performance.

Study goals and methodology
This study aims to evaluate the potential environmental impacts associated with the lycopene extraction from tomato residues by SFE, in a Portuguese context. The LCA methodology, as defined in the ISO standard 14040 [6,7], is followed in this essay. As a result, the main environmental impacts and the potential opportunities to improve the process environment performance can be identified.

Scope of the study and inventory analysis
An attributional approach is considered when applying the LCA methodology, taking into account only the energy and material flows needed to extract lycopene from the tomato residues. The system consists of drying the tomato residues and the SFE for lycopene recovery, and includes the production of solvents, electricity and steam. The generation and pre-processing of the tomato residues to obtain the tomato skins and the treatment of the residues generated in the extraction process are not considered.

Two case studies are considered in detail: i) case 1, in which tomato peels (TP) or mixed tomato residues (MTR) are compared for lycopene extraction using supercritical carbon dioxide (SC-CO₂) as solvent; ii) case 2, in which MTR are used for lycopene extraction, and the solvents SC-CO₂ and ethane are compared. The influence of transportation was also studied in case 1, for the lycopene extraction from TP.

The functional unit is 1 g of lycopene removed from tomato residues. The main process inputs are electricity, steam and solvent (SC-CO₂ or ethane). As no primary data is available for the extraction process, the inventory information was obtained by simulation using the chemical process simulation Aspen V7.3, based on process data available from the extraction process [8]. The operating conditions depend on the type of residues: for TP, a temperature of 500 °C and a pressure of 90 bar were used; for MTR, 60 °C and 300 bar were considered. The remaining information needed was taken from the Ecoinvent database V3.4 available in the software SimaPro™ V8.5.2.0.

The following impact categories were selected: climate change (CC), ozone depletion (OD), photochemical ozone formation (POF), acidification potential (AP), freshwater eutrophication (FE) and mineral, fossil & renewable resource depletion (MFRRD). The chosen characterization methodology for the environmental impacts is the International Reference Life Cycle Data System (ILCD) 2011 Midpoint+ V.1.10 [9].
Results
The results of case study 1 show that the nature of the tomato residues is a key aspect in the overall environmental performance of the lycopene extraction using SC-CO₂, stressing the need for a proper pre-treatment (tomato skins separation) to minimize the overall environmental impact. In particular, for MTR the electricity consumption is much larger, at least three orders of magnitude when compared with TP. The consumption of steam also increases but less when compared with the electricity increase, six to ninefold more. For MTR the electricity consumption is the dominant factor, accounting for more than 95 % contribution in all environmental impact categories with the exception of FE, in which steam generation is the main contributor. Moreover, steam and solvent consumptions are more than 80 % higher for MTR in comparison with TP. For TP, steam and electricity are the most important contributors to the environmental impacts, depending their relative importance on the impact category. In particular, steam is more important for CC, OD and AP, while electricity is more important for POF and FE. The transportation of tomato residues is not so relevant, as even for TP it represents no more than a 3 % contribution to each impact category. Similar conclusions can be obtained for the solvent, whose influence on the environmental impacts is stronger for AP and FE, but always lower than 15 %.

For case study 2, in which two solvents are compared for the lycopene extraction from MTR, the values obtained for the environmental impacts are compared for each solvent, normalized by the largest value obtained in each impact category. It can be seen in Figure 1 that in all impact categories ethane has a lower contribution to the environmental impacts. This is explained by the significantly lower energy consumption when ethane is used as solvent. Although similar amounts of solvent are used in the process, ethane is more efficient in extracting lycopene from MTR, as it extracts more quantity of lycopene from the same amount of tomato residues, when compared with SC-CO₂.

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References
Oxidation of vanillic acid for C₄ dicarboxylic acid production in the presence of TS-1 catalyst

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Introduction

C₄ dicarboxylic acids (DCA), which include succinic, fumaric, maleic and malic acids, have been identified as one of the 12 building blocks that will be the base of the biomass-derived chemical production. DCA are used for polyester production, as food additives, in the synthesis of pharmaceuticals, among others [1]. However, they are currently produced from petrochemical sources or from edible biomass fermentation. In this context, lignin, a byproduct of the pulping industry, does not compete with food sources. Although it is used as a low-value fuel, it can be depolymerized to serve as a source of a wide variety of chemicals [2,3]. Lignin structure is based in three main monomers (p-hydroxyphenyl, guaiacyl and syringil units) interlinked according to a wide arrange of possibilities, resulting in a heterogeneous tridimensional structure.

Lignin can be oxidized to produce several chemical compounds [3]. Oxidation with H₂O₂ can be done in the presence of several catalysts. Recently, titanium silicalite-1 (TS-1) has been reported to have a good catalytic activity for oxidation of organic compounds using H₂O₂, since it improves the nucleophilic attack capability of the oxidizer [4]. Guaiacol, due to its resemblance with guaiacyl units in lignin, was used as a model compound in oxidation studies. Oxidation was performed using H₂O₂ and TS-1 as catalyst, in alkaline mild conditions (25-85°C, 24 h) generating high percentages of maleic and oxalic acids [5]. However, based on lignin complex structure, other compounds should be screened to test the effect of TS-1 catalyst. Also, other parameters and ranges should be also studied.

In this work, vanillic acid (VA), a more complex model compound for lignin, will be oxidized in presence of TS-1. Several conditions will be evaluated, including temperature, pH and time.

Methods

Oxidation procedure. Oxidation was done using closed steel reactors (20 mL) with an inner PTFE vial, where 5.00 mL of a vanillic acid solution (10.0 g L⁻¹), 0.500 mL H₂O₂ solution (30 wt%) and 0.050 g of TS-1 were placed. Then, pH was adjusted with NaOH 2.0 mol L⁻¹. The reactors were placed inside an oven, using testing temperatures of 80-140°C (±0.1°C), for different times (0.5-11 h). After the defined reaction time, the reactors were quenched immediately in an ice water bath.

Carboxylic acid quantification. Aqueous samples were acidified with H₂SO₄ 2 mol L⁻¹ to pH-2, diluted as needed and filtered through a 0.22 μm pore-size filter. Carboxylic acids HPLC analysis was performed using a Knauer HPLC, equipped with a dual UV-VIS detector and Refractive Index Detector, and a Phenomenex® Rezex™ ROA H⁺ column (300 x 7.8 mm). The column was operated at 25°C, using an eluent mixture of solvent A (0.005 mol L⁻¹ H₂SO₄) and solvent B (15% acetonitrile in 0.005 mol L⁻¹ H₂SO₄), with a variable flow rate between 0.4-0.6 mL min⁻¹, and an injection volume of 20 μL. Carboxylic acids were identified and quantified using calibration curves prepared with standard solutions for each acid.

Results

The vanillic acid (VA) catalytic wet peroxide oxidation achieved better results over pH=9.0. At this pH, the hydroperoxide anion appears, increasing the reactivity of the oxidizer [6]. However, TS-1 was destroyed at pH=13, due to the catalyst’s siliceous zeolitic nature. Thus, the oxidation tests were done at pH=10.5. When performing the oxidation at different temperatures, the vanillic acid conversion was higher for the catalyzed reaction, as shown in Figure 1. The temperature increase resulted in an enhanced conversion for the non-catalyzed reaction, but never reaching the levels obtained in presence of TS-1.

![Figure 1. Vanillic acid conversion and C₄ dicarboxylic acid yield, in H₂O₂ oxidation at pH=10.5 and 3.0 h](image-url)

A higher C₄ DCA yield was achieved for the catalyzed reaction at T=120°C. However, over this temperature, the catalyzed reaction was not significantly different from the non-catalyzed one, as shown in Figure 1. This decrease in the yield was due to

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a lower production of succinic, maleic and malic acid, as shown in Figure 2.

Figure 2. C4 dicarboxylic acid yields, in H2O2 oxidation with TS-1 at pH=10.5 and 3.0 h.

DCA yield varies through time, as shown in Figure 3. Maleic acid is the first acid to be produced during the vanillic acid oxidation, which is converted then to the other acids, like malic and fumaric acids. Maleic acid is also the main acid obtained in the non-catalyzed oxidation, while the other acids are absent or scarce. This information confirms that vanillic acid follows the same chemical pathway as proposed for guaiacol, as suggested by Su et al. [5]. However, after several hours, the acid yield decreases because these compounds suffer degradation to lower molecular weight compounds (e.g. formic and oxalic acids).

Conclusion
The catalyst TS-1 helped to improve the vanillic acid conversion to C4 dicarboxylic acids. Moreover, temperatures lower than 120°C improved C4 DCA production. The main acids obtained were malic, succinic and maleic acids. It was also observed the need to have an alkaline pH to achieve higher reactivity. Nevertheless, too high pH promotes catalyst damage. The vanillic acid oxidation produced maleic acid as an intermediate, which then is converted to other C4 acids, being advisable to have short oxidation times to avoid product degradation. Vanillic acid is a suitable lignin model compound, due to its resemblance to guaiacyl units. However, it is recommended to expand studies by testing other model compounds and, in a second phase, lignin itself.

Acknowledgements
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References
Ionic liquids (ILs) have shown to be efficient and alternative solvents to extract a wide variety of compounds from biomass, in which the use of biomass residues plays a pivotal role within a sustainable framework. In this work, we demonstrate the ability of ILs to significantly improve the solubility of syringic acid in water, followed by the use of the most adequate aqueous solutions to extract phenolic compounds from *Rocha* pear peels. An increase in the solubility of syringic acid up to 85-fold was observed in presence of ILs when compared with its solubility in pure water. A response surface methodology was applied to optimize the extraction operating parameters using the best identified ILs, in which extraction yields of syringic acid up to 2.22 wt% were obtained. Finally, it was demonstrated that the target phenolic acid can be recovered from aqueous solutions of ILs by a dilution approach, in which water acts as anti-solvent.

The enhanced solvation ability of ionic liquids (ILs) for a wide range of solutes is well-known, being particularly relevant in the dissolution of poorly soluble compounds in aqueous media, which is relevant for the formulation of effective drugs, cleaning agents and personal care products. Furthermore, ILs aqueous solutions have shown to be remarkable solvents for the extraction of value-added compounds from biomass\(^1\). In both sets of applications, ILs have been proposed as potential substitutes for the commonly employed volatile organic solvents.

The aim of this work is to identify effective ILs to extract phenolic compounds from biomass residues, within a biorefinery approach. The solubility of syringic acid was first determined in several ILs aqueous solutions at 30°C to appraise the dissolution phenomenon and to identify effective ILs. Conventional volatile organic solvents were also investigated for comparison purposes. The collected data reveal a remarkable enhancement in the solubility of syringic acid in aqueous solutions of ILs up to 85-fold when compared to pure water. Afterwards, the best ILs aqueous solutions were used to extract phenolic compounds from *Rocha* pear peels, in which a response surface methodology was applied to optimize the operating conditions. An extraction yield of syringic acid up to 1.05 wt % was obtained under the optimized conditions. The sustainability of the extraction process was further optimized by carrying out several extraction cycles, reusing either the biomass or the IL aqueous solution. A maximum extraction yield of syringic acid of 2.06 wt% by reusing the solvent and of 2.22 wt% by reusing the biomass have been obtained. These values are markedly higher than those obtained with dichloromethane and methanol at the same operating conditions (1.51 wt.% and 1.68 wt.%, respectively). After the syringic acid extraction and taking advantage of its solubility data in aqueous solutions of ILs, water was added as an anti-solvent, allowing to recover 77 wt% of the extracted target compound with a purity of 93%. The results reported here have a significant impact on the understanding of the role of ILs to enhance the solubility of poorly water-soluble compounds in aqueous media, and on the design of effective and sustainable extraction and recovery processes for high-value compounds from biomass residues.

### Acknowledgements

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### References

Oxidative polymerization of magnesium-based lignosulphonates from acidic *Eucalyptus globulus* sulfite pulping by laccase: preliminary results

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Lignosulphonates (LS) are sulphonated lignin-derived by-products present in sulphite spent liquors (SSL). The valorization of LS represents an important profit for pulp companies and is a challenge towards biorefinery and circular economy concepts. LS are already used in concrete applications as water reducing admixtures. Their plasticizing properties could be enhanced by increasing their molecular weight (Mw) and functionality in order to substitute partially or even completely the superplasticizers from fossil sources. Accordingly, laccase-catalyzed oxidative polymerization of LS from SSL was carried out under pre-selected conditions (temperature, exposure time and enzymatic load) allowing the Mw increase up to 7 folds (from 3240 Da in initial LS to ca. 21.0 kDa). The modified LS fractions were fairly good soluble in water and contained an increased amount of $\cdot\cdot\cdot$-CO groups in conjugated phenolic structures as revealed by the UV-Vis analysis of the modified LS fractions.

Introduction

Worldwide, 60 to 90% of lignosulphonates (LS) derived from the sulphite pulping process are used as plasticizers and/or water-reduction agents in concrete formulations resulting in a concrete with good workability, lower water/cement ratio and higher compressive strength. The plasticizing and water-reducing effects of LS are limited, and, in this area of application, LS compete with petroleum-based polymeric formulations called superplasticizers, such as polycarboxylates (PCA). PCA are more efficient than LS giving rise to a concrete with superior fluidity or more noticeable water-reduction. However, PCA are up to 10 times more expensive than LS. Due to lacking efficiency, LS are usually used combined with PCA in concrete formulations. One of the strategies to improve LS performance consists of increasing their molecular weight through laccase polymerization maintaining their solubility in water [1]. Laccases (EC 1.10.3.2) consist of multinuclear copper-containing oxidoreductases that oxidize a variety of aromatic compounds using oxygen as the electron acceptor and produce water as by-product [2]. Laccases can be used as “eco-friendly” catalysts in the pulp and paper industry for the delignification of wood fibers, although, when used alone, they promote lignin polymerization with minor structural changes [1, 3].

LS from sulphite pulping of eucalypt wood are the least studied in polymeric applications; therefore this work is focused on evaluating the potential of LS for concrete applications. The chemical and structural characterization of LS from sulphite spent liquors (SSL) obtained from the acidic sulphite pulping of *Eucalyptus globulus* was completed. Hereafter, a preliminary study of LS polymerization using laccase without using any mediators was carried out.

Experimental

Industrial SSL from the magnesium-based acidic sulphite pulping of *Eucalyptus globulus* for the production of dissolving pulp was supplied by Caima-Indústria de Celulose S.A. (Constância, Portugal). Laccase Novozym® 51003 was kindly supplied by Novozymes ( Bagsvaerd, Denmark). All other chemicals were of analytical grade.

Lignosulphonates from SSL, designated as LSF, were purified by dialysis, followed by freeze-drying and exhaustive characterization by wet chemistry, quantitative $^{13}$C NMR, $^{1}$H-

$^{13}$C HSQC NMR and SEC, according to previously published procedures [4, 5].

Briefly, the enzymatic treatment consisted in adding laccase (loadings ranging from 42 to 166 U) to 10 mL of a 100 g/L LSF solution (pH 4.3) in a reaction vessel. The reactions were carried out at different temperatures (20-60 °C) with pure oxygen flow for 90 min. Samples were withdrawn from the reaction vessel at 10, 20, 30, 60 and 90 min of reaction time. The water-soluble LS samples were analyzed by SEC to assess the molecular weight (Mw) according to [4]. The fractions from the reaction at 40 °C with a laccase dosage of 166 U were analyzed by UV-Vis spectroscopy to identify the types of phenolic structures present in the modified LS. The LS samples were diluted 10000 times and measured against distilled water. Purified LS was analyzed as corresponding to $t = 0$ min.

Results

Characterization of purified lignosulphonates. Chemical characterization of LSF is provided in Table 1. SEC curves (not shown) confirmed the elimination of low molecular weight fractions from SSL during dialysis including sugars, xylo-oligosaccharides (XOS), and low molecular weight LS. After SSL dialysis, LSF still contain 5-6 % of carbohydrates suggesting that part of XOS are chemically linked to lignin structures. It is worth mentioning that the high standard deviation error of the molecular weight is due to the variability of structures of lignosulphonates.

**Table 1. Chemical analysis of LSF (% w/w).**

<table>
<thead>
<tr>
<th>LSF</th>
<th>Ash</th>
<th>Neutral sugars</th>
<th>SO$_3$H</th>
<th>Phenolic OH</th>
<th>Mw (Da)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>11.6 ± 0.5</td>
<td>6.1 ± 0.2</td>
<td>21.1 ± 0.7</td>
<td>3.0 ± 0.4</td>
<td>3240 ± 455</td>
</tr>
</tbody>
</table>

Structural assignments were confirmed by HSQC NMR spectroscopy and more than ten types of LS structures derived from different lignin structural units were identified, the major being β-O-4. Additionally, the main LSF structural elements were calculated per 100 phenylpropane unit (PPU) and are summarized in Table 2. As expected, LS structures from eucalypt wood are mainly constituted by syringyl units.
Table 2. Structural analysis of LSF by quantitative $^{13}$C NMR.

<table>
<thead>
<tr>
<th>Structural elements per 100 PPU LSF</th>
<th>LSF</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-O-4 structures</td>
<td>36</td>
</tr>
<tr>
<td>β-5 structures</td>
<td>7</td>
</tr>
<tr>
<td>Ar-H</td>
<td>196</td>
</tr>
<tr>
<td>Ar-C</td>
<td>180</td>
</tr>
<tr>
<td>Ar-O</td>
<td>222</td>
</tr>
<tr>
<td>S.G ratio</td>
<td>50:20</td>
</tr>
</tbody>
</table>

Lignosulphonates polymerization by enzymatic treatment. The optimum pH of the laccase is in the range 4-8 and, after purification, LSF solutions (100 g/L) exhibit a pH of 3.2. Hence, the pH of all LSF solutions was adjusted to 4.3-4.5 in order to ensure the oxidation efficiency of the laccase. The first approach consisted in assessing the optimal oxidation temperature of the laccase as well as the reaction time. The best results, i.e., the maximum Mw obtained was at 40 ºC as depicted in Figure 1, which is in accordance with the literature [1]. Also, it can be observed that beyond 90 min of reaction, there is no significant increase of the Mw.

Figure 1. Increase of the molecular weight of LSF (100 g/L) at different temperatures with a laccase dose of 42 U.

Then, the experiments proceeded with the optimization of the laccase dose added to 10 mL of LSF solution (100 g/L) at 40 ºC during a reaction time of 90 min. The results are depicted in Figure 2. Considering that the initial Mw of LSF is 3240 Da, adding 42 U led to a 2.3-fold increase of the Mw (7400 Da) while the addition of 166 U of laccase increased the final Mw up to 20800 Da (7-fold increase) and the modified LSF fractions were still soluble in water.

In order to assess the structural changes resulted from the LS oxidation, UV-Vis spectroscopy of the LS fractions withdrawn overtime from the reaction vessel was carried out (Figure 3). The band with maximum around 370 nm is assigned to conjugated phenolic structures [6] and, overtime, the intensity of this band increased due to higher abundance of this type of structures compared to unmodified LS. According to Zakis [6], this maximum is attributed to phenolic structures in lignin conjugated with a carbonyl group at the Cα-position.

Figure 2. Increase of the molecular weight of LSF (100 g/L) at 40ºC with different laccase doses.

Based on these results, future work will focus on concluding this study and carry out the complete characterization of the modified LS, namely the determination of the SO2H and phenolic OH contents, as well as 1D/2D NMR for structure elucidation.

Figure 3. UV-Vis spectra of modified LSF obtained at 40 ºC with a laccase dose of 166 U.

Conclusions
Results from the enzymatic treatment demonstrated the possibility to increase the molecular weight of eucalypt lignosulphonates up to 7 times, i.e., from 3240 Da up to a maximum of 20800 Da, without the use of any mediators in a considerable short reaction time (90 min) and moderate temperature (40 ºC). Simultaneously, the UV-Vis analysis of laccase-oxidized LS showed the formation of α-carbonyl groups in the conjugated phenolic structures. Modified LS need to be further structurally characterized and their plasticizing properties in concrete formulations evaluated with respect to non-modified LS and commercial PCA.

Acknowledgements
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References
Valorization of *Quercus cerris* cork by supercritical extraction with modified carbon dioxide as green and efficient solution in relation to the classical extraction with organic solvents

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The optimized production and the characterization of extracts from *Quercus cerris* cork using distinct solvents were accomplished in this work. Various extraction methods (batch solid-liquid extraction, Soxhlet extraction, and supercritical fluid extraction (SFE)) and solvents with different polarity (methanol, ethanol, dichloromethane, petroleum ether and modified supercritical carbon dioxide) were compared. The multidimensional scaling (MDS) technique was used to analyze the results, based on GC-MS and FTIR-ATR data. This statistical approach reinforced the proximity between weakly/non-polar extracts and pure friedelin, and between alcoholic extracts and the original biomass. From the SFE optimization, the best conditions that maximize total yield and selectivity towards friedelin were: 60 °C, 5 wt.% EtOH, 11 gCO₂min⁻¹. For friedelin concentration in the extracts, the maximum was attained for 40 °C, 0 wt.% EtOH and 5 gCO₂min⁻¹. In general, the optimum SFE conditions will always depend on whether an extract enriched in a higher diversity of compounds is preferred, or in a single target molecule like friedelin.

**Introduction**

The production of extracts from vegetable matrices has been one of the most explored pathways of bio-refinery due the richness of bioactive and functional compounds that can be found in natural raw materials. The great challenge within natural extracts research has been the supply of high quality products using innocuous solvents, reduced energy consumption and by maintaining the productivity associated with conventional extraction methods [1].

Due to its natural properties, cork has seen a great increase of interest and demand, mostly in the exploitation of *Quercus suber* species. In turn, *Quercus cerris* stands as an alternative source of cork for industrial applications, and it is mostly found in a geographical area ranging from Easter Europe to Minor Asia. The valorization of this raw material has focused on the extractives prone to be obtained from it [2–4], namely molecules from two main groups: phenolic compounds (more efficiently obtained with polar solvents), and aliphatic and terpenoid compounds (better suited for non-polar or weakly polar solvents), from which friedelin is an example of target compound [5].

Supercritical fluid extraction (SFE) is a green and sustainable technique for vegetable matrices processing. Besides enabling yields comparable to conventional organic solvent approaches, SFE allows a free-of-solvent extract at the end of the process. The most widely used solvent is carbon dioxide (CO₂) [6], whose mild critical properties are advantageous to cope with labile compounds, to operate at moderate pressure, and to preserve the natural label of the resulting product.

The present work aims at the optimized production and characterization of extracts from *Quercus cerris* cork produced by SFE. Accordingly, different extraction methods (batch solid-liquid extraction, Soxhlet extraction, and SFE) and solvents with different polarity (methanol, ethanol, dichloromethane, petroleum ether, and modified supercritical carbon dioxide) were compared. Multidimensional scaling (MDS) technique was used to establish proximity relations between the GC-MS and FTIR-ATR analysis of the various extracts. Then, the optimization of friedelin production through SFE was accomplished using the Response Surface Methodology (RSM).

**Methods**

*Q. cerris* bark was obtained from Kahramanmaras, Turkey, and was granulated and separated by density difference in distilled water. The floating fraction of cork-enriched granules was dried and ground into 20-40 mesh. The moisture content of the biomass was measured, being 5.6 wt.%.

Soxhlet extraction was carried out during 8 h, using ca. 3 g of 20-40 mesh *Q. cerris* cork and 120 mL of solvent (methanol (S1), ethanol (S2), dichloromethane (S3) and petroleum ether (S4)). For batch solid-liquid extraction, ca. 3 g of cork was extracted with each of the four organic solvents (30 mL) in a 1:10 w/v ratio. These extractions were performed in sealed and periodically manually shaken beakers for 24 h, using methanol (B1), ethanol (B2), dichloromethane (B3) and petroleum ether (B4). The obtained extracts samples were evaporated, weighed and analyzed by GC-MS and FTIR-ATR. The SFE assays were performed in a 0.5 L capacity Speed™ apparatus (Applied Separations, USA), using 45 g of cork per run and CO₂ modified with ethanol as cosolvent. The SFE extracts were analyzed after ethanol evaporation.

The multidimensional scaling (MDS) was applied to both FTIR-ATR spectra and GC-MS chromatograms. In the first case, the sixteen most relevant bands were selected and normalized in relation to methylene group stretching band. For the GC-MS, the sixteen most relevant peaks were selected, and the areas were normalized with the area of the internal standard (tetracosane). For SFE, the influence of the operating conditions was studied using a Box-Behnken design (BBD) of three factors and three levels, totalizing 15 experiments. The factors and respective levels were: extraction temperature (*T*), at 40-50-60 °C; ethanol concentration (EtOH wt.%), at 0.0-2.5-5.0 wt.%; and CO₂ flow rate (*G*CO₂), at 5.8-11 g min⁻¹. The pressure (*P* = 300 bar), extraction time (*t* = 8 h) and particle size (*d₀* = 20-40 mesh) were fixed along the experiments.
Results
Concerning the comparison between the various extraction methods and solvents, the highest total extraction yields ($\eta_{Total}$) achieved by Soxhlet show that the combination of fresh abundant solvent and temperatures close to the solvent boiling point are key advantages of the Soxhlet technique. For both Soxhlet and batch SLE, the use of petroleum ether led to the lowest yields, which contrasts with the maximum yield results for methanol and ethanol. These results highlight the influence of solvent power in the extraction of lipophilic/hydrophilic extractives. On the other hand, the extraction yields obtained by SFE are of the same order of magnitude of the results for batch SLE with different solvents.

In terms of friedelin concentration in the extracts ($C_{Friedelin}$), the best performing conditions were those involving weakly/non-polar solvents (petroleum ether, dichloromethane and supercritical CO$_2$). The great affinity of methanol and ethanol to solubilize other polar extractives led to a dilution of the friedelin in such extracts.

The FTIR-ATR spectra of the biomass show the presence of phloem material (i.e., non cork) in the extracted samples, which corroborates the previous results reported by Şen et al. [7]. Furthermore, the peaks associated to pure friedelin FTIR spectra have particularly acute shape for extracts obtained with weakly/non-polar solvents, which suggests the predominance of this molecule in those extracts.

MDS was applied to FTIR-ATR spectra (see Figure 1 as example) and to GC-MS chromatograms. The MDS method demonstrates that, more than the extraction method, what imposes major differences in chemical composition of extracts is the solvent polarity.

For the optimization of the SFE of friedelin, three responses were studied: $\eta_{Total}$, $C_{Friedelin}$, and selectivity towards friedelin ($\alpha_{F,N}$). For $\eta_{Total}$ and $C_{Friedelin}$, the highest values obtained were 2.2 wt.% and 36.2 wt.%, respectively. In terms of $\alpha_{F,N}$, all experimental values were higher than 1.0, which demonstrates that friedelin is selectively removed with supercritical CO$_2$.

After an analysis of variance (ANOVA), the reduced models (RM) were fitted to the data. For $\eta_{Total}$, a great impact of cosolvent content and CO$_2$ flow rate were observed. With the highest ethanol content (5 wt.%) one can obtain greater yields when compared with the CO$_2$ flow rate at its maximum. In terms of friedelin concentration, the positive effect of the combination of ethanol content and CO$_2$ flow rate is evident. The lowest friedelin concentrations are predicted at the intermediates values of each factor. For $\alpha_{F,N}$, the model predicts a real impact of ethanol on this response. In Table 1, the optimum conditions that maximize each response according to the reduced models are presented. For $\eta_{Total}$ and $\alpha_{F,N}$, the optimum was found for $T = 60\,^\circ C$, 5 wt.% EtOH; 11 $g_{CO_2}\, min^{-1}$, being 2.2 wt.% and 3.3 respectively. In contrast, the optimum conditions for a maximized $C_{Friedelin}$ (38.2 wt.%) are considerable distinct, pointing to 40 °C, 0 wt.% EtOH, and 5 $g_{CO_2}\, min^{-1}$.

Table 1. Optimum conditions for each response.

<table>
<thead>
<tr>
<th></th>
<th>$\eta_{Total}$ (wt.%)</th>
<th>$C_{Friedelin}$ (wt.%)</th>
<th>$\alpha_{F,N}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T$ (°C)</td>
<td>60</td>
<td>40</td>
<td>60</td>
</tr>
<tr>
<td>EtOH (wt.%)</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>$Q_{CO_2}$ (g min$^{-1}$)</td>
<td>11</td>
<td>5</td>
<td>11</td>
</tr>
<tr>
<td>Predicted values</td>
<td>2.2</td>
<td>38.2</td>
<td>3.3</td>
</tr>
</tbody>
</table>

Conclusions
Batch SLE reached friedelin concentrations lower than Soxhlet assays. Weakly/non-polar solvents showed a clear affinity to friedelin. In contrast, methanol and ethanol removed much polar compounds, giving rise to higher total extraction yields. The MDS results reinforced the proximity between pure friedelin and the weakly/non-polar extracts, and between alcoholic extracts and the original biomass. Concerning the SFE optimization, the optimum conditions for both $\eta_{Total}$ and $\alpha_{F,N}$ coincide: 60 °C, 5 wt.% EtOH, and 11 $g_{CO_2}\, min^{-1}$. For friedelin content in the extracts, the maximum was attained for 40 °C, 0 wt.% EtOH, and 5 $g_{CO_2}\, min^{-1}$. In general, the optimum SFE conditions will always depend on whether an extract enriched in a higher diversity of compounds is preferred, or in a single target molecule like friedelin.

Acknowledgements
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References
Life-cycle inventory analysis of microalgae-based biomass production

M. Branco-Vieira1,2*, M. Freitas2, T.M. Mata1, ... remains in the alga paste, because the centrifugation process results in a concentrated biomass

Microalgae have been reported as one of the most promising alternative feedstocks for industrial use. However, the use of microalgae for bioproducts production generates a significant amount of residual biomass, which can be used in integrated biorefineries for the extraction of added-value products. The analysis of the process using the Life-Cycle Assessment (LCA) permits to recognize the best routes and the technological processes with the lowest environmental impact, according to reduction and higher efficiency in feedstock uses. Therefore, in this study it was performed a Life-Cycle Inventory Analysis (LCIA), based on real process data, which was scale-up to a microalgae biomass production industrial plant. Values of energy, nutrients, water and materials consumption were used to create an inventory of inputs and outputs for the biomass production. This approach should support the decision-making, through the identification of critical factors to promote the development of sustainable pilot and large-scale algae-based biorefinery.

Introduction
Microalgae-based biorefineries consist of a holistic approach that have been studied for many authors [1]. Several bioproducts can be extracted and produced, depending on the particular microalga considered, its biochemical composition and the culture conditions [2]. Microalgae are lipid-rich species, containing also carbohydrates, proteins and other valuable compounds, that can be used as raw materials for a range of industries to produce high-value products [3].

Microalgae cultivation can be performed in open or closed bioreactors [4]. A number of factors must be considered to choose the best alternative for producing microalgae-based bioproducts. Both types of bioreactors show advantages for industrial applications; however, the final product(s) needs to be taken into account in order to define what kind of system will be better for each case. Generally, open systems are less expensive and easier to operate than closed systems. On the other hand, closed systems provide higher biomass productivities, better management of contamination and higher environmental controls [5].

In this study, data was used from a bubble-column photobioreactor (PBR), a type of closed PBR that is cheaper than those with different shapes [6,7]. The microalgae biomass produced was proposed, for which it is important to consider the utilization of the products and waste generated by each process in a circular economy approach. Therefore, the aim of this study is to evaluate a microalgae-based biomass production, using a Life Cycle Inventory (LCI), in order to support further environmental analysis of the process under a Life Cycle Assessment (LCA) perspective.

Methods
The present study proposes the construction of an industrial microalgae plant facility at Concepción, Chile. The proposed industrial facility is composed of total of 10,000 similar modules of 0.8 m³ PBR, totaling 8,000 m³ of cultivation, with a total area of 1.3 ha. This scenario was used to perform the LCI of the project, using experimental data obtained directly from one module of PBR, using the microalga strain *Phaeodactylum tricornutum*. The industrial plant is assumed to operate daily, for 24 hours. The proposed industrial microalgae production flowsheet is shown in the Graphical Abstract. The microalgae cultivation process starts by (1) pumping seawater to the reservoir, (2) mixing of the seawater with the nutrients required by the *P. tricornutum* growth, (3) feeding the culture medium to the PBR. After microalgae cultivation, the (4) microalgae culture is harvested through centrifugation and the recovered biomass, containing 15% of dry matter, can be used for the downstream process. The remaining wastewater from the culture medium, after biomass centrifugation is (5) filtered and returns to the seawater tank to be reused in another culture batch.

Results
The water, necessary to prepare culture medium for the microalgae growth is directly obtained from the sea in the amount of 26,501 m³ water.year⁻¹. The CO₂ required for microalgae growth is supplied from atmospheric air by air blower and contributes to mitigate about 891,558 kg of CO₂ annually. The same system that supplied CO₂ to the culture medium promoted the mixing of the culture, which corresponds to an energy consumption of 16,094 kW.h⁻¹.year⁻¹, while the centrifugation process required 316,818 kW.h⁻¹.year⁻¹. The major nutrients for the microalgae culture medium are nitrogen and phosphorus, which represent an annual consumption of respectively 16,721 and 1,128 kg. Freshwater is necessary for the cooling system, when the temperature is higher than that tolerated by *P. tricornutum*. In this case, the annual consumption of cooling water is 8,837,829 m³. The energy supplied to the culture system is about 111,968 GI.year⁻¹. The volume of biomass culture addressed to the harvesting process is estimated to be 240,781 m³, which corresponds to 231,150 kg of dried biomass. After biomass harvesting, an amount of 226,722 m³ of wastewater returns to the PBR recycling tank for feeding another microalgae culture batch, leaving an amount of residual wastewater of 25,191 m³, containing 2,519 kg of nitrogen and 504 kg of phosphorus. It is important to note that part of this residual wastewater remains in the alga paste, because the centrifugation process results in a concentrated biomass.
containing 15 % of dry matter. Lost biomass, represented by culture crashes, corresponds to 41,891 kg year\(^{-1}\) (Figure 1).

Figure 1. Inputs and outputs of the microalgae biomass production considered in this study, estimated on an annual basis. Blue arrows indicate the input parameters, red arrows indicate the output parameters and green arrow indicates the bioproduct potential.

**Considerations**

A wide range of products has been obtained from microalgae using the biorefinery concept. However, the analysis of sustainability plays an important role to evaluate the environmental feasibility of this process. The water and energy uses are the most important sustainability indicators on the microalgae-based industry, in order to make this approach economically and environmentally viable. In this study, the proposed reuse of harvesting water promoted a better water use performance and the harvesting process is the most energy intensive. Hence, the development of new technologies to decrease the energy intensity of the harvesting process should be the focus of the innovation and development studies in this field. Therefore, this LCI will support further analysis to conduct a LCA of the industrial plant proposed, using real data obtained in loco. This study is important for supporting the decision-making to implement an industrial microalgae facility on the Central-South of the Chile where aquaculture resources are an important economic factor of this region.

**Acknowledgements**

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Valorization of *Aurantiochytrium* sp. microalgae through supercritical fluid extraction: optimization of conditions, measurement and modeling of kinetic curves


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The valorization of *Aurantiochytrium* sp. biomass was accomplished through supercritical fluid extraction (SFE) aiming at the production of algal oil enriched in docosahexaenoic acid (DHA, 22:6 ω3). Pressure, temperature and flow rate conditions were optimized and kinetic curves were measured and modeled. The experimental optimization pointed to 300 bar, 40 °C, 12 g min⁻¹ of CO₂ as the preferable conditions to maximize both η_total and x_DHA. Under these conditions, η_total = 13.6 wt.% and x_DHA = 39.2 wt.%, with DHA standing as the most abundant individual fatty acid in the extracted oil. The SC-CO₂ modified with 5 wt.% of ethyl acetate is able to boost the extraction rate in the first two hours of extraction, which is confirmed by both the solubility and diffusional parameters fitted using the broken plus intact cells (BIC) model. Overall, the work supports the industrial exploitation of the *Aurantiochytrium* sp. microalgae by SFE for the production of algal oil enriched in DHA.

Introduction

In recent years, the research on algae based processes has strengthened driven by the expanding interest on implementing biotechnological approaches to produce valuable chemicals instead of their attainment by industrial synthesis or direct extraction from natural ecosystems. In this context, the microalgae of the genus *Aurantiochytrium* sp. (see Figure 1) has been object of an industrial effort to launch a tailored process devoted to the production of squalene. The strategy encompasses excretion of squalene to the culture media (from which it can be further refined) and the late recover of docosahexaenoic acid (DHA, 22:6 ω3) from the algal residues under the form of an algal oil. DHA is a valuable polyunsaturated fatty acid included in the omega-3 family, and is traditionally obtained from marine fish due to its beneficial features of preventing some cancer types, cardiovascular accidents, diabetes, antirheumatic processes, etc [1-3].

Methods

*Aurantiochytrium* spp. samples were obtained from the company DEPSIEXTRACTA - Tecnologias e Biológicas, Lda. SFE experiments were performed in a 0.5 L capacity Spe-ed™ apparatus (Applied Separations, USA; see Figure 2). The details of operation can be consulted elsewhere [6]. A total of 20 assays were performed here: 8 extractions at constant time (6 h), and 12 kinetic extraction curves (0-6 h).

In this work, the valorization of *Aurantiochytrium* sp. biomass was accomplished through the extraction with supercritical carbon dioxide, which is a benign technology very well positioned to answer green chemistry motivations towards circular and sustainable economy, while at the same time preserving the natural character of the ensuing products [4-5]. Accordingly, supercritical fluid extraction (SFE) operating conditions were optimized and kinetic curves were measured and modeled towards the disclosure of the best conditions to produce abundant and DHA rich oil from this microalgae.

Figure 1. *Aurantiochytrium* sp. microalgae.

Response surface methodology (RSM) was applied to optimize the extraction pressure (200-300 bar), temperature (40-80 °C) and flow rate (6-12 g min⁻¹) conditions. The chosen responses were Total extraction yield (η_total), DHA content (x_DHA), and antioxidant activity (AA). Upon establishment of optimum SFE conditions, kinetic curves were measured to unveil the yield and DHA content profiles along time. In addition, the kinetic and thermodynamic impact of the modification of the SC-CO₂ with different cosolvents (ethanol, isopropanol, n-hexane, and ethyl acetate) in 5 wt.% concentration was studied. Finally, the resulting extraction curves were modeled with the integrated broken plus intact cells (BIC) model of Sovová [7].

Results

The RSM optimization results showed that, within the experimental conditions studied, the total extraction yield (η_total) oscillated from 2.1 to 13.4 wt.%, and the DHA content (x_DHA) of the extracts ranged from 27.3 to 39.3 wt.%. For
ηtotal, the most influential factor is the extraction temperature, being preferable to operate at the minimum value of 40 °C. In turn, for xDHA, pressure and temperature share the same importance and while lower temperature is also preferable, higher pressure favors DHA concentration in the extracts.

Upon fitting first order polynomials to both responses, the reduced uncoded models were obtained (see Table 1) together with their coefficients of determination ($R^2$) and of adjusted determination ($R^2_{adj}$). The adjusted models demonstrate being able to correlate the experimental data.

Table 1. Reduced models fitted to the studied responses.

<table>
<thead>
<tr>
<th>$R^2$</th>
<th>$R^2_{adj}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.933</td>
<td>0.984</td>
</tr>
<tr>
<td>0.997</td>
<td>0.994</td>
</tr>
</tbody>
</table>

The optimum SFE conditions were 300 bar, 40 °C, 12 g min⁻¹ (Run 7), where the attained experimental values for the two responses reached $\eta_{total} = 13.6$ wt.% and $x_{DHA} = 39.2$ wt.%. In fact, under these conditions, the algal oil exhibits DHA as the major individual component, followed by palmitic, docosapentaenoic acid (DPA), and myristic fatty acids, with contents of 25.9, 15.4, 10.1 wt.% (see Figure 3).

Figure 3. Fatty acids profile of the Aurantiochytrium sp. algal oil under optimum SFE conditions (300 bar, 40 °C, 12 g min⁻¹).

The measured extraction curves (cumulative $\eta_{total}$) are shown in Figure 4 for Run 7 (pure SC-CO₂) and Runs 9 to 10 (each assay containing 5 wt.% of a different cosolvent). Accordingly, the impact of the chosen of cosolvents was particularly noticed in the first two hours of extraction, mainly in the case of ethyl acetate (Run 10). The BIC model confirmed solubility and diffusional nuances between the SFE assays with pure or modified SC-CO₂ can justify the observed kinetic differences.

Figure 4. SFE kinetic cumulative curves under optimum SFE conditions (300 bar, 40 °C, 12 g min⁻¹) with and without cosolvents.

**Conclusion**

The experimental optimization pointed to 300 bar, 40 °C, 12 g min⁻¹ as the preferable pure SC-CO₂ conditions to maximize both $\eta_{total}$ and $x_{DHA}$. Under these conditions, $\eta_{total} = 13.6$ wt.% and $x_{DHA} = 39.2$ wt.%, with DHA standing as the most abundant individual fatty acid of the oil. The SC-CO₂ modification with 5 wt.% of ethyl acetate is able to boost the extraction rate in the first two hours of extraction. Modeling results confirmed such experimental observations. On the whole, the work supports the industrial exploitation and valorization of the Aurantiochytrium sp. microalgae by supercritical fluid extraction for the production of algal oil enriched in DHA.

**Acknowledgements**

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R&D on biorefining and bioproducts: main achievements

In collaborative projects, high value extracts, sugars, lignin and new cellulosic materials represent the most intensive research [1-7]. Bark and other forest material were evaluated as source of bioactive compounds; R&D activity included process design for their extraction and purification [8,9] and scale-up studies [10]. For bark phenolic extractives, an integrated process of extraction, membrane processing and adsorption/desorption was designed and evaluated to obtain bioactive fractions in collaboration with Faculty of Engineering of the University of Porto – Figure 2 [11]. Purification of triterpenic acids has resulted in a joint patent between University of Aveiro and RAIZ [12]. Phenolic compounds and triterpenic acids were pointed out as promising ingredients in cosmetic and nutraceutical products.

Sugars production was investigated in different perspectives: i) as a process stream coming from the pre-extraction of wood aiming the hemicellulose recovery before kraft pulp, ii) resulting from enzymatic hydrolysis of primary sludge, iii) resulting from forest biomass deconstruction processes and enzymatic hydrolysis [1,2, 5-7]. These sugar solutions were then tested as commodity for biobased industry: Bioethanol, lactic acid, bacterial cellulose and polyhydroxybutyrate were produced, proving the suitability of cellulosic sugar solutions for bioproducts and biomaterials [2].

Lignins resulting from deconstruction process and from industrial kraft liquor were evaluated for their suitability for polyurethane foams, adhesives for technical applications and high added-value phenolic compounds [2, 13-15]. The potential of bleached eucalyptus kraft pulp were evaluated for the production of micro and nanofibrillated cellulosates (MFC and NFC) and xylans as advanced bio-based materials in high added-value applications [3, 16]. An integrated process for M/NFC and xylans production from bleached pulp was also developed. MFC derivatives were tested as reinforcement of composites, in electropositive materials, as anti-corrosion formulations additive and also in papermaking [3].

In this topic, RAIZ and The Navigator Company, in collaboration with the University of Coimbra, carried out several studies for the production and use of MFC / NFC in the fibrous matrix, as well as at the paper surface, for strength, barrier properties and printing quality improvement [3,17]. In this topic there is a PhD work programme ongoing.

Furthermore, sustainable composites based on polylactic acid/polypropylene and eucalyptus fibers with improved mechanical and environmental performance were investigated [4].

Impactus project: expected outcomes and challenges

Impactus project has started in 2018 and represents the R&D roadmap at The Navigator Company for the next 4 years in the areas: Market Pulp, UWF Paper, Tissue Paper and Biorefineries/Bioproducts – Figure 1.

The Consortium is composed by The Navigator Company, RAIZ - Forest and Paper Research Institute, the University of Coimbra and the University of Aveiro, in partnership with other Portuguese Universities and international RTOs. The human capital is intensive: 180 researchers/engineers plus 50 research grants and 2 Navigator invited chairs. Training 50 highly qualified young researchers and technicians for the promotion of Portuguese modern bioeconomy is one of the main goals. Among these, about 17 doctorate thesis should be completed by 2022. The expected outcomes are new 10 patent registration, 4 spin-offs on bioeconomy and 100 scientific publications. Each technical area has specific expected outcomes, although there is a close association between activities resulting from the integrated action plan for this project. This is the main asset of the impactus and, at the same time, the main challenge for the techno-economic evaluation of the new solutions, as well for the coordination, and management.

In biorefinery, the main expected outcomes are products from lignocellulosic biomass and technologies to support their production. Some examples are: new bioactive ingredients for added-value applications, clean technologies and integrated processes, biomass fractionation using optimized processes or using disruptive technologies, new materials/products based on resulting fractions according to their properties, thermochemical processes for biofuels, technologies for lignin isolation and new products, including composites, and new cellulosic materials based on BEKP, as thermoplastic materials and improved microfibrillated cellulose.

Impactus is an exemplary case of co-operation and co-creation between industry and academy, being a step forward to a green, global, sustainable and competitive bioeconomy in Portugal, based on eucalyptus pulp-and-paper industry.
Figure 1. Overview of project inpactus main R&D activities in Market Pulp, UWF Paper, Tissue Paper and Biorefineries/Bioproducts

Acknowledgements

Inpactus project – Innovative Products and Technologies from Eucalyptus, Project N.º 21874 funded by Portugal 2020 through European Regional Development Fund (ERDF) in the frame of COMPETE 2020 nº246/AXIS II/2017

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References

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Poster Session

REACTION AND SEPARATION PROCESS
Removal of antimony from water by iron-coated cork granulates

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Antimony is a metalloid with similar chemistry to arsenic but whose treatment is not as extensively studied. In this work, removal of antimony in both Sb(III) and Sb(V) oxidation states was performed using iron-coated cork granulates. It was found that Sb(III) adsorption was independent of pH in the range 4-10, while Sb(V) adsorption decreased with pH increase, being optimal at pH 3 when iron leaching was taken into account. Adsorption kinetics were slow for Sb(V) and equilibrium was only approached after 240 h, while for Sb(III) it was reached in about 16 h. The Elovich model fitted well to the experimental data. Both oxidation states followed a Freundlich isotherm for equilibrium data. Affinity was higher for Sb(V) than Sb(III). The Langmuir model allowed the estimation of the maximum adsorption capacities of 5.8 ± 0.5 mg g⁻¹ for Sb(III) and 12 ± 2 mg g⁻¹ for Sb(V). Iron-coated cork granulates proved to be adequate environmentally friendly adsorbents for Sb removal from water.

Introduction

Antimony (Sb) is a metalloid known since ancient times, when its powdered minerals were used in cosmetics and medicine, and which is applied in current times in fire retardants, alloys in batteries, solders, glassware, ceramics and ammunitions. Its chemistry and toxicity closely resemble that of arsenic, a metalloid of the same group in the periodic table, and they commonly occur together in the environment, namely in mining fields [1]. Just like As, Sb is usually found in two oxidation states in environmental matrices, Sb(III) and Sb(V), in anoxic and oxic conditions, respectively. However, there is a lack of extensive studies on the environmental behavior of Sb, probably due to the lower frequency of Sb presence in aquatic systems [2]. Nevertheless, removal of Sb from water sources is no less important, since antimony is identified as a possible carcinogen and targeted as priority pollutant by both EU and USA [3]. The World Health Organization guideline for antimony presence in drinking water is 20 µg L⁻¹ [4], but stricter limits have been imposed in the EU and USA, of 5 and 6 µg L⁻¹, respectively [1].

Treatment of antimony contamination in water matrices has not been extensively studied either. Possible techniques include coagulation/floculation, phyto remediation, bioremediation, ion-exchange, oxidation, electrochemical methods and membrane separation. Still, adsorption is one of the most sought out alternatives, due to advantages that include the simplicity of operation, cost-effectiveness, minimal sludge production and capability of regeneration [1].

Conventional adsorbents such as activated carbons, activated alumina and ion-exchange resins show either a lack of studies in the literature or very low adsorption capacities for Sb [1]. The best performing materials involve the use of natural and synthetic iron oxide minerals and (oxy)hydroxides [2,3], or iron modification of low-cost materials, for instance, saponified orange waste [5].

Previous studies by the research group have produced iron-coated cork granulates with optimized adsorption potential for As(III) and As(V) [6]. Cork is a natural, renewable material widely available in Portugal and the granulates are byproducts of its processing. Due to the similarities between the chemistry of As and Sb, it is proposed that iron-coated cork granulates may be also good adsorbents for Sb(III) and Sb(V) removal from aqueous matrices.

Methods

Iron-coated cork granulates were produced by contacting raw cork granulates of 0.8-1 mm size with aqueous suspensions of precipitated iron (oxy)hydroxides ([Fe] = 0.05 mol L⁻¹, pH = 7), at a solid/liquid (S/L) ratio of 20 g L⁻¹ in a rotating shaker at 20 rpm during 24 h, inside a temperature-controlled cabinet at 20 °C. The obtained iron content was 24 ± 2 mg g⁻¹. Sb(III) and Sb(V) adsorption experiments were carried out in similar conditions, but contacting the iron-coated cork granulates at a S/L ratio of 2.5 g L⁻¹ with aqueous solutions prepared from SbCl₃ or K₂Sb(OH)₆. The pH was adjusted with HNO₃ or NaOH solutions. The time of contact was varied for kinetic studies. Equilibrium studies have been done changing the S/L ratio (1-5 g L⁻¹) for Sb(III) and the initial concentration (1-40 mg L⁻¹) for Sb(V).

Sb concentrations were measured using flame atomic absorption spectroscopy (AAS) in the range 2-30 mg L⁻¹ and electrothermal AAS in the range 10-100 µg L⁻¹.

Results

The adsorption of Sb(III) and Sb(V) onto iron-coated cork was first assessed in terms of pH, in the range 2-10 for Sb(V) and 4-10 for Sb(III). It was found that adsorption from a 10 mg L⁻¹ Sb(III) solution was very resistant to pH, with a minimum value of 3.7 ± 0.1 mg g⁻¹ at pH 4 and a maximum value of 4.2 ± 0.1 mg g⁻¹ at pH 9. On the other hand, Sb(V) adsorption was highly dependent on pH with a decreasing trend, with a maximum adsorbed amount from a 25 mg L⁻¹ solution of 6.8 ± 0.2 mg g⁻¹ at pH 2 and a minimum of 0.6 ± 0.2 mg g⁻¹ at pH 10. As optimal operating pH for further studies, pH 6 was selected for Sb(III) as it is closer to natural conditions, and pH 3 was selected for Sb(V), to take into consideration the minimization of iron leaching, which was significant at pH 2 (1.7 ± 0.4 mg L⁻¹, corresponding to 0.7 ± 0.1 mg g⁻¹), but negligible at pH ≥ 3.

The adsorption kinetics showed that equilibrium was approached at about 16 h for Sb(III) but only after 240 h (10 d) for Sb(V), which indicated a very slow rate of adsorption for...
the latter. The pseudo-second-order [7] and Elovich [8] models were fitted to the kinetic data of both oxidation states:

\[ q_t = \frac{k_2 q_e^2 t}{1 + k_2 q_e} \]  
\[ q_t = \frac{b}{\ln(1 + abt)} \]  

Where \( q_t \) is the adsorbed amount (mg g\(^{-1}\)) at time \( t \) (h), \( q_e \) is the adsorbed amount at equilibrium (mg g\(^{-1}\)), \( k_2 \) is the pseudo-second-order kinetic constant (g mg\(^{-1}\) h\(^{-1}\)), \( a \) is the initial rate of adsorption (mg g\(^{-1}\) h\(^{-1}\)) and \( b \) is a coverage scale factor (g mg\(^{-1}\)) that corresponds to the reciprocal of the coverage at which the adsorption rate has fallen to 1/e of its initial value.

The kinetics of Sb(III) adsorption were studied at an initial concentration of 10 mg L\(^{-1}\) and of Sb(V) adsorption at an initial concentration of 25 mg L\(^{-1}\). Model fitting was performed with CurveExpert Professional. The results can be found in Table 1.

Table 1. Model fitting of adsorption kinetics.

<table>
<thead>
<tr>
<th>Model parameters</th>
<th>Sb(III)</th>
<th>Sb(V)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudo-second</td>
<td>( k_2 ) (g mg(^{-1}) h(^{-1}))</td>
<td>0.12±0.03 (5±2)×10(^{-3})</td>
</tr>
<tr>
<td>order</td>
<td>( q_e ) (mg g(^{-1}))</td>
<td>3.7±0.2 8.5±0.6</td>
</tr>
<tr>
<td>Elovich</td>
<td>( r^2 )</td>
<td>0.963 0.867</td>
</tr>
</tbody>
</table>

Both models fit the experimental data adequately, although the Elovich model slightly better, especially in the case of Sb(V). This is consistent with a first, fast step of adsorption and a second slower step governed by different mechanisms, since the model assumes a heterogeneous distribution of sites, varying with surface coverage. Adsorption equilibria were studied at 20 °C and also at optimal pH (3 for Sb(V) and 6 for Sb(III)) for both oxidation states. The range of equilibrium concentrations obtained for Sb(III) was slightly lower than for Sb(V), since in the former the S/L ratio instead of the initial concentration was varied, as the initial concentration had to be fixed at a maximum of 10 mg L\(^{-1}\). Sb(III), due to its lower solubility. The Langmuir [9] and Freundlich [10] models were fitted to the equilibrium data:

\[ q_e = \frac{K_L q_{max} C_e}{1 + K_L C_e} \]  
\[ q_e = K_F C_e^n \]  

Acknowledgements

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References

Study of the effect of the compensating anion on the CO2 sorption capacity of hydrotalcite-based sorbents

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In this work, several hydrotalcites (HTCs) with different compensating anions were prepared to be used as CO2 sorbents. It was found that the type of compensating anion, the partial substitution of Al3+ by Ga3+ and the impregnation with K2CO3 affect the CO2 sorption capacities of the materials. Besides the physicochemical characterization of the HTCs, the sorption equilibrium isotherms and breakthrough cycles were performed. It was found that some of the sorbents prepared present very good performance as compared to state-of-art materials, opening the possibility to be subsequently used in a sorption-enhanced reaction process where CO2 capture improves overall performance.

Introduction

CO2 emissions are an important environmental problem due to the impact they are causing on our planet. Therefore, there is a great interest in developing technological solutions for large-scale CO2 capture, sequestration or utilization [1]. Besides that, alternatives for improving processes like steel reforming have been investigated. One alternative is the sorption-enhanced concept: this reactor configuration allows shifting the thermodynamic equilibrium by removing for instance CO2 (a reaction product) from the reaction medium [1, 2]. Depending on the operating temperature, several sorbents may be used to capture CO2. It has been reported that hydrotalcites (HTCs) exhibit a good sorption capacity, stability and easy regeneration [1]. HTCs are layered double hydroxides, whose general formula is $[\text{M}^{2+}_{x}\text{M}^{3+}_{y}(\text{OH})_{2}]^{(3−x+2y)}[\text{A}^{n−}_{x/n}·\text{yH}_2\text{O}]^{n−}$, where M is a divalent metal cation and M3+ is a trivalent metal cation. An- is a compensating anion located in the interlayer space and x is generally between 0.2 - 0.4 [4], while y is the number of moles of water located in the interlayer.

Several layered structures of HTCs could be produced with several compensating anions and it was found that their physicochemical properties are highly influenced by such anions. Additionally, it was concluded that K2CO3-doped HTCs showed improved performance and that partial substitution of Al3+ by Ga3+ also improved the CO2 sorption capacity [1,3].

In this study, besides the physicochemical characterization of the materials (commercial and prepared by us with compensating anions never used before), the sorption equilibrium isotherms were determined. Also, the effect on CO2 sorption capacity caused by Ga3+ partial substitution and impregnation with K was evaluated for some samples and compared to that shown by the original HTC. Finally, several of the most promising materials were also submitted to breakthrough cycling tests.

Methods

HTCs with molar ratios Mg/Al = 2 or Mg/(Al+Ga) = 2 (Al/Ga ≈ 9) were synthesized using a co-precipitation method. A series of HTCs-A samples with different anions (A = CO32-, Cl-, SO42-, BrO3-, ClO4- or (CH2COOH)BH - the last anion called long in this work) were prepared by adding a Mg(NO3)2·6H2O and a Al(NO3)3·9H2O solution drop-wise into an anion precursor solution. The pH of the mixed solution was controlled at 9.5 using a NaOH solution (1 M). After, the mixture was stirred for 20 h at room temperature and filtered. Then, the solid was washed several times and dried (48 h at 40 °C). The HTCs with partial substitution of Al3+ by Ga3+ (called HTC-A(Ga)) were prepared using the same procedure. Some of the dry HTCs were impregnated with K (labeled as HTC-A_K or HTC-A(Ga)_K, where A is the compensation anion) with the aim of achieving a final K loading of 20 wt.% using K2CO3. These materials were dried again (48 h at 40 °C). To obtain a HTC-based sorbent the respective dry HTC was calcined (2 h at 400 °C in air).

The physicochemical characterization of the materials was realized through FTIR, TGA, XRD, AAS, BET and SEM/EDS. The CO2 sorption capacities were assessed using a magnetic suspension balance (static method); CO2 breakthrough runs were also carried out with a sorption step (with a feed of 15 vol.% of CO2 balanced in N2) and a regeneration step carried out under N2 flow at 300 °C. Some CO2 breakthrough runs with the HTC-long_K sample were performed in the presence of water (15 vol.%).

Results and Discussion

The CO2 sorption capacities of calcined HTCs (prepared by us) at 300 °C for different partial pressures of CO2 are shown in Figure 1. Among the HTCs-A prepared, HTC-CO3 showed the highest CO2 capture capacity of 1.08 mmol/g (Pco2 = 1 bar), which was much higher than other HTCs reported in the literature. The sorption capacities of the doped-sorbents can be seen in the Table 1. It was verified that the Ga3+ partial substitution in the HTC-CO3 sample enhanced the sorption capacity (1.15 mmol/g for HTC-CO3(Ga) at Pco2 = 1 bar) and by doping this HTC with 20 wt.% K (HTC-CO3_K), the CO2 sorption capacity was increased up to 1.43 mmol/g (Pco2 = 1 bar). These effects were not observed for HTC-long (sorption capacity increases with the impregnation of K and decreases with the Ga3+ partial substitution)

The thickness of the interlamellar layer (XRD analysis) for distinct compensating anions has the following trend: long > BrO3- > ClO4- ≈ Cl > SO42- > CO32-. It was found that the sorption capacity was in general higher for sorbents with HTC precursors that have higher interlamellar layer, except for the HTC-CO3 sample (see Figure 2) meaning that other properties are important in such a complex process.

In the breakthrough tests it was possible to see that the HTC-CO3 sample presents a higher sorption capacity than the HTC-
doping HTC–CO\(_3\) with 20 wt.% K, the CO\(_2\) adsorption capacity was further increased, up to 1.43 mmol/g (at 300 °C and P\(_{CO_2}\) = 1 bar) and it was verified that the Ga\(^{3+}\) partial substitution in this HTC also enhanced the sorption capacity. Besides that, it was possible to concluded that the presence of water in the breakthrough dynamic tests had a negative effect in the stability of the sorption capacity of the HTC-long\(_K\) samples, in opposition to the HTC based on carbonate. Some of the sorbents prepared present a very good performance to be subsequently used in a sorption-enhanced steam reforming process.

**Conclusions**

This study reports a detailed investigation on how the compensating anion affects the structural properties and CO\(_2\) sorption capacity of HTCs-based sorbents. It was found that the anions have an effect on the thermal stability and morphology, modifying the CO\(_2\) capture capacity of the sorbents. Among these HTCs, HTC–CO\(_3\) showed the highest CO\(_2\) sorption capacity of 1.08 mmol/g (at 300 °C and P\(_{CO_2}\) = 1 bar). By doping HTC–CO\(_3\) with 20 wt.% K, the CO\(_2\) adsorption capacity was further increased, up to 1.43 mmol/g (at 300 °C and P\(_{CO_2}\) = 1 bar) and it was verified that the Ga\(^{3+}\) partial substitution in this HTC also enhanced the sorption capacity. Besides that, it was possible to concluded that the presence of water in the breakthrough dynamic tests had a negative effect in the stability of the sorption capacity of the HTC-long\(_K\) samples, in opposition to the HTC based on carbonate. Some of the sorbents prepared present a very good performance to be subsequently used in a sorption-enhanced steam reforming process.

**Table 1.** Sorption Capacities of prepared hydrotalcites (at 300 °C and 1 bar).

<table>
<thead>
<tr>
<th>Sorbents</th>
<th>Sorption Capacity (mmol·g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTC–CO(_3)</td>
<td>1.05</td>
</tr>
<tr>
<td>HTC-CO(_3)(Ga)</td>
<td>1.15</td>
</tr>
<tr>
<td>HTC-CO(_3)(Ga)(_K)</td>
<td>1.45</td>
</tr>
<tr>
<td>HTC–CO(_3)(_K)</td>
<td>1.43</td>
</tr>
<tr>
<td>HTC–long</td>
<td>0.69</td>
</tr>
<tr>
<td>HTC–long(Ga)</td>
<td>0.17</td>
</tr>
<tr>
<td>HTC–long(Ga)(_K)</td>
<td>0.94</td>
</tr>
<tr>
<td>HTC–long(_K)</td>
<td>1.07</td>
</tr>
</tbody>
</table>

**Acknowledgements**

The authors acknowledge Laboratory for Process Engineering, Environment, Biotechnology and Energy, and the project NORTE-01-0145-FEDER-000005 – LEPABE-2-EKO-INNOVATION, supported by North Portugal Regional Operational Programme, under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund. C. Rocha is also grateful to the Portuguese Foundation for Science and Technology (FCT) for the Ph.D. grant (SFRH/BD/120179/2016), with financing from the European Social Fund (ESF) and national funds supported by the Ministry of Science, Technology and Higher Education. M. Soria is grateful to the FCT for the postdoctoral grant (SFRH/BPD/88444/2012), with financing from ESF and the Human Potential Operational Programme.

**References**

Separation of nadolol racemates by high pH reversed-phase preparative fixed-bed chromatography: Comparison of C18 materials

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Fixed-Bed technology will be used for the multicomponent preparative separation of a pharmaceutical beta-blocker chiral drug. New strategies using different achiral stationary phases will be presented. Nadolol is a quaternary mixture of equal amounts of four stereoisomers and will be used as case-study. A new methodology for the design, optimization and experimental implementation of the multicomponent separation will be introduced, including the use of three different achiral adsorbents, the screening and choice of the best adsorbent-solvent combination, taking in account the final preparative separation using the fixed-bed technology. Extensive experimental and simulation results will be presented, including solvent screening, measurement of equilibrium adsorption isotherms, breakthrough measurements, and fixed-bed (Azura prep HPLC unit) experimental preparative separation using C18 columns under reversed-phase mode.

Introduction

One of the main goals of the pharmaceutical industry nowadays, is to have more safe and efficient drugs. The purification of chiral pharmaceutical drugs is getting the interest from the industrial companies, particularly after the international regulations. Currently, more than 40% of marketed drugs have chiral active ingredients and almost half of these drugs are marked as racemic mixture.

Nadolol is one representative beta-blocker pharmaceutical drug prescribed worldwide for relieve of several diseases mainly related with the cardiovascular system. However, like other pharmaceutical drugs, it is also related with some severe risks, such as depression, insomnia and cardiovascular failure, among others. Some authors refer that these side effects could be related to the fact that nadolol drug is still marketed as a mixture of equal amounts of its four stereoisomers. Additionally, there are studies referring that some therapeutic effects of this drug are related to only one of the four stereoisomers. Despite the growing pressure of the international regulation agencies for pharmaceutical drugs’ safety, pure single nadolol stereoisomers are still not commercially available.

The nadolol pharmaceutical drug represents a very interesting case-study of multicomponent chiral separation since it is composed by four stereoisomers, being two pairs of enantiomers. In this way, it introduces the possibility of alternative strategies, using different kind of separation sequences and techniques, the use of different packings (chiral and achiral stationary phases), and the correspondent mobile phase optimization at both normal and reversed phase modes [1-3].

The design of the complete separation of nadolol stereoisomers asks for a global experimental and simulation methodology considering both the characterization and the optimization of each separation step and its sequences, to achieve the four nadolol components pure.

The present work will scope on optimizing the enantiomers separation of nadolol using different achiral C18 adsorbents. For this case, an extensive set of experiments were carried out using achiral C18 columns, such as, XBridge, Shield and XSelect, all the three achiral adsorbents obtained from Waters.

Materials and methods

The mixture of the four nadolol stereoisomers was obtained from Sigma-Aldrich (Schnelldorf, Germany). The HPLC-grade solvents, ethanol, acetonitrile and the basic modifier diethylamine (DEA) were obtained from Fluka (Bunchs, Switzerland). Three types of analytical (4.6mm ID x 250mm L; particle size diameter of 5 μm) and preparative (19mm ID x 100mm L; particle size diameter of 10 μm) Waters C18 achiral columns were used: XBridge, Shield and XSelect, all obtained from Waters. The columns’ efficiency characterization, screening of the mobile-phase composition, loading experiments, adsorption isotherms and breakthroughs measurements were carried out using a preparative Knauer HPLC system equipped with a Smartline UV detector 2520 set at 270 nm wavelength, two Smartline 1050 pumps with 50 mL/min pump heads and a XBridge Prep OBD C18 10 μm (250x30 mm) column with a 10 μm particle size diameter [2]. Experimental results presented in this work will stress the advantage of using an intermediate step based on achiral reversed-phase liquid chromatography to perform the separation of the two racemates of nadolol.

New strategies using different packings (chiral and achiral stationary phases) will be presented in this work as well. Using C18 columns, such as, XBridge, Shield and XSelect, all the three achiral adsorbents obtained from Waters. The experimental work focus on screening of mobile phase composition, solubility of nadolol racemates using different pure solvents and solvent mixtures, pulses under analytical and preparative conditions, equilibrium adsorption isotherms and breakthrough measurements. Additionally, experimental results will include the preparative separation by fixed-bed chromatography using an Azura Prep LC unit equipped with two 250 mL/min pump heads and a XBridge Prep OBD C18 10 μm (250x30 mm) column with a 10 μm particle size diameter [2]. Experimental results presented in this work will stress the advantage of using an intermediate step based on achiral reversed-phase liquid chromatography to perform the separation of the two racemates of nadolol.

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The system was equipped with two preparative HPLC pumps P2.1L model with 250 mL/min pump heads, and one UV/VIS detector UVD2.1L model set at 270 nm wavelength. This preparative system was equipped with a Waters XBridge Prep C18 column of preparative diameters (30 mm ID x 250 mm L and particle size diameter of 10 μm). A flow-rate between 25 and 75 mL/min was used with this preparative column.

Results

The set of experimental and simulation results will include the screening of the mobile phase composition using the tree types of achiral adsorbents. Several reversed-phase solvents based on ethanol-water mixtures were tested in terms of resolution and dispersion, by means of loading pulses. Results presented will include the experimental measurement of the equilibrium adsorption isotherms (Fig. 2) and breakthrough experiments for all the three types of adsorbents and using the most promising solvent compositions.

Modelling and its validation is a crucial step to the accurate equilibrium and kinetic data estimation. Some simulation results for the preparative separation of the nadolol racemates by simulated moving bed technology will be also presented (See Fig. 3). Finally, some experimental results concerning the preparative separation of nadolol racemates using the Azura Fixed-Bed preparative HPLC system will be also presented.

Figure 1. The Azura Pilot Prep HPLC (Brigantia EcoPark).

Figure 2. Comparison between experimental and model results for the adsorption equilibrium isotherms (left) and selectivity (right) for the two pairs of nadolol racemates, as a function of their feed concentrations, using 30%ethanol/70%water mobile phase composition with 0.005% diethylamine (pH=11) and the three different C18 Waters columns: XBridge (diamonds), Shield (circles) and XSelect (triangles). All fittings use the linear + Langmuir competitive model.

Figure 3. SMB productivity (left) and solvent consumption (right) for the separation of nadolol racemates using the XBridge (solid lines), Shield (dashed lines) and XSelect (dotted lines) columns using a 30%ethanol/70%water with 0.005% diethylamine as mobile phase (pH=11) as a function of the nadolol feed concentration.

Conclusions

The optimization of preparative fixed-bed chromatography depends on the proper choice of the mobile phase composition. The separation of nadolol racemates was studied using different ethanol/water compositions with three different achiral C18 Waters materials (XBridge, XSelect and Shield) at both analytical and preparative scales. The design of the preparative separation process was studied, by means of loading pulses, the measurement of the adsorption equilibrium isotherms, breakthrough experiments using a 30%ethanol/70% water mobile phase composition. A linear+Langmuir model was found to describe well the adsorption behavior. Breakthrough experiments were also performed to validate the equilibrium model and to predict axial dispersion and mass transfer resistance. The equilibrium data was also used to predict the operating conditions for future extra simulated moving bed (SMB) operation. Additional experiments were carried out on a fixed-bed preparative system in order to optimize the separation of nadolol racemates. A mobile phase composition of 20% ethanol/80% water/0.1% diethylamine was selected to perform a sequential five-injection experiment to confirm the viability of fixed-bed operation for obtaining pure nadolol racemates.

Acknowledgements

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References

Solketal production from glycerol ketalization with acetone: Thermodynamic and reaction kinetic study

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*ruifaria@fe.up.pt

The main results of a thermodynamic and kinetic study of glycerol ketalization to produce solketal in the presence of ethanol, as solvent, are presented in this work, using Amberlyst-35 ion-exchange resin as catalyst for this reaction. Reaction equilibrium and kinetic parameters were determined in a batch reactor, in the absence of external mass transfer limitations. Concerning reaction thermodynamic equilibrium, it was obtained a standard enthalpy of \( \Delta H^\circ \approx -13.2 \pm 3.8 \text{ kJ mol}^{-1} \). Eley–Rideal (ER), Langmuir–Hinshelwood–Hougen–Watson (LHHW) and Pseudo-Homogeneous (PH) kinetic models were proposed to fit the obtained experimental data. LHHW reaction rate law could accurately describe the experimental results, considering the presence of internal mass-transfer resistances. The activation energy for the overall reaction was found to be 74.7 ± 17.0 kJ mol\(^{-1}\).

Introduction

The continuous demand of fossil-based fuels is leading to their depletion, resulting in a search for alternative fuels, such as biodiesel [1]. Biodiesel is mainly produced by a transesterification reaction using animal fats and vegetable oils as raw materials [2, 3]. Glycerol is obtained as the main by-product from this process. Consequently, in order to solve one problem, another is formed. The continued production of biodiesel led to a continued production of glycerol at such a rate that the sustainability and the economy of the biodiesel industry is becoming compromised.

In this background, a glycerol valorization strategy must be developed. There are several value-added applications to transform glycerol into other chemical commodities. One of particular interest is the glycerol ketalization reaction with acetone, converting glycerol into solketal (4-hydroxymethyl-2,2-dimethyl-1,3-dioxolane). Solketal is useful as oxygenated fuel additive, since it is capable of reducing particle emission and gum formation in gasoline, can improve the liquid properties for low temperature fuel transportation and optimize the octane number when blended with gasoline [4, 5].

There are several catalysts used for ketalizations. Among them, ion-exchange resins have been widely used for glycerol ketalization [6]. Therefore, ion-exchange resin Amberlyst-35 was selected as catalyst given its potential for sorption-enhanced processes comprising ketalization reactions according to the data reported in the open literature.

Objectives

In this work, an extensive study of glycerol ketalization with acetone was performed, with the intention of developing a glycerol valorization strategy, by converting this chemical commodity into solketal. Consequently, data was collected in order to describe the chemical equilibrium constant dependence with temperature and to estimate the reaction standard Gibbs free energy, and standard enthalpy, considering a nonideal liquid-phase model, determining the activity of each species through the UNIFAC group contribution method. The influence of several operating variables on the reaction rate, namely temperature, acetone/glycerol feed composition and solvent concentration, were experimentally studied, allowing to determine a model that describes the reaction kinetics over a wide range of operating conditions. Langmuir-Hinshelwood-

Hougen-Watson (LHHW), Pseudo-homogeneous (PH) and Eley-Rideal (ER) models were considered to describe reaction rate laws of the glycerol ketalization with acetone.

Experimental

The reaction experiments were carried out in a glass-jacketed autoclave, model Buchiglaustaster, from Germany, with a maximum capacity of 1 dm\(^3\). The mechanical stirrer has an adjustable speed between 0 and 2200 rpm. A thermostatic bath was coupled to the unit, controlling the system temperature, by continuous circulation of oil through the jacket of the autoclave.

The temperature and pressure of the experiment was measured by a thermocouple and a pressure sensor, respectively, which were monitored by a computer.

Results and Discussion

The study of external mass-transfer limitations consisted in evaluating the reaction at different stirring speeds, from 600 to 900 rpm, with an acetone/glycerol molar ratio of 2, dissolved in 30% ethanol (in molar basis), for 8h. After 200 minutes, the reaction reaches the equilibrium, with a maximum conversion of 70%. The reaction rate had the same evolution for 900 and 750 rpm, leading to the conclusion that external mass transfer issues are suppressed at 750 rpm. Therefore, subsequent experiments were carried out at 750 rpm.

Concerning the effect of temperature (in the range of 303 K to 323 K) on glycerol ketalization, it was possible to observe that a higher temperature led to a lower equilibrium conversion, as expected for an exothermic reaction. It was also clear that the initial rate of the ketalization reaction increases with temperature.

Feed composition was also studied. Experiments with different Acetone/Glycerol molar ratios, ranging from 0.5 to 2, showed that molar ratio above and higher than 1 led to a higher initial reaction rate and higher equilibrium conversion.

From the results obtained regarding the effect of solvent concentration, it was possible to observe that initial ethanol concentration is not very relevant for the solketal yield, within the concentration range studied.

Regarding reaction equilibrium, through the experiments carried out, it was possible to determine the dependence of the equilibrium constant on temperature, which is expressed as \( K_\text{eq} = 5.938 + 1584.1 / T \). Through the result obtained, it was

\[ \text{[C]} \]
possible to predict the standard Entropy, Enthalpy and free Gibbs energy values, which are given in Table 1.

Table 1. Standard Entropy, Enthalpy and free Gibbs for glycerol ketalization reaction with acetone.

\[
\begin{array}{lcc}
\Delta S^0 \text{ (kJ mol}^{-1}\text{ K}^{-1}) & 4.94 \times 10^{-2} \pm 1.20 \times 10^{-2} \\
\Delta H^0 \text{ (kJ mol}^{-1}\text{)} & -13.2 \pm 3.8 \\
\Delta G^0 \text{ (kJ mol}^{-1}\text{)} & 1.54
\end{array}
\]

As mentioned previously, 3 different reaction rate laws were considered to described the kinetic behavior of the studied reaction, namely ER, LHHW and PH. A comparison between a concentration based reaction rate law and an activity based reaction rate law was performed for all cases, as well as presence and absence of internal mass-transfer resistances. The results obtained are depicted in Table 2 and it was possible to observe that the value of the reaction activation energy increases when resistance to the diffusion of the compounds through the catalyst pores was considered, for both LHHW, ER and PH models. This result is an indication that this phenomenon must be taken into account. The error associated to the activation energy of the different reaction rate laws was higher for ER and PH models than for LHHW model, corresponding to 24% and 29%, respectively. From the results presented in Table 2, one can conclude that the LHHW model including intraparticle mass-transfer limitations can describe the experimental results more accurately than the other models tested, as it presents the highest coefficient of determination. This model considers a surface reaction between the adsorbed reactants, where the water formation is considered to be the rate limiting step. For the LHHW model, it was possible to determine a reaction activation energy of 74.7 ± 17.0 kJ mol\(^{-1}\).

**Conclusion**

The work presented intends to report a detailed study of the glycerol ketalization reaction, for the synthesis of solketal, describing its thermodynamic equilibrium and kinetics. Chemical equilibrium data was gathered for temperatures ranging from 303 K to 323 K. The standard enthalpy and free Gibbs energies at 298 K were found to be ∆H\(^0\)= -13.2 ± 3.8 kJ mol\(^{-1}\) and ∆G\(^0\)= 1.54 kJ mol\(^{-1}\), respectively, using the UNIFAC model to determine the activities of each species. All the reaction kinetics results were obtained after ensuring that the experiments were performed in the absence of external mass transfer limitations. It was concluded that external mass transfer resistances are negligible if the stirring speed was set to 750 rpm or faster. The experimental results revealed that the reaction kinetics behavior could be accurately described by the LHHW reaction rate law, considering the presence of internal mass-transfer resistances. The activation energy for the overall reaction was found to be 74.7 ± 17.0 kJ mol\(^{-1}\).

<table>
<thead>
<tr>
<th>Model</th>
<th>(E_a) (kJ mol(^{-1}))</th>
<th>(k_{eq}) (mol kg(^{-1}) s(^{-1}))</th>
<th>(K_{S,W})</th>
<th>(R_{model}^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LHHW with mass transfer resistance and activity coefficients</td>
<td>74.7 ± 17.0</td>
<td>6.84 ± 2.92</td>
<td>17.0 ± 6.7</td>
<td>0.960</td>
</tr>
<tr>
<td>LHHW without mass transfer resistance and with activity coefficients</td>
<td>49.4 ± 10.0</td>
<td>0.67 ± 0.15</td>
<td>9.9 ± 2.7</td>
<td>0.946</td>
</tr>
<tr>
<td>LHHW with mass transfer resistance and concentration</td>
<td>72.1 ± 16.8</td>
<td>5.65 \times 10^{-2} \pm 2.13 \times 10^{-2}</td>
<td>1.1 ± 0.4</td>
<td>0.955</td>
</tr>
<tr>
<td>LHHW without mass transfer resistance and concentration</td>
<td>45.5 ± 9.6</td>
<td>5.94 \times 10^{-1} \pm 1.12 \times 10^{-1}</td>
<td>0.8 ± 0.2</td>
<td>0.943</td>
</tr>
<tr>
<td>ER with mass transfer resistance and activity coefficients</td>
<td>74.0 ± 17.4</td>
<td>7.82 ± 5.30</td>
<td>61.7 ± 52.8</td>
<td>0.956</td>
</tr>
<tr>
<td>PH with mass transfer resistance and activity coefficients</td>
<td>64.0 ± 18.6</td>
<td>1.88 ± 0.33</td>
<td>-</td>
<td>0.935</td>
</tr>
</tbody>
</table>

**Acknowledgements**

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**References**

Spinel type – carbon based nanocomposites for the magnetically assisted removal of Hg and As species from different aqueous matrices

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Introduction

Two of the most harmful toxic elements among the natural and anthropogenic contaminants, especially regarding to their bioaccumulative character, are As and Hg, which occupy, respectively, the first and third place on the list of priority hazardous substances of 2015 provided by the Agency for Substances and Toxic Diseases. Whereas the World Health Organization (WHO) drinking water guidelines for As and Hg are 10 μg/L and 6 μg/L, respectively. There some studies reporting the co-occurrence of mercury and arsenic in groundwater. These cases mainly concern tailings of mining sites with mercury and arsenic. Remediation technologies for natural waters contaminated with toxic elements, include catalytic processes, membrane processes, ion-exchange, adsorption and ionizing radiation processes. However, it is important that water purification methods are affordable to developing nations, hence they should be available at a low cost, which for most of these techniques is not the case. For this reason, the adsorption process is generally preferred since it meets this need due to its easy handling and the wide variation in available natural and synthetic sorbents. During the past decade magnetic nanoparticles as separating agents have attracted world-wide research attention [1]. Reason to this are not only the remarkable magnetic properties that make them easily separated from water with an external magnetic field, but also the high surface area, adaptable morphology and high efficiency [2]. Among these nanoparticles, spinel ferrites (SF) received most attention because of their superparamagnetic properties, high surface to volume ratio and their nanometer size. However, there are some challenges in the application of SF for water remediation. First, due to their high surface energy, van der Waals forces and magnetic interactions, spinel particles tend to form aggregates. This results in a decrease on the amount of active sorption sites. Second, only little is known about the stability of spinel particles, and thus little can be stated about the leaching of metal ions into solutions from spinel particles during the sorption or desorption processes. Third, although the nanosize of spinel particles is a property that makes them ideal for the sorption of toxic elements, the small size also has some disadvantages. Due to the small particle size, it is difficult to use the SF particles in a continuous flow system (Chandra et al., 2010). Even more important to mention, is the ongoing debate among scientists on the safety aspects of nanoparticles. In general, there is still a lack of known about the toxicity of nanoparticles, and consequently little is known about their environmental impact, hence risk assessments cannot be made (Mueller et al., 2010).

An approach to overcome these problems that has been chosen in some studies, is the creation of composites by attaching the SF particles to a supporting material.

Objectives

Therefore, the aim of this research was to synthesize different composites by attaching spinel ferrites on supporting material, in this case exfoliated graphite, and subsequently to evaluate their capability to uptake Hg(II) and As(III) from different aqueous matrices that (partly) represent realistic environmental scenarios.

Methods

Exfoliate graphite was obtained by ultrasonic treatment (5 h) of graphite (5 g) in 100 mL of dimethylformamide. The colloidal spinel ferrites (MFeO₄, M=Fe, Mn or Co) were prepared via oxidative hydrolysis [3]. The MFeO₄/EG composites were prepared by two different techniques. In the first approach, the composites were obtained by electrostatic assembly of the MFeO₄ NPs to the sheets of exfoliated graphite, while in the second the composites were obtained by in situ oxidative hydrolysis. The capability of the composites and their respective SF NPs, to remove Hg(II) and As(III) from model solutions was studied for binary systems at pH 6. The removal capability of each material was assessed by exposing equal amounts of material (40 mg/L) to solutions of Hg(II) or As(III), with an initial element concentration of respectively, 50 μg/L and 1000 μg/L. Batch experiments were conducted at a maximum period of 24 h in 100 mL.

Six magnetic composites were synthesized by two methods, using 3 types of spinel ferrites (SF: MFe₃O₄· M=Fe, Co or Mn) and exfoliated graphite(EG). In the first method(EG1), the SFs were synthesized prior to addition to EG and the composites were prepared by electrostatic assembly, while in the second one (EG2), the SFs were synthesized in the presence of EG by in situ oxidative hydrolysis process. The composites were studied as sorbents for the removal of two toxic elements (As and Hg), from different aqueous matrices. The removal efficiency of all composites was evaluated in batch conditions, at Hg and As wastewater guideline concentration, at pH 6. In general, most Hg removal was seen for the SF@EG1 composites, while most As removal was seen for the SF@EG2 composites. The lowest Hg and As removal was seen for all the Fe₃O₄ materials. As a proof of concept, composites were applied in As contaminated groundwater, and >90% removal was seen for both CoFe₃O₄@EG2 and MnFe₃O₄@EG2.

As removal (%)
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mL-Schott® glass flasks at 22 °C, with stirring (250 rpm), after dispersion of the sorbent in an ultra-sound bath. Quantification of Hg(II) in solution was performed by cold vapor atomic fluorescence spectroscopy and the quantification of As was performed by hydride generation atomic fluorescence spectroscopy.

As a proof of concept, the most promissory sorbents were applied to treat a real groundwater sample, collected from a well in Estarreja (Portugal), an area with a historical record of contamination with mercury and arsenic.

Results

At neutral pH values, which in this study is the most representative for real water matrices, generally the CoFe₂O₄ and MnFe₂O₄ particles showed the highest removal rates for As and Hg, from unary model solutions. From the composites, generally the @EG2 composites showed higher removal percentages, and from all materials, the MnFe₂O₄ particles and the MnFe₂O₄@EG2 composites showed the highest removal percentages of As and Hg from the solutions at neutral pH.

The removal of Hg and As from binary model solutions was investigated for the materials that had better performance for unary solutions: CoFe₂O₄@EG2 and MnFe₂O₄@EG2 composites.

For arsenic, as in the mono element experiment, the CoFe₂O₄@EG2 composites showed a larger removal percentage than the MnFe₂O₄@EG2 composites (Figure 1). In the multi element system, the removal percentage of CoFe₂O₄@EG2 was larger (91%) than in the mono element system (84%). Also, according to the mass balance calculations more arsenic was adsorbed per mass of adsorbent in the multi element system (23.6 mg/g) compared to the mono element system (19.8 mg/g). The arsenic removal percentage of MnFe₂O₄@EG2 however was slightly larger for the mono element experiments (73%) than the multi element experiments (66%). According to the mass balance calculations however, an approximately similar amount of arsenic was adsorbed by MnFe₂O₄@EG2 in the mono- (17.0 g/mg) and multi element system (17.1 g/mg).

For mercury, as in the mono element experiment, in the multi element system removal percentage of the MnFe₂O₄@EG2 was larger than the removal of the CoFe₂O₄@EG2 composites. As was seen for arsenic, the removal percentage of CoFe₂O₄@EG2 was slightly larger in the multi element system (57%) than in the mono element system (50%), as well as the adsorbed mercury per sorbent mass (0.7 and 0.6 mg/g, respectively). For the MnFe₂O₄@EG2 composites, the removal percentage in the multi element system (84%) was lower than in the mono element system (91%), as well as the calculated amount of adsorbed mercury per sorbent mass (0.98 and 1.1 mg/g, respectively).

As a proof-of-concept CoFe₂O₄@EG2 and MnFe₂O₄@EG2 composites were applied to a groundwater collected from a well in the region of Estarreja (Portugal). This water had an As content of around 200 µg/L. The levels of Hg were low (<1µg/L), so we only look to the removal of As. As display in Figure 2, both CoFe₂O₄@EG2 and MnFe₂O₄@EG2 showed an almost complete removal of arsenic (91 and 97%, respectively). In control flasks only, a decrease of 2% in the arsenic concentration was seen that corroborates the efficiency of these composites in real water samples.

Figure 2 Arsenic removal from groundwater collected from a well in Estarreja region (Initial As concentration = 200µg/L, pH=6.8, sorbent concentration 40g/L)

Conclusions

Six types of composites were successfully synthesized according to two distinct methods, containing one of the three types of spinel ferrites (SFs; Fe₂O₃, MnFe₂O₄ and CoFe₂O₄) combined with exfoliated graphite. It was found that synthesis via in situ oxidative hydrolysis (EG2), results in composites that were less stable and contained relatively large amounts of the SF component compared to the composites synthesized via an electrostatic assembly (EG1). In general, arsenic removal was mainly governed by the SF component of the composites whereas for mercury removal the EG played an important role as well. Among all the composites examined in this study, the CoFe₂O₄@EG2 and MnFe₂O₄@EG2 composites can be considered as the most promising sorbents for As(III) and Hg(II) removal, respectively.

Acknowledgements

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References

Carbon dioxide ennoblement via catalytic bi-reforming of methane

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Introduction
The current dependence on non-renewable resources, in particular oil, to obtain energy and most chemicals needed to support modern lifestyles has resulted in growing environmental impacts. Climate change, due to the emission of greenhouse gases (GHG), in particular CO2 and CH4, is considered one of the most significant impacts. Nowadays, strategies to minimize its effects involve the capture of CO2 and the utilization of renewable energy to obtain other compounds that can be further used as fuels or as basic feedstocks [1].

To avoid the impacts resulting from increasing emissions of greenhouse gases, in particular carbon dioxide and methane, there is a need to develop new processes that may reduce net carbon emissions and contribute to a more circular economy. Bi-reforming of methane (BRM) is a promising method for syngas production, with a hydrogen-to-carbon monoxide ratio of two, relevant for example, when the purpose is methanol synthesis. In this work, reaction studies were carried out over a nickel-based catalyst varying the temperature (798-1123 K). Results show that syngas gas can be successfully produced using this process. For the range of operating conditions studied, the carbon dioxide and methane conversions increase with temperature, reaching 100 % and 40 %, respectively. Future work will analyze the influence of other factors, in particular using higher amounts of catalyst or different feed mixtures, with the goal of approximating the hydrogen-to-carbon monoxide ratio to levels more suited for methanol production.

Experimental work
A commercial 15 wt % Ni/γ-Al2O3 catalyst (Octolyst 1001), supplied by Evonik (earlier Degussa AG) in form of extrudates was used in this work as catalyst [4]. A detailed discussion on the characterization of the catalyst can be found in previous reports [4,6]. Its most relevant properties are presented in Table 1. The experiments were performed at temperatures in the range of 798 to 1123 K, in 25 K intervals, and at atmospheric pressure, in a continuous flow tubular reactor. The feed had a carbon-to-water molar ratio of 2 ([3CH4 + 1CO2): 2H2O] corresponding to flows of 30 cm³/min of CH4, 20 cm³/min of H2O and 10 cm³/min of CO2 with no inert. A mass of catalyst of 15 g was used in all experiments.

Table 1. Properties of catalyst used [4].

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Ni (wt%)</th>
<th>Al2O3 (wt%)</th>
<th>S BET (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Octolyst</td>
<td>15.4</td>
<td>84.6</td>
<td>250</td>
</tr>
</tbody>
</table>

Conversions of the reactants (CH4, CO2 and H2O) were calculated as described in eq. (4):

$$X_i[\%] = \frac{n_{i,0} - n_i}{n_{i,0}} \times 100\%$$

(4)

where $n_{i,0}$ represents the molar flowrate of CH4, CO2 and H2O in the feed, respectively. $n_i$ are the outlet molar flowrates of these reactants. The molar fractions $y_i$ (or partial pressures) were calculated for all species present in the outlet gas stream according to eq. (5).

$$y_i = \frac{n_i}{\sum_i n_i} = \frac{p_i}{P} = \frac{\sum_i p_i}{P}$$

(5)

where $n_i$ represents the molar flowrate of one species and $\sum_i n_i$ represents the total molar flowrate of the species in the outlet stream. Equilibrium conversions and reactant/product composition are provided directly in the experimental figures. The reactor setup for BRM is depicted in Figure 1. Details about the reactor setup can be found in a previous study [3].

Results and Conclusion
CH4, H2O and CO2 conversion versus space time, at different temperatures, are shown in Figure 2 for the catalytic vapor phase reaction. Partial pressures of CH4, H2O and CO2, CO and H2 are also shown in Figure 2.

The results highlight that, although the reactions occur simultaneously, they occur in different extents - different conversions relative to each of the 3 reactants were reached under different operating conditions, being the conversion relative to CO2 the lowest one, this shows that the DRM is the
limiting step. This is particularly important at the lower temperatures used in the tests. As expected for endothermal reversible reactions, increasing temperatures and space time lead to increasing conversions of the reactants (Figure 2a). At temperatures of 873 K and higher, CH₄ is easily converted, while H₂O and especially CO₂ require higher reaction temperatures. At a reaction temperature of 1123 K, almost all CH₄ is converted at a space time of 0.024 s, while H₂O and CO₂ only reach conversion levels of 90 and 35 %, respectively. With increasing space time, the conversions of the reactants also increase. On the other hand, the partial pressures (p) of the reactants decrease while the partial pressures of the products increase (Figure 2b). Examining the logarithm of the equilibrium constants as function of the temperature of the reactions considered [2] it can be seen that the steam CH₄ reforming (SRM), water gas shift, catalytic CH₄ decomposition, carbon gasification and especially dry CH₄ reforming (DRM) are favored with increasing temperatures at low pressure. The moderate H₂O conversions or the lower decrease in partial pressure of H₂O at lower reaction temperatures is typical in catalytic reactions. This is a consequence of the relative high bond energy (O-H) in the H₂O molecule that requires high activation energy to break. A relative low CO₂ or a low decrease of CO₂ partial pressure is observed due to the very strong and stable bond energy of the mentioned molecule. One can clearly observe that for a catalyst mass of 15 g and a reaction temperature of 1123 K (the highest used in this work) the conversion of CO₂ did exceed the 40% barrier.

Figure 2: Conversion vs. reaction temperature (a) associated with product distribution (b) vs. reaction temperature at steady-state conditions. Operating conditions: m_{cat} = 15.0 g; P = 101.325 kPa; \dot{\text{inert}} = 0 Ncm/min.

Future work
Future work will focus on analyzing the effects of varying the mass of catalyst and the feed mixture, with the main goal of determining the most adequate conditions to obtain, in the reaction products, a H₂ to CO ratio more suited for methanol production, thus facilitating the integration of the BRM with methanol production processes.

Acknowledgements
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References
Nowadays, the development of effective methodologies for the separation of alcohol and alkane mixtures is a challenge for chemical engineers. The main reason is due to the proximity of the boiling points of these azeotropic mixtures [1]. One of the most common separation techniques is the azeotropic or extractive distillation. However, extreme conditions (high temperatures and high pressures) are required to carry out this kind of processes. Therefore, a large amount of energy is necessary to successfully achieve the separation, which makes it economically unattractive due to the increase in production costs. Liquid-liquid extraction is a technique based on the immiscibility of two liquid phases which can have different economically advantages when solvents that are not volatile are used. In this case, this solvent can be recovered, and no special equipment is required. This process is a respectful alternative with the environment.

In recent years, the azeotropic separation of an alcohol and an aliphatic hydrocarbon (ethanol-hexane and ethanol-heptane) have been studied using ionic liquids (ILs) as extraction solvents because ILs have emerged as a more ecological alternative to replace the volatile organic solvents used in the chemical industry in this type of processes. However, one of the great disadvantages of using ionic liquids as extraction solvents on an industrial scale is the high cost, and complex synthesis [2], compared to those of traditional organic solvents. In this matter, the aprotic ionic liquids have attained a greater attention to be used in separation processes. These ionic liquids derive from the neutralization reaction between organic acids and ethanolamine, reducing the costs and complexity of the synthesis methodology [3].

In this work, three aprotic ionic liquids (2-hydroxyethylammonium formate, [N002001][HCO2], 2-hydroxyethylammonium propanoate, [N002001][C3H4CO2], and 2-hydroxyethylammonium butyrate, [N002001][C3H5CO2]) were used in the separation of azeotropic mixtures ethanol + hexane and ethanol + heptane. The liquid-liquid equilibria (LLE) of the aforementioned systems were determined at 101.2 kPa and 298.15 K and are represented in Figure 1. In addition, the binary parameters of NRTL were determined with the Aspen Plus V.9.

Figure 1 shows the triangular diagram for ternary system heptane + ethanol + [N002001][HCO2] where the tie-lines have positive slopes, indicating that the ethanol has affinity with these aprotic ionic liquids. The values of the distribution coefficient and selectivity for the separation of ethanol and heptane are represented in the Figure 2 for all ionic liquids studied in this work. The results show that these values increase in the following trend [N002001][HCO2] > [N002001][C3H4CO2] > [N002001][C3H5CO2].

In summary, the use of aprotic ionic liquids as extraction solvents for the separation of the azeotropic mixtures ethanol-hexane and ethanol–heptane by liquid–liquid extraction was performed. The results demonstrated a high efficiency in the separation of these two azeotropes where the [N002001][HCO2] IL showed the best results. The increment of the hydrogenated alkyl side chain of the anion reduces the efficiency in the extraction of alkanes from these azeotropic mixtures. Moreover, NRTL model can adequately correlate the experimental LLE data. Finally, the three ionic liquids investigated can be suitable solvents in the liquid + liquid extraction for the separation of azeotropic mixtures heptane/ethanol and hexane/ethanol.
Figure 2. Selectivity (S) versus molar fraction of ethanol in the hydrocarbon phase for the ternary system heptane + ethanol + ionic liquid at 298.15 K.

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References
New extension of the Liu-Silva-Macedo model to multicomponent Lennard-Jones intradiffusivities

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Transport properties such as molecular diffusion coefficients ($D_{12}$) are very important for the accurate design and optimization of industrial processes involving mass transfer. However, there is a lack of experimental diffusion coefficients, especially for multicomponent systems, and accurate models for $D_{12}$ estimation.

In this work, a new extension of the Liu-Silva-Macedo model to multicomponent Lennard-Jones intradiffusivities (New-LSM) is presented. The model was devised and the embodied parameters optimized using a database containing 1457 experimental points spanned across 131 ternary systems. Two model variants were developed, one for prediction and another for correlation. The New-LSM predictive model achieved an average relative deviation of 7.93 % that range from 0.73 to 41.34 %, while the New-LSM 1-parameter correlation attained an average deviation of 4.15 % ranging from 0.23 to 15.75 %.

Knowledge of transport properties is extremely important for accurate modelling and equipment design for processes involving mass transfer phenomena such as supercritical fluid extraction (SFE) [1]. In SFE it is increasingly used CO2 modified with a co-solvent in order to tune the affinity of the solvent mixture to specific solutes. Hence there is a need for transport properties such as diffusion coefficients ($D_{12}$) for solutes in solvent mixtures [2]. Currently, the experimental data for multicomponent systems, both supercritical and liquid, are scarce. Moreover, there is an absence of accurate models for these cases [3].

One of the most important models for diffusivity estimation is that of Liu-Silva-Macedo [1], originally developed for self-diffusion coefficients and later extended to binary tracer diffusion (TLSM) [1]. The model was later extended to multicomponent Lennard-Jones (LJ) intradiffusivities by Merzlikin and Pfennig [1], who introduced a set of mixing rules and five embodied constants optimized using molecular dynamics (MD) data.

The new extension of the Liu-Silva-Macedo model to multicomponent intradiffusivities (New-LSM) is based on the same mixing rules proposed by Merzlikin and Pfennig (LSM-MP), but an extensive database was used, namely, 1457 experimental points spanning across 131 ternary systems, namely 90 liquid systems and 41 supercritical systems.

In this essay, the number of constants was reduced, the mixing rules were revised, and an empirical parameter was introduced. The resulting predictive and 1-parameter correlation forms of the New-LSM model both show an improved performance when compared to the LSM-MP model, which showed relative deviations from 4.81 to 86.22 % with an average error of 36.40 %.

The predictive form of the New-LSM model showed relative deviations comprehended between 0.73 to 41.34 % with an average of 7.93 %, while the 1-parameter correlation of New-LSM achieved relative deviations between 0.23 to 15.75 % and an average of 4.15 % (see Table 1). In Figure 1 can be seen a graphical representation of the relative error dispersion as function of the experimental $D_{12}$.

![Graphical representation of the relative error dispersion as function of the experimental $D_{12}$](image)

Figure 1. Relative error of both New–LSM predictive model and 1-parameter correlation versus datapoints ($D_{12}^{exp}$).

Furthermore, the Dymond–Hildebrand–Batschinski (DHB) [1] correlation and the predictive Wilke-Chang equation [4] were also tested using the same database. The DHB achieves relative deviations between 0.10 and 22.92 % with an average of 8.05 %, while the Wilke-Chang equation shows higher relative deviations, in the range 1.40-146.13 % with an average of 15.47 %. When comparing the relative deviations achieved by the Wilke-Chang equation to the ones obtained with the new model,
the last one exhibits better performance. Regarding the DHB equation, this one shows lower relative deviations than the new correlative model, but attains higher relative deviations. It should be noted that the new proposed correlation has only one adjustable parameter while the DHB equation has two, showing the robustness of the New-LSM. Although the new models exhibit a good performance it is important to mention the shortage of the database used in this studied. All the main results presented are summarized in Table 1.

Table 1. Number of parameters, and minimum / maximum (average absolute relative deviations, AARD) achieved by each model evaluated for $D_{12}$ with an extensive database.

<table>
<thead>
<tr>
<th>Model</th>
<th>No. param</th>
<th>AARD$_{min}$ (%)</th>
<th>AARD$_{max}$ (%)</th>
<th>AARD$_{mean}$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LSM-MP</td>
<td>0</td>
<td>4.81</td>
<td>86.22</td>
<td>36.40</td>
</tr>
<tr>
<td>New-LSM predictive model</td>
<td>0</td>
<td>0.73</td>
<td>41.34</td>
<td>7.93</td>
</tr>
<tr>
<td>Wilke-Chang</td>
<td>0</td>
<td>1.40</td>
<td>146.13</td>
<td>15.47</td>
</tr>
<tr>
<td>New-LSM 1-parameter correlation</td>
<td>1</td>
<td>0.23</td>
<td>15.75</td>
<td>4.15</td>
</tr>
<tr>
<td>DHB</td>
<td>2</td>
<td>0.10</td>
<td>22.92</td>
<td>8.05</td>
</tr>
</tbody>
</table>

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References
Experimental and modeled diffusivities of metal acetylacetonates in liquid ethanol, and comparative insights with their diffusivity in supercritical CO₂

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Diffusion coefficients ($D_{12}$) are important for the modeling, design and optimization of rate-controlled processes. In this essay, the $D_{12}$ of five metal acetylacetonates (Me(acac)$_n$), namely palladium(II) acetylacetonate, chromium(III) acetylacetonate, vanadyl(II) acetylacetonate, nickel(II) acetylacetonate and titanium(IV) oxacyclacetinate, were measured in liquid ethanol using the chromatographic peak broadening technique. The measurements were performed at atmospheric pressure at temperatures between 303.15 and 333.15 K. The obtained $D_{12}$ values ranged from 0.789x10$^{-5}$ to 2.00x10$^{-5}$ cm$^2$ s$^{-1}$. Furthermore, our $D_{12}$ values in ethanol were compared with the corresponding Me(acac)$_n$ in supercritical CO$_2$ (SC-CO$_2$) compiled from the literature. Both SC-CO$_2$ and ethanol data were modeled with well-known literature equations, having achieved relative deviations from 0.32 to 34.71%.

Transport properties such as thermal conductivity, viscosity and diffusivity are essential for proper modeling, design, scale-up and optimization of processes involving heat, momentum and heat transfer phenomena [1]. Since no proper universal theory exists for estimation of diffusion coefficients ($D_{12}$), due to the complexity of intermolecular potentials involved, it becomes essential to determine $D_{12}$ experimentally.

Metal acetylacetonates, usually represented as Me(acac)$_n$, are organometallic complexes consisting of anions and metal cations. Due to their high solubility in organic solvents and thermal stability, these compounds can be found in a series of industrial applications such as semiconductors and as catalysts in organic reactions [2-4].

In recent years, the trend is to replace traditional organic solvents by so-called green solvents such as ethanol but also supercritical CO$_2$ (SC-CO$_2$). The combination of supercritical fluids with organometallic complexes currently offers some interesting applications such as in the preparation of nanomaterials [5].

In this work, the $D_{12}$ of five Me(acac)$_n$ were determined by the chromatographic peak broadening (CPB) [6] technique. The CPB technique comprises injecting a solute impulse in a capillary column where a solvent stream is passing under laminar regime. The pulse will broaden due to the effects of both axial convection and axial dispersion (Figure 1). The $D_{12}$ value can be obtained by fitting the response curve to:

$$C(L,t) = \left( \frac{m}{4\pi D_{12} t} \right)^{1/2} \exp \left[ -\frac{(L-u_0 t)^2}{4 D_{12} t} \right]$$  \hspace{0.5cm} (1)

and

$$D_{12} \equiv \frac{R^2 u_0^2}{48 \epsilon}$$  \hspace{0.5cm} (2)

where $C$ (mol cm$^{-3}$) is the solute concentration, $m$ (mol) the quantity of injected solute, $R$ (cm) the column radius, $L$ (cm) the column length, $t$ (s) time, and $u_0$ (cm s$^{-1}$) the average linear velocity. The fitting process consisted on the minimization of the root mean square error, $\epsilon$:

$$\epsilon(\%) = \left\{ \frac{\sum_{i=1}^{n}[C_{\text{calc}}(L_i) - C_{\text{exp}}(L_i)]^2}{\sum_{i=1}^{n}[C_{\text{exp}}(L_i)]^2} \right\}^{1/2} \times 100$$  \hspace{0.5cm} (3)

where $C_{\text{exp}}$ is the experimental concentration and $C_{\text{calc}}$ the calculated by equation 1.

Figure 1. Typical response of CPB technique [7].

The $D_{12}$ measurements of palladium(II) acetylacetonate, Pd(acac)$_2$, chromium(III) acetylacetonate, Cr(acac)$_3$, vanadyl(II) acetylacetonate, VO(acac)$_2$, nickel(II) acetylacetonate, Ni(acac)$_2$, and titanium(IV) oxacyclacetinate, TiO(acac)$_2$, were performed in ethanol at temperatures ranging from 303.15 to 333.15 K, and at atmospheric pressure. In a preliminary stage, a set of exploratory runs were carried out to determine the optimum wavelength ($\lambda$) for $D_{12}$ determination. For the five solutes in study, this was found to be in the range of 236 and 367 nm. The determined diffusivities scored from 0.789x10$^{-5}$ to 2.00x10$^{-5}$ cm$^2$ s$^{-1}$. Furthermore, the results were modeled with well-known expressions from the literature, namely, Wilke-Chang, the semi-empirical correlations of Magalhães et al., and a modified Stokes-Einstein [7] equation, achieving relative deviations from 0.32 to 34.71%.

These measurements were then compared with experimental diffusivities in supercritical CO$_2$: for Pd(acac)$_2$ and Cr(acac)$_3$ [7-9], retrieved from the literature for the following operating conditions: 90-400 bar, and 308.2-343.2 K. Under these conditions, the $D_{12}$ values in SC-CO$_2$: varied from 0.448x10$^{-6}$ to 1.628x10$^{-4}$ cm$^2$ s$^{-1}$. Moreover, with a hydrodynamic based equation, these experimental results achieved relative deviations from 3.15 to 5.02 %. In fact, the supercritical results show the same trend as in ethanol for the corresponding Me(acac)$_n$ being...
possible with the same expression to estimate $D_{12}$ in any of the two solvents.

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References
A new test to measure the degree of deionization required for tartaric stabilization of wines by electrodialysis

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In this work, a controlled freeze-thawing test for the evaluation of the deionization degree (DD), which is associated to wine tartaric stabilization by electrodialysis, is proposed. Wine samples are frozen at -20°C in a single direction, from bottom to top, thereby ensuring their controlled nucleation, maintained frozen for 24 hours and finally thawed at 0°C. Results of this test were compared with those obtained from the well-known mini-contact test. The deionization degrees obtained with the mini-contact test show an under-prediction between 5 and 9% when compared to the new test. Based on these results this new method proved to be more reliable and reproducible than the mini contact test, giving more precise DD values to be used as a set-point in the tartaric stabilization of wines by electrodialysis.

Introduction
Tartaric stabilization of wines by Electrodialysis is a process that has been used for more than one decade by many wine makers worldwide. As this process removes potassium and bitartrate ions from the wine, it is necessary, beforehand, to predict the deionization degree (DD) that renders the wine stable [1]. This DD has been estimated, so far, through the measurement of the degree of tartaric instability, which can be defined as the percent reduction of the conductivity of wine in a mini-contact test during 4 hours at -4°C, with inoculation of 4 g/L of micronized KHT [2,3]. To improve the robustness of the method, an exponential decay curve is usually fitted to the time curve of the conductivity and the final conductivity of the wine is estimated through extrapolation to infinite time [4]. This approach is, however, far from ideal, for different reasons: (i) it may under-predict the DD of the wine. Also the final stability of the treated wine must again be checked using a more robust mini-contact test, called ISTC50 [5]; (ii) when the wine fails this check, it is necessary to stabilize it again, which introduces further delay and additional costs in the wine production chain; (iii) requires a well-equipped laboratory that must be operated by a skilled operator capable of preparing the wine samples and analyzing the results; (iv) its complexity renders the technology less familiar to winemakers and is an additional obstacle to the adoption of electrodialysis, specially by small wineries.

Bearing in mind the unquestionable importance of having a precise and reliable method to determine the DD to be imposed to electrodialysis it is here proposed a robust and simple test to measure the tartaric instability of wines based upon a technique of freezing and thawing the wine in controlled conditions. The precise control of the freezing step is essential to obtain reproducible results, as the wine always follows the same space-time freezing pattern. Different types of wines were tested and the predicted DD was compared to results obtained from the mini-contact test with extrapolation to infinite time.

Methods
Controlled freeze-thawing test
This test is performed using a specially designed device. It consists on a metal reservoir containing an interior support where the bottom of glass flasks rest. A superior perforated plate (12 holes) keeps the flasks still. Water is poured into the metal reservoir, until it reaches the bottom of the flasks, and the device is then placed in a regular freezer with a temperature of -20°C overnight to form ice. The added water, once frozen at -20°C, has sufficient sensible heat to ensure a fast and complete freezing of the 12 wine samples [6]. Once the ice is formed, the device is removed from the freezer and placed inside an open insulated box. A volume of 100 µL of water is added to each flask to ensure that ice starts to grow immediately after the addition of the wine. This procedure ensures that ice nucleation within the wine is always controlled. Then, 10 mL of wine are added and the freeze-thawing device is placed back in the freezer during 6, 24 and 48 hours. After this time, it is placed again in an open insulated box and previously cooled water (in a regular refrigerator) is poured until near the top of the flasks in order for the wine samples to thaw at 0°C. After 30 minutes, the specific conductivity, κ, of the thawed wine and of the reference wine samples is measured. The degree of deionization of each wine sample is then computed by Eq.1.

\[
DD(\%) = \left( \frac{k_{\text{initial}} - k_{\text{final}}}{k_{\text{initial}}} \right) \times 100 \quad (\text{Eq.1})
\]

Mini-contact test. The mini-contact test consists on the inoculation of the wine with 4 g/L of micronized KHT, at -4°C. Wine samples are kept inside a thermostated jacketed glass vessel that is continuously stirred and the specific conductivity of the wine is measured and registered at regular time intervals during 5 hours. Eq. 2 is then fitted to the experimental results [4] to predict the conductivity at infinite time.

\[
\ln \kappa = \frac{a + ct}{1 + bt} \quad (\text{Eq.2})
\]

Where κ is the specific conductivity of the wine in µS.cm⁻¹, t is the time in minutes and a, b and c are fitting parameters.

Results
The deionization degrees obtained from the controlled freeze-thawing test increase with the time during which the wine is kept frozen, as seen in Figure 1. For each of the five types of wine it was necessary to increase the freezing holding time to 24 hours to obtain higher deionization degrees in agreement with typical reported values [7, 8]. These results indicate that this test sub-estimates the DD when the freezing holding time is less than one day. For 24 and 48 hours the triplicate results are consistent, the
difference being no greater than 3% between the maximum and minimum DD values (black dashes at each bar).

![Graph comparing DD values](image)

Figure 1 - Comparison of the DD from the controlled freeze-thawing test for freezing holding times of 6, 24 and 48 hours.

In order to understand the DD difference between freezing holding times of 24 and 48 hours, a sample of 30 mL of the wine Rosé 2, submitted to the controlled freeze-thawing test and completely thawed, was then moved to a jacketed glass vessel at 0°C, with magnetic stirring, and the conductivity was measured at regular time intervals during 3 hours. As shown in Figure 2, the limiting specific conductivity of the wine tends to the same value for both freezing holding times. The wine that was kept frozen during 48 hours has an initial deionization degree of 40%, which decreases, however, to about 34% after 20 min of continuous agitation at 0 °C. On the other hand, the DD of the sample submitted to a freezing holding time of 24 hours shows practically no variation when stirred. This result suggests that the excess of potassium or calcium bitartrate that precipitates while the wine is frozen needs some time to redissolve when the wine thaws.

In the graphical abstract is compared the DD obtained from the controlled freeze-thawing test with that of obtained through the mini-contact test. For six different wines, the DD predicted by the last is at least 5,7% lower than the corresponding value obtained by the new proposed test. For the white wines, in particular, this difference can reach 9%. All the limitations associated with the mini-contact test, these results suggest that the new test predicts a more precise DD. First of all, the extrapolation of the mini-contact test results to infinite time cannot be used as an absolute method to predict the DD. By common sense, it is known that any extrapolation using an empirical equation is prone to prediction errors. This is particularly true when the mini-contact test is trying to predict in only 4 hours a natural phenomenon that may take several weeks to complete, when the wine is cooled down to negative temperatures [3]. Another intrinsic limitation of the mini-contact test associated with the extrapolation to infinite time is related with the fact that, for some wines, it is possible to fit the experimental data using different parameters, obtaining, in the end, different DD. For all these reasons, comparing the results of both methods is unquestionable that the controlled freeze-thawing test presents more reliable DD values, which makes it more appropriate to be used in conjunction with electrodialysis when to decide what DD must be imposed for tartaric stabilization of wines.

**Acknowledgements**

We thank Dr. Rui Estrela, from the company Wineinova, Lda (wineinova.com), for supplying the controlled freeze-thawing device (StabilPro System) and the wine samples.

**References**

Packed shell and tube heat exchanger for the separation and recovery of unreacted monomer in polyvinyl chloride plants

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Introduction
VCM is the main monomer in the production of PVC plastics. However, VCM is a toxic and carcinogenic agent, and poses an environmental and safety risk for the personal exposure of plant workers and product consumers, alike, if the resulting polymer is not duly treated. As such, the removal and recovery of this monomer must adhere to strict safety and purity requirements. From a report elaborated by Foster D. Snell, Inc. [1], it was estimated that 10-15% of VCM remains after the polymerization step, as such, the unreacted monomer must be stripped from the polymer product, separated, and recycled. In PVC plants, most VCM emissions originate from the reactor purge, the slurry stripping step and other equipment purges. These streams are characterized by the high concentration of VCM, low volume and low temperature, which are the optimal conditions for the use of adsorption based separation methods [2]. Patents [3, 4] have been published that detailed the use of activated carbon for adsorption separation of a VCM/inert gas mixture stream. One of the main drawbacks from this method pertains to the high adsorption enthalpy of VCM on activated carbon, which results in a high temperature profile during the adsorption step as well as requiring a high temperature to regenerate the carbon bed. Prior art describes the use of steam to regenerate the packed bed followed by a condensation step to separate the VCM from the resulting liquid water. Additionally, an internal coil can also be implemented to help heat up the packed bed during regeneration and to cooldown the bed during the adsorption step. Besides the disadvantage of creating a liquid stream that may or may not require further purification, a hot and cold inert gas stream is needed to dry and to cooldown the adsorbent, respectively [4]. As such, instead of direct heating, thermal regeneration by the Joule effect, microwaves and indirect heating is preferred [5]. Heating jackets and coils are traditional examples of indirect heating, however, as the heat transfer is mostly done in the radial direction, the performance of these equipment limits the scale up of fixed-bed adsorption columns, which would otherwise result in adsorption columns with an exceedingly high column height and cycle duration. A solution then consists in increasing the heat transfer performance of the equipment, which reduces the cycle time and consequently the column height. One of the proposed designs consists on a shell and tube heat exchanger, with the adsorbent phase on the tube side and the heat transfer fluid flowing on the shell side. Patents and studies [6-8] have been published that reported the successful use of these designs in similar situations. In this work, a 2-dimensional (2D) mathematical model of a Temperature Pressure Swing Adsorption (TPSA) was developed for the equilibrium adsorption separation of VCM and nitrogen (N₂), on activated carbon. In order to increase the process heat transfer rate, a shell and tube geometry was studied and implemented into the TPSA model. Using this geometry, a 5-step system of 3 adsorbent packed shell and tube heat exchangers was designed to separate and purify a 40-60 % (v/v) VCM/N₂ gas mixture. This system was designed to produce a 95 % (v/v) VCM rich stream and a N₂ stream with a VCM limit concentration of 8 ppm (w/w).

Most of the produced vinyl chloride (VCM) is used in the polymerization towards polyvinyl chloride (PVC). As this reaction does not have a total conversion, the unreacted monomer must be purged from the reaction slurry, purified, and recycled back to the polymerization reactor. This monomer recovery gives answer to both economical, and health and safety requirements. In this work, a 2-dimensional (2D) mathematical model of a Temperature Pressure Swing Adsorption (TPSA) was developed for the equilibrium adsorption separation of VCM and nitrogen (N₂), on activated carbon. In order to increase the process heat transfer rate, a shell and tube geometry was studied and implemented into the TPSA model. Using this geometry, a 5-step system of 3 adsorbent packed shell and tube heat exchangers was designed to separate and purify a 40-60 % (v/v) VCM/N₂ gas mixture. This system was designed to produce a 95 % (v/v) VCM rich stream and a N₂ stream with a VCM limit concentration of 8 ppm (w/w).

Objectives
Develop a 2D TPSA mathematical model coupled with a shell and tube heat exchanger design for the high adsorption enthalpy separation of VCM and N₂ on activated carbon. Design a TPSA cycle setup that complies with the process and environmental product purity requirements.

Methods
To simulate a 2D multicomponent TPSA model in a packed shell and tube heat exchanger, a set of mass, energy, and momentum balances must be specified, and some considerations must be made. The following assumptions used in the model are built upon the framework established by Da Silva et al. [11].

1. The gas phase follows the ideal gas behavior;
2. The internal mass transfer resistance is expressed with the Linear Driving Fore model;
3. The external mass and energy transfer resistances are expressed with the film model;
4. The column porosity, cross section and particle density are constant along the column;
5. The temperature profile is homogeneous inside the particle;
6. The Ergun equation is valid locally, only the pressure and velocity variations are considered in the momentum balance;
7. The header tube arrangement is able to achieve a uniform flow distribution;
8. The shell heat transfer performance is similar over all the tubes in the shell cross section;
9. The resistance in the shell side energy transfer in the close neighborhood of each tube is negligible;

Based on these considerations, the shell and tube heat exchanger geometry can be represented by the translation symmetry of each packed tube following, for example, a square pitch. As such, to calculate the performance of the proposed equipment, only the tube side of the heat exchanger needs to be simulated. The dimensions of this equipment were mainly based on standard values commonly found in literature to both guarantee an executable design and to facilitate size and parameter estimations.

Results
As illustrated by Figure 1, the proposed TPSA cycle consists of five steps. For the first step (Feed), the gas mixture is feed into the packed tubes to produce a N₂ stream at a pressure $P_{\text{high}}$. During this step, the exothermic high-pressure adsorption of VCM heats up the adsorbent material, and, therefore, must be continually cooled down by the shell side heat transfer fluid at $T_{\text{low}}$. In the Blowdown step, the tubes are radially heated up by the shell side heat transfer fluid at $T_{\text{high}}$, and blown down counter currently to the low-pressure $P_{\text{low}}$, producing a VCM stream. In the Purge step, the packed tube is further regenerated by a countercurrent desorption with a N₂ product purge. In the Cooldown step, the tubes are cooled down back to $T_{\text{low}}$ and are repressurized to $P_{\text{high}}$ using the N₂ product, in the Pressureization step.

For the proposed separation, it was decided that each heat exchanger should have approximately 230 tubes with an outer diameter of 32 mm, thickness of 1.2 mm and 4 m length. Following a square pitch design with a clearance of 8 mm, the shell diameter was estimated at 60 cm. Following a time schedule of 2000 s for the Feed step, 1200 s for the Purge and Cooldown steps and 800 s for the Blowdown and Pressureization steps, the present model can be extended to a triple packed heat exchanger system to guarantee a continuous feed consumption and purified stream production, as exemplified in the Graphical Abstract.

Conclusion
To ensure the VCM product purity requirement, for a feed flow rate of 193 Nm³/h, and an inlet velocity of 0.35 m/s, a N₂ purge flow rate of 12 Nm³/h, and an inlet velocity of 0.02 m/s was used. For this system, the high and low pressure, and temperature were, respectively, 100 kPa and 50 kPa, and 463 K and 293 K. Under these specifications, the purified N₂ rich stream contained a VCM concentration of 7.4 ppm (w/w), a N₂ recovery of 96.4 % (v/v) was achieved, with a thermal energy consumption of 4.07 MJ/kgVCM. Considering that no energetic integration was performed for this system, the reported thermal energy consumption is additive.

References
Catalytic performance of rare-earth doped HBEA zeolites over Friedel-Craft acylation reactions

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In this work a comparison of the catalytic performance of HBEA zeolite, doped with different percentages of rare-earth cations, in the Friedel-Crafts acylation of furan with acetic anhydride is performed. Kinetic /equilibrium constants were obtained from the mathematical treatment of experimental results with a previously proposed simplified version of the Langmuir-Hinshelwood model. Kinetic constants were also correlated with earlier characterization data of these zeolites namely with Brönsted acidity, quantified by pyridine adsorption followed by FTIR, and catalytic acidity estimated by the model catalytic cracking of n-heptane.

Introduction

Friedel-Craft acylations remain still today as very important reactions in organic synthesis, being used not only at a laboratory scale but also in industrial processes, namely in the pharmaceutical industry. Classical Friedel-Craft acylations depend on the use of Lewis acids like AlCl₃ or strong protonic acids like HF. However, these catalysts are not re-usable and can also generate toxic residues during the work-up reaction procedures.

This fact directed these studies towards the substitution of the initial catalysts by others more “environmentally friendly”. One of the most efficient approaches was the use of solid acid catalysts, namely zeolites. These materials present several advantages such as easier separation procedure from the reaction mixture (by simple filtration), lower toxicity, and possibility of being re-generated.

The use of zeolites in Friedel-Craft acylations is now decisively present in organic chemistry research, either in the laboratory or in the industry and, in this context, several studies have tested many zeolitic structures with different substrates [1].

However, much of the studies do not perform a systematic analysis of the catalytic performance. Following a previous work [2], we now present a kinetic comparison of HBEA zeolite samples doped with different percentages of rare-earth cations, in the acylation of furan with acetic anhydride (Figure 1).

![Reaction scheme](image)

Figure 1. Reaction scheme.

The study of the influence of different material characteristics (e.g., acidity) on the catalytic performance of these zeolites, is also performed using previous experimental data [3].

Methods

All HBEA zeolitic rare-earth doped materials were prepared according to the procedure previously described [3], involving an ionic exchange methodology. Material characterization using different techniques is also described in the same reference.

In the catalytic experiments, a mixture of furan (0.71 g) and acetic anhydride (5.4 g) was added to a sample of zeolite (100 mg). The suspension was heated at 60 ºC in a heating plate with in situ temperature control under vigorous stirring and reflux. Periodically, small aliquots were withdrawn from the reaction mixture using a hypodermic syringe, filtered using a Millipore Swinnex support and a Millipore Durapore (0.45 μm HV) membrane filter. The reaction progress was analyzed by gas chromatography on a Perkin Elmer auto-system, equipped with a DB-5MS capillary column and a flame ionization detector. The identification of the products was achieved by injecting standard samples. The kinetic data was treated using a simplified form of the Langmuir-Hinshelwood model (Equation 1), described in detail in a previous work [2]:

\[
\text{Rate} \equiv k \frac{[A][S]}{([A]+[S]+K_r[P])^2}
\]  

(Equation 1)

where [A], [S] and [P] are the concentrations of acetic anhydride, furan and 2-acetylfuran respectively, \(k\) represents the rate constant and \(K_r\) the ratio between the adsorption equilibrium constants of the product and the normalized equilibrium constants of the reagents.

Values for \(k\) and \(K_r\) were obtained through nonlinear regressions and iterative numerical methods.

Results

Figure 2 represents a typical kinetic experimental curve. The modified Langmuir-Hinshelwood model was applied to all kinetic data of the different tested samples. Results are summarized in table 1.

![Kinetic curve](image)

Figure 2. Kinetic curve of the acylation of furan with acetic anhydride using HBEA zeolite.

Original HBEA zeolite and rare-earth doped samples were previously characterized, namely regarding their acidity. From literature it is known that acidity is related with the catalytic performance in Friedel-Craft acylations [1]. Table 2 summarizes...
the experimental data of Brönsted acidity measured by FTIR pyridine adsorption and of “catalytic” acidity measured by a model reaction (n-heptane cracking) [3].

Table 1. \( k \) and \( K_r \) obtained from the Langmuir-Hinshelwood model.

<table>
<thead>
<tr>
<th>Sample</th>
<th>( k ) (mmol min(^{-1}) g(^{-1}))</th>
<th>( K_r )</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBEA</td>
<td>0.0400</td>
<td>4.1</td>
</tr>
<tr>
<td>La1</td>
<td>0.0117</td>
<td>4.5</td>
</tr>
<tr>
<td>La2</td>
<td>0.0096</td>
<td>10.0</td>
</tr>
<tr>
<td>Yb1</td>
<td>0.0111</td>
<td>6.0</td>
</tr>
<tr>
<td>Yb2</td>
<td>0.0012</td>
<td>5.0</td>
</tr>
<tr>
<td>Nd1</td>
<td>0.0170</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Table 2. Acidity characterization and rare-earth composition (data quoted from [3]).

<table>
<thead>
<tr>
<th>Sample</th>
<th>Rare Earth (%))</th>
<th>Brönsted Acidity (cm(^{-1}))</th>
<th>n-heptane cracking (mol h(^{-1}) g(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBEA</td>
<td>---</td>
<td>234</td>
<td>5.9</td>
</tr>
<tr>
<td>La1</td>
<td>Lanthanum (0.29)</td>
<td>217</td>
<td>5.0</td>
</tr>
<tr>
<td>La2</td>
<td>Lanthanum (0.71)</td>
<td>161</td>
<td>5.2</td>
</tr>
<tr>
<td>Yb1</td>
<td>Ytterbium (0.98)</td>
<td>136</td>
<td>5.1</td>
</tr>
<tr>
<td>Yb2</td>
<td>Ytterbium (2.13)</td>
<td>129</td>
<td>4.9</td>
</tr>
<tr>
<td>Nd1</td>
<td>Neodymium (0.43)</td>
<td>221</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Figures 3 and 4, show the correlations between the rate constants of the studied Friedel-Craft acylations and the acidity parameters previously obtained (note: some outliers were removed in each case).

Figure 3. Correlation between the Brönsted acidity and the rate constant of the Friedel-Craft acylation.

Figure 4. Correlation between the rate constant of n-heptane cracking (model reaction) and the rate constant of the Friedel-Craft acylation.

Conclusions
It was possible to calculate values of both rate constants and adsorption equilibrium constants for the acylation reactions of furan with acetic anhydride in the presence of HBEA zeolite, doped with different percentages of rare-earth cations. All the rare-earth doped zeolites have lower catalytic performance when compared with the HBEA zeolite, and those with lower percentage of rare earths showed relative better performance. A high correlation between the rate constants of the studied Friedel-Craft acylations and two different measures of acidity was demonstrated.

Acknowledgements
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References
Effect of dispersant on the stabilization of calcium carbonate nanoparticles

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In this work, the effects of the temperature and of different dispersants on the stability over time of a suspension of calcium carbonate nanoparticles were studied. A full recrystallization was obtained, for both analysed temperatures (7 and 20 °C), at the end of one week when the nanoparticles were in a suspension of distilled water. The use of dispersants improved the stability of the suspension. For the case where a sodium citrate solution was used a clear improvement on the particles’ stability was observed: at 7 °C, recrystallization of the particles was practically non-existent after 3 weeks; however, a total recrystallization occurred by week 16. A twice more concentrated solution of sodium citrate, at 7 °C, prevented recrystallization for a minimum of 16 weeks. For a storage solution of sodium oleate, stability was achieved for, at least, 16 weeks. Furthermore, it was seen that lower temperatures had a retarding effect on the recrystallization of the particles.

Introduction
Nano and microtechnology are currently fields of great relevance, so much that the global market for the commercialization of micro-nanoproducts and systems is expected to exceed one trillion dollars by 2018 [1]. Currently, one of the compounds of interest in micro/nanoscience is calcium carbonate (CaCO₃). It is a natural and abundant mineral, that represents about 4% of the earth’s crust [2] and is the main constituent of limestone, marble and chalk [3]. It is known in the form of three anhydrous crystalline polymorphs – calcite, aragonite and vaterite [3] – or in its two hydrated forms – monohydrocalcite (CaCO₃·H₂O) and ikaite (CaCO₃·6H₂O) [4, 5]. This compound can also present various amorphous forms [4], being the amorphous calcium carbonate a nanoparticle, poorly ordered, metastable precursor to crystalline CaCO₃ [6].

Calcium carbonate has a wide range of application areas that include paint, paper and plastic industries, the use in adhesives, sealants, caulks, putty and printing inks, in the process of flue gas desulfurization (set of processes that trap the sulphur-oxygen compounds produced in the combustion of coal) and in biomedical and food applications (as an antacid, as a calcium supplement in foods, as a slight abrasive toothpaste, in chewing gum and in both paper and plastic for contact with food) [3]. It can also be applied in drug delivery, as template for encapsulation of bioactive compounds, for encapsulation of proteins, for insoluble powder formulation in nasal drug delivery system, as bone replacement material in human periodontal osseous defects, in the construction of biosensors and for enzyme immobilization [7, 8].

This compound global demand is driven by the replacement of kaolin in the paper industry, the use of nano-calcium carbonate in the plastic and rubber sectors, its application as drug templates, the consumption associated with the paint industry and the growing focus on increasing mineral loadings in paper. Thus, global production is expected to reach 98.7 million metric tons by 2020 [9].

Objectives
The aim of this work is to study the stability of calcium carbonate nanoparticles over time when they are in a suspension at different conditions, i.e. under distinct storage solutions and/or temperatures.

Methods
Calcium carbonate was produced in a semi-batch reactor with a high-speed dispersing and homogenizing device (MICCRA Homogenizer Disperser D-9) at 11 000 rpm. A stoichiometric proportion of calcium chloride was added to the potassium carbonate, already inside the reactor, using a peristaltic pump (Ismatec MCP) and an injector (that ensures that the fluid is injected directly below the dispersing device). After the precipitation of calcium carbonate, the suspension was washed and filtered, in order to remove the potassium chloride formed in the reaction, and the product was then stored in distilled water or in a solution containing a dispersant. Two distinct dispersants were studied: a hydrophilic (sodium citrate) and a hydrophobic (sodium oleate). Table 1 shows the storage conditions used in the various experiments performed.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Solution</th>
<th>Temperature / °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>B1</td>
<td>Distilled water</td>
<td>20</td>
</tr>
<tr>
<td>B2</td>
<td></td>
<td>7</td>
</tr>
<tr>
<td>B3</td>
<td>Sodium oleate</td>
<td>20</td>
</tr>
<tr>
<td>B4</td>
<td>(9.3 g·L⁻¹)</td>
<td>7</td>
</tr>
<tr>
<td>B5</td>
<td>Sodium citrate</td>
<td>20</td>
</tr>
<tr>
<td>B6</td>
<td>(2.2 g·L⁻¹)</td>
<td>7</td>
</tr>
<tr>
<td>B7</td>
<td>Sodium citrate</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>(4.5 g·L⁻¹)</td>
<td></td>
</tr>
</tbody>
</table>

Results
The particles produced in the semi-batch experiment are shown in a transmission electron microscope (TEM) image, present in the graphical abstract. In experiments B1 and B2 (when the product was stored in distilled water), recrystallization was observed at the end of one week (Figure 1b and Figure 1d). Comparing scanning electron microscope (SEM) images of both experiments for 48 hours after the production of the particles (Figure 1a and Figure 1c), it can be seen that a lower recrystallization is observed when the particles are stored in a suspension at lower temperatures. Therefore, it is concluded that lower temperatures slow down surface reactions and the
consequent recrystallization of the particles, allowing the stability of the product for a longer period.

Figure 1. Scanning electron microscope images of sample B1 for (a) 48 hours and (b) 1 week and of sample B2 for (c) 48 hours and (d) 1 week.

In the case of sodium oleate (9.3 g·L⁻¹) being used as a storage solution (experiments B3 and B4), no recrystallization was observed for at least 16 weeks (data not shown). For a 2.2 g·L⁻¹ sodium citrate storage solution (experiments B5 and B6), recrystallization was delayed at lower storage temperatures (data not shown) and it was concluded that, although not as effective as the sodium oleate storage solution, improvements in the stability of the particles resulted from the storage in a sodium citrate solution. At 7 °C, two solutions of different concentrations were analysed (experiments B6 and B7), and the results are presented in Figure 2. Figure 2a shows that there is practically no recrystallization of the particles after three weeks, however, Figure 2b shows that, after 16 weeks, the particles recrystallized to a different morphology than they did when stored in distilled water. Figure 2c and Figure 2d allow concluding that the use of a twice more concentrated storage solution has a positive effect on the stability of the produced particles over time.

Figure 2. Scanning electron microscope images of sample B6 for (a) 3 weeks and (b) 16 weeks and of sample B7 for (c) 3 weeks and (d) 16 weeks.

Conclusion
When CaCO₃ nanoparticles were stored in a suspension of distilled water, recrystallization occurred completely after one week. The addition of dispersants improved the behaviour of the suspensions: for a storage solution of 9.3 g·L⁻¹ of sodium oleate at both 7 and 20 °C and for a solution of 4.5 g·L⁻¹ of sodium citrate at 7 °C, there were no signs of recrystallization of the nanoparticles for, at least, 16 weeks. Current work includes the optimization of the process variables for the continuous production of calcium carbonate nanoparticles using the NETmix technology.

Acknowledgements
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References
Mine waters are considered important environmental pollutants but are also potential sources of valuable metals. The mine of São Domingos is an abandoned open-pit mine located in the southeast of Portugal, in Alentejo. This mine was active for more than 100 years until 1966, when the activity was ceased due to resource depletion. São Domingos mining activity created some issues in this region, such as the presence of toxic acid mine drainage waters, with pH < 2 and rich in sulfate anions and valuable metals.

Aqueous biphasic systems (ABS) appeared as a more benign alternative for conventional liquid-liquid extraction, which involves the use of volatile organic compounds. These systems are composed of two aqueous-rich phases and their formation can be induced by the mixing of two salts, two polymers or a salt and a polymer. ABS composed of ionic liquids (ILs) - salts with melting points below 100 °C - present additional advantages, such as low viscosity, fast phases separation and high extraction efficiency for a large range of compounds.[1] Several authors[2,3] studied the application of ILs for the selective extraction and purification of metals from different streams, with remarkable results. Due to their ionic nature, the use of ILs allows metal extraction mechanisms that are not possible when conventional organic solvents are applied.[4]

In this context, the objective of the present work is to study the use of ABS composed of ILs to the extraction of metals from São Domingos acid mine drainage water, envisaging not only the detoxification of mine tailing site but also the recovery of value-added metals to be reused and recycled.

With this aim, the present work starts with the characterization and the pre-treatment of the acid mine drainage water provided from São Domingos mine. Then, the effect of the IL nature in the extraction of metals as cobalt, nickel and copper through ABS composed of different ILs and Na₂SO₄ salt is evaluated by using standard aqueous solutions of these metals. Finally, the systems that present the best results in the initial screening are applied for metals extraction on the acid mine drainage water sample. The obtained results demonstrated that ILs anion presents a high influence in metals extraction, with thiocyanate-based ILs showing the best extraction efficiencies. Thus, the potential use of thiocyanate-based salts as additives in ABS composed of lower cost and less toxic ILs - which demonstrated a lower ability to extract metals - was also evaluated. Remarkably, a significant increase of the extraction efficiencies (>100%) was observed. Furthermore, the selective separation of Ni and Co was also attained.

In conclusion, it is here demonstrated the high ability of IL-based ABS to extract metals from acid mine drainage waters, allowing the recovery and recycling of valuable metals.

Acknowledgements

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References

Deep eutectic solvents (DES) are considered green solvents that result from the formation of hydrogen bonding complexes between a hydrogen-bond acceptor (HBA) and a hydrogen-bond donor (HBD). DES share some of the ionic liquids well known characteristics and additionally present high biodegradability, easy synthesis and renewable character. [1] Due to these properties, DES have been applied as phase forming components of aqueous biphasic systems (ABS) [2,3,4] allowing to obtain high extraction levels of a large range of compounds. However, its application in ABS can present some limitations, due to the non-stoichiometric partition of DES’ components between the two aqueous phases. In this context, pseudoternary DES-based ABS, in which DES’ components stoichiometry is maintained, appear as potential liquid-liquid systems to the extraction and separation of biomolecules.[4]

In this context, the goal of this work is evaluate the pH effect on systems equilibrium, HBA:HBD molar ratio and biomolecules partition on DES/salt-based ABS. For this, DES/salt-based ABS composed of potassium citrate salt (K$_2$H$_3$O$_7$) and DES constituted by tetrabutylammonium chloride ([N$_{4444}$][Cl]) as HBA and the alcohols ethanol and n-propanol as HBD, at different molar ratio (2:1, 1:1 and 1:2), are here studied. Moreover, three biomolecules (gallic acid, l-tryptophan and caffeine) are used as molecular probes to evaluate the partition coefficient and the extraction efficiency at different pH values.

The obtained results suggest that the HBD (ethanol/n-propanol) promote changes on the binodal curves, which seem to be dependent of the alcohol nature. In what concerns the pH effect on the binodal curves, the slight changes observed are induced by the chemical speciation of the citrate salt. Furthermore, at pH 9, on the top phase, which is richer in [N$_{4444}$][Cl] (HBA), the initial molar ratio is maintained, while, at pH 5 a larger amount of alcohol is partitioned to the salt-rich phase and consequently the HBA:HBD molar ratio is not maintained in any of the phases of the ABS. In what concerns the biomolecules partition, the system’ properties seems to be the major factor of influence on the partition coefficients ($K$) values. However, to manipulate the partition behavior a combination of hydrophobic/hydrophilic and electrostatic interactions as well as the salting-out effect need to be considered.

In conclusion, the results of this work show the versatile behavior of DES-based ABS, where external factors such as pH can be used to drive the DES components partition and consequently to change the phases properties and tune the partition behavior of a target biomolecule.

Acknowledgements

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References

Computational simulation of the aromatic extraction process: Evaluation of thermodynamics and sulfolan selectivity

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In the aromatic extraction operation, a combination of operations is required to obtain products with a high degree of purity. Aromatic hydrocarbons are obtained from the cut range of naphtha. In general, the target of the extraction is called BTX’s (Benzene, Toluene and Xylene) and one of the solvents most used in the process is sulfolane. A unit of aromatic extraction was simulated based on the parameters reported in the literature, using as solvent the sulfolane in the ratio of 1.6 (Solvent/Feed). The conceptual design was carried out with the simplification of the feed with the objective of thermodynamic evaluations and solvent efficiency. The creation of a database with parameters of binary interaction was carried out to characterize the best model to be used in the simulation, excelling NRTL model. A stream of aromatics was obtained in 97% purity. The selectivity of the solvents was evaluated in relation to the solutes of interest, revealing the high potential of the solvent.

Introduction
In the process of fractionation of the oil, several cuts are obtained among which is the naphtha. In this section are allocated the aromatic compounds, undesirable in the process that aim at obtaining lighter constituents, but they are essential compounds in the petrochemical industry [1]. The most used operation to obtain these constituents is the liquid-liquid extraction, because the various constituents present in the naphtha with very close boiling points. Some solvents are being widely used, but there is still great potential for research aimed at improving the process [2]. Both operational and conceptual aspects should be evaluated correctly. In this sense, a good thermodynamic evaluation becomes necessary for coherent analysis of the process. According to literature research, there are several problems evaluated regarding the convergence of the simulated process [3], [4], [5], [6].

The specific objectives of this work were the following ideas to:
- Carry out a study around the mass and energy balance, to verify the degree of purity obtained for the aromatics and energy required in solvent recovery;
- Create a liquid-liquid equilibrium database, using data from the literature;
- Perform thermodynamic modeling from the regression of the collected data, integrating the values obtained to the AspenPlus software database.

Materials and methods
The process chosen in this work to simulate aromatic extraction was based on the models proposed by [5] and [6], since they are simplified models, but they represent in detail the characteristics of the process. For the solvent recovery section, the operating conditions were used from the work developed by [4].

Characterization of the feed. The feed used for the simulation is described in Table 1.

Thermodynamics. Two packets of binary interaction parameters were considered, the UNIQUAC and NRTL models were the most representative for ELL presented in the systems. For both, the UNIFAC model (thermodynamic model of group contribution) was used, with the objective of estimating the unavailable interaction parameters. In order to represent better the interactions of the components in the liquid phase, the thermodynamic modeling was performed. The modeling was made possible by the regression of existing experimental data in the literature and involving aromatic-aliphatic-solvent-type systems. To perform the comparison between the experimental values and calculated by the modeling the RMSD was used according to Equation (1).

\[\text{RMSD} = \frac{1}{N} \sum \left( \frac{x_{\text{calc}} - x_{\text{exp}}}{x_{\text{calc}}} \right)^2 \]  \hspace{1cm} (1)

where the envelopes I and II characterize the phases in which the components are; calc and exp are abbreviations of calculated and experimental, respectively. N and C are the numbers of tie lines and components, respectively, and x is the molar fraction, also be able to be replaced by the mass fraction (w).

Table 1. Characterization of the project load in terms of: mass flow rates, mass fractions and boiling point description.

<table>
<thead>
<tr>
<th>Component</th>
<th>Mass flow (kg/h)</th>
<th>% mass</th>
<th>Boiling point (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heptane</td>
<td>1000</td>
<td>0.02</td>
<td>98.4</td>
</tr>
<tr>
<td>Hexane</td>
<td>8600</td>
<td>0.196</td>
<td>68.0</td>
</tr>
<tr>
<td>Toluene</td>
<td>12160</td>
<td>0.243</td>
<td>110.6</td>
</tr>
<tr>
<td>Benzene</td>
<td>27040</td>
<td>0.541</td>
<td>80.1</td>
</tr>
<tr>
<td>Sulfolane</td>
<td>80000</td>
<td>1</td>
<td>285.0</td>
</tr>
</tbody>
</table>

The database used for modeling and obtaining parameters to the simulation can be found in [1]. The binary interaction data between sulfolane / toluene, toluene / hexane, sulfolane / hexane, sulfolane / benzene were reproduced from the experimental data of [7]. The other parameters were regressed with the experimental data of the created database.

Structure of the simulation. In the Aspen Plus simulator, the blocks were connected to each other through the chains, according to the flowchart of Figure 1.
Figure 1 - Flowchart of the aromatic extraction process proposed in the simulator.

**Results**

Figures 2 (a) and 2 (b) show these results for the hexane (Hex) / toluene (Tol) Sulfolane (Sulf) at 303.15K and Heptane (Hep) / Toluene (Tol) / Sulfolane (Sulf) blends at 298.15K, respectively. The low values of mean squared deviations (RMSD) and deviations between the estimated and experimental tie lines demonstrate a satisfactory prediction of the ELL and greater safety in the estimation step of the other equilibrium data and subsequent application in the extractor simulation.

**Distribution coefficient (K) and Selectivity (β)** First, the calculations were performed for the main E-101 extraction tower. It’s observed in evaluating such values that the selectivity (β) of Toluene (β-Tol) and Benzene (β-Benz) were much larger than unity in the order of magnitude of tens. Thus, the high selectivity of the system is confirmed using Sulfolane as solvent. The values obtained for the secondary extractor of the raffinate E-101, also present high value for selectivity of water in relation to sulfolane (desired solute) that was much higher than unity in the order of magnitude of many powers which is characteristic for this tower, considering the high affinity of Sulfolane in relation to quite polar water. The extremely high values can be explained by the reproduction of the operating conditions of the plant from the work [5].

**Acknowledgements**

To the fuel laboratory (LAC) of UFPE, for all support and financial support.

**References**


Studies on the solubility of syringic, vanillic and veratric acids in water and organic solvents

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Phenolic acids are a chemical family of significance in the health and food industries, among others. Despite its importance for the design of separation processes, solubility data of phenolic acids are seldom available in literature. In this context the solubility of syringic, vanillic and veratric acids was measured in water and eight organic solvents (methanol, ethanol, 1-propanol, isopropanol, ethyl acetate, acetonitrile, 2-butane and dimethylformamide) at 298.15 K and 313.15 K using the isothermal analytical shake-flask method. Calorimetric studies were also carried out by differential scanning calorimetry (DSC) to determine the melting properties of the phenolic acids. Finally, the semi-predictive Non-Random Two-Liquid Segment Activity Coefficient (NRTL-SAC) model [1], combined with the reference solvent approach (RSA) [2], was applied to correlate and predict the solid-liquid equilibria. The average relative deviations were 30% for the correlation and 44% for the predictions.

Introduction and Objectives
Phenolic acids are an important class of naturally occurring compounds with different applications in the pharmaceutical, food, oil and cosmetic industries. In this context, the solid-liquid equilibrium data of phenolic acids are fundamental in the design of their separation processes, such as extraction, precipitation or crystallization.

In this work, the solubility of three phenolic acids (syringic, vanillic and veratric acids) in water and eight pure organic solvents (methanol, ethanol, 1-propanol, isopropanol, ethyl acetate, acetonitrile, 2-butane and dimethylformamide) was studied, at 298.15 K and 313.15 K, following previous work carried out by our research group focused on phenolic compounds [3–6]. The chemical structures of the phenolic acids are shown in Figure 1.

![Chemical structures of phenolic acids](image1.png)

**Figure 1.** Chemical structures of: (a) syringic acid; (b) vanillic acid and (c) veratric acid.

Experimental Methods
The solubility experiments were carried out by the isothermal shake-flask method combined to the gravimetric method of analysis, described elsewhere in detail [3,6].

The temperatures and enthalpies of fusion were determined by Differential Scanning Calorimetry (DSC) in a 204 F1 Phoenix, NETZSCH, device equipped with a nitrogen feeding system. Samples of 2 up to 10 mg were hermetically sealed into aluminum pans and placed along with a reference cell to be heated at a heating rate of 1 K/min. For each compound, at least three runs were considered for the final results.

Results and Discussion
The solubility data for syringic, vanillic and veratric acids in water and organic solvents are presented in Figure 2.

![Solubility data](image2.png)

**Figure 2.** Experimental solubility of the phenolic acid in water and organic solvents at 298.15 K and 313.15 K: (a) syringic acid; (b) vanillic acid; (c) veratric acid.

As can be seen, the solubility of the acids is considerably higher in organic solvents compared to water. In systems containing alcohols, the solubility increases with the decrease of the alkyl chain of the alcohol. Additionally, the solubility of these acids is the highest in dimethylformamide (DMF).

In general, for water, dimethylformamide and the selected alcohols, vanillic acid is the most soluble, followed by syringic acid and finally the least polar veratric acid.

For 2-butane and ethyl acetate, the solubility of veratric acid is higher than values found for syringic acid, but lower than those measured for vanillic acid. In the case of binary systems containing acetonitrile, the solubility follows the ranking:
veratric acid > vanillic acid > syringic acid. In all cases, the solubility increases with temperature. The melting properties measured by DSC in this work were compared with the available literature data [7–12]. The fusion temperatures measured in this work are in agreement with the following average values from literature: 483.9 K ± 2.1 for vanillic acid, 481.4 K ± 1.6 for syringic acid, and 453.1 K ± 0.2 for veratric acid. Regarding the enthalpies of fusion, the results are less consistent, as the values obtained in this work are 17% to 30% higher than the average literature values, depending on the acid.

Finally, the NRTL-SAC model [1] was applied to describe the solid-liquid equilibria of these systems. First, the solubility in only seven solvents (water, methanol, ethanol, 2-propanol, 2-butane, ethyl acetate and acetonitrile) was used in the correlation step. Then, the parameters obtained were used to predict the solubility in 1-propanol and DMF. The average relative deviations (ARD) of the correlation step was 33%. The ARD were 22% for solubilities in 1-propanol and 94% for DMF. The correlation results are shown in Figure 3. Due to the uncertainty of the melting properties, an alternative methodology was followed in which the reference solvent approach (RSA), proposed by Abildskov and O’Connell [2], was coupled to the NRTL-SAC model. In this case, slightly lower ARDs were found: 30% in the correlation results and 44% in predictions.

Acknowledgements

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References

Activation of persulfate and peroxymonosulfate by heterogeneous catalysis for the degradation of organic pollutants

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The activation of persulfate and peroxymonosulfate to generate sulfate radicals and degrade organic pollutants was evaluated. The selected pollutants were a dye, Lissamine Green B, and an ionic liquid, 1-butyl-1-methylpyrrolidinium chloride. The key parameters were optimized and both homogeneous and heterogeneous catalysis were assessed, getting better results with the second mode. For the persulfate activation, iron adsorbed in amberlite was used as catalyst. Different concentrations of iron were tested, achieving the best results with 5 g of catalyst (93% of dye removal). For the peroxymonosulfate activation, a system combining iron and electric field was evaluated. The concentration and source of iron (pyrite, goethite and magnetite), the oxidant dosage and the current applied were studied. A TOC decay of 80% was obtained with the activation of peroxymonosulfate (10 mM) with pyrite (1 mM of iron) at 150 mA within 300 min. Moreover, the reusability of both catalysts was demonstrated.

Introduction
Lately, sulfate radicals-based advanced oxidation processes (AOPs) has attracted the attention of the scientific community. These processes are based on the generation of powerful oxidants, sulfate radicals (SO₄²⁻), which attack the pollutants until its mineralization. Sulfate radicals have higher selectivity and longer half-life than hydroxyl radicals [1]. They can be generated through the activation of persulfate (PS; S₂O₅²⁻) or peroxymonosulfate (PMS; HSO₅⁻). Up to date, different methods of activation have been reported in the literature, such as metal ions, heat, UV radiation, activated carbon, ultrasound and electric field [2,3].

Regarding the transition metals for PS and PMS activation, the mechanism is shown in Eqs. 1 and 2 [1]. Homogeneous catalysis involves some limitations, being the most important the difficulty of the catalyst recovery. Thus, heterogeneous transition metals systems have achieved a great interest [4].

\[ S_2O_7^{2-} + M^{n+} \rightarrow M^{n+1} + SO_4^{2-} + SO_4^{2-} \] (1)

\[ HSO_5^- + M^{n+} \rightarrow M^{n+1} + SO_4^{2-} + H^+ \] (2)

Conversely, one of the most recent strategies of activation is the application of the electric field [5]. Sulfate radical can be produced by electron transfer reaction (Eqs. 3 and 4) [1].

\[ S_2O_7^{2-} + e^- \rightarrow SO_4^{2-} + SO_4^{2-} \] (3)

\[ HSO_5^- + e^- \rightarrow SO_4^{2-} + H^+ \] (4)

The combination of different activation methods is a promising system, since it can enhance the removal capacity for organic compounds. Several studies have been reported in the literature, such as Lin et al. [5], who demonstrated the efficiency of an electro/Fe³⁺/PMS process to degrade clofibric acid.

The aim of this work was to evaluate the activation of PS and PMS through different activation methods for the degradation of organic pollutants. To select the target compounds, we focused on a traditional industry as textile, since it is one of the oldest industrial procedures, and the use of new solvents as ionic liquids. Therefore, the chosen pollutants were the dye Lissamine Green B and the ionic liquid 1-butyl-1-methylpyrrolidinium chloride ([bmpyr][Cl]).

Materials and methods
Reactants. All reagents were acquired by Sigma-Aldrich in analytical grade and they were used without further purification. Oxone® (KHSO₅·1/2K₂SO₄·1/2K₂SO₃) is the commercial formulation of PMS (Figure 1). The solutions were made using milli-Q grade water.

![Chemical structure of Oxone® salt.](image)

Figure 1. Chemical structure of Oxone® salt.

Experimental assays. On one side, the removal of Lissamine Green B was assessed through the activation of PS with Fe³⁺. The experiments were made in homogeneous and heterogeneous mode. In the latter case, the catalyst was prepared by fixing the Fe³⁺ by adsorption on a cationic resin (Amberlite IR120 Na⁺ form). The initial concentration of the dye was 7.5 mg/L and 1 mM of PS was added to the solution. The operational volume was 150 mL and all the tests were carried out at room temperature.

On the other side, the degradation of [bmpyr][Cl] was evaluated through the activation of PMS with iron-based mineral catalysts (pyrite, goethite and magnetite) under the electric field. The experiments were carried out in a cylindrical reactor with an operational volume of 150 mL. The initial concentration of [bmpyr][Cl] was 1.82 mM and different ratios of PMS and Fe³⁺ were evaluated. Two electrodes were connected to a direct current power supply, carbon felt (CF) as cathode and boron-doped diamond (BDD) as anode. Sodium sulfate 10 mM was added to the solution as supporting electrolyte.
Analytical methods. The dye degradation was monitored by a spectrophotometer Jasco V-360, whereas the [bmpyr]Cl removal was followed by ionic chromatography with a conductivity detector (Metrohm 733 IC) equipped with a column Dionex™ IonPac™ CS12 (CACTI, University of Vigo). The mineralization was evaluated through the total organic carbon (TOC) decay. It was measured employing a Lange cuvette test (LCK 380) in a Hach Lange DR 3900 spectrophotometer, or via catalytic high-temperature combustion by multi N/C 3100 equipment (Analytic Jena) coupled with a non-dispersive infrared (NDIR) detector (CACTI, University of Vigo).

Results and discussion

Dye Lissamine Green B. In order to evaluate the PS activation, the dye degradation was carried out using iron as activator. A control using only PS was made. The initial concentration of dye was 7.5 mg/L, PS 1 mM and Fe²⁺ 0.1 mM. The efficiency of dye removal was improved 8% using Fe²⁺, achieving 66% of dye removal at 120 min. Then, different concentrations of Fe²⁺ were tested (0.1-0.4 mM), obtaining the best results with 0.3 mM (over 54% within 20 min). When Fe²⁺ 0.4 mM is used, the concentration is too high and the iron precipitates.

In the case of heterogeneous catalysis, the iron was adsorbed in amberlite. As in homogeneous catalysis, different concentration of Fe²⁺ were tested (0.5-5 g catalyst), achieving the best results with 5 g of catalyst (93% dye removal in 15 min). The reusability of the catalyst was evaluated (Figure 2).

![Figure 2. Reusability of heterogeneous catalyst in order of dye removal. Experimental conditions: Lissamine Green B 7.5 mg/L, PS 1 mM, 3g amberlite.](image)

Ionic liquid [bmpyr]Cl. To activate the PMS a combination of iron and electric field was employed. Different experiments were assessed for setting the optimal parameters, such as PMS concentration, source and concentration of iron and current applied. Among the sources of iron used (pyrite, goethite and magnetite), the best results were achieved with pyrite and the ratio 1:10 (Fe²⁺:PMS). Next, the effect of the applied current was evaluated in the range from 25 mA to 150 mA. As it can be observed in Figure 3, highest mineralization levels were achieved when greater current was applied.

![Figure 3. Effect of current on TOC decay (%). Experimental conditions: [bmpyr]Cl = 1.82 mM, PMS = 10 mM, Na₂SO₄ = 10 mM, pyrite = 1 mM Fe.](image)

The stability of the catalyst was evaluated due to it is one of the critical factors for its application in a continuous process. The concentration of [bmpyr]Cl was 1.82 mM and 1 mM Fe²⁺ as pyrite and 10 mM PMS were added to the solution. The experiment was carried out at 150 mA and the catalyst was utilized for five consecutive cycles. The [bmpyr]Cl degradation and TOC decay decreased slightly with the cycles.

Conclusions

In this study, it was demonstrated the potential of sulfate radicals as mighty oxidants capable of degrade complex organic pollutants. These radicals were generated through the activation of PS and PMS by using heterogeneous catalysis. In the case of PS, the addition of iron adsorbed in amberlite was proposed as a viable process to degrade the dye Lissamine Green B. As regards PMS, the electro-activation in presence of iron-based mineral as catalyst was proved to degrade the ionic liquid [bmpyr]Cl. Therefore, the application of these processes is a promising technology in the treatment of wastewaters.

Acknowledgements

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References

Development of a molecular model for [Ch]Cl aiming at the thermodynamic modelling of deep eutectic solvents

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Introduction
Deep eutectic solvents (DESs) are a class of green solvents that consist on mixtures of two (or more) compounds that due to strong and highly complex hydrogen bonds established between a hydrogen bond donor (HBD) and hydrogen bond acceptor (HBA), exhibit a eutectic point much lower than that expected for an ideal system [1], forming stable liquids at relatively low temperatures that can be used as solvents in a wide range of applications and whose properties can be tailored aiming at a specific application.

Cholinium chloride ([Ch]Cl) is the most used compound in DES formulations, usually combined with harmless HBDs, such as carboxylic acids, alcohols, amines, urea, and many others. Therefore, a reliable knowledge of the thermodynamic behavior of systems containing [Ch]Cl becomes vital for the simulation and design of industrial processes involving DESs.

Recently, SAFT-type equations of state (EoSs) due to their capability to explicitly account for the association phenomenon, have been proposed to describe the complexity of DESs-based systems [2]. Aiming at applying such EoSs, a reliable coarse-grained model able to represent the physical features of the different compounds is required. However, when proposing a model for [Ch]Cl two major problems arise: Firstly, the two-sites association schemes commonly used to model ionic liquids and salts seem to be too simplistic to capture the highly complex behavior of [Ch]Cl in DESs. Secondly, [Ch]Cl, as many other DESs’ constituents, is solid at room temperature and thus, vapor pressures and liquid densities, usually used to obtain the model parameters, are not available and alternatives are required.

Objectives
The main objective of this work is the development of an accurate and physically sound model for [Ch]Cl in the framework of soft-SAFT EoS, overcoming the limitations of the current approaches. Hence, the new model is intended to abolish the erroneous use of pseudo-pure component approaches to model DESs, having a more complex association scheme to better represent the role of [Ch]Cl in DESs, without increasing the number of parameters to be fitted and avoid the use of experimental data from very diluted aqueous solutions to obtain the molecular parameters. Once the model is developed, its performance and versatility are assessed through the successful modelling of a wide variety of systems containing [Ch]Cl, such as its binary mixtures with ethylene glycol (EG), glycerol, ethanol, H2O, carboxylic acids, and ternary mixtures of a DES with either CO2 or H2O.

soft-SAFT EoS [3]
soft-SAFT views molecules as a number of spherical segments tangentially bonded forming chains that might associate at specific bonding sites (see Fig. 1). The residual Helmholtz energy of the system (Ares) is thus obtained as a sum of different contributions (eq. 1): a reference term (Aref) that accounts for the monomer-monomer interactions; a chain term (Achain) to account for the formation of chains from the individual segments and an association term (AAss) that considers short range and highly directional forces such as hydrogen-bonding. When dealing with strong polar molecules like CO2 an additional term (Aelec) can be added. Once Aref is obtained, one can easily derive all the thermodynamic properties.

Figure 1. Schematic representation of the physical foundation of SAFT-type EoSs.

To fully characterize an associating compound i, like those present in DESs, five physically meaningful pure-component parameters are required by the model, namely the number of segments constituting the chain (m), the diameter of the segments (σ), the dispersive energy between segments (εka) and the association energies (εHB) and volumes of the sites (sHB).

Completing the molecular models, an association scheme needs to be defined a priori, specifying the number and type of association sites and the interactions allowed in the system.

In this work, considering the MD simulations and ab initio calculations for [Ch]Cl-based DESs available in literature, [4] five association sites were assigned to the salt (as illustrated in the graphical abstract): two sites (A and B) representing the
hydroxyl group as commonly done for alkan-1-ols,[5] two sites (C and D) to mimic the cation-anion interactions as typically done for ionic liquids[6] and an additional positive site E to represent the methyl groups that were found to be able to establish strong doubly ionic hydrogen bonds with the chloride anion.[4]. The association energies and volumes were transferred from other species involving the same functional groups decreasing to three the number of unknown parameters to be regressed from experimental data. To avoid the fitting to aqueous solutions, experimental data for the vapor-liquid equilibria (VLE) and high-pressure liquid densities (ρpT measurements) of [Ch]Cl + EG (a typical DESs) were measured and used in the parameterization procedure. The molecular models and parameters for the other compounds investigated in this work were readily available from previous publications.

Results

The three unknown parameters for [Ch]Cl (m, σ, ε/kB) were regressed from the experimental ρpT data measured for [Ch]Cl + EG (1:2) and boiling temperatures of [Ch]Cl + EG binary mixtures at different compositions. A state-independent binary interaction parameter (BIP) was fitted simultaneously to ensure a quantitative agreement with the experimental data. The results from the fitting are depicted in Fig. 2 and Table 1.

![Figure 2](image-url)

Figure 2. (Left) VLE of [Ch]Cl-EG. (Right) ρpT of [Ch]Cl-EG (1:2). Symbols represent experimental data while the solid lines depict the soft-SAFT results.

<table>
<thead>
<tr>
<th>m (Å²)</th>
<th>σ (Å)</th>
<th>ε/kB</th>
<th>BIP</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.358</td>
<td>3.598</td>
<td>417.62</td>
<td>1.062</td>
</tr>
</tbody>
</table>

The parameters obtained following this procedure were found to be in good agreement with those reported by Lloret et al. [6] for the symmetrical tetraalkylammonium chlorides and can thus be used to predict thermophysical properties and phase equilibria of other [Ch]Cl-based DESs. An example is the prediction (without using binary interaction parameters) of the ρpT data for [Ch]Cl + glycerol shown in Fig. 3 (left) where the excellent agreement with the experimental data demonstrates that the soft-SAFT model proposed here can correctly capture the effect of both temperature and pressure on the DESs densities. Similarly, the effect of composition is being well reproduced by the model as an excellent agreement was observed for the liquid densities of [Ch]Cl + phenol at different compositions (Fig. 3. Right).

![Figure 3](image-url)

Figure 3. (Left) ρpT of [Ch]Cl+Glycerol (1:2) (Right) Densities (@1atm) of [Ch]Cl + phenol at different HBD-HBA ratios.

One of the most challenging systems involving [Ch]Cl is its binary mixture with water. As reported by Zubeir et al. [7], using PC-SAFT, the five-pure component parameters of [Ch]Cl and a temperature-dependent binary parameter were necessarily fitted to VLE data and densities of aqueous solutions to achieve a reasonable agreement with the experimental data. Here, using the model developed for [Ch]Cl an excellent agreement with VLE and density data of aqueous solutions of [Ch]Cl was obtained as shown in Fig. 4. The need for temperature-dependent parameters being successfully eliminated.

![Figure 4](image-url)

Figure 4. Binary system [Ch]Cl + H2O. (Left) Boiling temperatures (Right) Liquid densities at different compositions.

Reliable results were also obtained for a variety of other systems involving [Ch]Cl such as the VLE of [Ch]Cl + ethanol, the solid-liquid equilibria (SLE) of [Ch]Cl + carboxylic acids or the CO2 solubilities in different DESs which are vital for CO2 capture applications commonly reported for DESs.

Conclusions

In this work a new coarse-grained model for [Ch]Cl was proposed in the framework of soft-SAFT EoS by successfully transferring the association parameters from other species and using experimental data for a typical DESs to obtain the remaining pure-component parameters for [Ch]Cl. Accurate results were obtained for the VLE, SLE and ρpT calculations of different DESs and their mixtures with H2O or CO2 using no more than one state-independent binary interaction parameter.

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References

Methylcycloalkane/benzene separations by extractive distillation with ionic liquids

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Ionic liquids (ILs) are potential neoteric solvents to design new advanced separation processes. Among several separation cases studied so far, the good performance of ILs regarding the deaeromatization of liquid fuels has received especially attention. However, liquid-liquid extraction alone seems not to be enough to fulfill current aromatic commercial standards, i.e. extreme aliphatic and aromatic purities. Extractive distillation with ILs stands as a new process configuration to overcome these limitations by enhancing the aliphatic/aromatic relative volatilities. In this work, a complete experimental and modelling study is presented to further explore extractive distillation with ILs for methylcycloalkane/benzene challenging separations.

Introduction

Ionic Liquids (ILs) are non-conventional salts that are liquid under 373 K and have negligible vapor pressure, showing an interesting liquid range of use. Among other purposes, ILs have been successfully used in a high number of separation cases as solvents in liquid-liquid extraction processes or as mass agents in vapor-liquid separation technologies [1]. Among other separation processes, liquid-liquid extraction and extractive distillation stand as the two more extended options to separate liquid compounds with similar boiling points by adding a solvent or mass agent, respectively. Liquid-liquid extraction stands as a preferably option due to the lower energy consumption to perform the separation but, unless the solvent has outstanding extractive properties, this process will demand a purification section to completely purify the two hydrocarbon classes. On the other hand, extractive distillation permits to separate and purify the compounds and avoids further purifications; nevertheless, the main separation will require more energy than in the liquid-liquid extraction case. Therefore, the effectiveness of the added compound, as solvent or mass agent, will determine the best technology [2].

The development of the aromatic/aliphatic separation by liquid-liquid extraction using ILs has been proved technically feasible, although with several limitations. The extractor inability to separate and purify aromatics and aliphatics demands further separation and purification steps at high vacuum conditions (very low pressures) [2]. The use of an extractive distillation configuration, using ILs, stands out as an alternative to the liquid-liquid extraction, with improved aromatic/aliphatic separation and several other advantages [3]: the separation and purification of the aromatics occurs in the same equipment; no solvent losses are acceptable in the distillate due to the negligible vapor pressure of ILs; aromatic/solvent separation can be flexibly planned working with a flash distillation unit under vacuum conditions or with an stripping column. After finding the first evidences on the extractive distillation suitability to separate toluene and n-heptane [2], more complex and challenging separations must be found and tested.

Regarding this, methylcyclopentane/benzene separation remains as one of the most difficult separation identified in the literature with hardly difference in the boiling of the two mixed hydrocarbons. Due to the interest of this mixture in the reformer naphtha [4], in which benzene is mainly mixed with methylcyclopentane and methylcyclohexane, an effective aromatic/aliphatic separation can allow to incorporate an additional benzene source to the petrochemical industry.

Objectives

The main goal of this work is to select suitable ILs and develop an experimental-modelling strategy in the search of an IL-based extractive distillation process to separate benzene from methylcycloalkanes.

Methods

Experimental. The vapor-liquid-liquid equilibrium (VLLE) determination was performed by an HS-GC technique, as fully explained in our last work [2]. After achieving the equilibrium, the characterization of the vapor phase and IL-rich liquid phase compositions and amounts allows one to know the hydrocarbon-rich compositions in equilibrium and calculate the equilibrium pressure.

Modelling. CPA EoS [5,6] is expressed in terms of compressibility factor (Z) as follows:

\[ Z = Z_{\text{phys}} + Z_{\text{assoc}} = \frac{1}{1-b\rho} - \frac{a\rho}{RT(1+b\rho)} \]

(1)

where \( a \) and \( b \) are energy and co-volume parameters, respectively, for the physical term, whereas \( g \) refers to a simplified hard-sphere radial distribution, and \( X_{\text{A}} \) is the mole fraction of component i not bonded at site A for the association contribution.

Imidazolium- and pyridinium-based ILs have been reported as associative compounds with two association sites for CPA EoS modelling [7]; hence, the same approach was selected in this work.
Results
Six cyano-based ILs were pre-selected considering their performance in the toluene/n-heptane separation, selecting those with the best combination of toluene/n-heptane selectivity and toluene distribution ratio. The six ILs were tested for the methylcycloalkane/benzene separation at solvent to feed (S/F) ratios of 1, 5 and 10 and temperatures of 323.2 and 363.2 K. As depicted in Table 1, the combination of both dicyanamide ([DCA]) and thiocyanate anions ([SCN]) with 1-ethyl-3-methylimidazolium cation ([C3C1im]) allows to achieve the best results within aforementioned conditions, enhancing methylcycloalkane/benzene relative volatilities.

[C3C1im][DCA] and [C3C1im][SCN] impact on the methylcycloalkane/benzene separation was deeply analyzed in the whole range of compositions and in a wider range of temperatures, aiming at completely define heterogeneous and homogeneous extractive distillation performance and boundaries. Although homogeneous extractive distillation is independent from composition over 403.2 K, the effectiveness of the ILs allows to think also in heterogeneous extractive distillation as a feasible technology with lower energy and solvent consumptions.

Conclusions
[C3C1im][DCA] and [C3C1im][SCN] stand as very efficient mass agents to perform the complex benzene separation from methylcycloalkanes, especially from methylcyclopentane. The experimental-modelling strategy using HS-GC and CPA-EoS has allowed to propose a robust model capable of delimiting homogeneous and heterogeneous extractive distillation with enough accuracy to be used in a process simulator to optimize a new technology to separate a challenging mixture in the chemistry industry.
Cyclohexane/cyclohexene separation by extractive distillation with cyano-based ionic liquids

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Introduction

The cyclohexane/cyclohexene conventional distillation demands high number of equilibrium steps since both cyclic hydrocarbons have very close boiling points, i.e. 353.9 K and 356 K for cyclohexane and cyclohexene, respectively [1]. Several approaches have been tested until now, covering mainly adsorption, extractive distillation, and membrane-based processes using conventional solvents and separation conducted with advanced materials [2]. Recently, liquid-liquid extraction has been extensively studied using ionic liquids (ILs) as new solvents to separate cyclohexane from cyclohexene [3,4].

ILs are liquid salts at temperatures below 373 K with non-volatile character that have shown highlighted effectiveness in a wide variety of separation cases regarding compounds with near boiling points. Although most of the cases the use of ILs refers to a liquid-liquid extraction technology, extractive distillation with ILs also stands as a suitable technology that should be evaluated together with liquid-liquid extraction as a necessary task for designing chemical processes [5,6].

From the liquid-liquid equilibrium (LLE) data available in the literature, it seems that all ILs have shown low cyclohexene/cyclohexane selectivities (< 5) and mass-based cyclohexene distribution ratios (< 0.20). Accordingly, liquid-liquid extraction technology would require large amounts of solvent to hardly separate the two species; thus, liquid-liquid extraction would demand further purification for extract and raffinate to assay commercial standards. As a result of liquid-liquid extraction limitations, the evaluation of the extractive distillation technology stands as the most convenient way to improve the knowledge of the cyclohexane/cyclohexene separation with ILs.

Objectives

In this work, both experimental and modelling tasks were carried out as a first step to establish the technical feasibility of the extractive distillation with ILs to separate cyclohexane from its mixtures with cyclohexene. VLE data for systems composed by cyclohexane, cyclohexene and cyano-based ILs were determined at 323.2, 363.2 and 403.2 K. All systems were properly modelled with Perturbed Chain Statistical Association Fluid Theory (PC-SAFT), regressing the ILs’ molecular parameters from their high-pressure density literature data and ensuring the well-known non-volatile character of these solvents.

Methods

Experimental. The VLE determination was performed by an HS-GC technique, as fully explained in our last work [7]. After achieving the equilibrium, the characterization of the vapor phase compositions and amounts allows one to know the liquid phase composition in equilibrium and calculate the pressure.

Modelling. PC-SAFT equation of state [8] can be written in residual Helmholtz energy as the sum of hard-chain (hc), dispersive (disp) and associative forces:

\[ a_{\text{res}} = a_{\text{hc}} + a_{\text{disp}} + a_{\text{assoc}} \]

\[ RT \]

\[ RT \]

\[ RT \]

\[ RT \]

\[ RT \]

Tricyanomethanide-based and dicyanamide-based ILs were considered as association fluids, as reported in the literature, with 3+3 and 3+2 schemes [9]. Accordingly, five parameters are required, namely the segment number (m), the segment diameter (σ), the energetic parameter (ε/kB), the association volume (k) and the association energy (ε/kB).

Results

Three tricyanomethanide and two dicyanamide-based ILs were selected due to their compensate and high extractive properties shown for the cyclohexane/cyclohexene separation. From experimental VLE, cyclohexane/cyclohexene relative volatilities (α1, 2) were calculated and are depicted in Table 1, evidencing high effectiveness of all ILs.

As shown, it is clear that [4-C4C6Im][TCM] and [C4C6Im][TCM] ILs solubilize more cyclohexene and are more effective at low temperatures, whereas dicyanamide-based ILs show the lower cyclohexene retention, being more effective...
than the others only at 403.2 K. Overall, \([\text{C}_2\text{C}_1\text{im}]\text{[TCM]}\) stands as a commitment mass agent in the temperature range from 323.2 to 403.2 K; in addition, this IL also presents the lowest viscosity among all promising ILs proved so far in this separation, 7.7 mPa·s at 323.2 K [6].

| Table 1. Cyclohexane/cyclohexene relative volatility (\(\alpha_{1,2}\)) in the cyclohexane (1) + cyclohexene (2) + IL (3) systems for equimolar hydrocarbon mixture in feed and solvent to feed ratio of 10 |
|-----------------|-----------------|
| IL              | \(\alpha_{1,2}\) |
| \(T = 323.2\) K |                 |
| \([\text{C}_2\text{C}_1\text{im}]\text{[DCA]}\) | 1.4             |
| \([\text{C}_2\text{C}_1\text{im}]\text{[DCA]}\) | 1.6             |
| \([\text{C}_2\text{C}_1\text{im}]\text{[TCM]}\) | 1.7             |
| \([\text{C}_2\text{C}_1\text{im}]\text{[TCM]}\) | 1.9             |
| \([4\text{-C}_2\text{C}_1\text{py}]\text{[TCM]}\) | 2.1             |
| \(\text{without IL}\) | 1.03           |
| \(T = 363.2\) K |                 |
| \([\text{C}_2\text{C}_1\text{im}]\text{[DCA]}\) | 1.9             |
| \([\text{C}_2\text{C}_1\text{im}]\text{[DCA]}\) | 3.0             |
| \([\text{C}_2\text{C}_1\text{im}]\text{[TCM]}\) | 3.2             |
| \([\text{C}_2\text{C}_1\text{im}]\text{[TCM]}\) | 3.0             |
| \([4\text{-C}_2\text{C}_1\text{py}]\text{[TCM]}\) | 2.8             |
| \(\text{without IL}\) | 1.03           |
| \(T = 403.2\) K |                 |
| \([\text{C}_2\text{C}_1\text{im}]\text{[DCA]}\) | 2.0             |
| \([\text{C}_2\text{C}_1\text{im}]\text{[DCA]}\) | 2.0             |
| \([\text{C}_2\text{C}_1\text{im}]\text{[TCM]}\) | 2.0             |
| \([\text{C}_2\text{C}_1\text{im}]\text{[TCM]}\) | 1.6             |
| \([4\text{-C}_2\text{C}_1\text{py}]\text{[TCM]}\) | 1.8             |
| \(\text{without IL}\) | 1.02           |

On the other hand, PC-SAFT has properly described the vapor-liquid equilibria regarding binary and ternary systems, using transferable binary interaction parameters, as well as simultaneously represented the high-pressure densities and negligible vapor pressures for the mass agents, with molecular parameters with physical meaning. Overall, PC-SAFT was revealed as a proper model to further explore in a process simulator the boundaries of the extractive distillation with ILs, aiming at propose new technologies to separate cyclohexane from cyclohexene.

**Conclusions**

In the search of a new process for the cyclohexane/cyclohexene separation and taking advance of the well-known properties of ILs, promising cyano-based ILs have been tested here. Although extractive distillation approach is sound for the five ILs tested, \([\text{C}_2\text{C}_1\text{im}]\text{[TCM]}\) stands as the most potential mass agent to further explore a new technology capable of enhancing better hydrocarbon standards in addition to improve current technologies. The suitable experimental-modelling combination of HS-GC and PC-SAFT has allowed to propose a model ready to be implement in a process simulator.

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**References**

Separation of cyclohexene from cyclohexane by liquid-liquid extraction with ionic liquids

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Finding an energy-efficient process for the separation of olefins from paraffins is one of the major challenges in the petrochemical industry. Currently, extractive distillation is the most used technology due to the close boiling points of these compounds; however, this process has several drawbacks and large energetic consumption. Liquid-liquid extraction with ionic liquids (ILs) can overcome these limitations due to the mild operating conditions of this technology and the unique properties of ILs. Five promising ILs have been tested here in the cyclohexane/cyclohexene separation by liquid-liquid extraction. The liquid-liquid equilibria (LLE) for the ternary mixtures {cyclohexane + cyclohexene + IL} were determined at optimized temperatures and atmospheric pressure, regressing the data to Non-Random Two Liquids (NRTL) model.

Introduction

The separation of olefins from paraffins by distillation process involves large energetic costs and capital investment due to the close relative volatilities of these compounds [1]. To overcome this drawback, the separation of close boiled compounds is commonly performed via extractive or azeotropic distillation [2,3]. In the search of new technologies at mild conditions, liquid-liquid extraction with ILs rises as a new alternative. The use of ILs as extractive solvents would improve capacities and selectivities with respect to conventional organic solvents and would decrease solvent losses in the process [4].

Domanska et al. have reported that imidazolium- and pyridinium-based ILs with cyan and bis(trifluoromethylsulfonyl)imide anions show high extractive properties in the liquid liquid extraction for the cyclohexane/cyclohexene separation [3,4]. However, some interesting ILs have not been proved up to the date and are identified as potential species to separate cyclohexane and cyclohexene. First, 1-alkyl-4-methylpyridinium cation stands as the structure that offers the most compensate performance between capacity and selectivity as demonstrated in a previous work regarding aliphatic/aromatic separation [5], whereas new anions as tetraethylammoniumcobaltate incorporates a transition metal, which is known to enhance the separation of paraffins from olefins [6].

Objectives

The aim of this work is the separation of cyclohexene/cyclohexane by liquid-liquid extraction using new promising ILs, namely 1-butyl-4-methylpyridinium tricyanomethanide ([4bmpy][TCM]), bis(1-ethyl-3-methylimidazolium) tetrathionyanatomobaltate ([emim][Co(SCN)4]), bis(1-butyl-3-methylimidazolium) tetraethionyanatomobaltate ([bmim][Co(SCN)4]), 1-ethyl-4-methylpyridinium bis(trifluoromethylsulfonyl)imide ([4empy][Tf2N]), and 1-butyl-4-methylpyridinium bis(trifluoromethylsulfonyl)imide ([4bmpy][Tf2N]).

Methods

Hydrocarbons and ILs were gravimetrically added to 8 mL vials using a Mettler Toledo XS 205 balance with a precision of ±1·10⁻⁵ g. The LLE was reached in a Labnet Vortemp 1550 shaking incubator for 5 h at T = (298.2, 313.2 and 328.2 ± 0.3) K. To reach the complete phases splitting, the vials were moved to a Labnet Accublock dry bath for 12 h at T = (298.2, 313.2 and 328.2 ± 0.5) K. Triplicate samples from the raffinate phases were analyzed in an Agilent 7890A gas chromatograph (GC) equipped with a flame ionization detector (FID). The extract phases were analyzed following the multiple headspace extraction (MHE) method. Triplicate samples of 100 µL from the extract phases were added to 20 mL vials and analyzed using an Agilent 7890A GC coupled with a Headspace Sampler Agilent 7697A. A detailed description of both analytical methods can be found in our previous work [7].

Results

First, LLE data have been determined for the ternary systems {cyclohexane (1) + cyclohexene (2) + IL (3)} at optimized temperatures and atmospheric pressure. From the experimental data, the hydrocarbon distribution ratio (Di) and the cyclohexene/cyclohexane selectivity (α2,1) for the ternary systems {cyclohexene (1) + cyclohexane (2) + IL (3)}, depicted in Figure 1, were calculated from the experimental compositions of the raffinate and extract phases with the following equations:

\[
D_i \left( \frac{g}{g} \right) = \frac{w^I_i}{w^I_j}
\]

(1)

\[
\alpha_{2,1} = \frac{D_2}{D_1}
\]

(2)

where \(w_i\) is the mass fraction for the hydrocarbon i, and superscript I and II refer to the raffinate and extract phases, respectively.

As can be seen in Figure 1, the five studied ILs show values of distribution ratio and selectivity similar or even higher than those previously reported in the literature standing out as the benchmark solvents for the studied case. Among the new ILs, [4bmpy][TCM] showed the highest mass-based cyclohexene distribution ratio up to the date with a selectivity near the highest reported for the cyclohexane/cyclohexene separation.
In addition, the NRTL model has been used to correlate the experimental data of the five ternary systems. Figure 2 shows the obtained LLE data and the accurate NRTL regression for the [4bmpy][TCM].

![Liquid-liquid equilibrium at T = 298.2 K and P = 0.1 MPa. Solid lines and full points represent experimental tie-lines whereas dashed lines and empty squares are the regressed data using the NRTL model.](image)

**Figure 2.** Liquid-liquid equilibrium at $T = 298.2$ K and $P = 0.1$ MPa. Solid lines and full points represent experimental tie-lines whereas dashed lines and empty squares are the regressed data using the NRTL model.

**Conclusions**

The [4bmpy][TCM] IL has shown the highest values of distribution ratio and the [emim]$_2$[Co(SCN)$_4$] IL has shown the highest cyclohexene/cyclohexane selectivity. However, the [4bmpy][TCM] IL has shown the best combination of extractive properties among all ILs proved so far. The experimental LLE data were successfully fitted to the NRTL model.

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**References**

Impact of water on the CO₂ solubility in [C₄C₁im][Ac]

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It is widely accepted that the presence of water, even at very low concentrations, has a negative impact on the carbon dioxide solubility in ionic liquids (ILs) but, few studies have focused on evaluating water real impact on the sorption capability over the entire concentration range. Here, the water impact on the CO₂ solubilities in the promising 1-butyl-3-methylimidazolium acetate ([C₄C₁im][Ac]) IL was evaluated for [C₄C₁im][Ac]:H₂O molar ratios of 5:1, 2:1, 1:1, and 1:2, for temperatures ranging from (293 to 363) K and pressures up to 70 MPa. Water is shown to improve the IL capability to solubilize CO₂, with equimolar molar ratios leading to enhanced solubilities for pressures up to 5 MPa, whereas [C₄C₁im][Ac]:H₂O molar ratios of 5:1 and 2:1 present higher CO₂ solubility at higher pressures.

Introduction

Ionic Liquids (ILs), a new class of solvents with outstanding properties, present high CO₂ solubility (in mole basis), high selectivity, low volatility, good thermal stability, and have a very tunable structure that allows the design of compounds that can meet the requirements of a given process. There has been an increasing interest from both academia and industry, and it is widely accepted that ILs are good candidates for CO₂ capture;¹ however, only ILs that present chemical reaction with CO₂ have shown to be solvents with promising capabilities at low temperatures and moderate pressures, to fulfill industrial requirements.² Among the continuously growing number of ILs that present chemisorption the [C₄C₁im][Ac] has attracted most interest.³,⁴ Shifflett et al.³,⁵ have demonstrated the viability of its use at industrial scale and an interesting advantage of [C₄C₁im][Ac] when compared with other chemical solvents, such as amines, is that presents also a good sorption capability at high pressures on the physical absorption regime.

In previous works,⁶,⁷ we have shown that water plays a vital role on promoting the chemical reaction between the species for the system [C₄C₁im][Ac] + CO₂. In the presence of water, [C₄C₁im][Ac] and CO₂ react forming 1-butyl-3-methylimidazolium-2-carboxylate, involving also the isomerization process and the formation of a “transient” CO₂-carbene complex, and acetic acid. Moreover, the formation of bicarbonates (HCO₃⁻) was also observed and their concentration increases with the water content, not affecting the carbamate formation. However, the water impact at moderate to high pressures, out of the chemisorption region, remains unclear and worth exploring.

In this context, Anderson et al.⁸ developed a new acid gas stripping solvent for removal of carbon dioxide from natural gas sources based on ionic liquids with carbamate anions where the water content was carefully controlled at near equimolar amounts. The impact of water in the solubility stands as extremely relevant, especially if one considers that most potential streams to be treated have some water in their composition. The concept of water as an enhancer of the absorption rather than an impurity stands as a paradigm shift capable of changing ways of thinking and contributing to the development of new methods for CO₂ capture.

Objectives

In this work the impact of water on CO₂ solubilities in the [C₄C₁im][Ac] IL is evaluated. [C₄C₁im][Ac]:H₂O molar ratios of 5:1, 2:1, 1:1, and 1:2 were experimentally investigated for temperatures ranging from (293.2 to 363.2) K.

Methods

The high pressure equilibrium cell is based on the synthetic method and consists of a horizontal hollow stainless-steel cylinder, closed at one end with a movable piston and at the other end with a sapphire window, from which the operator follows the behavior of the sample with pressure and temperature. Other details were fully described in previous publications.⁴ An exact mass of IL, determined using a high weight/high precision balance (Sartorius LA200P), was introduced in the cell followed by an exhaustive in situ drying procedure (1 Pa, 303 K and stirring) to ensure the removal of any atmospheric gas and water. For the aqueous mixtures preparation, water was added, after the IL, to the cell through a flexible pressure capillary and its mass measured with the mentioned balance. The gas was introduced under pressure, from an ultra-lightweight composite cylinder, by means of a flexible pressure capillary and its mass measured also with the balance cited above.

Once the mixture with known composition reaches the desired measurement temperature, the pressure is increased until the system becomes monophasic. The pressure is then slowly decreased until a phase change is observed and then slowly increased until the last bubble disappears. The methodology is repeated several times aiming at determine the minimum equilibrium pressure necessary to solubilize the gas. The pressure was measured by a piezoresistive silicon pressure transducer (Kulite HEM 375) fixed directly inside the cell, to reduce dead volumes, with an accuracy better than 0.2%.

Results

Firstly, it is seen that increasing water content, even over [C₄C₁im][Ac]:H₂O molar ratios of 1:2, does not inhibit the chemical reaction between [C₄C₁im][Ac] and CO₂ and enhance equal or even better CO₂ molarities than that for the neat IL in the same conditions. As depicted in Figure 1, the 1:1 mixture shows the higher solubility independently of the temperature and at 5 MPa (or lower pressures). The 1:2 mole ratio presents equilibrium pressure similar to those of the neat IL, whereas the remaining mixtures present solubilities that range between those reported for the neat IL and the 1:1 mole ratio. Above 5
MPa and molalities higher than 5 mol·kg$^{-1}$, 5:1 and 2:1 proportions stand out as the most favorable.

Figure 1. $pTm$ diagrams for the systems CO$_2$ + [C$_4$C$_1$im][Ac] + H$_2$O. Data for neat compounds were taken from literature.$^9$

Figure 2 shows the CO$_2$ molality dependence on the water molar fraction in the solvent, at 313 K, to clearly establish that the molality shows a maximum with water that moves from equimolar proportions, at pressures below 5 MPa, to IL: H$_2$O ratio of 2:1 (water mole fraction of 0.34). If one aims to design a process to capture CO$_2$ at low or moderate pressures the equimolar IL:H$_2$O ratio stands out as the target condition (with an enhancement in molality, compared to the pure IL, of around 38%), while for processes operating at higher pressures the carbon dioxide capture is enhanced when using a IL:H$_2$O molar ratio of 2:1 (with an enhancement in molality of around 40% and 16% compared to the pure and the 1:1 molar ratio, respectively). Finally, the well-known decrease on the IL viscosity by adding water is an additional advantage for process design.

Conclusions

Water has shown to improve the IL capability to solubilize CO$_2$ with IL + H$_2$O mixtures, with water mole fraction ranging between 0.17 to 0.66, showing better performance than the neat [C$_4$C$_1$im][Ac]. Equimolar ratios have shown to allow enhanced solubilities at pressures up to 5 MPa, whereas [C$_4$C$_1$im][Ac]:H$_2$O proportions of 5:1 and 2:1 have shown higher CO$_2$ solubility at higher pressures. Thus, water can be considered an advantage rather than an impurity.

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Simulation and optimization of the ethanol dehydration process by extractive distillation using imidazolium-based ionic liquids as solvents

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Ionic liquids (ILs) represent a new class of solvents as promising surrogates to conventional organic solvents (volatile and toxic) as well as alternatives to reduce environmental pollution. One use of ILs of great importance to industry is in breaking azeotropes, as in the separation of ethanol/water mixtures. In this work, the extractive distillation process to produce dehydrated ethanol was evaluated for three imidazolium-based ILs as solvents, namely [Emim][Cl], [Bmim][Cl] and [Hmim][Cl]. The Aspen Plus® software was used to simulate the process and multivariate statistical techniques were employed to optimize the separation. The multivariate statistical techniques were effective in the optimization of the process as operational conditions could be determined to minimize the energetic consumption while holding ethanol purity according to specification (greater than 99.3%).

Introduction

The inclusion of liquid biofuels in the energy matrix is a consequence of the increasing concern with environmental issues and the adoption of public policies in response to the oil crises. One of the main biofuels used is ethanol, obtained from sugarcane, which can be used, directly or by modification, in internal combustion engines with spark ignition. The technology employed on a larger scale in the production of anhydrous alcohol is based on azeotropic or extractive distillation with the use of conventional solvents that are generally volatile and toxic. The ethanol production has been studied with the objective of replacing these conventional solvents by cleaner solvents, as well as aiming at energy savings and increasing the purity of anhydrous ethanol.

In this scenario, a new trend arises for the use of ionic liquids (ILs), which show the advantage of having a good ability for separation, easy handling and low contamination of the distilled products. In this work, the extractive distillation process for production of anhydrous ethanol was simulated and optimized for the ILs 1-ethyl-3-methylimidazolium chloride ([Emim][Cl]), 1-butylyl-3-methylimidazolium chloride ([Bmim][Cl]) and 1-ethyl-3-methylimidazolium chloride ([Hmim][Cl]) as solvents. Experimental design techniques and the desirability function were employed to identify the best operating conditions for the process in order to maximize the purity of anhydrous ethanol fuel and solvent recovery as well as to minimize the energy consumption.

Methodology

The process was simulated using the Aspen Plus software, version 10. The NRTL (Non-Random Two-Liquid) model was used to calculate vapor-liquid equilibrium due to the presence of strongly polar components. The extractive distillation unit was initially set up based on data from [1, 2]. The ILs used were inserted in the simulator as pseudocomponents. The thermodynamic properties of the pure ILs and the NRTL binary interaction parameters were taken from [3, 4, 5].

Simultaneous evaluation of process factors was carried out using Statistica® software, version 8. A full factorial design (with central point) with 6 factors was initially employed. Table 1 shows the factor levels studied, where S/F is the solvent-to-feed ratio, column P is the solvent recovery column and column C is responsible for the ethanol dehydration. The process flow sheet is presented in the graphical abstract.

The desirability function [6] was used to simultaneously optimize the energy of the C (E - Col C) and P (E - Col P) columns and maximizing the concentration of ionic liquid recovered (C LI-rec) and anhydrous ethanol fuel (C AEF), maintaining the purity of the ethanol according to the specifications (above 99.3%).

Results and Discussion

For the process using the ILs [Emim][Cl] and [Hmim][Cl], factor 3 did not present significant effects on the responses. Hence, the starting set up column P feed stage was kept throughout the simulations (third plate for [Emim][Cl] and seventh plate for [Hmim][Cl]). In the case of [Hmim][Cl], the best results were obtained at values 0.46 and 0.2 for factors 5 and 6, respectively. Therefore, the desirability function was computed using a 2n design for the [Bmim][Cl]. In the case of [Emim][Cl], a five-factors design was employed and a central composite design with 3 factors was employed for the [Hmim][Cl]. According to the results of the total desirability function (Figure 1), the optimized levels for each factor studied could be identified, presenting the best condition to meet all specifications initially proposed.

The process conditions and optimized responses to each solvent are organized in Table 2. The starting configuration is also presented in Table 2 for comparison purposes.

It can be observed that the specification purity of the anhydrous ethanol was achieved as well as more than 99% by mass of IL could be recovered for all processes. In terms of energy consumption in column C, a decrease of 11.6, 41.2 and 31.6 % was observed for the solvents [Emim][Cl], [Bmim][Cl] and [Hmim][Cl], respectively. In the case of the energy of column P, a decrease of 23.5, 31.9 and 26.7 % in the same order was observed.

As can be observed in Figure 2, the combination of process simulation with multivariate statistical techniques was effective in the process optimization. The optimized energy consumption was less than the energy reported by the majority of the studies found in literature.
Table 1. Design factors and levels.

<table>
<thead>
<tr>
<th>Factors</th>
<th>[Emim][Cl]</th>
<th>[Bmim][Cl]</th>
<th>[Hmim][Cl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>1) Hydrous ethanol feed stage</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2) Solvent Feed Stage</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3) Feed stage of column P</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4) Reflux ratio of column C</td>
<td>-0.7</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>5) Reflux ratio of column P</td>
<td>0.5</td>
<td>1.5</td>
<td>2.0</td>
</tr>
<tr>
<td>6) Ratio S/F</td>
<td>0.2</td>
<td>0.28</td>
<td>0.31</td>
</tr>
</tbody>
</table>

Table 2. Simulation results before and after optimization and factor modifications.

<table>
<thead>
<tr>
<th>Factors</th>
<th>[Emim][Cl]</th>
<th>[Bmim][Cl]</th>
<th>[Hmim][Cl]</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>b</td>
<td>a</td>
<td>b</td>
</tr>
<tr>
<td>1)</td>
<td>20</td>
<td>24</td>
<td>20</td>
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<td>2)</td>
<td>6</td>
<td>2</td>
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<tr>
<td>3)</td>
<td>1</td>
<td>3</td>
<td>9</td>
</tr>
<tr>
<td>4)</td>
<td>1</td>
<td>0.7</td>
<td>2</td>
</tr>
<tr>
<td>5)</td>
<td>2</td>
<td>0.5</td>
<td>2</td>
</tr>
<tr>
<td>6)</td>
<td>0.26</td>
<td>0.25</td>
<td>0.23</td>
</tr>
</tbody>
</table>

Responses:
- C EAF (% m/m): 99.3, 99.4, 99.6, 99.5, 99.7, 99.8
- E: Col C (MW-h): 2.184, 1.93, 3.143, 1.848, 3.642, 2.491
- E: Col P (MW-h): 0.885, 0.677, 0.543, 0.37, 0.845, 0.619

Initial condition (a) and after optimization (b)

Figure 1. Global desirabilities.

Figure 2. Comparison of the results obtained with the literature.

Acknowledgements
The Authors are grateful to CAPES, CNPq, NUQAAPE, FACEPE and INCTAA for the financial support.

References
Binderless shaped metal-organic framework particles

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Metal-organic framework (MOF) particles were shaped by binderless mechanical compression of commercial ZIF-8 and MIL-53(Al) powders. Two compression pressures were employed — 62 MPa and 125 MPa. The impact in the mechanical, structural, and textural characteristics of the MOFs was evaluated, as well as the influence in carbon dioxide (CO2) adsorption capacity at 303 K. The obtained MOF particles are robust. The procedure has more impact on the crystalline and textural properties of MIL-53(Al) than on those of ZIF-8. The shaping procedure also has minor impact on the CO2 adsorption capacity of the ZIF-8 while the MIL-53(Al) granules yielded higher CO2 adsorption capacity losses: up to 24% at 15 bar of CO2 pressure. The method employed in this work proves to be a viable and straightforward alternative for the preparation of ZIF-8 and MIL-53(Al) particles to be applied in gas-phase separation processes.

Introduction

Metal-Organic Frameworks (MOFs) are crystalline materials built up by metal ion clusters connected through functional organic linkers presenting large specific surface areas and pore volumes [1]. Although an increasing number of novel MOF structures are being synthesized yearly [2], their application in gas-phase separations at industrial scale is not feasible due to the lack of millimetre-scale MOF particles (cylindrical pellets, spheres or granules). Shaped MOF particles can overcome the large pressure drops and consequent energy consumption of using adsorbent columns with powder-like particles. Thus, shaping adsorbents is an important step for their use in industry, although the shaping procedure can affect their properties, e.g. adsorption capacity and selectivity towards a specific gas separation.

In this work, the formulation of binderless MOF granules by mechanical compression of two distinct MOF powders is studied. For this purpose, commercial powdered samples of ZIF-8 (Basolite® Z1200) and MIL-53(Al) (Basolite® A100) were employed. The impact of the compression pressure (62 and 125 MPa) in the mechanical, structural, and textural properties of the produced particles is evaluated and compared with the pristine powders. The influence of the shaping procedure on carbon dioxide (CO2) adsorption uptake is assessed, evaluating the potential of using the produced particles in adsorption-based separation/purification processes.

Methods

Binderless MOF disk tablets were prepared using a bench-top manual hydraulic press (Graseby Specac, UK). MOF disks with 10 mm of diameter (~0.5 g) were prepared by applying a pressure of 62 MPa or 125 MPa for 10 minutes. After the disks formation, these were crushed and sieved to select particles with sizes within 1 and 2 mm.

The mechanical resistance of the produced MOF particles was evaluated by performing pressurization/depressurization cycles mimicking the typical steps of a Pressure Swing Adsorption (PSA) process.

The crystallinity of the MOF particles was evaluated through Powder X-Ray Diffraction (PXRD) performed in an X-Ray Diffractometer (Rigaku MiniFlex™ II, Japan). Nitrogen (N2) adsorption/desorption isotherms at 77 K were measured using an ASAP® 2420 (Micromeritics, USA).

Adsorption/desorption equilibria of CO2 at 303 K were experimentally determined within the pressure ranges of 0–30 bar and 0–15 bar for ZIF-8 and MIL-53(Al) samples, respectively. The adsorption equilibrium data of CO2 on ZIF-8 were measured using a standard static gravimetric method; for the MIL-53(Al) samples a volumetric/manometric static procedure was employed.

Results

The ZIF-8 and MIL-53(Al) MOFs particles prepared are shown in Figure 1 [3].

The pressurization/depressurization essays performed suggest that both the compressed particles of ZIF-8 and MIL-53(Al) can be employed in adsorption processes, since, for pressures up to 18 bar, the MOF weight loss was always below 5% of the initial mass packed into the column. This demonstrates that the produced MOF particles are robust.

The experimental PXRD spectra obtained for the ZIF-8 powder and the compressed granules are similar, and identical to the theoretical pattern [4]. On the other hand, the PXRD results for MIL-53(Al) show that the granules patterns changed more significantly, in relation to the pristine powder, than ZIF-8’s subjected to the same mechanical compression.

The N2 adsorption equilibrium isotherms at 77 K of the ZIF-8 and MIL-53(Al) granular samples are lower than the isotherms for the original powders. This is due to the MOF crystals compression and consequent decreases of the specific surface areas and pore volumes. This effect is much more significant in the case of MIL-53(Al), while the ZIF-8 pore volume and specific surface are is only slightly affected by the compression.
Regarding the CO$_2$ adsorption equilibria isotherms, the obtained results showed that the ZIF-8 particles produced show no difference with the powder up to 8 bar, and above this pressure only a slight decrease in adsorption capacity is observed when compared with the pristine powder. This is accordance with the data obtained from the N$_2$ adsorption at 77 K. On the other hand, the MIL-53(Al) prepared particles have observable CO$_2$ adsorption capacity losses above 2 bar, and at 15 bar the decrease in CO$_2$ uptake, when compared with the original powder, is as high as 24$\%$ [3, 5].

**Conclusions**

MOF particles have been prepared through binder-free mechanical compression of commercial ZIF-8 and MIL-53(Al) powders. The MOF particles produced are robust and mechanically resistant. PXRD and N$_2$ adsorption at 77 K showed that the mechanical compression impact in the crystalline and textural properties of the MOFs is much more important in the case of MIL-53(Al) than in ZIF-8. This conclusion is confirmed by the CO$_2$ adsorption equilibria isotherms. The results obtained suggest that the methodology employed produced ZIF-8 and MIL-53(Al) particles that can be used in adsorption-based gas separations.

**Acknowledgements**

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**References**

Production and characterization of biodiesel obtained by transesterification catalysed by ionic liquids based on imidazolium

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Introduction

Biodiesel is a fuel produced from fats or oils, vegetable or animal, consisting of esters of fatty acids. Its main advantages are due to the fact that they can be obtained from renewable resources, to have an ecological emission profile, in addition to being biodegradable and environmentally friendly, but has the disadvantage of a high cost of production. The International Energy Agency reported that biodiesel produced with current technology is about two to three times more expensive than petrol and diesel oil due to the high cost of the raw material used. Several studies show that the value added of the raw material represents about 75% of the biodiesel production cost [1, 2]. The production of biodiesel from waste cooking oils and the application of ionic liquids as catalysts have been applied as a possible alternative to overcome the disadvantages in the traditional production process. Ionic liquids can be recovered and the recycled, making the industrial process economically and environmentally friendly, in addition to the fact that there is no conflict with the food industry due to the reuse of oils that are no longer edible. The reutilization of waste oils to biodiesel production has the potential to decrease approximately 45% of the all cost of production, even taking into account the coast increase with pre-treatments necessary for the reduction of water content and free fatty acids [3].

The biodiesel produced from oils and fats from vegetable and animal sources are a promising alternative for replacing the use of fossil fuels, but their high viscosity can be a disadvantage to the engine ignition. The most widely used processing option is the transesterification reaction, which is favorable to obtain fuel from triglycerides, and the esterification reaction, which is a great option for the conversion of free fatty acids (FFA) present in oils, in esters of fatty acids. In both reactions, the presence of catalysts for the production of biodiesel is required for an effective conversion [2, 4-5].

Basic catalysts are widely used because they have lower costs and high availability requires low amounts of alcohol, with acceptable reaction times. However, this base-catalyzed process is very sensitive to the presence of water and FFAs, which leads to saponification reactions. The saponification, besides causing an unproductive consumption of the catalyst hinders the purification process of the final product. Therefore, the use of basic catalysts in oils which have high rates of FFAs, as is the case of waste oils, is complex, and some pre-treatments are mandatory, which makes the process time-consuming and therefore expensive. On the other hand, acid catalysts are not sensitive to FFAs, which makes them excellent alternatives to use in oils with high levels of acidity. However, these catalysts lead to reaction times up to 4000 times higher, higher alcohol/oil molar ratios; higher reaction temperatures and can lead to corrosion of equipment, increasing the costs. Ionic liquids used in catalytic systems, come as an alternative to solve this issue in biodiesel production [4-5].

Ionic Liquids

By definition, ionic liquids are organic salts with exceptional thermal and chemical stability, high catalytic activity and solvability, low viscosity, low toxicity, non-flammable and are easily handled. One of the most significant characteristics of ionic liquids is the possibility of designing the molecule according to its specific application, tracing a certain set of properties. Since these compounds are high priced, this property makes it highly attractive. The recycling process, most of the time, occurs through distillation, solvent extraction, adsorption or the application of separation membranes [5].

When it refers to the production of biodiesel linked to ionic liquids, some published studies shows that the acidic character of the ionic liquid has a high influence on the catalytic action of biodiesel production. In addition, ionic liquids are able to reduce significantly the number of purification stages in the process of producing the fuel, reducing the costs of its production [6].

Ionic Liquids in Biodiesel Production

Imidazolium-based ionic liquids have been the most studied for biodiesel production due to their ionic standards, low pressure and capacity for self-organization in different states, including hydrogen sulfate 1-butyl-3-methylimidazolium [BMIM][HSO4], which has been showing promising results.
Elsheikh et al. (2011) carried out a study on the production of biodiesel from the transesterification reaction. They used crude palm oil and investigated ionic liquids containing imidazolium, [BMIM][HSO₄], [BIM][HSO₄], [MIM][HSO₄], to study which one had the highest catalytic potential. The best results were obtained once again, with the [BMIM][HSO₄] ionic liquid, reaching a conversion of 91.2% in its optimum reaction conditions, represented by 4.4% catalyst concentration, molar ratio of alcohol/oil of 12:1, reaction temperature of 160°C and reaction time of 120 min [7].

Hafidiz et al. in 2013, performed a multi-objective optimization in the esterification reaction using oleic acid and methanol catalyzed by [BMIM][HSO₄]. They study some reaction parameters such as, the reaction temperature, reaction time, molar ratio between methanol and oleic acid and the dosage of catalyst. They concluded that optimal reaction conditions occurred with a temperature of 87°C, a reaction time of 5.2 hours, alcohol/oil molar ratio of 9:1 and catalyst charge of 0.06 mol, resulting in 80.4% acid conversion oleic acid and 81.8% yield of methyl oleate [8].

In 2013, the same group studied the production of biodiesel from the esterification reaction of oleic acid with methanol, using as catalyst [BMIM][HSO₄]. They identified as the reaction optimal conditions an alcohol/oil molar ratio of 9:1, the catalyst load was 3.4% by mass, a reaction time of 4 hours and a reaction temperature of 90°C, resulting in an oleic acid conversion of 84.4% [9].

Li et al. (2014) also carried out a study of biodiesel production from the transesterification reaction. They used Campsottheta acuminita seed oil in the presence of several imidazolium-based acidic liquids acting as catalysts. Using a methanol/oil ratio of 6:1, catalyst concentration of 5%, temperature of 60°C and a reaction time of 30 min, [BMIM][HSO₄] was only the third of the five catalysts studied with the best result, with a conversion of 38.5% [10].

Ullah et al., in 2015 published a research on the production of biodiesel from a two-step process. In the first step, an esterification reaction was carried out, using palm oil and methanol, catalyzed by an ionic liquid with the purpose of reducing the acidity of this oil. In the second step, KOH was used to catalyze the transesterification reaction. Three ionic liquids were used in the first phase of the process [BMIM][HSO₄], [BIM][HSO₄] and [MIM][HSO₄]. The best result was obtained with hydrogen sulfate 1-butyl-3-methylimidazolium, [BMIM][HSO₄]. The best reaction conditions for this step used a catalyst concentration of 5% by weight, an alcohol/oil molar ratio of 15:1, a reaction time of 60 min and reaction temperature of 160°C. In the second phase, the KOH-catalyzed transesterification was carried out at 60°C, 1% by weight catalyst load and a reaction time of 60 min. At the end of the two steps, the overall yield reached 95.65% [11].

Irana Alimova, in 2016 studied the production of biodiesel in the esterification reaction of oleic acid and methanol, using the same ionic liquid, [BMIM][HSO₄]. The optimum operating conditions were time and reaction temperature of 4 hours and 90°C respectively, a molar ratio alcohol/oil of 10:1 and a catalyst loading of 10%, thus resulting in a conversion of 89.7% [12].

In 2017, Arevik Tadevosyan, in a similar study, of the production of biodiesel by esterification reaction with, [BMIM][HSO₄] as catalyst, determined as optimal conditions the alcohol/oleic acid ratio of 10:1, catalyst load of 20%, reaction temperature of 90°C and reaction time of 6 hours. It resulted in an oleic acid conversion of 84.8% [13].

Conclusions
With these information brought from the literature we can conclude that [BMIM][HSO₄] shows promising results in the catalytic system of biodiesel production. Therefore, the objective of this work is the study of the application of hydrogen sulfate 1-butyl-3-methylimidazolium ionic liquids in the catalysis of transesterification reactions of mixtures of triglycerides with high free fatty acid contents in transesterification reactions of high acidity waste cooking oils. Besides performing the characterization of the quality of the biodiesel produced, the kinetic study of the reaction and the recovery of the catalyst employed will be extensively studied.

References
Esterification process catalyzed by ionic liquids for fatty acid methyl esters production

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Due to the massive use of energy from non-renewable sources as well as to environmental problems it is important to find green alternatives. In this context the biodiesel production arises, as a possible diesel substitute which can be produced using basic or acid catalysts. Despite these traditional catalysts present high production yields, the problems that come from its use and the environmental impacts associated, result in the need for the search of more sustainable alternatives. The ionic liquids (IL) as possible catalysts represents an interesting alternative because IL are reusable and environmentally friendly. In present work, the main goal is to study biodiesel production using the ionic liquid 1-buthyl-3-methylimidazolium methyl sulfate [BMIM][MeSO₄] as catalyst, by the assessment of its kinetic activity. The results of kinetic study by the integral method, showed low activation energy of 5.16 kJ/mol and a pre-exponential factor (A) of 0.0624 L².mol⁻².min⁻¹.

Introduction

The high consumption of natural resources has increased the demand for renewable energy sources which have been gaining more importance to establish a world energy balance and to reduce existing energy needs. Thus, the demand for new renewable energy sources from biomass, such as biodiesel and bioethanol, are appointed as excellent options for substitution of fuels derived from petroleum. These types of energies can contribute to an improvement in different levels: environmental, social and economic. However, the development of its production processes requires improvements that are related to the implementation of profitable methods of production. So, the main goal is to establish biodiesel production using raw-materials such as non-edible oils and used cooking oils (UCO) using environmentally “friendly” catalysts, allowing the reduction of carbon dioxide emissions into the atmosphere.

The introduction of ionic liquids as catalysts in the production of biodiesel is a promising solution to solve the problems derived from the use of traditional catalysts, since the ionic liquids present properties which may give them the designation of “green” catalysts, namely the possibility of reuse without a significant loss in the yield of reaction. With the goal of studying the ionic liquid 1-buthyl-3-methylimidazolium methyl sulfate, [BMIM][MeSO₄] performance as a catalyst in the esterification reaction of oleic acid with methanol, it is important to establish a set of reaction parameters that define the optimal operational conditions of the process. These parameters are: reaction time, temperature, oleic acid/methanol molar ratio, and catalyst concentration in the reaction media.

However, in a first approach, a kinetic study was implemented in order to verify the sensitivity to temperature of the esterification reaction.

Material and Methods

Reagents. Oleic acid, tech 90% was used as obtained from Thermo Fischer (Germany). The reagents obtained from Sigma Aldrich (Switzerland) were ionic liquid 1-buthyl-3-methylimidazolium methyl. Methanol, ethanol and n-heptane were obtained from Fisher and anhydrous sodium sulfate was obtained from Carlo Erba (France). To prepare the solutions for the determination of acid value it was used hydrochloric acid from Fisher Chemical (United Kingdom), potassium hydroxide, diethyl ether and borax of Riedel-de-Haën (Germany). Acid-base indicators phenolphthalein and methyl red were obtained from Panreac (Spain) and Riedel-de-Haën (Germany), respectively.

Kinetic study of esterification reaction of oleic acid. The study of kinetic activity was performed in a flask with two nozzles, used as a 100 mL reactor, immersed in a paraffin bath. An automatic heating plate with magnetic stirring (VWR, model VMS-C4), equipped with a temperature sensor, and a reflux condenser were used. The needed quantities of ionic liquid, oleic acid and methanol were always added in this order into the reactor.

All kinetic studies of esterification reaction of oleic acid with methanol have been carried out using a time reaction of 8 hours. The catalyst quantity was 10% w/w and molar ratio oleic acid/methanol 1:10. Throughout the reaction, in pre-determined times (0, 15, 30, 60, 90, 120, 180, 240, 300, 360, 420 and 480 min), 1 mL of sample was removed from the flask using a micropipette and stored in 4 mL vials. The acidity value was determined according to the European Standard EN 14104 [1]. The kinetic study was performed using four different reaction temperatures (60, 80, 100 and 110 °C) with the main goal of estimate experimentally the activity energy.

Results

Kinetic study of esterification reaction of oleic acid. The kinetic activity of the esterification reaction of oleic acid with methanol was studied using the [BMIM][MeSO₄] ionic liquid as catalyst. In order to determine the order of the reaction, different measurements were carried out using different temperatures using 10% w/w of catalyst, 1:10 oleic acid/methanol molar ratio. For the determination of the order of reaction and the activation energy, the acid value, expressed in mg KOH/g biodiesel, (see Equation 1), was determined immediately after the sample collection in pre-determined times using the procedure according to European Standard EN14104 [1] using the following equation:

\[
AV \left( \frac{mg \ KOH}{g \ biodiesel} \right) = \frac{V_{KOH} \times C_{KOH} \times MM_{KOH}}{m_{biodiesel}} \tag{1}
\]

Where AV is the acid value, V_{KOH} is the volume of the KOH solution used in the titration, in mL, C_{KOH} is the concentration
of the standard KOH solution, in mol/L, \( \text{MMKOH} \) is the molar mass of KOH, which is 56.1 g/mol, and \( m_{\text{biodiesel}} \) is the sample mass measured in g.

The conversion of oleic acid in percentage, was estimated by comparing the initial and final acidity values (see Equation 2). It was considered for the calculation of the initial acid value, the sample collected at time 0 min.

\[
\text{Conversion (\%)} = \left( \frac{AV_0 - AV_1}{AV_0} \right) \times 100
\]

(2)

Where \( AV_0 \) is the acidity for the oleic acid in initial instant and \( AV_1 \) is the final value acidity for a certain time, both in mg KOH/g sample.

Figures 1 and 2, show the evolution of acidity value and conversion with reaction time for different temperatures, respectively.

![Figure 1](image1.png)

Figure 1. Effect of the time reaction in the acidity value using four different temperatures.

![Figure 2](image2.png)

Figure 2. Effect of the time reaction in the conversion using four different temperatures.

Using these results, it was possible to estimate the order of reaction regarding to oleic acid. So, the integral method was applied for 0\( ^{th} \), 1\( ^{st} \), 2\( ^{nd} \) and 3\( ^{rd} \) order kinetics relating to oleic acid, for all temperatures. The results were studied for each reaction order and it was expected that if a particular order should be respected, the corresponding data would be distributed in a straight line. Afterwards, the regression coefficients (\( R^2 \)) were compared and the order of reaction was selected taking account the highest correlation coefficient value attained. Arrhenius theory was used for calculation of activation energy, for the esterification of oleic acid using the previously referred ionic liquid. Equation 3 was used to represent a quantitative basis for the relationship between the activation energy, temperature and reaction rate constant.

\[
k = A e^{-E_a/RT}
\]

(3)

The logarithmic form of Equation 3 can be expressed as:

\[
\ln k = \ln A - \frac{E_a}{R} \times \frac{1}{T}
\]

(4)

Where \( k \) is the reaction rate constant, \( A \) is the Arrhenius constant or pre-exponential constant, \( R \) is the universal gas constant (kJ.mol\(^{-1}\).K\(^{-1}\)), \( T \) is the absolute temperature (K) and \( E_a \) is the activation energy (kJ/mol).

Fig. 3 shows the \( \ln k \) values plotted against 1000/T, in order to determine the activation energy (\( E_a \)), as presented in Equation 4.

![Figure 3](image3.png)

Figure 3. Arrhenius plot of the rate constant (\( \ln k \)) versus the inverse of temperature (1000/T) for the esterification of oleic acid catalyzed by [BMIM][MeSO_4] ionic liquid.

The highest regression coefficient was obtained for the analysis considering a 2\( ^{nd} \) order kinetics for all temperatures, with a regression coefficient \( (R^2) \) of 0.9968. Therefore, the activation energy (\( E_a \)) and the pre-exponential factor (\( A \)) were estimated assuming the type of kinetics referred above. The obtained value for the activation energy (\( E_a \)) was 5.16 kJ/mol and the pre-exponential factor (\( A \)) was estimated at 0.0624 L\(^2\).mol\(^{-2}\).min\(^{-1}\).

Roman [2] presented a similar study using the IL 1-buthyl-3-methylimidazolium hydrogen sulfate ([HMIM][HSO_4]), and estimated the activation energy (\( E_a \)) as 6.80 kJ/mol and the pre-exponential factor as 0.0765 L\(^2\).mol\(^{-2}\).min\(^{-1}\).

**Conclusion**

This work aims to study the use of [BMIM][MeSO_4] ionic liquid as a catalyst for the esterification reaction of oleic acid with methanol. The kinetic study showed a low activation energy of 5.16 kJ/mol, estimated by the application of the integral method, for a 2\( ^{nd} \) order kinetics relating to oleic acid. The relative low value found for the activation energy, indicates that the ionic liquid used in this study may be a viable catalyst for this type of reactions, showing a relative low sensitivity to temperature changes for the considered temperature range.

**References**


Biodiesel production through transesterification applying ionic liquids as catalysts

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Introduction
Historically, economic growth was always dependent on energy generation, causing pressure on fossil energy sources. During the 70’s, with a decrease in the supply and an increase in the price of non-renewable fuels, Humanity was faced with the need to involve, in a beneficial way, the economic development and conservation of natural resources, thus seeking alternative and viable forms of energy [1-3].

According to the Brazilian legislation introduced in 2005 [4], biodiesel is a biofuel obtained from renewable biomass for internal combustion engines or energy generation, which can partially or totally replace fossil fuels. Biodiesel is chemically composed of FAME (fatty acid methyl ester), obtained from the chemical reaction of triglycerides with an alcohol, in the presence of a catalyst.

Different raw materials can be used to produce biodiesel, such as used edible vegetable oils (soybean oil, rapeseed oil) or inedible oils (jatropha oil, castor oil), animal fat, waste cooking oils and oil extracted from algae [5].

Acid and basic catalysts are applied to accelerate the reaction rate. For transesterification reaction, basic NaOH or KOH catalysts are the most commonly used. Alternative options for these catalysts are ionic liquids, which are being studied since they enable a more environmentally sustainable biodiesel production process. Among its properties, such substances have potential for recyclability, high catalytic activity, simple operating conditions and high conversion rates for short reaction times [6].

Objectives
The objective of this work is the study of the influence of applying 1-methylimidazolium hydrogen sulfate [HMIM][HSO₄] IL on the catalysis of the transesterification reaction of a highly acidic waste vegetable oil (WVO), in order to assess the viability of the use of acidic imidazolium based IL as catalysts in biodiesel production processes, with further optimization of the main operational conditions: reaction time, reaction temperature, catalyst percentage and oil/methanol molar ratio.

Metodology
Chemicals and raw material. Samples of simulated oils with variable acidity were used as raw material for the transesterification reaction by the incorporation of different contents of oleic acid (90%) in a previously qualitatively and quantitatively characterized WVO. For the reaction, methanol was used. As the catalyst it was used the IL [HMIM][HSO₄]. Transesterification reaction. In the following order, IL, oleic acid, waste cooking oil and methanol were added, in different proportions, to a 100 mL reaction vessel. The vessel was immersed in a paraffin bath, on an automatic heating plate under selected temperature and agitation velocity, coupled with a water reflux condenser. After the reaction time, the mixture was removed from the bath and transferred to centrifuge tubes and stored in the refrigerator for 20h at 4°C. After this period, the samples were centrifuged at 3000rpm for 20 minutes until the organic and the aqueous phases were completely separated and ready to be splitted. Both phases were stored in vials at 4°C waiting for further analysis.

Experimental design. An experimental design based on a total factorial was generated with three parameters at two levels (2³) in duplicate: incorporated oleic acid (20% and 40% wt), methanol/simulated oil molar ratio (1:20 and 1:40) and reaction time (4h and 8h). Table 1 describes the 3 parameters chosen, the code applied, and the 2 levels used. The experimental results were analyzed with Design Expert 11 software.

The used methodology defines 16 runs for understanding the influence of each factor on the response. Each run was carried out accordingly to the generic transesterification procedure already presented. One response was evaluated: the conversion of simulated oil, measured according to the procedure described in the European Standard EN14104/2008. The fixed reaction parameters were: temperature, 90°C, and percentage of catalyst, 10% wt.

Table 1. Levels chosen for experimental design.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Code</th>
<th>1</th>
<th>2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oleic Acid incorporated (%)</td>
<td>A</td>
<td>20</td>
<td>40</td>
</tr>
<tr>
<td>Molar ratio oil/methanol (mol/mol)</td>
<td>B</td>
<td>1:20</td>
<td>1:40</td>
</tr>
<tr>
<td>Time (h)</td>
<td>C</td>
<td>4</td>
<td>8</td>
</tr>
</tbody>
</table>

Results and Discussion
The factors that influence the conversion of simulated oil were evaluated by using factorial plots: interaction effect and cube plots. ANOVA and p-value significant levels were used to...
check the significance of the effects on the conversion (R). The experimental results are displayed in Table 2. The conversion of simulated oil can be expressed using the following equation:

\[
R = 88.31 - 1.47A + 4.08B + 4.79C + 0.8410AB + 0.0400AC - 1.16BC
\]  
(1)

This function describes how the experimental variables and their interactions influence the reaction. The model presented an adjusted square correlation coefficient \( R^2 \) of 96.7% for the conversion of simulated oil, adjusting the experimental data well. For the conversion, the reaction time parameter (C) was the most significant, followed by the molar ratio oil/methanol (B), the oleic acid incorporated in the residual oil (A), the BC interaction, the AB interaction and the least significant parameter was the AC interaction. The variables presented the value of p-value, respectively, of 9.45×10⁻², 3.74×10⁻², 1.10×10⁻², 4.90×10⁻², 2.48×10⁻² and 9.01×10⁻¹. It is noteworthy that the lower the value corresponding to p-value bigger is the significance of the parameter for the presented result.

Fig. 2 corresponds to interactions of effects between variables. A significant interaction results when the lines are not parallel. These plots indicated that interaction between molar ratio oil/methanol and time (BC) was stronger than between oleic acid incorporated and molar ratio oil/methanol (AB) and the interaction between oleic acid incorporated and time (AC) was statistically significant but much smaller.

**Conclusion**

The factor of greatest influence in the conversion was the reaction time, followed by the molar ratio oil/methanol. The factorial experiments showed a significant interaction between the two variables of greater influence. These parameters had a positive influence on the conversion rate studied.

![Figure 1. Cube plot for conversion (R).](image1.png)

**Table 2. Experimental responses.**

<table>
<thead>
<tr>
<th>Run</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>R1 (%)</th>
<th>R2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>93.96</td>
<td>96.80</td>
</tr>
<tr>
<td>2</td>
<td>-1</td>
<td>-1</td>
<td>1</td>
<td>90.85</td>
<td>93.91</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>74.89</td>
<td>76.87</td>
</tr>
<tr>
<td>4</td>
<td>-1</td>
<td>1</td>
<td>1</td>
<td>96.92</td>
<td>96.41</td>
</tr>
<tr>
<td>5</td>
<td>1</td>
<td>-1</td>
<td>-1</td>
<td>87.09</td>
<td>88.83</td>
</tr>
<tr>
<td>6</td>
<td>-1</td>
<td>-1</td>
<td>-1</td>
<td>79.86</td>
<td>81.51</td>
</tr>
<tr>
<td>7</td>
<td>-1</td>
<td>1</td>
<td>-1</td>
<td>89.34</td>
<td>89.41</td>
</tr>
<tr>
<td>8</td>
<td>1</td>
<td>1</td>
<td>-1</td>
<td>88.55</td>
<td>87.76</td>
</tr>
</tbody>
</table>

R: Values obtained for the conversion of simulated oil; R1 and R2 correspond to duplicates.

![Figure 2. Interaction effects plot for conversion (R).](image2.png)

**References**


Phosphorus recovery from aqueous solutions with an eco-friendly adsorbent

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This work aims to evaluate the feasibility of recovery phosphorus by adsorption processes in batch and fixed-bed column, using thermal modified industrial waste (eggshell) as an adsorbent. The screening phase revealed that calcined eggshell at 700 °C (CES700) is the best material. This adsorbent was characterized through nitrogen adsorption, XRD, SEM-EDS and thermogravimetric analysis. Through batch process, the influence of initial pH and adsorbent dosage were investigated. Langmuir-Freundlich model showed the best fitting to the equilibrium data, and the maximal adsorption capacity for CES700 was 39.0 mg P-PO4/g. The kinetics of the process is controlled by a pseudo-first order model. Fixed-bed column experiments were conducted for studying the influence of most relevant operating conditions (feed concentration, superficial velocity). Yoon-Nelson, Thomas and Bohard-Adams models properly described the saturation curves, with values of R² equal or greater than 0.98.

Introduction

The uncontrolled growth of the world population associated with the increase of consumption have contributed to environmental imbalances. Despite not being as widely studied as water shortage or climate change, phosphorus scarcity (P) is an emerging problem in the 21st century [1]. In fact, in 2017 the List of Critical Raw Materials for the European Union (EU) included this element as critical. Besides the relevance of P in the area of fertilizers, its removal from liquid effluents is a global concern considering the deleterious environmental effects posed by this element. Phosphorus can lead to eutrophication when its concentration in water bodies is high. Industry, domestic effluents, and agriculture are the main sources of P in the environment [2]. Among the diverse technology to capture P from wastewaters, adsorption, precipitation and constructed wetlands are the main methods used [3]. Adsorption will be the process considered in this study due to its high efficiency, fast kinetics, selectivity, and the possibility of recovering adsorbate and reuse the adsorbent [4]. Several studies have addressed the removal of P through adsorption using calcite [5], calcined alkaline residue [6] and eggshell [7-8]. Eggshell may have diverse applications, while it has revealed good properties as adsorbent [9].

Objective

This study aims to recover phosphorus from aqueous solutions by adsorption in batch and in fixed-bed column, using thermal treated industrial eggshell (CES700) as adsorbent.

Methods

An eggshell (ES) waste sample was collected from a domestic source. The sample was washed, and the membrane was removed. After drying at 105 °C, the sample was milled (0.15<d<0.42 mm). A screening phase allowed to identify ES treated at 700 °C for 2 h, in a muffle furnace, led to the best adsorbent (CES700). The raw material (ES105) and CES700 were characterized in respect to several parameters: pH, pHPC, A500 and pore volume (N2: adsorption/desorption), mineralogy (XRD), morphology (SEM-EDS) and thermal behavior (Thermogravimetry). The aqueous solutions were prepared with KH2PO4, and the concentration of phosphorus in phosphate form (P-PO4) was measured by EDXRF. Batch experiments were carried out by keeping under agitation, a certain amount of adsorbent in contact with a known volume of solution, for a predetermined period. Then, the adsorbent was separated from the fluid using a 0.45 µm pore filter. This procedure was adopted either for equilibrium and kinetic tests, both modeled by common equations (Langmuir: Eq.1; Freundlich: Eq.2; Langmuir-Freundlich: Eq.3; pseudo-first order: Eq.4; and pseudo-second order: Eq.5, kinetics models):

\[
q = \frac{q_m K_C}{1 + K_C C}
\]

(1)

\[
q = K_L C^n
\]

(2)

\[
q = \frac{q_m (K_{LF} C)^n}{1 + (K_{LF} C)^n}
\]

(3)

\[
\frac{q}{q_m} = \left(1 - \frac{1}{K_C q_m} + \frac{C}{q_m}ight)
\]

(4)

where q is the sorption capacity, qm the equilibrium sorption capacity, C the concentration, t the time, qm, KL, K LF, n, qmLF, KLF, nLF, k1 and k2 are parameters.

Results

The initial screening phase led to the materials showed in Figure 1, depending on the temperature of calcination. Indeed, its appearance was modified once at 600 °C the small amount of organic matter was in a charred state (black color); then at 700 and 800 °C the char was decomposed, showing a main phase of CaCO3 (calcite). At 900 °C most of CaCO3 was decomposed to CO2 (a loss of 45 % of material was observed), remaining CaO. Figure 2 depicts the removal of P-PO4, defined by Eq.6 for each of the materials shown in Figure 1.

\[
R(P-PO_4)(\%) = \left(1 - \frac{C_t}{C_0}\right) \cdot 100
\]

(6)

Results of Figure 2 revealed that the initial raw material (ES105) does not have affinity to capture P-PO4. In contrast, CES600 allows a removal of 23.5% P-PO4, corresponding to a removal capacity of 2.19 mg/g. However, CES700, CES800 and CES900...
led to a significant removal, reaching values of 80.1, 83.4 and 77.7%, respectively. In these cases, the capacities were 7.38, 7.71 and 7.24 mg/g, correspondingly. Table 1 shows the main properties of ES105 and CES700. Both materials are characterized by low BET areas and low porosity, alkaline pH, low total dissolved solids (TDS) when in contact with solutions, and low organic matter (OM). CES700 was the screened material for further studies. Figure 3 shows the experimental equilibrium isotherm and the fitting with three models.

Figure 1. Eggshell after drying at 105 °C (ES105); and after calcination at 600, 700, 800 and 900 °C.

Figure 2. Influence of ES calcination temperature on the removal of P-PO₄ (%) (C₀=100 mg P-PO₄/L; 10 g ads/L; 2 h in agitation; 25 °C).

Figure 3. Equilibrium isotherm (at 25 °C; agitation for 2 h; L/S=100 mL/g; pH_initial =8) and fitting with three models.

From Figure 3 it seems clear that the Langmuir-Freundlich is the best isotherm to describe the experimental data (R²=0.979), despite Langmuir isotherm is also acceptable (R²=0.959). In contrast, the Freundlich model is not suitable (R²=0.839). According to the Langmuir-Freundlich isotherm, the maximum adsorption capacity is 39.02 mg/g. Indeed, the maximum adsorption capacity measured in the lab was 42.45 mg/g. Moreover, dynamic batch tests (results not shown) revealed a very fast adsorption kinetics, since at the end of 30 min the equilibrium is already reached. These data were well fitted by a pseudo first order kinetics (R²>0.97).

Figure 4 displays, as an example, the breakthrough curves for flow rates of 5 and 10 mL/min.

Comparing these experimental tests, it can be observed that the adsorbent saturates more rapidly to higher inlet flows (high superficial velocities). These data allowed the calculation of important operating parameters, such as breakpoint time, stoichiometric time, exhaust time, fraction of saturated bed, capacity factor, etc. All empirical Bohard-Adams, Thomas and Yoon-Nelson models described well the breakthrough curves (R²>0.989).

Preliminary germinations tests with *Lepidium Sativum* seeds showed that after adsorption, the loaded adsorbent with P-PO₄ promotes the growth of the roots.

**Conclusion**

This study showed that ES calcinated at 700 °C (CES700) is an adsorbent with very interesting properties for phosphorous recovery, in the form of P-PO₄. The maximum capacity of CES700 is about 39 mg/g. The kinetics is fast, and the material is stable during batch or column tests. Thus, in order to promote a circular economy, this work shows that the recovery of phosphorus from liquid effluents is possible using a low-cost and eco-friendly adsorbent. In the end, a waste is diverted from landfill, liquid effluents may be depurated and, potentially, a fertilizer is obtained.

**References**

The quest for a circular economy developing green processes has swelled the interest on the end-of-life products processing. This paper proposes an alternative of the REEs recovery from leach solutions from fluorescent lamp wastes optimizing the leaching process and adding a SLM step. Red phosphor waste was undergone a first leaching to remove the main non-ferrous impurities (mainly Ca). Unfortunately, a small REEs amount was lost in this step. Thus, the leachate from L1 was treated in a SLM step using Cyanex 923 as a carrier. Nevertheless, when NaEDTA 0.05 M is used as receiving phase, the permeability coefficients were only linearly for low carrier concentrations (0.30-0.60 M) because a third phase is formed using high Cyanex 923 concentrations (0.90-1.20 M). This third phase can be avoided using a modifier such as the 2,6-dimethy-1,4-heptanone. Finally, the REEs recovery is possible with a second leaching step using HNO₃ or HCl 2 M.

**Introduction**

Nowadays, the recovery and separation of rare earth metals (REEs) has become one of the main interests subjects into the scientific community due to the need of developing alternatives to relieve their increasing consumption and supply risk. Moreover, the REEs growing consumption tendency does not seem to change in the coming years because their use is essential for the advanced technologies (wind turbines, hybrid cards, technologic devices, fluorescent lamps, etc.) development. Neodymium, terbium, dysprosium, yttrium and europium have been considered as critical metals by several technical reports [1].

In order to provide a new way to recover and recycle REEs, the use of the end-of-life products and the processing of their waste streams has turned into a promising alternative. Hence, this research was initiated to develop a process which improved the recovery of these metals from the leaching solutions of the fluorescent lamp wastes applying supported liquid membranes. Due to the high content of REEs, especially yttrium and europium, our investigation is focused on the halophosphate phosphors rich on red phosphors (YOX) [2].

The characterization and leaching of a fluorescent lamp waste for the REEs recovery have been carried out in the current study. The fact that a real waste has been used, force us to propose step by step all the processes required to achieve a complete REEs recovery, starting from a sample containing different impurities (mainly Ca and PO₄³⁻). However, the disadvantage of working with a real waste is how to remove these impurities. The high added value provided by this investigation is the use of supported liquid membranes (SLM) to recover the REEs from the liquor obtained after a first leaching (L1) which contain REEs at low concentrations.

**Material and methods**

The end-of-life fluorescent lamp wastes were obtained from Recyberica Ambiental S.L. Cyanex 923 was used as extractant and it was kindly supplied by Cytec Canada Industries. Kerosene from Sigma-Aldrich as a diluent (Ref. 607010) and NaEDTA from PanREac AppliChem (Ref. 131669) and hydrochloric acid as stripping agents were used.

![Diagram](image)

**Results**

Different acidic leaching agents have been carried out to evaluate the leaching efficiency from the fluorescent lamp waste for the REEs recovery. Graphical Abstract shows the flow sheet process used to recover the REEs from the end-of-life fluorescent lamp wastes. After magnetic separation to remove the ferrous impurities, a leaching using low concentration of acid (L1) was carried out to separate non-ferrous impurities, mainly calcium. The REEs losses in the L1 stage were recovered by SLM using Cyanex 923 as carrier. As previous studies recommend, if Cyanex 923 is used, nitric acid was the chosen media [6]. The solid obtained in the L1 stage has undergone to a second leaching (L2) using high acid concentration to obtain a liquor reach in REEs which will be mixed with the permeate fraction coming from the SLM experiment.

Experiments were also conducted to evaluate the effect of Cyanex 923 concentration in the liquid membrane. If hydrochloric acid 0.1 M as receiving phase is used, the
permeability coefficients increase when the carrier concentration also increase. However, using Na$_2$EDTA 0.05 M, the permeability coefficients were linearly for the low Cyanex 923 concentration (0.30–0.60 M). This means that the metal transport is only controlled by the diffusion through the membrane. Then, the permeability remains almost constant in the range 0.90–1.20 M of the carrier. This may be due to the apparition of a third phase. In order to elucidate it, different modifiers such as tributylphosphate (TBP) and 2,6-dimethyl-1-4-heptanone were used. Comparing the results obtained using Cyanex 1.20 M without modifier and when these modifiers were used, it can be asserted that the TBP has a negative effect because the permeability decreases. However, using 2,6-dimethy-l-4-heptanone as modifier the REEs transport is higher.

Conclusions
This publication reports a new potential route to reclaim REEs from fluorescent lamp wastes. The experiments were carried out at bench scale to evaluate the recycling potential based on two steps of leaching and a supported liquid membrane process.

The optimal conditions for the L1 stage are HNO$_3$ 1 M and 10 min of contact time to maximize the Ca and PO$_4^{3-}$ separation and minimize the REEs losses. The SLM experiments showed that Cyanex 923 allow us the selective recovery of the REEs. However, a modifier such a 2,6-dimethyl-4-heptanone is required when Na$_2$EDTA 0.05 M is used as receiving phase if the concentration of the carrier is higher than 0.60 M to avoid the third phase formation. Finally, for the L2 step the REEs recovery is possible using HNO$_3$ or HCl 2 M for 48 h.

To sum up, the REEs can be completely recovered from their leachates optimizing the leaching process and adding a supported liquid membrane step.

Acknowledgements
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References
Sludge free solar photo-Fenton combined with biofiltration for the degradation of phenolic compounds from olive mill wastewaters

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Fenton’s process is an interesting wastewater treatment, while its main drawback is the dissolved iron remaining after the treatment that must be removed before releasing the effluent. This generally leads to the production of iron sludge that constitutes a second source of pollution. The aim of this work is to study the efficiency of solar photo-Fenton on the degradation of olive mill wastewater. The use of light reduces the iron loads required for the reaction. In addition, the research also aims to infer about the possibility of combining solar photo-Fenton’s with biofiltration using as hosts Corbicula fluminea to remove the iron remaining after treatment. This is an invasive species with high biofiltration capacity and tolerance to toxic compounds that can have an important role on wastewater treatment. Thus, an environmental application will be given to an alien species combining effluents depuration with pest management strategies.

Agro-industries (such as olive oil production) are important companies in Mediterranean Sea area countries such as Portugal and Spain. The economic growth observed is leading to a higher demand for these products. Consequently, the amount of olive mill wastewater produced is increasing [1]. In fact, these industries are intense water consumers and generate liquid effluents with a high organic load. In this context, the treatment of these wastewaters is an essential step aiming at the environmental protection and water recovery.

The traditional biological treatment systems are unable to offer a suitable solution for such effluents. The main drawback is associated with the presence of toxic compounds in their composition (such as phenolic substances). Moreover, their seasonal character reduces the microorganisms efficiency since a continuous adaptation to the feed characteristics would be required. Also, the evaporation pounds usually applied are not a suitable solution since their efficiency is dependent upon the climacteric conditions. Moreover, the unpleasant odors produced as well as the possibility of groundwater contamination constitute important drawbacks.

Sustainable solutions based on advanced treatments are required for these environmental problems. Advanced oxidation processes, based on the oxidant power of hydroxyl radicals, may be an interesting approach. Among them, Fenton’s process, based on the oxidant power of hydrogen peroxide catalyzed by iron ions, is of particular interest. In fact, this treatment process operates at ambient conditions of pressure and temperature with high efficiency which makes it industrially interesting. Besides, generally a biodegradability increase and a toxicity removal is observed which means that such chemical process may be integrated with a biological system which may reduce the operating costs.

One of the main drawbacks of this process is the amount of iron used to catalyze the reaction. This homogeneous catalyst is always present at a concentration higher than the legally regulated for the discharge of an effluent to the natural water courses. Thus, separation techniques are required to remove the excess of iron. Typically, the treated effluent is alkalized and iron is removed by the precipitation of Fe(OH)₃. These sludges entail a new source of pollution requiring a suitable management which will increase treatment costs.

The use of light to enhance Fenton’s reaction (photo-Fenton process) increases the reaction rate and the required load of iron is reduced [2]. Moreover, the potential use of Sun as a cost free light source is an important feature of this process [3]. The low amount of iron applied may be then removed by a biofiltration system using invading clams as hosts. Corbicula fluminea is an example of such species that shows interesting results on the depletion of several contaminants from water [4, 5]. The use of this alien species for wastewater treatment will give an environmental application combining effluents depuration with pest management.

Bearing in mind the high industrial interest in Fenton’s process for wastewater treatment and the fact that olive oil producer countries are mainly located in Mediterranean Sea basin (where the sunlight is plentiful all year) the aim of the present work is the optimization of solar photo-Fenton’s process for the detoxification of olive mill wastewater. Moreover, this process was combined with biofiltration using C. fluminea so that the iron content of the discharged wastewater can be lowered.

A simulated effluent encompassing 5 phenolic compounds (trans-cinnamic, 3,4-dimethoxybenzoic, 4-hydroxybenzoic, 3,4,5-trimethoxybenzoic and 3,4-dihydroxybenzoic acid) usually present in this effluent was prepared. The concentration was 100 mg/L of each of the pollutants. Fenton’s experiments were carried out in a cylindrical borosilicate photo-reactor with a working volume of 0.5 L. The tube was equipped with a porous stone axially placed to disperse air during the reaction which promotes mixture. The reactor was loaded initially with the mixture of phenolic compounds. The predetermined amount of iron (FeSO₄) was dissolved into the solution and the reaction started when the pre-determined amount of hydrogen peroxide (33% w/V) was introduced. During the dark experiments, the reactor was covered with aluminum foil to avoid the interference of light. Photo-Fenton experiments were carried out using a high-pressure sodium grow lamp and natural sunlight. In all cases, the spectrum of light entering the reactor was recorded using an Ocean Optics USB4000 fiber optic spectrometer. Samples were periodically withdrawn and the required amount of Na₂S₂O₃ (0.1 N) was added to quench the remaining H₂O₂ in solution. Total phenolic content (TPH) was determined by the Folin-Ciocalteu method as described elsewhere [4]. The concentration of the individual phenolic...
acids was obtained through HPLC following the method proposed by Gomes et al. (2018) [5]. Biofiltration experiments were performed using 500 mL biofilters with a support for the Asian clams (20 clams per liter). Constant temperature (20 °C) and aeration were applied as well as a photoperiod cycle of 16th light: 8h dark. C. fluminea individuals were collected from an infested site in Mira, Portugal. The iron content in the samples collected was analyzed by atomic absorption (Perkin-Elmer 3300).

Preliminary experiments involving sunlight and hydrogen peroxide, sunlight and iron as well as photosynthesis showed negligible effect on the phenolic acids degradation.

As an example, Figure 1 shows the normalized concentration of 3,4,5-trimethoxybenzoic acid along time during dark and photo-Fenton’s reaction ([H₂O₂] = 55 mM and [Fe²⁺] = 16 mg/L). Photo-Fenton’s reaction were performed using either a high-pressure sodium grow lamp and natural sunlight.

As it can be observed from the Figure 1, for all the experimental conditions a high initial reaction rate is observed. In fact, with the 3,4,5-trimethoxybenzoic acid normalized concentration reaching 20% for dark Fenton and photo-Fenton using the high-pressure grow lamp after 20 min of reaction. However, the reaction rate was much higher when sunlight was applied. Moreover, for the same experimental time the normalized concentration of the acid reached 2% (corresponding to a degradation of 98%) when natural sunlight was applied. While the high-pressure grow lamp only emits photons on the visible light region, the sunlight also comprises a fraction of UV radiation that can be used by hydrogen peroxide and iron complexes to enhance the reaction rate.

It should be also noticed that while the initial pH of the effluent was of about 5.5, the addition of Fenton’s reactants automatically lowered it to ~ 3.2, which is within the optimal range for Fenton’s process.

After the initial high reaction rate, for all the conditions applied a plateau was reached corresponding to 20% to dark and photo-Fenton using the high-pressure grow lamp and 2% when solar photo-Fenton was applied. Thus, the reactants load applied for the process was further optimized.

Furthermore, biofiltration using C. fluminea hosts showed interesting results regarding the removal of the remaining dissolved iron after Fenton’s process. Besides, some further organic matter removal was observed. This is explained by the high biofiltration capacity of Asian clam which, allied with their reasonable tolerance to toxic substances, makes it suitable low cost biofilters.

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References
Ionic liquids as solvent for the extraction of phenols from effluents of biomass fast pyrolysis

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The goal of this work is to analyze the ability of 2 ionic liquids (ILs) as solvents to extract phenols from the aqueous fraction obtained after water washing of the oil generated in the fast pyrolysis of the biomass. This aqueous phase contains sugars that can be fermented to obtain bioethanol but also has phenols that must be removed before fermentation to prevent any damage to the microorganisms. In this work, the extraction efficiency of phenolic compounds using ILs as solvent is proposed as an alternative to the use of volatile organic compounds. This work was performed through equilibrium distribution studies. The phenol concentration was determined by absorbance measurements. The effects of phenol initial concentration and the chemical structure of phenols and ILs on the extraction process were analyzed and discussed. In all cases, high extraction efficiencies were obtained which has demonstrated the ability of these ILs to remove the phenolic compounds in this kind of streams.

Introduction

Lignocellulosic biomass has become an alternative source for the production of chemicals and bio-fuels such as bio-ethanol. Fast pyrolysis is an emerging technology to transform the lignin into gases, liquids, and char. Among the many compounds obtained during this process, sugars are growing in importance since they can be converted into bioethanol by fermentation. A known method to separate sugars from pyrolysis oil is based on liquid extraction using water, which leads to two fractions, a sugar-rich aqueous fraction and an oil fraction. Nevertheless, the presence of aromatic compounds, specifically phenolic compounds, in the aqueous phase is a drawback because these compounds are toxic and strong inhibitors for growth microorganisms [1]. In order to ferment the pyrolytic sugars to bioethanol, removal of these aromatic compounds is crucial.

Although solvent extraction using volatile organic compounds (VOCs), such as ethyl acetate or methyl isobutyl ketone, has been reported as an effective method to remove aromatic compounds in this kind of streams, the use of these toxic and flammable compounds should be avoided. As an alternative, solvent extraction with ionic liquids (ILs) has recently emerged as an efficient method to carry out this purpose [2,3].

Objectives

The aim of this work is to evaluate the extraction of three phenolic compounds (phenol, guaiacol, and syringol) from water using ILs as solvent. For that, 1-propyl-1-methylpyperidinium bis(trifluorosulfonyl)imide, [PMpip][NTF₂], and 1-propyl-1-methylpyrrolidinium bis(trifluorosulfonyl)imide, [PMpyr][NTF₂] ILs were tested. From the obtained results, the effect of phenolic compounds and IL structures on the efficiency of the extraction process was analyze and discussed.

Methods

The ILs used in this work were supplied by Iolitec (Germany), and phenolic compounds were purchased from Sigma-Aldrich (Steinheim, Germany). All compounds were used as received without any further purification. Deionized water was used to prepare the aqueous solutions.

The extractions were carried out in glass vials in which 2 mL of ionic liquid and 3 mL of water solution were introduced (VIL:Vw= 2:3). To analyze the effect of the initial phenol concentration on the extraction, all systems were studied at three different initial compositions of phenolic compounds (100, 500 and 1000 mg/L). The samples were vigorously stirred in a magnetic stirrer during 30 min, and then they were introduced for 2 h in a thermostatic metal block (with an accuracy of ± 0.5 °C) at selected temperature to achieve equilibrium state. These conditions were taken from a preliminary study previously published by us [4]. After phase separation, samples of the aqueous phase were collected with a syringe and phenolic composition was obtained by UV-visible absorbance measurements with an accuracy of ± 0.002 Abs (from 0 to 0.5 Abs). This results in a maximum uncertainty of ± 3.5 % for the extraction efficiency values (% E), which was calculated as % E = (Cₒ - Cᵣ)/(Cₒ), where Cₒ and Cᵣ are the concentrations of phenolic compounds in aqueous phase before and after the extraction process, respectively.

Results

The results obtained in this work for [PMpip][NTF₂] and [PMpyr][NTF₂] are plotted in Figures 1 and 2, respectively. As can be seen in Figures 1 and 2, similar extraction efficiencies were obtained with both ILs. Therefore, in this case, the structure of the cation does not play an important role on the ability extraction of these non-aromatic ILs. Similar results were obtained in our previous work comparing aromatic and non-aromatic ILs [4].

Figure 1. Extraction efficiency percentage (%E) versus initial phenol for the systems (Phenolic compound + water + [PMpip][NTF₂]) Phenolic compounds: phenol (black), guaiacol (grey), and syringol (white).
On the other hand, extraction efficiencies increase with the initial phenol concentration and they also increase in the following order: phenol < syringol < guaiacol. This last behavior is related to the presence of methoxy groups in syringol and guaiacol which decreases their solubility in water, hence enhancing the extraction process.

**Conclusions**

In all cases, high extraction efficiencies were obtained supporting that the proposed ILs (i.e. [PMpip][NTF$_2$] and [PMpyr][NTF$_2$]) could be used as solvents to extract phenolic compounds from effluents of biomass fast pyrolysis. While the nature of the IL cation does not seem to have a determinant role, the structure of the phenolic compounds shown to have impact on the extraction efficiencies. As this preliminary work was performed using phenol + water binary samples, further studies including more complex mixtures (also sugars) should be performed to test the real efficiency of these ionic solvents.

**References**

Heterogeneous solar photo-Fenton using red-mud as low cost catalyst for olive mill wastewater treatment

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Red mud (a waste from the Bayer process) is an iron-rich material. The aim of the present research was to test it as a low-cost heterogeneous catalyst for dark and photo-Fenton’s reaction on the abatement of the pollutant character of olive mill wastewater. Red mud showed low efficiency regarding the pollutants adsorption. However, the use of visible light was able to promote the photocatalytic oxidation of the phenolic acids tested. Moreover, the use of simulated visible light was able to truly improve Fenton’s efficiency. In fact, the improvement was even (56%) observed when Sun was applied as light source. In addition, low iron leaching was observed after the treatment processes. Red mud seems to be an interesting low-cost catalyst for heterogeneous photo-Fenton avoiding the production of iron sludges after the treatment. This work successfully combines waste management with wastewater treatment.

Fenton’s process is based on the oxidant power of hydrogen peroxide catalyzed by iron ions, which will lead to the production of hydroxyl radicals able to decompose a wide range of organic compounds. Since it operates at room conditions of temperature and pressure and requires reactants easy to handle, this technology is highly appreciable for industrial applications. However, its main disadvantage is related with the need of separating the dissolved iron from the liquid bulk at the end of the process. In fact, the amount of iron required for an effective catalysis of the reaction rate is usually much above the legal limit for an effluent discharge [1]. Usually, a precipitation step is implemented where the solution pH is increased to promote the precipitation of Fe(OH)3 sludges. Thus, besides a second source of pollution (due to the sludges produced), the catalyst recover and reuse is jeopardized. To overcome this shortcoming, iron-rich heterogeneous catalysts have been studied [2]. An interesting alternative is the application of iron-rich solid wastes as catalysts in Fenton’s process [3].

Alumina industry generates a problematic iron rich waste, the so-called red mud. This mud is a residue from the aluminum oxide production by the bauxite leaching process (Bayer process). Around 1 to 1.5 tons of this waste is produced by 1 ton of alumina and globally up to 66 million tons are produced annually [4]. Its pollutant characteristics makes it difficult to handle. Usually it is landfilled requiring a vast area of land and groundwater. Red mud is rich in iron and aluminum as well as some rare metals [5]. Thus, it can be acendaminate as low-cost catalyst for Fenton’s process. This way waste management can be combined with wastewater treatment.

Olive oil industrial sector is increasing due to the well-known benefits of olive oil to health. However, from the extraction process a large amount of wastewater is produced. It is estimated that up to 10 million ton of olive mill wastewater (OMW) are produced every year [6] with special incidence at the Mediterranean region. These streams are rich in phenolic compounds that attribute toxicity and antibacterial character. Therefore, the traditional biological wastewater treatment systems are not suitable to handle it. In this context, Fenton’s process can be an interesting alternative.

The aim of the present research was the analysis of the catalytic activity of red mud in the degradation of a simulated OMW by heterogenous Fenton’s process. Moreover, dark experiments were compared with heterogeneous photo-Fenton using a high-pressure sodium grow lamp and natural sunlight. The influence of main operating parameters was evaluated.

Red mud from the Bayer process was obtained from a Greek company. The material was dried overnight at 105°C. Before use, it was milled in a ceramic ball mill until a homogenous powder was obtained. The red mud was analyzed regarding its chemical composition through XRD using a PANalyticalX’Pert PRO diffractometer (CuKα radiation, 20 = 20 – 80º). Phases quantification was performed with PanalyticalHighScore Plus 4.1 (PDF-4) software. The material morphological characteristics were analyzed by SEM (Hitachi S4100).

The simulated OMW was prepared by dissolving 100 mg/L of each of the following phenolic acids in ultrapure water: trans-cinnamic, 3,4-dimethoxybenzoic, 4-hydroxybenzoic, 3,4,5-trimethoxybenzoic and 3,4-dihydroxybenzoic acid.

Dark and photo-Fenton’s experiments were carried out in a cylindrical borosilicate reactor (500 mL). The vessel is axially equipped with a porous stone to promote air bubbling guarantying the homogeneity of the reaction mixture. 500 mL of the synthetic OMW were initially loaded. The required amount of dried red mud is added and the reaction started when hydrogen peroxide (33% w/V) was introduced. Aluminum foil was used to cover the reactor during dark experiments. A high-pressure sodium grow lamp (LUMATEK 600 W) was used for photo-Fenton’s experiments. The solar photo-Fenton’s assays were performed in the roof top of the Department of Chemical Engineering from University of Coimbra (40.1865243°, 8.41797950°). The light intensity and spectrum was followed using Ocean Optics USB4000 fiber optic spectrometer. Samples were periodically withdrawn filtrated (0.45 µm syringe cellulose acetate membranes) and the required amount
of Na$_2$S$_2$O$_3$ (0.1 N) was added to quench the remaining H$_2$O$_2$ in solution.

The concentration of the individual phenolic acids was obtained through HPLC (Shimadzu) using a C18 column (SiliaChrom) at 40 °C. The mobile phase (0.5 mL/min) consisted in a 50:50 mixture of methanol and acidified water (0.1% orthophosphoric acid). The detection was performed at 255 nm. The mineralization was determined by following total organic carbon (TOC) using a TOC-V CPN (Shimadzu) analyzer coupled with an autosampler /V-ASI, Shimadzu). Dissolved iron was determined by atomic absorption (Perkin-Elmer 3300) to check the leaching of metal from the heterogeneous catalyst.

The major crystalline phases contained in the material include iron oxides, mostly hematite (Fe$_3$O$_4$) and aluminum hydroxide, especially diaspore (AlO(OH)) and gibbsite (Al(OH)$_3$). These compounds are present in bauxite. Moreover, also silicon oxide, titanium oxide, sodium oxide and calcium oxide were detected.

Figure 1 shows SEM image of the fresh red mud used in the heterogeneous Fenton’s experiments. Figure 2 compares the depletion of cinnamic acid (as an example) in four situations: when only in contact with 1 g/L of red mud (RM); red mud and light from the grow lamp (RM + Light); dark Fenton’s experiment using 55 mM of H$_2$O$_2$ (RM + H$_2$O$_2$); dark Fenton’s experiment using 55 mM of H$_2$O$_2$; photo-Fenton with the grow lamp (RM + H$_2$O$_2$+ Light) and solar photo-Fenton (RM + H$_2$O$_2$+ Sun).

Figure 1. Morphology of the fresh red mud.

Figure 2. Cinnamic acid normalized concentration along the experiments.

It can be concluded that RM is not able to adsorb the initial compound since negligible variations were observed when RM was putted in contact with the effluent. The use of light (from the grow lamp that essentially emits visible light) and the RM was able to remove only 12% of the initial pollutant. This means that visible light may be enough to promote electron-hole separation in the red mud that will lead to the production of oxidant moieties (such as hydroxyl radicals) that are able to oxidize the organic compounds. Dark- Fenton (RM + H$_2$O$_2$) led to 28% of cinnamic acid removal in 1h. However, this efficiency was truly improved when a light source was applied. A high-pressure sodium grow lamp was used as light source leading to 48% of the initial pollutant degradation in 60 min. However, the photo-Fenton’s efficiency decayed when natural sunlight was applied (37%). This must mean that visible light shouldbe the most responsible by the photocatalytic activity of the red mud. Still, the low mineralization results reveal that the reaction mainly follows partial oxidation. An important point is that only a low amount of dissolved iron was detected at the end of the experiments (0.3 mg/L) which shows that red mud may be a stable material for heterogeneous Fenton’s reaction. Therefore, waste management can be successfully combined with wastewater treatment.

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References

Lagrangian mixing simulation and quantification of scales

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Lagrangian Mixing Simulation (LMS) is a new method that uses particles to track the mixing of two fluids. The line formed by the particles enables the calculation of interfacial area generation and the prediction of segregation scales. LMS is applied over 2D CFD simulation in a Confined Impinging Jets (CIJ) reactor for Re=100, 300 and 500. LMS shows that interfacial area generation is exponential for chaotic flows (Re=300 and 500) and linear for steady flows (Re=100). LMS is able to compute mixing scales smaller than 10⁻⁸ m, using desktop computers.

Introduction
Mixing efficiency in transitional flows relies on chaotic advection mechanisms such as stretching, folding and rupture of the material, associated to an exponential growth of interfacial area and a reduction of segregation scales [1]. Lagrangian techniques were developed and applied to characterize mixing in 2D and 3D such as the mapping and extended mapping methods [2,3]. With the evolution of computational facilities, CFD simulations have been used to study mixing, using Eulerian framework [4]. Following this line, a hybrid approach between Lagrangian and Eulerian methods, the volume-of-Fluid (VOF) model, was used by Fonte et al. [1] to determine the segregation scale in a CIJ model. A CIJ mixing chamber is composed of two injectors opposite placed near the chamber’s closed top and an outlet at the open end; 2D and 3D models of CIJ’s have been validated in previous works [4]. For Reynolds numbers larger than 120, CIJ presents a chaotic flow regime characterized by an extreme dynamics of vortex shedding. This chaotic behaviour was stated by Ottino [5], “the best achievable mixing corresponds to exponential stretching nearly everywhere and occurs in some regions of chaotic flows”, in his book where he tries to characterize mixing: “the basic objective here is to compute the length (or area) corresponding to a set of initial conditions”, where he pointed out that the mixing of two phases could be quantified by the line that defines the boundary between phases (interfacial area). However, small scales associated to a reduction of the laminae dimensions and the exponential area growth demand computational resources which at that time were not powerful enough; in fact, Ottino concluded that “the manifolds will not appear as continuous lines which they are, but as a series of disconnected dots”; moreover, he said that “computations based on discretization of the velocity field provide only a crude picture of the flow”. In 2012, Fonte [6] used an adaptive grid scheme, yet he could not achieve the smallest scales. Another way to simulate lamellar flow structures is the use of particle tracking [7]. In this work, a new approach using Lagrangian Mixing Simulation (LMS) is used to quantify mixing: particles are initially placed over an Eulerian velocity field completely comprising the inlet of a CIJ chamber and along with the simulation time, they change their coordinates; the line formed by the particles grows in order to go along with the fluids injection. In this way, two parameters can be measured: the interfacial area generation and the striation thickness which will enable to quantify mixing.

Case study: CIJ reactor
Geometry. A 2D CIJ model validated in previous works [4] was used to simulate the mixing dynamics (Figure 1). The chamber’s width and height are $H=5.0\times10^{-2}$ m and $D=1.0\times10^{-2}$ m, respectively and the injectors diameter is $W=1.5\times10^{-3}$ m. The residence time is $\tau=\frac{H D}{2Wv_{ inj}}=4.2\times10^{-2}$ s.

![Figure 1. 2D CIJ reactor.](image)

Boundary conditions. At the inlets, a parabolic profile was considered,

$$v(x) = \frac{3}{2} v_{ inj} \left( 1 - \left( \frac{x}{W/2} \right)^2 \right)$$

with the inlet jets’ velocity, $v_{ inj}=4$ m/s. It was also assumed: no slip condition at the walls, constant uniform pressure at the outlet and symmetric state solution for the initial conditions, considering no shear wall at the chamber symmetry axis. The fluid density was 1000 kg/m³ and the viscosity was changed with the Reynolds number: Re=100, $\mu=60$ mPas; Re=300, $\mu=20$ mPas; Re=500, $\mu=10$ mPas.

Computational fluid dynamics model. The 2D domain was discretized with a structured orthogonal mesh of quadrilateral elements with maximum edge length, $\Delta x=5.0\times10^{-3}$ m. The commercial finite volume CFD package ANSYS Fluent™ was used to solve steady and transient states. The time step, $\Delta t$, was chosen, according to the Courant number

$$C = \frac{v_{ max}}{\Delta x} = 1$$

where $v_{ max} = \frac{3}{2} v_{ inj} = 6$ m/s. The time step was then, equal to $8.3\times10^{-6}$ s.

Simulation results. Steady-state of 2D CIJ model was simulated for Re=100 and transient state was simulated up to 10 $\tau$ for Re=300 and 500. As an example, the vorticity map for Re=500 is shown in Figure 2.

![Figure 2. Vorticity sign (white for negative values and black for positive values) in a CIJ 2D reactor simulation for time equal to 10 $\tau$ at Re=500.](image)

Lagrangian simulation
CFD simulations were used to apply LMS. The particles are initially positioned in the chamber’s inlet over CFD data and its
coordinates will change according to 2D transient data. The particles will then form a growing line that tracks the flow. The particles position vector at each time step, \( \mathbf{X}_{i,j} \), is given by

\[
\mathbf{X}_{i,j} = \mathbf{X}_{i-1,j} + \mathbf{v}_{i-1,j} \Delta t
\]

in which \( \Delta t \) is the time step and \( \mathbf{v} \) is a vector with the velocity vectors, \( \mathbf{u} \). \( i \) represents the time and \( j \) the particle. As particles are advected by the chaotic flow, they become apart from each other; to prevent this distancing (which would not enable an accurate tracking of the flow features), a new particle is inserted when

\[
|\mathbf{X}_{i,j+1} - \mathbf{X}_{i,j}| > r
\]

where \( r \) is a given value (preliminary simulations have shown that \( r=1.0 \times 10^{-4} \) m was an adequate value [8]).

The initial particles (\( np=18 \)) were equally distributed over the velocity field provided by the CFD simulation (Figure 3), forming a line that completely comprises the inlet jet. Each simulation was performed at least until the particle’s line tip attains the chamber’s outlet (as shown in the Graphical Abstract).

**Mixing Quantification: Interfacial Area Generation**

The interfacial area generation is measured by the length of the line formed by the particles.

\[
s = \sum_{i=1}^{np} |\mathbf{X}_{i,j} - \mathbf{X}_{i,j+1}|
\]

where \( np \) is the total number of particles. The interfacial area generation is given by

\[
\frac{s}{s_0} = \frac{s}{s_0}
\]

in which \( s_0 \) is the initial length (\( s_0 = W \)). For transient-state (Re=300 and 500) the values of \( \alpha \) have an exponential growth. Each LMS was fitted to an exponential fitting \( \alpha = a e^{b \Delta t} \) where \( a \) and \( b \) are the fitting parameters. An average of 14 LMS results was considered and the exponential fittings can be written for Re=300 and 500, as \( \alpha = 14.5 e^{0.0145 \Delta t} \) and \( \alpha = 8.4 e^{0.0051 \Delta t} \), respectively, showing that when the Reynolds number increases, the value of \( \Delta t \) decreases and the value of \( b \) increases. For Re=100, LMS has shown that the interfacial area generation is linear and can be expressed by \( \alpha = c t \) with \( c=6.6 \times 10^{-1} \) m/s.

**Conclusions**

An algorithm to perform LMS was developed. LMS method was applied over 2D CFD simulations for Re=100, 300 and 500 in a CFI model. LMS was used to characterize mixing through the measure of interfacial area generation and calculation of the striation thickness, for the latter, a calculation procedure was proposed. LMS has shown to be a very efficient method and it was shown that: interfacial area generation is exponential for transient flows (Re=300 and 500) and linear for steady flows (Re=100); Segregation scales could be measured up to magnitudes smaller than \( 10^{-4} \) m. Moreover, CPU time of an LMS is less than a minute which makes it a strong tool to study the mixing, especially in chaotic systems.

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**References**

Modeling of carbonation and calcination reactions of Ca-looping process for CO₂ post-combustion capture in gPROMS®

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In this work, the Overlapping Grain Model (OGM) [3] and A rate equation theory for the pore size distribution of calcined CaCO₃ (RThPSPD) [4] were implemented in gPROMS® software. The OGM models the carbonation of CaO and the RThPSPD the calcination of CaCO₃ and respective pore dynamics. The OGM was validated using data from fluidized bed reactor experiments, and accurately models the reaction regimes observed. The pore structure of the CaCO₃ particle was added as an initial condition to the RThPSPD, and the calculation of surface area of vacancies was changed to avoid overestimating total pore area. The vacancy balance equation was also corrected to avoid undue pore volume disappearance. This work lays the basis for the simulation of sorbent deactivation in Ca-Looping by integration of both models, to be done after the validation of the modified RThPSPD model.

Introduction
Calcium-looping (CaL), is a post-combustion technique for carbon capture and storage (CCS), that relies on the carbonation reaction of CaO(s) to capture CO₂(g), and on the calcination reaction of the resulting CaCO₃(s), to generate a stream of highly concentrated CO₂(g), effectively separating it from the stream of flue gases. The CaL process is a very promising candidate for industrial post-combustion CO₂ capture because widely available and low-cost natural limestone can be used as a sorbent; it is also a suitable process for retrofitting existing plants [1], lowering the overall cost and complexity of implementation. The CaReC – Carbon emissions Reduction in the Cement Industry – project, which includes the work here presented, aims to mitigate CO₂ emissions via cooperation with the Portuguese cement producer CIMPOR, to perform a techno-economic evaluation of the implementation of a CaL reactor in the producer’s industrial plant. The CaL process is especially interesting when the cement industry is concerned, due to the possibility of using the deactivated sorbent from the CO₂ capture as feedstock for cement production [2].

Objectives
The objective of the present work is to model the carbonation, the calcination reaction and corresponding pore dynamics using the gPROMS ModelBuilder® software. Both models are implemented and the OGM is validated for various sorbents, laying the groundwork for the simulation of sorbent deactivation along multiple cycles using the pore dynamics and the relation between loss of pore area and loss of sorbent reactivity.

Methods
Two different models were used: the Overlapping Grain Model (OGM) [3] for the carbonation reaction and a modified version of A rate equation theory for the pore size distribution of calcined CaCO₃ (RThPSPD) by P. T. Liang et al. [4]. A brief explanation of both models ensues, with emphasis on the changes done to the RThPSPD.

Overlapping Grain Model – Carbonation. The OGM is a grain model that considers each CaO particle to be comprised of solid and spherical grains randomly distributed throughout the particle. The formation of CaCO₃ during the reaction is assumed to happen at grain surface, causing the grains’ volume to expand concentrically, increasing the grains’ radius, and shrinking the radius of the unreacted CaO core.

One characteristic of the carbonation reaction is that it exhibits two distinct main reaction regimes: a kinetic controlled profile for small reaction times, and a transitional and a diffusional regime for longer times [2]. Modeling the particle as an aggregate of dynamic grains with shells of CaCO₃ – allowing for particle porosity - adds two mechanisms that can control the rate of reaction besides the intrinsic chemical reaction. Particle porosity allows for the inclusion of the diffusion of CO₂ through the solid, and the layer of CaCO₃ around the grain adds an additional resistance to the transport of CO₂ between the pore and the reaction front. These two effects and the intrinsic reaction rate allow for a very accurate simulation of the carbonation reaction, and its two reaction regimes. The OGM, however, cannot estimate the maximum carbonation conversion, and uses an input parameter \( V_d \), the deactivated fraction, to achieve the desired maximum conversion.

A rate equation theory for the pore size distribution of calcined CaCO₃ – Calcination. The RThPSPD models the calcination reaction with a shrinking core model, where a CaCO₃ core gives way to a shell of CaO. It also models the dynamics of the porous structure using a rate equation based on a balance to the number of vacancies of any given size. The RThPSPD considers that two phenomena alter the porous structure during calcination: the release of CO₂ from the reaction, which creates vacancies by leaving the lattice; and the sintering process, which occurs naturally in CaO due to the high temperatures necessary for calcination.

The model, as presented in [4], has some shortcomings, for example, the lack of initial conditions for the rate equation derived from the experimental pore size distribution (PSD) of the initial unreacted CaCO₃. Thus, the parameter values that result from model validation will not accurately represent the decomposition and sintering processes. The original model assumes that the particle is initially a solid with no porosity and that the porous structure present in the final PSD originates solely from the release of CO₂; which is not true, due to CaCO₃ being porous [2]. The PSD after calcination is thus inextricably linked to the PSD of the original unreacted CaCO₃. Our version of the model adds initial conditions, obtained by assuming that the experimentally measured porous volume for a given pore diameter is equivalent to the total volume of vacancies of the respective size, assuming these vacancies to be spherical.

Furthermore, in the original model, the authors calculate the total surface area of the vacancies in the particle as the surface area of a sphere. However, sorbents for CaL are typically
analyzed using N$_2$ adsorption-desorption experiments in conjunction with the BJH model [3,5], where the assumption that pores are cylindrical, amongst others, leads to $V_{pt} = D_{eq}S/v_{0.4}$ [6], a relationship between pore volume, average pore diameter and pore area. Since, for a sphere of volume $V$ and radius $r$, the superficial area $S$ is $3V/r$, assuming the vacancies as spherical when calculating their surface area will generate values exactly 150 times larger than when using the BJH equation. As total pore area plays a very important part in the reactivity of the sorbent [5], overestimating this property will skew future simulations towards less conservative values of conversion, which is not desired. In our version of the model, we assume the pore area to be correspondent to a cylinder with a total volume equal to the total volume of vacancies of a given diameter $d_i$; and thus the pore area is calculated using the BJH equation presented previously. 

Along these improvements, the pseudo steady-state approximation originally assumed for the CO$_2$ partial pressure has been forgone; and the balance has been modified to make the effects of CaCO$_3$ decomposition and CaO sintering simultaneous, instead of sequential. The vacancy balance was also changed to correct an error where the total volume of vacancies would decrease, which does not occur during calcination [4], as no pore straightening mechanism is present.

**Results**

The OGM was validated using experimental data from a fluidized bed reactor experiments for three different sorbents: commercial CaCO$_3$, dolomite, and waste marble powder (WMP), a residue from the Portuguese marble industry recently shown to have economic potential as a sorbent by Pinheiro et al. [7]. As can be seen on the graphical abstract (Fig. 2), the OGM was fitted to the experimental results with a great degree of accuracy, being able to replicate both reaction regimes for the all sorbents, highlighted for the Dolomite series as line segments $i$ and $ii$. A slight deviation exists for the commercial CaCO$_3$, where the transition to the diffusional regime is sooner than expected. This can be related to the grain size distribution used to model the results for this sorbent, which was estimated using the experimental PSD as a basis. The relation between both is not straightforward, and it seems that a reallocation of the CaO volume present in higher grain sizes to lower sizes would benefit the fitting, as the CaO would be more easily available – CO$_2$ transport-wise – and thus the diffusion would only hinder the reaction rate for higher conversions.

As for the improved RThPSD model, it is still undergoing validation, since somewhat extensive experimental work is required. Notwithstanding, the changes made, especially the inclusion of initial conditions for the vacancy distribution, make the RThPSD apt to more realistically model the effects of calcination on the pore structure of a given sorbent and eventually estimate changes in pore area for different starting pore structures. The original model’s lack of proper initial conditions for the vacancy distribution circumscribe it to single calcinations that result in PSD’s with bimodal distributions (as can be seen on the Results section of [4]).

As for the correction made to the vacancy balance, simulations were ran using the values for the fitting parameters originally obtained in [4] using both the original balance and our modified version. The results for total vacancy volume along time for both simulations are plotted in the figure below.

For the original balance, not only is the total volume always below that of the modified version, as it can be observed that around 35 minutes, when the reaction terminates, the pore volume has been decreasing for approx. 10 min due to the reaction generation not being enough to offset the artificial volume consumption in the original balance. When using the modified balance the volume is conserved, as expected.

![Total Pore Volume (m³/g)](image)

**Figure 3.** Total pore volume (m³/g) using the original and the modified vacancy balance.

**Conclusion**

The implementation of OGM in the gPROMS ModelBuilder® software and subsequent validation with data from a fluidized bed reactor was successful, with the OGM being able to accurately replicate the experimental conversion profiles. The modified RThPSD model is yet to be validated, but several improvements were implemented in the same software, and simulations were made using values from the validation of the original model [4], which allowed verifying the improvements were correctly implemented. Seeing as the OGM cannot predict maximum CaO conversion, the relationship between loss of pore area and loss of sorbent reactivity is especially important and must be studied in order to be considered in the model. The improvements made to the RThPSD model will allow for the proper modeling of the changes to the pore structure during calcination which, upon validation, will make possible the estimation of loss of area for any given temperature, pressure and initial pore structure. The next step will be the validation of the improved RThPSD followed by the use of both models to estimate sorbent deactivation during cycling between carbonation and calcination reactions for various sorbents. The objective of the CaReCI project is to create a multi-scale model of a CaL process, where the models described in this work serve as particle-scale models.

**Acknowledgements**

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**References**

Modulating behavior of ionic liquid on micellization behavior in aqueous surfactant

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The intermicellar interaction study is of a great importance regarding its fundamental effect on micellar stability, which affects applied aspects of surface activity and micellar aggregation. Generally, the overall behavior of Ionic liquids (ILs) toward surfactants is determined by their electrostatic and hydrophobic interactions that are controlled by the structural properties of IL molecules including head group and counterion type and hydrophobic chain length. Besides, some ionic liquids are well-known for forming hydrogen bonding with water as well as with counterions and this can also influence ILs interaction with surfactants considerably and make it even more complicated. Thus, it is so difficult to generalize and predict about the effect of ILs on interfacial and aggregation properties of surfactants and any IL/surfactant mixture needs separate study as a specific case. To overcome this complexity, this review briefs the effect of IL with different chain lengths, cation and anion types on the surface activity, self-aggregation behavior, and aggregate morphology of cationic, anionic, zwitterionic and gemini surfactants in aqueous solution. Further, a comparative overview of all effects including ionic strength, temperature, electrolyte conditions such as salts and ionic liquids on intermicellar interaction has been discussed in terms of hydration, hydrophobic, and steric interactions on micellar stability.

The interaction between ionic liquid (IL) and surfactants depend on the ions of surfactants and controlled by the intra-molecular interactions. Addition of IL in aqueous surfactant solution alters the behavior of interaction between the cation/anion of the IL and surfactant head group. The ability of IL in changing the properties of aqueous solution of surfactant may be attributed to extent of interactions between the cation/anion of the IL and surfactant head group. Aggregation of surfactants supported by an addition of ILs have wider application in many fields such as extraction process, absorption and separation process, practical applications in interface science [1].

Critical micelles concentration (CMC) indicates the concentration range over which surfactant solutions show an abrupt change in a physical property which reflects the balance between the hydrophobic interaction of the hydrocarbon parts of amphiphilic molecules and the hydration and electrostatic repulsive effects of hydrophilic head groups. CMC of ionic surfactants in aqueous solutions decreases with the increase in ionic strength of the solution. The CMC value depends on the extent of electrostatic and hydrophobic interactions of ILs with the surfactant head and tail groups which can either lead to decrease or increase in CMC of the surfactant system, respectively. The electrostatic attractive interactions between the ILs and the surfactant charged head groups reduce the repulsions among surfactant head groups, thus allows surfactant molecules to come closer to each other for micellization at a lower concentration than CMC. On the other side, the hydrophobic interactions due to long chain ILs, solubilizes surfactant monomers in the aqueous media and delays the aggregation process, so micelle formation takes place at a higher concentration than CMC. The dominance of either of these interactions due to ILs decides the CMC of surfactant system [2]. However, it seems that other factors rather than electrostatic and hydrophobic effects are also there that influence the micellization behavior of surfactants in the presence of ILs, since the effect of the IL chain length on CMC is somehow found unusual. The hydrophobic chain part of the surfactants interacts loosely with the water molecules in aqueous environment, but in ILs media the physicochemical properties of surfactants significantly enhance because of the polar or ionic head group which interacts strongly via., dipole-dipole or ion-dipole interaction. The overall effect of IL on the CMC for surfactants seems to be a combination of various factors, including hydrophobic effect, electrolyte effect, hydrogen bonding and steric factor effect in both the solution and micellar phases [3]. Table 1 reports the CMC of mixture of ILs with each type of surfactants (not possible to report all mixture of IL and surfactant mixture in the extended abstract).

Tremendous efforts have been made worldwide to explore the aggregation behavior of the surfactants in the presence of a suitable IL. And, still the mechanism of interactions in aqueous surfactant with IL is not very well understood since it’s not very systematically studied. Thus, to generalize and predict about the effect of ILs on interfacial and aggregation properties of surfactants and any IL/surfactant mixture needs separate study as a specific case. To overcome this complexity, this review briefs the effect of IL on anionic, cationic, non-ionic, gemini and zwitterionic surfactant by summarizing their CMC. Further, a comparative overview of all effects including ionic strength, temperature, electrolyte conditions such as salts and ionic liquids on intermicellar interaction has been discussed in terms of hydration, hydrophobic, and steric interactions on micellar stability.
Table 1. CMC of mixture of ILs with various surfactants reported in the literature.

<table>
<thead>
<tr>
<th>IL</th>
<th>Surfactant</th>
<th>Conc (IL wt %)</th>
<th>CMC at $T = 298$ K</th>
<th>Reference</th>
</tr>
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<tr>
<td>[C₂mim][Br]</td>
<td>SDS</td>
<td>0.00</td>
<td>8.2</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.05</td>
<td>5.5</td>
<td></td>
</tr>
<tr>
<td>[C₄mim][C1]</td>
<td>CTAB</td>
<td>0</td>
<td>0.91</td>
<td>[5]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>0.42</td>
<td></td>
</tr>
<tr>
<td>[C₄mim][BF₄]</td>
<td>CTAB</td>
<td>0</td>
<td>0.36</td>
<td>[5]</td>
</tr>
<tr>
<td>[C₆mim][Br]</td>
<td>TTAB</td>
<td>0</td>
<td>3.4</td>
<td>[6]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.2</td>
<td>3.32</td>
<td></td>
</tr>
<tr>
<td>[C₄C₁mim][Br]</td>
<td>TX-100</td>
<td>0</td>
<td>2.27</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>2.21</td>
<td></td>
</tr>
<tr>
<td>[C₆mim][BF₄]</td>
<td>Tween-20</td>
<td>0</td>
<td>0.63</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>0.93</td>
<td></td>
</tr>
<tr>
<td>[C₆mim][Br]</td>
<td>(14-6-14,2Br-)</td>
<td>0.2</td>
<td>1.3</td>
<td>[9]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>[C₆mim][Br]</td>
<td></td>
<td>0.2</td>
<td>1</td>
<td>[9]</td>
</tr>
<tr>
<td>[C₆mim][BF₄]</td>
<td>SB-12</td>
<td>0</td>
<td>3.5</td>
<td>[10]</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.1</td>
<td>2.8</td>
<td></td>
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References
Rigorous modelling of the NOx absorption process: steady state sensitivity and validation of the dynamic behavior

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The absorption of nitrous oxides in nitric acid plants is a current industrial challenge due to stricter emission constraints and more demanding regulations, expected in a near future. To cope with this, the optimization of NOx absorption systems is required. Here we describe the behavior of an industrial NOx absorption column, using a simulation based strategy relying on a detailed mechanistic model. To demonstrate the usefulness of this tool, a sensitivity analysis of the steady state operation relative to changes in a diluted feed stream are considered. Next, the model is extended to simulate the dynamic behavior of the unit, and the simulation results are compared with industrial data. This tool evidences potential use both for dynamic optimization and control to reduce the NOx emissions, as well as for process design and troubleshooting of existing industrial units.

Introduction

Nitric acid plants still face problems relative to NOx emissions during transient operation periods, start-up and shutdown. Despite the presence of downstream NOx abatement reactors, aiming at reducing their concentration to nearly zero, these systems cannot be used during start-up and shutdown, since explosive byproducts can be formed. The transient emissions do not violate current environmental regulations; however, they have a perceptual visual impact. Additional regulations in the near future are also expected to include more stringent limits during transient periods, requiring additional operational measures. Therefore, the development of a rigorous model capable of reproducing both the steady state and the dynamic regime of these units is very appealing, since it can help industrial players in the optimization and control of these process units.

The advantages of using dynamic modelling tools in process optimization are recognized by various researchers. Schneider and Górak [1] developed a dynamic model of a reactive absorption column using the two-film theory, and demonstrate its utility by simulating the reactive absorption of sour gases in a packed column. In 2003, Kenig et al. [2] published a review on rigorous modelling of reactive absorption processes. Their model was applied to several cases for validation, including the NOx absorption in steady state. The authors note that rate based simulations present several computational challenges. These models require high computational effort which may lead to significant numerical errors and consequently limit their application to industrial problems. Hence, the authors suggested that improvements both in the computational approach and the numerical methods should be studied in order to provide “reliable, consistent, robust and user-friendly simulation tools for reactive absorption operations”. Later, Kenig and Seferlis [3] studied the implementation of rate based models for design and control applications, using an approach based on orthogonal collocation on finite elements (OCFE). This simulation was implemented in gPROMS, and the results were validated with experimental data from a NOx packed column in steady state operation at 600 kPa, producing nitric acid with 32%wt concentration. Their dynamic simulation demonstrated the response of the control system with increasing inlet NOx concentrations.

Despite these previous studies, some open issues remain in the reliable and efficient simulation of the NOx absorption process. Among these, we can point to: (i) the rigorous simulation of absorption units operating at nitric acid azetotropic conditions, near 68%wt.; (ii) the difficulties in simulating units operating at higher pressures, e.g. near 1000 kPa; (iii) model validation of industrial data; and (iv) the simulation of the start-up operations. This work describes a rigorous rate based dynamic model that overcomes these gaps. Our simulation of the steady state operation is presented in [4] and herein we complement the study with a sensitivity analysis and extend the model to simulate the dynamic behavior of the column.

Rigorous Rate-based Model

The present model relies on the two-film theory to describe the mechanism of the nitrogen oxides absorption in water, where the mass transfer between liquid and gas phases is limited due to gas and liquid phase resistances in their respective films; see [4] for details. To accurately describe the mechanistic behavior of the unit, the model includes the fluxes of species between phases, together with the transfer resistance and kinetic terms. The dynamic rate-based model leads to a DAE system of the form:

\[
\dot{x} = f(x, z, u) \quad \text{(eq. 1)}
\]

\[
0 = g(x, z, u)
\]

This corresponds to a semi-explicit Hessenberg index-1 system of DAEs. Various methods can be used for the numerical integration of these type of equations [5]: (i) Backward Differentiation Formulae (BDF) or the Gear method, (ii) Extrapolation methods, or (iii) Implicit Runge-Kutta (Orthogonal Collocation) methods. Depending on the modelling assumptions, our model can present high stiffness. Among the options available, the BDF class of methods have been widely used due to their good stability properties [6]. After discretization of the open space between trays, the proposed dynamic NOx absorption model is a large system formed by approximately 25000 equations. Its steady state version was implemented both the GAMS modelling environment and in the Wolfram Language, using Mathematica. The dynamic version is implemented in Mathematica only, using the built-in NDSolve numerical routine, due to a number of factors: (i) it takes advantage both

![Graph showing the relationship between liquid and gas concentrations.](image-url)
of the matrix sparsity and uses symbolic manipulation; (ii) uses the high quality IDA solver, that relies on efficient BDF methods, providing numerical solutions with high accuracy; and (iii) all software components are well maintained. For the integration of DAE system, the consistency of the initial solution is provided by the steady state version of the model.

**Simulation Results**
A sensitivity analysis is commonly used for several purposes such as: (i) identifying key optimization parameters and; (ii) investigate the impact of the parameters, their uncertainty and possible adjustment ranges.

The Graphical Abstract presents a sensitivity analysis of the HNO₃ concentration profile in the column (wl), relative to the concentration of the diluted lateral feed (LFT), where Pos refers to its Position in the column. Here, the reference scenario R is used for comparison, and five additional scenarios are simulated. Cumulatively, they show that higher concentrations of acid in the lateral feeding stream leads to higher concentrations also in the column. Higher feed concentrations result in lower driving-force below the LFT which decreases the efficiency of this section of the column. Consequently, fresh water entering the top is not sufficient to absorb the remaining NOₓ and results in the peaks observed after the LFT. The comparison of curves wlR-0.06 and wlR-0.06, PosR-2 demonstrates that there is an optimal position for the LFT, which can also depend on the concentration and flowrate of the lateral stream. We notice that a lower LFT decreases the reaction volume available and increases the column efficiency, as the NOₓ pressure leaving the absorption column decreases (202 ppm vol. vs 210 ppm vol.).

The results for scenarios wlR+0.02 reveal that when more concentrated acid enters the LFT, the profile along the column shows peaks, commonly seen as an indication of inefficiency. However, they can be avoided/minimized if additional reaction volume is made available which, on turn, requires moving the position of the LFT towards the bottom of the column.

A comparison of the dynamic simulation results with industrial data is considered in Figure 2, where NPNO is stands for the NO normalized pressure. Although the operating conditions are not completely identical, the industrial unit response was monitored using an onsite NO concentration meter placed before the EnviNO₃ reactor. This may cause some differences when comparing the results of the model with those of the plant, due to the reactions extents between those two points. Although some noticeable deviations are observed, the response trend is quite similar and the model discrepancy is less than 4.7%.

![Figure 2. Comparison between model results and industrial data.](image)

**Conclusions**
The NOₓ absorption in nitric acid plants is a current problem due to the NOₓ emissions, since stricter regulations are expected in a near future, with application also to transient regimes. This work considered a steady state sensitivity analysis and a dynamic simulation of a NOₓ absorption column, and its comparison with industrial data. The model can be used to reduce the NOₓ emissions during the start-up and shut-down operation, through optimization of the operation procedures during these regimes.

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**References**
Sequential extraction of phenolic compounds from *Libidibia ferrea* fruits using pressurized fluids

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The phenolic compounds present in *Libidibia ferrea* (jucá) extracts are recognized to be responsible for the well-known biological activities. In this work, different pressurized fluids were sequentially employed for the fractionated extraction of jucá fruits. Supercritical carbon dioxide (scCO₂), water, ethanol and acetone were employed in the subsequent and sequential extraction steps. Results showed that extracts rich in fatty acids, phytosterols, lupeol and lupenone, were mainly obtained at the first scCO₂ extraction step, while pressurized acetone/CO₂ extractions were more selective for gallic acid and quercetin-3-β-D-glucoside. Obtained extract compositions are justified by the complex effects that operational temperature/pressure have on the relevant thermophysical properties that are known to affect solvent-based extraction methods, as well as by the raw material properties and chemical compositions after each extraction step that was performed.

**Introduction**

Jucá (*Libidibia ferrea* Mart.) fruit extracts (usually in the form of teas, decoctions and infusions) have been widely used in Brazil as wound healing, anti-inflammatory and anti-bacterial folk medicines. These biological activities were reported to be associated with the large amounts and wide variety of phenolic compounds that are present in different parts of jucá, particularly in its fruits [1]. A similar species, *Caesalpinia spinosa*, was previously studied by Seabra et al. 2012, using supercritical CO₂ (scCO₂), ethanol and water, as well as some of their pressurized mixtures at different relative compositions, as extraction solvents and in order to obtain phenolic-rich extracts from *Caesalpinia spinosa* seed coats [2].

The aim of this work is to study the effects of type of extraction solvent, temperature, and pressure, on the sequential and fractionated extraction of jucá fruits, namely on extraction yields and extract compositions, and in order to obtain phenolic-rich extracts. This complete study will be published soon [3].

**Methods**

Sequential and fractionated extractions were performed in a high pressure extraction apparatus previously described by Gañán et al. [4]. Supercritical carbon dioxide (scCO₂, 5h, 8.92×10⁻⁵ kg/s) was employed in a first extraction step, and water (1.5h, 3.4×10⁻⁵ kg/s), ethanol (1.5h, 1.3×10⁻⁵ kg/s) and acetone (2h, 1.5×10⁻⁵ kg/s), all modified with CO₂, 10% (w/w), were employed in the 3 subsequent and sequential extraction steps (in this order). Extraction pressure/temperature conditions were varied according to previously established factorial design (2²) of experiments, replicated in a central point (318 K/25 MPA). Sequential extractions were performed without removing the solid raw material from the extraction vessel.

Extraction yields obtained by scCO₂ (1st extraction step) were processed and analyzed qualitatively by GC-MS, while the extracts obtained from the subsequent steps (2nd, 3rd and 4th extraction steps) were analyzed quantitatively by HPLC-DAD, according to Guan et al. [5]. Analyses were performed in duplicate. Obtained mass spectra were analyzed by comparison with data available in PBM and NIST databases. Total phenolic contents were determined by the Folin-Ciocalteu method, in triplicate [6]. Results are expressed as gallic acid equivalents (GAE, %).

**Results**

Depending on the specific pressure and temperature extraction conditions that were employed, in the 1st step (scCO₂), 2nd step (pressurized water/CO₂), 3rd step (pressurized ethanol/scCO₂) and 4th step (pressurized acetone/scCO₂) extraction, the obtained extraction yields were found to be between 0.3 to 0.5%, 0.2 to 1.1%, 0.1 to 0.83% and 0.1 to 3.8% (m/m, d.b.), respectively. An example is presented in Figure 1.

The above indicated results can be explained by the complex effects that temperature and pressure conditions have on the relevant thermophysical properties that are known to affect solvent-based extraction methods (e.g., density, viscosity, diffusivity, dielectric constant), and by the raw material properties and chemical composition after each extraction step that was performed (e.g., previous depletion of specific chemical substances, changes in raw material morphological properties).

In the 1st extraction step (scCO₂), and considering constant temperature, results showed that increasing pressure/density will lead to a yield increase, being this effect less pronounced both at lower and higher pressures/densities. On the other hand, and at constant pressure, the effects of temperature/density in obtained extraction yields seem to be negligible (results not presented).

In the 2nd extraction step (pressurized water/CO₂, 90:10 w/w), and considering constant temperature, the obtained results showed that increasing pressure/density will lead to a small extraction yield increase (at lower temperatures), or to a small extraction yield decrease (at higher temperatures) (results not presented). The observed yield variations are not as drastic as those observed for the 1st extraction step (scCO₂), certainly due to the smaller effects of pressure on the solvent density of liquids (and if compared to the pressure effects on the density of a supercritical fluid). On the contrary, and at constant pressure, a decrease in density (or an increase in temperature) will lead to higher extraction yields. These results also show that, and in addition to density (which typically controls the solubility of substances in extraction solvents), the operational
pressure and temperature conditions are also affecting other relevant thermophysical properties that are important for the overall extraction process, particularly at the highest solvent density regions (> 900 kg/m³): viscosity (which increases at higher densities) and diffusivity (which decreases at higher densities), both of them detrimental for the extraction process.

However, other factors should also be taken into consideration in the extraction yield results discussion: the specific and distinct density/viscosity/diffusivity and polarity/dielectric constant behavior of all the involved liquid solvents (with temperature and pressure). For example, in the 3rd extraction step (when using pressurized ethanol/CO₂, 90:10 w/w), and at the same pressure and temperature conditions that were employed at the 2nd extraction step (with water), it is possible to see that the density range of this liquid solvent mixture (735-820 kg/m³) is well below the density achieved for the water/CO₂ solvent mixture (990-1015 kg/m³) (results not presented). The viscosity, diffusivity and polarity of this solvent mixture are also certainly quite different from the water/CO₂ liquid solvent mixture. The same justifications can be employed to explain the different trends of pressure/temperature effects that were observed for the performed 4th extraction step (pressurized acetone/CO₂, 90:10 w/w) (results not presented). Therefore, all these different properties and their distinct and intricate pressure/temperature dependencies will surely impact the obtained extraction yields. Finally, the raw material properties and its chemical composition after each extraction step that was carried out, will also have a strong influence on subsequent extraction yield results. This is mostly due to the previous depletion of some specific chemical substances and to the consecutive pressurization/depressurization cycles, which will result in appreciable changes in the chemical and morphological properties of the raw material to be extracted. All the above justifications will also explain the observed differences that were found in the relative compositions of the extracts obtained at the different extraction steps. With the exception of the 1st extraction step (with scCO₂), and in which less polar compounds are expected to be extracted (fatty acids, phytosterols, as well as lupeol and lupenone) [7], all the subsequent extractions carried out using pressurized liquids and performed at different pressure/temperature conditions, originated extracts containing different phenolic compounds and at different relative concentrations. For example, and in general terms, the extracts obtained by pressurized water/CO₂ presented lower concentrations of gallic acid, whereas the extracts obtained by pressurized acetone/CO₂ presented the highest concentrations of this phenolic compound. On the other hand, the presence of quercetin-3-β-D-glucoside had not been yet reported for jucá, and its highest concentrations were also obtained with pressurized acetone/CO₂. Therefore, these results show that the use of this methodology, and namely of this pressurized liquid mixture presents relevant selectivities towards gallic acid and quercetin-3-β-D-glucoside. These results will be comprehensively detailed in a future work [3].

Conclusions
This work employed a tunable, versatile and selective extraction method, based on the sequential use of different pressurized fluids, for the fractionated extraction of jucá fruits. Extracts rich in fatty acids, phytosterols, lupeol and lupenone, were mainly obtained at the 1st scCO₂ extraction step, while those extracts rich in phenolic compounds were obtained mainly in the subsequent pressurized liquid-based extraction steps. Pressurized acetone/CO₂ extractions were more selective for gallic acid and quercetin-3-β-D-glucoside. Obtained extraction yields and extraction selectivities are justified by the complex effects that temperature/pressure extraction conditions have on the relevant thermophysical properties that are known to affect solvent-based extraction methods, as well as by the raw material properties and chemical compositions after each extraction step.

Acknowledgements
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References
Solketal, a fuel additive produced from the valorization of glycerol

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Considering heterogeneous catalysts applied for the valorisation of glycerol, especially through its acetalization/acetylation reactions, several solid silica-alumina catalysts, like zeolites or natural clays (e.g. montmorillonites), seems to be very active catalysts in these processes, because they are cheap, non-corrosive, economically benign, with micro porosity and with high surface area and, easy to handle. Heterogeneous catalysis shows the typical processual advantages, when compared with the traditional homogeneous catalysts. In the present work, montmorillonite MMK10 with an atomic ratio of Si/Al = 10 obtained from natural sources, was activated to increase their acidity, to enhance its activity in the production of a fuel additive in diesel engines, like Solketal, an acetal obtained from the reaction of glycerol with acetone. This catalyst was characterised by SEM-EDS, FTIR and other structural and textural characterisation techniques. Catalytic tests of MMK10 were applied to produce Solketal, optimizing the several operating conditions, like temperature, reaction time, molar ratio of acetone/glycerol/ethanol (ethanol as co-solvent) and, the weight percentage of catalyst related with glycerol, the main raw-material in this process.

Introduction

With the depletion of fossil fuel reserves and aiming towards the reduction of greenhouse gases, such as CO₂ and NOₓ, more ecological and renewable sources have been sought, with the most attractive and wanted to be the use of used frying oils, in the production of biodiesel. Biodiesel is produced by transesterification of those vegetable oils with an alcohol, like methanol, and can be used after blending with fossil-based diesel.

Glycerol, a by-product of the transesterification process, is produced on a large scale with little market value, which has led to the search for alternatives in using this by-product. This means that new applications for this polyol are being developed, such as, its ketalization, producing glycerol ketals as bio-based diesel additives, like Solketal. Solketal can be used as a fuel additive to reduce the NOₓ emissions and to improve the cold flow properties of liquid transportation fuels.

Objectives

The aim of this experimental work is the study of the Solketal’s production by catalytic reaction of glycerol with acetone, under heterogeneous catalysis. With this reaction 2 enantiomers are formed, one of them being Solketal (98%). Besides that, all the Solketal produced was quantified by, among other techniques, through refractometry. In this process, various reaction conditions, types of solid catalysts and calcination temperatures of those catalysts were tested. The Solketal produced, mixed with biodiesel/diesel mixtures, was evaluated, to analyse the effect of its physical properties, such as density and viscosity and, also, its effect on atmospheric monitoring emissions, coming from a diesel engine. The solid catalysts used were characterized by several methods, to perform surface, structural and textural analysis.

Materials and Methods

Catalyst Preparation. The catalysts used were prepared from zeolite Y (Faujasite) and, also, from silica-alumina natural clays (montmorillonites). Both catalysts were prepared equally, which the first step was their activation with an aqueous solution of NH₄NO₃ at 80 °C, with magnetic stirring, for 6 hours. After this activation process, to increase catalyst acid sites, the solid were filtrated, using vacuum filtration, dried in an oven overnight at 90 °C and, finally, calcinated at 500 °C for 8 hours, with a heating rate equal to 5 °C/min.

Solketal production experiments. For each catalyst used, they were performed several experiments (catalytic tests) to produce Solketal from the acetalization of glycerol, according with the following chemical equation:

\[
\text{H}_2\text{O} + \text{CH}_3\text{OH} + \text{HOCH}_2\text{CH(OH)}\text{CHOHCH}_2\text{OH} \rightarrow \text{H}_2\text{O} + \text{CH}_3\text{OCH(OH)CH(OH)CH}_2\text{OCH(OH)CH(OH)CH}_2\text{OH}
\]

The operating conditions like temperature, catalyst/glycerol mass ratio and acetone/glycerol/ethanol (A/G/E) molar ratio were changed, in the reactor, with mechanical stirring, for both catalysts (see figure 1). The use of ethanol as co-solvent, to improve glycerol solubilization and increase Solketal yield was also studied. Acetone is used as large excess (6:1 acetone/glycerol molar ratio) to increase, also, glycerol conversion. Table 1 summarizes some of the operating conditions applied, for both catalysts.

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>% (w/w) (catalyst/glycerol)</th>
<th>Time (h)</th>
<th>A/G/E molar ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>40,0; 50,0</td>
<td>1,0; 3,0</td>
<td>2 - 4</td>
<td>6/1/1</td>
</tr>
<tr>
<td>60,0</td>
<td>5,0; 7,5</td>
<td></td>
<td>6/1/2</td>
</tr>
</tbody>
</table>

The solid catalysts were characterised by, among others, Scanning Electron Microscopy with Electron Diffraction Spectroscopy (SEM-EDS), infrared spectroscopy (FTIR-ATR), X-ray Diffraction (XRD) and, performed textural characterisation, using adsorption/desorption isotherms with liquid nitrogen, at 77 k.

Solketal purification. After the production of Solketal, already described, for each experiment, the catalyst was separated from the liquid phase, through vacuum filtration, recovering the solid. Then, it was performed several distillations to...
separate acetone, ethanol and water, from the mixture Solketal/non-reacted glycerol (see figure 1).

Figure 1: Batch reactor apparatus for Solketal production (left) and Solketal purification step (right).

**Solketal quantification.** After the purification step, Solketal was quantified by refractometry, using, as standards, to plot the calibration curve, liquid mixtures of reference Solketal in pure glycerol. Standards and samples were placed in an Abbé refractometer, at 25 ºC, using a thermostatic bath with PID controller. A minimum of two concordant index refraction measures were collected for each sample.

**Results**

Catalyst preparation CHARACTERISATION. HY zeolite and montmorillonite catalysts were analysed by SEM-EDS. Figure 2 shows the correspondent SEM images of the MMK10 montmorillonite clay catalyst, before and after the activation process, with the addition of NH₄NO₃ aqueous solution, followed by drying and calcination steps previously described. It’s possible to see some changes in the surface catalyst while occur the activation of catalyst with acid sites. Figure 3 shows EDS diagrams of MMK10 catalyst, before and after activation process also, where it’s possible to observe the lines ascribable with Si and Al, besides K, typical of this catalyst. After the activation process, the atomic ratio Si/Al decreases. Nevertheless, the activity data on the catalytic tests achieved was significantly.

Figure 2. SEM analysis of MMK10 catalyst: a) Before activation process. b) After activation process.

**Solketal acetalization process.** Figure 4 shows a graph with some main results of Solketal yield, measure through refractometry, for some operating conditions. It’s possible to confirm the significantly activity of MMK10 in the acetalization of glycerol, to produce Solketal.

Figure 3. EDS diagrams of MMK10 catalyst: a) Before activation process. b) After activation process.

Figure 4. Catalytic activity (yield – wt%) of MMK10 in the acetalization of glycerol, measure through refractometry.

**Conclusions**

The valorization of glycerol can be enhanced to produce fuel additives in diesel engines, like Solketal. The acetalization of glycerol to produce acetals, like Solketal, through heterogeneous catalysis shows to be an interesting way to produce these compounds, avoiding the typical disadvantages of homogeneous catalysis, like the production of liquid wastes. MMK10 solid catalyst shows high activity in the production of Solketal, mainly, with the presence of ethanol as co-solvent, enhancing glycerol mixing with acetone, thus minimizing mass transfer limitations. The best operating conditions achieved were 40 ºC, 2h, %wt (catalyst/glycerol) = (5,0-7,5%) and, a molar ratio of A/G/E = 6/1/2.

More experimental work must to be done to validate all work objectives, like, verifying Solketal quality parameters.

**Acknowledgements**

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**References**


Reactive LIF test reaction for micromixing studies: definition of suitable operational conditions and validation in a T-jets mixer

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This work aimed to test and optimize one reactive PLIF method for (micro)mixing studies, consisting on an acid-base reaction with uranine as reactive tracer. The test-reaction was studied in a stopped-flow equipment to define suitable operational conditions, namely: chemical composition of the inflow streams to mix, concentration of reagents and fluorophore, excitation light wavelength and degradation of the streams (over a 24h-period) in increasing viscosity media. The most suitable operational conditions were tested in a T-jets mixer, illuminated in the axial plane with a laser light sheet, enabling to obtain fluorescence images inside this mixer. After adapting some of the operational conditions originally suggested in the literature, the test reaction became easier and suitable to be used in micromixing studies. Experiments performed in the T-jets mixer validated those operational conditions, showing the usefulness of this test reaction for micromixing studies.

Introduction and Objectives
Mixing processes of liquid–liquid components are of great technical interest in many industries. In fact, for some industrial processes, mixing degree may be the most important factor affecting product yield and influencing the economics of such processes [1]. This consciousness has led many authors during the last decades to focus on finding chemical methods to assess (micro)mixing degree. Among the studied methods, the most cited ones are probably the so-called Bourne’s reaction [2, 3] and Villermaux’s “iodide-iodate system” [4]. In recent years, optical methods have gained importance in this research field. Laser induced fluorescence (LIF) is a non-intrusive, optical technique based on the stimulation of fluorescence by laser and the measurement of the emitted light. For low tracer concentrations the fluorescent intensity is proportional to the local concentration [5].

The goal of this work was to test, adapt and optimize one reactive luminescence method for micromixing studies. The studied test reaction has already been addressed in the literature [6,7], and this work intended to study its applicability and limitations, validating it in a pilot-scale T-jets mixer.

Methods
Phosphoric acid, H₃PO₄, and sodium hydroxide, NaOH, were used in this work as acid-base pair. When the two streams mix, the local pH modification promotes a change in the fluorescence intensity of a fluorophore dissolved in one of the streams (in this work, uranine dissolved in the acid stream), acting as a marker of the reaction kinetics:

\[ \text{H}_3\text{PO}_4 + 3\text{NaOH} \rightarrow \text{Na}_3\text{PO}_4 + 3\text{H}_2\text{O} \]

Aqueous solutions of different concentration of uranine were prepared and the emission spectra (excitation light wavelength ranged from 250 to 700 nm) were determined in a stopped-flow equipment (Applied Photo-Physics SX, 18MV). Linearity of emission against concentration was then established for the following excitation light wavelengths, \( \lambda \): 350, 476 and 500 nm; these wavelengths are within and out of the uranine’s maximum emission range. The spectra readings were repeated after 24h to assess whether the ageing of the reagents had some influence on spectra that would limit the usability of the method within that period, corresponding to the preparation of the reagents from one workday to the following. All essays were carried out at 25°C. This 24h-degradation assessment was replicated for uranine solutions prepared in glycerine:water ratios of 0:100, 50:50, 60:40, 70:30 and 80:20% (wt.). In short, suitable operational conditions were defined for the studied test reaction, namely: chemical composition of the inflow streams to be mixed, concentration of reagents and fluorophore, and suitable excitation light wavelength.

The operational conditions determined in the previous stage as suitable for mixing studies were tested (validated) in a T-jet mixer, consisting of two opposite feeding channels connected to a mixing chamber at an angle of 90°. The T-jets were made in a transparent acrylic block and were illuminated in the axial plane with a laser light sheet, enabling to obtain fluorescence images inside this mixer.

Results and Discussion
The relationship between uranine concentration and emission intensity is shown in Figure 1a and the influence of pH on uranine’s emission intensity in Figure 1b. Figure 1a shows that emission intensity displays a linear trend with uranine concentration up to 30 mg/L, at all wavelengths tested (\( R^2 > 0.97 \)), although for \( \lambda = 476 \) or 500 nm the intensities and the slope of the straight line are higher, meaning higher sensitivity to small concentration changes at local scale. Figure 1b shows that uranine emits basically for pH above 3, reaching its maximum at pH ≈ 8. For pH between 5 and 8 the curve sharply increases, meaning maximum sensitivity of emission depending on local pH and, thus, on reaction course. This is an encouraging result since that range overlaps with a pH range where the H₃PO₄:NaOH titration curve is smooth, allowing the detection of small differences of mixing degree at the local scale.

The emission spectrum did not change over 24h. Thus, uranine test reaction can be scaled up for mixing studies without frequent preparation requirements of (large) reagent stocks during a workday, which in turn would be a considerable drawback.
Regarding more viscous liquids, the results show a stability of the reagents solutions in glycerine up to 60% (wt.), corresponding to approximately 13 cP. Above that value, uranine’s emission spectrum undergoes significant changes, and so this study did not proceed to higher viscosity values. The images depict these changes.

The images show light laminas throughout the flow domain, which correspond to regions of the formation of a product from the chemical reaction. From Figure 3 it is clear the role of the onset of vortices that mix both flow streams, at Re=100, on the increase of chemical reaction throughout the mixer. Thus, Reactive LIF enables to visualize the patterns of chemical reaction inside a reactor, i.e., it enables the visualization of micromixing.

**Conclusions**

After adapting some of the operational conditions originally suggested in the literature, the studied test reaction became easier and suitable to be used in micromixing studies. The recommendable conditions for employing uranine test reaction are: 20 mg/L uranine concentration in the acid stream; acid and base concentration in the streams so that pH=7 after reaction in the mixing chamber; excitation wavelength in the range 475-500 nm.

Experiments performed in the T-jets mixer showed that the test reaction is suitable for mixing studies, enabling clear visualization of the streams’ impinging point and the reaction course as well, through the increase of the emission intensity inside the mixing chamber.

The findings of this work are a useful contribute to this research field, by the adaptation and improvement of a test reaction that could be used as efficient, safe and cost-saving method for (micro)mixing assessment studies in media with viscosity up to ca. 13 cP (25°C), using glycerin as thickener.

**Acknowledgements**

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**References**

CO₂ and CH₄ adsorption on MIL-160(Al) from dry and wet streams

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The presence of contaminants in natural gas/biogas leads to a reduction of heat value. Adsorption is one of the technologies used to upgrade the natural gas and the biogas, by removing the carbon dioxide (CO₂). In this study, the adsorption properties of different adsorbents, such as CO₂, methane (CH₄), and water (H₂O), on MIL-160(Al), were measured at three different temperatures. CO₂ and CH₄ adsorption equilibrium isotherms are of Type I. While the adsorption of water presents a Type IV shape. The dynamic experiments performed in a fixed-bed set-up included single component (CO₂, CH₄, and H₂O) adsorption and desorption experiments, in a bed initially filled with helium, as well as co-adsorption of CO₂ (or CH₄) with H₂O. Adsorption and desorption of CO₂ in a bed initially saturated with H₂O at 50% relative humidity was also studied. The breakthrough curves of CO₂ and CH₄ present a compressive front during the adsorption branch, and then a dispersive front during the desorption branch.

Introduction
Methane is the main specie-present in natural gas (80-95%) and biogas (55-70%) [1, 2]. Both fuels contain CO₂ as the second main component, besides other contaminants, such as H₂O [1, 3]. The presence of these contaminants, mainly the CO₂, leads to a decreasing of heat value of the referred fuels [4]. The upgrade of the heat value can be achieved by adsorption, adsorption, membrane, and cryogenic separation processes [5]. However, the presence of water can be limiting to some of these CO₂ removal technologies, like the adsorptive separation processes. In the present work, we study the adsorption behavior of water, carbon dioxide, and methane on shaped MIL-160(Al), to evaluate its potential for the upgrade of (bio) methane in dry or wet streams.

Experimental
The MOF MIL-160(Al) is isostructural to CAU-10 and is built up from inorganic aluminum helical chains which are connected by 2,5-furan dicarboxylate ligands [6]. The material was synthesized at the Korea Research Institute of Chemical Technology (KRICT). The adsorption equilibrium isotherms were performed in a Rubotherm magnetic suspension balance (precision of 0.01 mg), in batch mode. CH₄ and CO₂ adsorption equilibrium isotherms were measured at 293 K, 303 K, and 323 K. While the adsorption equilibrium isotherms of H₂O were measured in a manometric set-up equipped with a pressure sensor of 100 mbar, at 303 K, 323 K, and 343 K. MIL-160(Al) samples were activated at 423 K during 12 h under vacuum. Breakthrough experiments were performed in a bench-scale fixed bed unit at 298 K and 1 bar. The stainless-steel column was packed with MIL-160(Al) and both ends of column were capped with a layer of glass wool. Prior to breakthrough experiments, material was activated by heating up to 423 K under helium flow for 12 h. Single and binary breakthrough curves (H₂O, CH₄, and CO₂) were performed.

Results
The CH₄, CO₂, and H₂O adsorption equilibrium isotherm at 303 K on MIL-160(Al), are presented in Figure 1 and Figure 2. CO₂ and CH₄ adsorption equilibrium isotherms present type I isotherm according IUPAC classification (Figure 1). In CH₄ adsorption equilibrium isotherm is visible a hysteresis loop.

![Figure 1. Adsorption-desorption equilibrium isotherm of CO₂ and CH₄ on MIL-160(Al), at 303 K (filled symbol: adsorption; open symbol: desorption).](image1)

![Figure 2. Adsorption equilibrium isotherm of H₂O on MIL-160(Al), at 303 K.](image2)

Figure 2 presents the adsorption equilibrium isotherm of H₂O at 303 K. This isotherm has a type IV shape according to IUPAC. The material is slightly hydrophilic when compared to other MOFs, since the sharp increase in H₂O uptake happens at low p/p₀ values (about 5-15%).

The adsorption breakthrough curve of 50% of CO₂ in helium (P = 0.5 bar, dashed line in Figure 1) presents a compressive front during the adsorption and dispersive pattern during the desorption which is typical for systems with favourable adsorption equilibrium isotherms (Figure 3) (Type 1). The adsorption breakthrough curve of 50% of CH₄ in helium (P = 0.5 bar, dashed line in Figure 1) presents the same pattern, however is visible a faster CH₄ saturation comparatively with CO₂.
As expected from the adsorption equilibrium isotherms for both components.

Figure 3. Breakthrough curve of 50% CO$_2$ in helium, on MIL-160(Al) at 298 K (The dashed line represents the beginning of the regeneration step).

Figure 4. Breakthrough curve of 50% CH$_4$ in He, on MIL-160(Al) at 298 K (The dashed line represents the beginning of the regeneration step).

The experimental breakthrough curves of CO$_2$ and H$_2$O co-adsorption on MIL-160(Al) is shown in Figure 5. Initially, H$_2$O vapour was transported with CO$_2$ at 50% relative humidity (RH), and fed into column, mixed with a dry helium (50%) stream.

Figure 5. Breakthrough curve of CO$_2$ in a binary mixture of dry He with saturated CO$_2$ (50% RH), on MIL-160(Al).

It can be observed that the adsorption capacity towards CO$_2$ is not much affected by the co-adsorption of water. However, it was observed a drastic reduction on the CO$_2$ adsorption capacity if the material is pre-saturated with water at 50% RH.

Conclusion

CO$_2$ and CH$_4$ isotherms present a Type I isotherm shape, being visible hysteresis in CH$_4$ isotherms. H$_2$O isotherms present a type IV isotherm. Breakthrough curves present a compressive front (adsorption), followed by a dispersive front (desorption). The presence of water can be detrimental towards the material performance (CO$_2$ adsorption), if the material is pre-saturated saturated with water. However, the co-adsorption of water seems to not affect the materials capacity to capture the CO$_2$.

Acknowledgements

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References

Study of triterpenic acids isolation by simulated moving bed at two distinct scales

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In this work the chromatographic separation of betulinic, oleanolic and ursolic acids (naturally occurring triterpenic acids with relevant bioactive properties) was studied at both analytical and preparative scales with the objective of implementing their separation by simulated moving bed (SMB). Preliminary HPLC experiments were conducted to select appropriate mobile and stationary phases. Equilibrium and mass transport parameters were determined through breakthrough experiments with pure compounds in a single analytical column. This information was then applied in the modeling of ternary breakthrough experiments conducted both at analytical and preparative scales. Finally, the SMB separation of the three compounds was simulated using a phenomenological rigorous model and applying data from analytical and preparative scale experiments, comparing the obtained results.

Triterpenic acids, namely betulinic (BA), oleanolic (OA) and ursolic (UA) acids, are high value compounds, with promising biologic and pharmacologic activities such as antioxidant, anti-HIV, anti-inflammatory, anti-tumor, anti-microbial, among others [1]. These compounds are readily available from multiple natural sources, e.g. in *Eucalyptus globulus* bark [2], however they are difficult to isolate due to their very similar chemical structures, especially in the case of oleanolic and ursolic acids which are positional isomers (Figure 1).

![Molecular structures of betulinic, oleanolic and ursolic acids.](image)

Figure 1. Molecular structures of betulinic, oleanolic and ursolic acids.

The simulated moving bed (SMB) chromatography is a well-established technique [3]. Although initially developed for large-scale processes such as for separations of petroleum components and sugars, its applications have been extended towards fine chemistry and even enantiomers isolation [4]. The continuous countercurrent operation of SMB units maximizes the mass transfer driving forces enabling the effective separation of components even when selectivities approach the unitary value, contrary to batch elution chromatography, in which high selectivities are a mandatory requirement. Moreover, this operation mode reduces significantly mobile phase consumption and stationary phase utilization. Hence, SMB appears as an attractive candidate for the efficient separation of triterpenic acids.

In this work the separation of betulinic, oleanolic and ursolic acids was studied at analytical and preparative scales with the objective of implementing their SMB separation. For this purpose, pulse experiments were performed to determine appropriate mobile and stationary phases, selecting a C18 column (250 × 4.6 mm, 5 µm) as adsorbent and methanol/water 95/5 (%. v/v) as the best mobile phase, allowing a good compromise between resolution and solubility of the target compounds (see chromatogram in Figure 2).

![HPLC chromatogram of betulinic, oleanolic and ursolic acids in Apollo C18 column and 95/5 (% v/v) methanol/water.](image)

Figure 2. HPLC chromatogram of betulinic, oleanolic and ursolic acids in Apollo C18 column and 95/5 (% v/v) methanol/water.

Breakthrough adsorption experiments for each acid were carried out at analytical scale to measure their unary isotherms and mass transfer coefficients (see Figure 3 for the fitting of chromatographic model to betulinic acid breakthrough runs at three different feed concentrations). The obtained parameters were then validated by comparing experimental and purely simulated breakthrough curves for the stringent case of a ternary mixture separation.

Breakthrough assays were also carried out at preparative scale (250 × 22 mm, 5 µm column) using the same adsorbent as the one used thus far. Modelling of chromatographic curves at preparative scale using data obtained from the analytical column revealed significant discrepancies, marking the importance of the proper characterization of each column.

The SMB separation of the three triterpenic acids was designed taking into account data obtained from preparative experiments and using a cascade of two SMB units: in the first, betulinic acid was isolated from a mixture of oleanolic and ursolic acids; and in the second, oleanolic and ursolic acids were separated. The triangle theory and a general strategy based on a Design of Experiments & Response Surface Methodology (DoE-RSM) [5] were used to optimize the operation conditions (flow rates and switch times) with the aim of obtaining 99 % purity on all compounds. Simulation results, of which the concentration...
profile in the SMB at cyclic steady state is presented in Figure 4, for the isolation of betulinic acid from oleanolic and ursolic acids, showed that the SMB unit successfully separates the compounds with the desired purities from a feed mixture representative of a natural extract.

Figure 3 – Adsorption breakthrough curves of betulinic acid at three different feed concentrations in an Apollo C18 analytical column with 95/5 (% v/v) methanol/water. Points – experimental data; Lines – model.

Figure 4. Simulation results at the end of the 45th cycle of SMB operation (cyclic steady state already established) for the isolation of betulinic acid (continuous line) from oleanolic and ursolic acids (dashed lines). (E – eluent; F – feed; X – extract; R – raffinate).

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References

Molecularly imprinted polymers (MIPs) are a class of materials that have artificially created receptor structures. MIPs have attracted considerable interest in several applications, such as adsorption, ion exchange, membranes, sensors, catalysis, and drug development [1].

Generically, MIPs are cross-linked polymers with specific binding sites for a particular compound. These sites are tailor-made by the copolymerization of crosslinking monomers and functional monomers in the presence of the target molecule, called the template. After polymerization, the template is removed from the polymer leaving behind recognition sites that are complementary to the target molecule in terms of size, shape and functionality. This leads to the MIP selectively binding to the template or its analogues in preference to other molecules (see Figure 1) [2].

Figure 1 – The molecular imprinting process: a) monomers, b) cross-linker, c) template molecule, 1-2) imprinting, 3) template extraction, 4) rebinding [3].

Conventional sorbents, like silica gel based adsorbents, have been employed to separate and purify triterpenic acids, such as betulinic (BA), oleanolic (OA) and ursolic acids (UA) [4-5]. However, the separation process can be difficult and inefficient due to poor affinity and selectivity, particularly in cases of compounds of very similar chemical structures such as OA and UA which are positional isomers. In this context, the development of a MIP specifically designed for the separation of betulinic, oleanolic and ursolic acids can greatly improve the purification process of these triterpenoids allowing their application in the pharmaceutical industry.

In this work, several MIPs were synthesized by thermal precipitation polymerization following a similar procedure to that reported by Granado et al. [6]. Different formulations were tested using various combinations of monomers (methacrylic acid or 4-vinylpyridine) and solvents (e.g., 1-butanol, dioxane, and chloroform). Oleanolic acid was chosen as the template molecule from among the three triterpenic acids. Solvent selection had to take into account the very low solubility of oleanolic acid in most organic solvents. For each imprinted polymer the respective non-imprinted material was also synthesized using the same procedure but without adding the template molecule.

The synthesized MIPs were analyzed by scanning electron microscopy (SEM) to determine particle size and morphology corresponding to distinct reaction conditions as well as compare the different characteristics of imprinted and non-imprinted polymers. An example is shown in Figure 2.

Figure 2 – SEM image of MIP synthetized with methacrylic acid as monomer and 1-butanol as solvent.

The most uniform particles were obtained using methacrylic acid as monomer and 1-butanol as solvent. These polymers resulted in a white powder, and SEM analyses confirmed they were microparticles of mostly uniform size. Remaining formulations did not appear to provide successful MIPs.

The removal of the template molecule by Soxhlet washing is intended to free the binding sites with high affinity to the solute. However, imprinted polymers are not homogeneous and contain both selective and non-selective binding sites, which affects the affinity of the overall material. Batch adsorption experiments
were carried out to study the viability of using the previously synthesized MIPs as stationary phase by evaluating their adsorption capacity and selectivity towards betulinic, oleanolic and ursolic acids. Batch adsorption experiments were conducted using ethanol or acetonitrile as solvent. The best MIP showed adsorption ability of oleanolic acid using ethanol and no adsorption towards the remaining two triterpenic acids. Several isotherms were fitted to the experimental data, including linear, Langmuir, Freundlich, linear-Langmuir, bi-Langmuir, and Toth models, and the best fit was found to be a Freundlich isotherm.

The infinite selectivities of this material towards oleanolic acid, in conjunction with great adsorption ability, highlight the large potential of this polymer under the scope of chromatographic separation of the triterpenic acids. Nonetheless more experiments are required to confirm this assertion.

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References
Development of integrated processes applying reversible aqueous biphasic systems

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The ability to induce reversible phase transitions, e.g. between homogeneous solutions and biphasic liquid-liquid systems, is of paramount relevance in the development of integrated separation processes. Both pH- and temperature-triggered aqueous biphasic systems (ABS) composed of ionic liquids (ILs) are here disclosed as switchable liquid-liquid systems, and their application as integrated separation platforms is demonstrated.

The ability to induce reversible phase transitions between homogeneous solutions and biphasic liquid-liquid systems, at pre-defined and suitable operating conditions, can be applied to design integrated production-separation processes. Switchable systems allow to carry out reactions or extractions in the monophasic region, which after the application of an external stimulus results in the creation of two-phase liquid-liquid systems feasible for separation and/or purification steps. This approach also contributes to a more feasible recovery of the target products and phase-forming components, allowing their recyclability. In the past few years, ionic-liquid-based aqueous biphasic systems (IL-based ABS; ternary systems formed by water, ILs, and a salting-out species) have been largely investigated for the separation and purification of a broad range of biocompounds. Nevertheless, their stimuli-responsive behavior and potential as integrated production-separation platforms were scarcely addressed. In this context, the objective of the current work is to characterize novel IL-based ABS and their phases’ transitions by changes in pH and temperature, and to evaluate their applicability as integrated production-separation processes.

It will be shown that pH-driven ABS can be prepared by changing the speciation of the organic salt (potassium citrate) used in the ABS formulation, in which alkaline pH values are favorable to induce two-phase formation. These systems can act as efficient integrated production-purification platforms, in which hydroxymethylfurfural (HMF) is produced from fructose at acidic pH, and then separated from the unreacted precursor by an increase in the pH and consequent formation of a two-phase system [1]. Temperature-driven ABS composed of ILs will be additionally shown. Unlike classical liquid-liquid systems, these ternary systems allow to work in a wide range of temperatures values and compositions which can be tailored to fit the requirements of a given separation process. These systems can be used in the separation of proteins (cytochrome c and azocasein) [2], while keeping their biological functions, as well as in the development of integrated biocatalytic processes [3]. Enzyme-catalyzed reactions occur in the homogenous solution, after which small changes in temperature induce the two-phase formation and the complete separation of enzymes from the respective products. These systems also allow the recovery and reuse of the ABS phase-forming components, contributing towards the development of sustainable production and separation processes. Although additional research on these solvents and systems scale-up feasibility is still required, the results here presented unveil the relevance of IL-based ABS as efficient integrated production-separation processes.

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References
Production of clean synthetic fuels using nanocrystalline MFI-based micro/mesoporous zeotypes synthesised via bottom-up approaches

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The oligomerisation of 1-butene was studied under high-pressure and continuous-flow operation (200-250 ºC, 30-40 bar), in the presence of micro/mesoporous zeotypes based on the MFI topology, prepared via different non-destructive bottom-up strategies, which influenced the material properties (morphology, textural and acid properties) and consequently the catalytic performances. In general, the zeotypes outperformed commercial zeolite ZSM-5, typically used in commercial oligomerization processes. The best-performing zeotype led to 77% conversion at 200 ºC and 30 bar.

Research efforts have been focused on developing efficient, sustainable production routes to quality fuels with reduced environmental impact. Olefin oligomerisation represents an alternative process to produce clean synthetic fuels, with low content of sulfur and aromatics, using olefins from renewable or non-renewable sources. Zeolites of the MFI type are used as acid catalysts in olefin oligomerization processes. Zeolites possess micropores, which may cause important diffusion limitations, especially when the desired products are relatively bulky molecules (e.g. oligomers). In recent years, materials research has focused on improving the catalytic properties of zeolites, by enhancing the active sites accessibility. An interesting strategy is to introduce secondary porosity in zeolites. While the micropores may impose shape-selectivity, the mesopores may facilitate internal diffusion, enhancing the reaction rate and avoiding severe coking and consequently catalyst deactivation.

In this work, the oligomerisation of 1-butene was studied under continuous-flow, high-pressure conditions, in the presence of MFI based zeotypes possessing mesoporosity. The zeotypes were prepared via different bottom-up approaches [1,2,3] (Figure 1). The catalytic tests were carried out in a fixed-bed reactor, at 200 ºC, 30 bar and weight hourly space velocity of 2.2 g cat\(^{-1}\) h\(^{-1}\). The products were analysed using a gas chromatograph, assembled on-line with the reactor.

The introduction of mesoporosity by non-destructive bottom-up approaches led to MFI-based zeotype catalysts with favourable morphological, textural and acid properties for 1-butene oligomerisation that allowed high conversion of butenes and yield of liquid products, characteristic of diesel cuts. The best-performing zeotype was prepared via crystallization of silanized protozeolitic units, and led to 77 % conversion of butenes, and an average space time yield of liquid products of 791 mg g\(^{-1}\) cat h\(^{-1}\) (7 h on-stream), and a mass ratio diesel cut/naphtha cut = 2. These results compared favourably to commercial zeolite ZSM-5, as well as literature data for several aluminosilicates tested under similar 1-butene reaction conditions.

![Hierarchical zeolites (Multiple levels of porosity)](image)

Figure 1. Bottom-up approaches and synthesis procedure used for the preparation of the MFI-based zeotypes (AiP = aluminium isopropoxide, SDA = structural directing agent).

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References

Mesoscopic solid acid catalysts based on the BEA topology were explored for olefin oligomerisation, which is an attractive synthetic route to produce clean, sulphur-free fuels with reduced aromatics content. Specifically, the oligomerisation of 1-butene, which may derive from renewable sources, was carried out under high pressure and continuous-flow operation. The mesostructured catalysts consisted of a hierarchical zeotype (BEA-hier) synthesized via one-pot approach using a dual function template, and a composite (BEA/TUD) possessing zeolite nanocrystallites embedded in a mesoporous matrix synthesized under hydrothermal conditions. The influence of the material properties, catalyst activation temperature, and reaction conditions (pressure and temperature) were investigated, combining materials characterization, GC×GC-ToFMS and catalytic studies. The catalytic performances were compared to commercial nano- and microcrystalline zeolites possessing different topologies.

Introduction

Olefin oligomerisation is an alternative route to produce clean, sulphur-free fuels or fuel additives with reduced aromatics content and other products, such as detergents, resins, plasticizers, drugs, flavours, perfumes, dyes, etc. Light olefins, such as 1-butene, may derive from non-renewable or renewable sources [1,2]. During the last decade, great efforts have been made in materials science in order to improve the acid catalysts used in oligomerisation reactions. The focus is to minimize steric hindrance and diffusion limitations inside the microporous structures of zeolite or zeotype materials, and enhance the active site accessibility and catalyst stability. The reduction of the crystallite size down to the nanoscale is an interesting approach to avoid diffusion limitations, since the diffusion path lengths are shortened. However, the use of catalyst nanoparticles in catalytic processes may present several environmental and technical drawbacks, which may be avoided by dispersing and stabilizing the catalyst nanoparticles in porous inorganic matrices with high specific surface area and narrow pore size distributions.

In the present work, mesostructured materials based on the BEA topology were explored for the oligomerisation of 1-butene, under high pressure and continuous-flow operation. Specifically, a composite consisting of BEA zeolite nanocrystallites embedded in a siliceous mesoporous matrix of the type TUD-1 was synthesized under hydrothermal conditions (BEA/TUD) [3], and a hierarchical material (BEA-hier) was synthesized using a relatively cheap, commercial polymer as dual function template [4]. The catalytic performances of the mesostructured materials were compared to commercial nano- and microcrystalline zeolites possessing BEA topology (BEA-nano, BEA-micro, respectively), and benchmark catalysts possessing MFI topology, namely ZSM-5 and COD-900 (related to the Conversion of Olefins to Distillates (COD) industrial process). The influence of material properties and reaction conditions on the oligomerisation reaction system was investigated by combining characterization, catalytic and comprehensive two-dimensional gas chromatography (GC×GC) combined with time-of-flight mass spectrometry (ToFMS) studies.

Experimental

The materials were characterized prior and after the catalytic reaction by powder XRD, nitrogen adsorption-desorption isotherms (-196 °C), FT-IR spectroscopy of adsorbed pyridine, $^{27}$Al MAS NMR spectroscopy, thermal analyses, electronic microscopy (SEM, TEM), ICP-EAS for Si and Al, and elemental analysis for C. The catalytic tests were performed using a fixed-bed reactor, as represented in Figure 1.

Results

The materials were tested for the oligomerisation reaction of 1-butene (1C4), for 8 h on-stream, at 200 °C, 30 bar and weight hourly space velocity (WHSV) of 2.2 g g$^{-1}$ h$^{-1}$. All the materials were active for the conversion of 1-C4 to products with boiling points (b.p.) in the range of 170-390 °C (C10-C24, characteristic of diesel; Dcut) and products with b.p. below 170 °C (C6-C10, characteristic of naphtha; Ncut). Without catalyst, the reaction was sluggish, ascertaining the catalytic role of the materials in the oligomerisation process, and negligible thermal reactions.
The catalyst activation temperature (T_{act}) was evaluated for all materials at 200 and 450 ºC. The BEA-based materials allowed better catalytic performances in terms of butenes conversion (X_{C4}) and space-time yield (STY) of liquid products, when activated at 200 ºC than at 450 ºC. The MFI-based catalysts showed the opposite effect. The activation temperature had no significant effect on the mass fraction of Dcut/Ncut of the products. The $^1H$ NMR spectra of the products indicated extremely weak resonances due to aromatics (spectral region 6.5-9.2 ppm), which is consistent with the GC×GC-ToFMS results. Thus, the liquid products were essentially composed of aliphatic compounds. The BEA-hier material allowed better catalytic results in terms of X_{C4} (73 %) and STY to liquid products (849 mg g_{cat}^{-1} h^{-1}). BEA-hier led to a mass ratio of Dcut/Ncut of the products of 1.2 and relatively low ratio of aromatic to aliphatic products (ca. 0.06), at 8 h on-stream, 250 ºC, 30 bar, WHSV of 2.2 h^{-1}, and T_{act} of 200 ºC. This catalyst was calcined and re-used in the oligomerisation reaction for another 8 h on-stream. The X_{C4} decreased to 46 % and the STY decreased to 357 mg g_{cat}^{-1} h^{-1}, whereas the mass fraction of Dcut/Ncut of the products remained similar. The characterization studies of the BEA-hier catalyst, before and after the catalytic reaction, showed that the morphological and textural features did not suffer considerable modifications.

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References

Lycopene (C\(_{40}H_{56}S\)) is an open chain, unsaturated, red-colored carotenoid that possesses bioactive properties. It occurs in fruits such as tomatoes, watermelons, papayas and pink grapefruits. It has been shown in several studies that high lycopene consumption can prevent heart diseases and cancer [1,2].

Astaxanthin (C\(_{46}H_{52}O_4\)) is also an interesting bioactive compound with relevant antioxidant properties. Over the years, the benefits of this carotenoid have been associated with reduced ischemic diseases. Astaxanthin can be found in many microorganisms and marine animals, such as shrimp, crayfish, salmon, trout, krill, microalgae or even in yeast [3,4]. Many industrial separations, like solid-liquid and supercritical fluid extractions, are often limited by film and/or intraparticle resistances. The design and optimization of their industrial extraction and purification require equilibrium and kinetic data. Hence, in this work, their diffusion coefficients at infinite dilution were measured using the chromatographic peak broadening technique in the range 1-100 bar and 303.15-333.15 K. The solvents used were ethanol and ethyl acetate. The obtained diffusivities ranged from \(3.447 \times 10^{-6}\) to \(6.679 \times 10^{-6}\) \(cm^2\) \(s^{-1}\) in the case of lycopene, and from \(8.172 \times 10^{-6}\) to \(1.218 \times 10^{-5}\) \(cm^2\) \(s^{-1}\) in the case of astaxanthin. These data were modeled using several predictive and correlation models, with relative deviations between 5.00 and 91.29 % (lycopene) and 3.94 and 79.38 % (astaxanthin).

The first method determines \(D_{12}\) by fitting the response curve given by Eq. (1) to data:

\[
\tilde{c} = \frac{m}{\pi R_0} \frac{1}{2\sqrt{\pi Dt}} \exp\left(\frac{L - \tilde{u}t}{4Dt}\right)
\]

and

\[
D = D_{12} + \frac{R_0^2 \tilde{u}^2}{48D_{12}}
\]

where \(\tilde{c}\) (mol cm\(^{-3}\)) is the solute concentration at column outlet, \(m\) (mol) is the injected amount of solute, \(R_0\) (cm) is the tube inner radius, \(L\) (cm) is the tube length, \(\tilde{u}\) (cm s\(^{-1}\)) is the average linear solvent velocity and \(t\) (s) is time.

The non-linear fitting is accomplished minimizing the square root of the mean square error, \(\varepsilon\), given by:

\[
\varepsilon (\%) = 100 \times \sqrt{\frac{\int_{t_1}^{t_2} [c_{\text{calc}}(t) - c_{\text{exp}}(t)]^2 dt}{\int_{t_1}^{t_2} c_{\text{exp}}(t)^2 dt}}
\]

where exponents \(\text{exp}\) and \(\text{calc}\) denote experimental and calculated concentrations, and \(t_1\) and \(t_2\) are the interval limits to perform the correlation. This range was selected so that the measured curve was higher than 10 % of the peak height [7].

The second method is based on moments analysis, by which \(D_{12}\) is obtained via the theoretical plate height, \(H\):

\[
D_{12} = \frac{\tilde{u}}{4} \left[H \pm \sqrt{H^2 - \frac{R_0^2}{3}}\right]
\]

and

\[
H = \frac{\tilde{u} W_{0.607}}{L}
\]

where \(W_{0.607}\) is the half width of the peak, in time units, measured at 60.7 % of the peak height [6,7].

In this work, the diffusivity of lycopene (85 % purity, HPLC) was measured in liquid ethanol, while the diffusivity of astaxanthin (97 % purity, HPLC) was measured in liquid ethyl acetate. These solvents were chosen according to their solvent power and availability for industrial extractions [8,9].

The obtained diffusivities ranged from \(3.447 \times 10^{-6}\) to \(6.679 \times 10^{-6}\) \(cm^2\) \(s^{-1}\) for lycopene and from \(8.172 \times 10^{-6}\) to \(1.218 \times 10^{-5}\) \(cm^2\) \(s^{-1}\) for astaxanthin. The obtained results were modeled with various predictive and correlation models from the literature, whose performance was assessed by the average absolute relative deviation (AARD):

\[
AARD (\%) = \frac{1}{N_{DP}} \sum_{i=1}^{N_{DP}} \left| \frac{D_{12}^{\text{exp}} - D_{12}^{\text{calc}}}{D_{12}^{\text{exp}}} \right| \times 100
\]
where NDP is the number of data points. The selected models were: the Stokes–Einstein based expressions of Wilke–Chang, Tyn–Calus and Reddy–Doraiswamy [7]; the Dymond-Hildebrand-Batschinski (DHB) [5] and Catchpole-King correlations [7]; the predictive model of Tracer Liu-Silva-Macedo (TLSM) and Tracer Liu-Silva-Macedo (TLSM) correlation; and the four empirical and semi-empirical correlations of Magalhães et al. [10]. The ranges of calculated relative deviations were 5.00-91.29 % for lycopene and 3.94-79.38 % for astaxanthin.

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References
Model of a Formaldehyde Absorption System

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Introduction
The aim of the work was to develop a model that described the behaviour of the above-described absorption system. The model will be used together with a previously developed model of an industrial reactor for formaldehyde production [1] to simulate the entire formaldehyde production unit. The final process model will allow a better understanding of the system as a whole and find the optimal operating conditions that reduce waste and increase the production profitability.

Model development
Reactions
The simulation of formaldehyde absorbers is highly complex, mainly due to the reactions occurring in the liquid phase and the exothermicity of the process. Formaldehyde reacts with water to form methylene glycol according to reaction (1) and higher poly(oxyethylene) glycols by a series of reversible reactions, by reaction (2) [2].

\[
\begin{align*}
\text{CH}_2\text{O} + \text{H}_2\text{O} &\rightleftharpoons \text{CH}_2\text{OH} \quad (1) \\
\text{CH}_2\text{(OH)}_n + \text{HO(CH}_2\text{O)}_m\text{H} &\rightleftharpoons \text{HO(CH}_2\text{O)}_n\text{H} + \text{H}_2\text{O}; \quad (n = 2...n_{\text{max}}) \\
\end{align*}
\]

Additionally, instead of reacting with water, formaldehyde can also react with the methanol in solution and form hemiformals through reaction (3), which in turn can polymerize by means of reaction (4) [2].

\[
\begin{align*}
\text{CH}_2\text{O} + \text{CH}_2\text{OH} &\rightleftharpoons \text{CH}_3\text{OCH}_2\text{OH} \quad (3) \\
\text{CH}_2\text{O} (\text{CH}_2\text{O})_n\text{H} + \text{CH}_2\text{OCH}_2\text{OH} &\rightleftharpoons \text{CH}_2\text{O} (\text{CH}_2\text{O})_n\text{H} + \text{CH}_2\text{OH}; \quad (n = 2...n_{\text{max}}) \\
\end{align*}
\]

Phase equilibria
Because formaldehyde has a tendency to react with water, the phase equilibrium will be significantly influenced by these reactions. Furthermore, formaldehyde has more affinity for methanol than for water, meaning that the effect of methanol in aqueous formaldehyde solutions cannot be neglected even for very small concentrations [2].

\[
\begin{align*}
\frac{y_i P}{y_j P} = \gamma_j \beta_i, \quad i = F, W \\
\end{align*}
\]

\[
\begin{align*}
K_{\text{MG}} &= \frac{y_{\text{MG}} y_{\text{W}}}{y_{\text{MG}} y_{\text{W}}} \times \frac{P_{\text{MG}}}{P} = \frac{y_{\text{MG}} y_{\text{W}}}{y_{\text{MG}} y_{\text{W}}} \times \frac{P_{\text{MG}}}{P} \\
K_{\text{MG}2} &= \frac{y_{\text{MG}_2} y_{\text{MG}_1}}{y_{\text{MG}_2} y_{\text{MG}_1}} \times \frac{P_{\text{MG}_2}}{P} \\
K_{\text{ip}} &= \frac{y_{\text{ip}} y_{\text{ip}_1}}{y_{\text{ip}} y_{\text{ip}_1}} \times \frac{P_{\text{ip}_1}}{P} \\
K_{\text{ip}2} &= \frac{y_{\text{ip}_2} y_{\text{ip}_1}}{y_{\text{ip}_2} y_{\text{ip}_1}} \times \frac{P_{\text{ip}_2}}{P} \\
\end{align*}
\]

Figure 1. Scheme of the vapour-liquid phase and chemical equilibrium for aqueous solutions of formaldehyde and methanol [2].

The thermodynamic model includes physical phase equilibria of formaldehyde, water, methanol, methylene glycol and hemiformal, described by equations (6) and (7), and chemical equilibria of the methylene glycol, hemiformal, poly(oxyethylene) glycols and poly(oxyethylene) hemiformals formation, described by equations (8) and (9).
Rate-based model

Usually, the absorption column profiles are determined assuming the equilibrium state between the streams leaving each stage and by using HETP-values (Height Equivalent to a Theoretical Plate). However, this method is difficult to implement in a process driven by rate-controlled phenomena, as it the case of reactive absorption.

A rate-based stage model is considered to be a more consistent modelling approach [3]. The model is based on a description of a single stage, which relates to either a tray or a packing segment. The gas and liquid phase balances are calculated separately, where the mass and heat transfer resistances are considered according to the film theory. Figure 2, the film model equations are combined with the relevant reaction and diffusion kinetics.

Figure 2. Two-film model for the stage description.

The model major assumptions are: (1) The absorption system operates in steady-state; (2) A plug-flow regime is considered for both liquid and gas phase, i.e. there is no axial dispersion for mass and heat transfer; (3) The absorption column operates adiabatically; (4) The packing is fully wetted, i.e. the interfacial area is the same for mass and heat transfer; (5) There is only gas to liquid mass transfer of water and formaldehyde; (6) The liquid in the distributor above the bottom packing of column T-1 and at the bottom of both columns is ideally mixed and the heat and mass transfer between liquid and gas is negligible.

Results and conclusions

The absorption system presented in Figure 1 was simulated using data supplied by the licensor of an industrial unit for formaldehyde production as input values of the model. The temperature and the formaldehyde mass percentage profiles are presented in Figure 3. Although these profiles were not yet compared with the real plant data, the simulation results are in agreement with what would be expected. Table 1 compares the simulation results with the licençor data and presents the absolute relative deviation between them.

The temperature results present some considerable deviations, that the authors expect to improve with a more detailed model of the heat removed in the column T-2, as well as the revision of some of the correlations used throughout the model, namely for the calculation of the interfacial area and the mass transfer coefficients on the trays sections.

Acknowledgements

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References


Synthesis and characterisation of aluminosilicate ZSM-5 membranes

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Aluminosilicate ZSM-5 membranes have been prepared on a tubular porous α-alumina support using a seeding by suspension method, followed by hydrothermal secondary growth synthesis. These membranes have been subsequently characterised by X-Ray Diffraction and Scanning Electron Microscopy. Permeation experiments have been performed under fixed and programmed temperature in the range of 298-383 K, using He, N$_2$ and O$_2$. The permeance results (expressed in mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$) vary between 1.08×10$^{-6}$ and 1.93×10$^{-5}$ for He, from 1.04×10$^{-7}$ to 2.20×10$^{-7}$ for N$_2$, and between 5.69×10$^{-8}$ and 1.36×10$^{-7}$ for O$_2$. While the results for O$_2$ and N$_2$ are comparable with the ones found on the literature, the permeance of He is higher than expected. The quality of the membrane was proved, discarding the existence of macro and meso-defects.

Membrane technology is an increasingly important and versatile way for gas separation, when compared to more conventional approaches like pressure swing adsorption (PSA) and cryogenic distillation [1]. Membranes provide several advantages [2] as for instance: (i) simplicity of operation, (ii) the possibility to be coupled to other processes, (iii) low energy consumption (phase changes are not necessary, and the pump and/or compressor costs are relatively low), and (iv) various environment friendly features (no solvents are involved, no wastes are generated, no chemical reactions occur during the process avoiding secondary products [3]).

Synthetic membranes may be classified in five main groups, depending on the nature of the material and/or its arrangement: organic, inorganic, organic-inorganic (MOFs and coordination polymers), composite and liquid membranes [4][5].

Currently, organic membranes dominate the global membrane market due to their advantages. However, they are generally limited to a moderate temperature range of operation, approximately 363-373 K [5]. Inorganic membranes, characterised by exhibiting high thermal and mechanical stabilities, surpass not only in terms of operating temperature but also in pressure conditions [6]. Consequently, they have been the target of an increasing number of studies.

Zeolites represent a group of inorganic microporous aluminosilicate materials, which are composed by AlO$_6$ and SiO$_4$ connected by oxygen atoms, leading to a 3D crystalline structure. Due to the dimensions of their channels in the order of molecular size, and taking into account their high thermal and mechanical stabilities, zeolites are particularly interesting rather than other microporous materials. Therefore, zeolites are widely used both in catalysis and separation processes [7][8].

In this work, several zeolite membranes with MFI structure (ZSM-5) were synthesised and subsequently characterised. The characterisation included not only the study of the structure and morphology of the membranes, but also permeation assays using several gases.

Regarding the synthesis, all membranes were prepared on a tubular porous support of α-alumina (α-Al$_2$O$_3$). A seeding by suspension method was adopted, followed by hydrothermal secondary growth.

Considering the structural characterisation, X-Ray Diffraction (XRD) and Scanning Electron Microscopy (SEM) analyses were accomplished.

The dynamic characterisation was possible by performing two types of permeation experiments: (i) at fixed temperature; and (ii) at programmed temperature under fixed transmembrane pressure difference. These tests were accomplished using He (Praxair, 99.999%), N$_2$ (Praxair, 99.995%) and O$_2$ (Praxair, 99.999%). Figure 1 presents the experimental set-up used for the permeation tests, including an electric oven, a membrane module, an instrumentation and control element, and a graphical interface.

Figure 1. Lab unit for permeance measurements including, from left to the right: a graphical interface, an instrumentation and control box, and an electric oven containing the membrane module.

XRD patterns confirmed that the synthesized phase was ZSM-5. On the other hand, SEM images showed the growth of a continuous polycrystalline layer.

With respect to the permeance measurements, carried out under the temperature range of 298-383 K, it was possible to confirm the hydrophilic behaviour of the membranes. Furthermore, the permeance of each gas was determined at several temperatures, and the results, expressed in mol m$^{-2}$ s$^{-1}$ Pa$^{-1}$, ranged from 1.08×10$^{-6}$ to 1.93×10$^{-5}$ for He, from 1.04×10$^{-7}$ to 2.20×10$^{-7}$ for N$_2$, and from 5.69×10$^{-8}$ to 1.36×10$^{-7}$ for O$_2$.

While the N$_2$ and O$_2$ permeances are comparable with the information available in the literature, the results for He are higher than expected [9].
Permeance measurements are also important to evaluate the quality of the synthesized membrane. Figure 2 represents the collected values of permeance, over the studied temperature range, using N\textsubscript{2}. The results show the increase of N\textsubscript{2} permeance with temperature, which indicates the absence of macro and mesodefects.

These experimental values allowed us to establish a relationship between the kinetic diameter of each gas (0.260 nm for He, 0.364 nm for N\textsubscript{2} and 0.346 nm for O\textsubscript{2}) and the permeances themselves. Helium, characterised for having the lowest kinetic diameter, presents the highest permeance values.

Acknowledgements
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References
During the past decade there were significant efforts on the study of ionic-liquid-based aqueous biphasic systems (IL-based ABS) as alternative separation platforms [1]. Although ABS have been described as benign and biocompatible alternative separation systems, most of the studied IL-based ABS are formed by pairing imidazolium ILs and inorganic salts, which may raise some biodegradability and toxicity concerns. This fact triggered the research on ILs derived from natural sources and/or by combining ILs with polymers, carbohydrates or amino acids, aiming at creating more bio- and environmentally-friendly ABS [2–4]. ABS composed of ILs and carbohydrates have been previously reported [5]. These studies were focused on offering a greener character to ABS using carbohydrates, non-toxic and renewable compounds, instead of high charge density inorganic salts. While promising advances towards more sustainable separation techniques have been achieved, all these studies employed ILs comprising aromatic cations and fluorinated anions. These fluoride-based ABS are expensive, some are not water-soluble, and in general they are environmentally persistent [6].

Focusing on addressing novel ABS, a range of carbohydrates was studied with tetraalkylphosphonium- and tetraalkylammonium-based ILs, with higher biodegradability, lower toxicity and lower cost. The carbohydrates investigated comprise: (i) monosaccharides, such as D-(−)-fructose, D-(+)-glucose, xylose, and D-(+)-mannose; (ii) disaccharides, such as D-(−)-maltose, and sucrose; and (iii) polysols, such as maltitol, xylitol, and D-sorbitol. These were combined with aqueous solutions of several ILs, namely [P444]Br, [P444][MeSO₄], [P444(OC)₂]Br, [P444][Cl], [P444][But], [P444][Hex], [P444][Oct], [N444][Br], and [N444][OCl]Br, envisaging the creation of novel ABS. The respective ternary phase diagrams, tie-lines, and tie-line lengths, were determined at several temperatures.

The investigated set of non-aromatic and non-fluorinated ILs demonstrated to be able to form ABS with carbohydrates, including more benign ILs, namely analogues to glycine-betaine (AGB-ILs).

Based on the obtained results it was found that the more hydrophilic carbohydrates (i.e., those with more −OH groups and with higher ability for hydrogen-bonding with water) combined with more hydrophilic ILs (as defined by their hydrogen-bond basicity) are more prone to form ABS. The extraction performance of the novel ABS was evaluated for antioxidants, namely vanillic (VA), gallic (GA) and syringic (SA) acids. The extractions experiments were carried out at two mixture compositions in the biphasic regime: 35 wt% of carbohydrate + 40 wt% of [P444]Br and 50 wt% of carbohydrate + 25 wt% of [P444]Br, [P444][MeSO₄] or [P444(OC)₂]Br. Extraction efficiencies ranging between 65 and 99% were obtained for all antioxidants to the IL-rich phase, in a single-step. These values are similar or higher than those obtained with ABS formed by fluorinated ILs and inorganic salts, reinforcing the competitive extraction performance of this set of more benign and lower ionic strength ABS. Finally, we demonstrated that phenolic acids can be recovered from the IL-rich phase, and that the employed IL can be recovered and reused with no losses in the ABS extraction performance. All studies reported hitherto on the development of sustainable IL-based ABS by the use of carbohydrates focused on ILs constituted by aromatic cations combined with fluorinated anions – a crucial requirement given the weak salting-out of carbohydrates. Herein, we demonstrate the possibility of creating more sustainable ABS formed by carbohydrates and tetraalkylphosphonium- and tetraalkylammonium-based ILs. These novel ABS display an outstanding performance to extract moderately hydrophilic compounds, such as phenolic acids. In summary, the novel systems disclosed in this work are competitive in terms of extraction performance, even when compared with ABS formed by ILs and strong salting-out salts, and their use in the separation of other high-value compounds is thus envisioned.
Acknowledgements
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References
UVC coupled tube-in-tube membrane microreactor using on-demand H$_2$O$_2$ injection for oxytetracycline degradation

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With the purpose of increasing the efficiency of UVC/H$_2$O$_2$ coupled systems for the treatment of contaminants of emerging concern, an innovative setup is proposed. The principle novelty of the new tube-in-tube membrane microreactor relies on the continuous, uniform and on-demand dosing of H$_2$O$_2$. An ultrafiltration membrane is used as a dosing system to deliver on demand H$_2$O$_2$ into the annulus of the reactor. In the annulus, where the liquid to be treated flows, UVC light is provided via four mercury lamps located externally to the outer tube. Keeping a constant gradient of the chemical across the whole length of the reactor, process efficiency could be increased, leading to a reduction in the use of the chemical, as well as a decrease in treatment time. Besides providing a better performance, the reactor has the advantage of an easy upscaling into a real plant.

Introduction

The presence of microcontaminants in aquatic ecosystems has become an emerging concern due to the inability of current treatment methods to remove such compounds [1]. These contaminants of emerging concern (CECs), even in concentrations of nanograms or micrograms, constitute a potential threat to both the ecosystem and human health [2]. Consequently, more efficient wastewater treatment methods are required to reduce the discharge of such compounds into the environment. The use of advance oxidation processes in general and particularly the combination of UVC/H$_2$O$_2$ have proved to be a suitable approach for the removal of CECs [3, 4]. Based on the potential of such a combined system, the authors aim to present a new setup (Figure 1), combining UVC radiation and on-demand H$_2$O$_2$ injection using a ceramic ultrafiltration membrane to degrade oxytetracycline (OTC), as a model CEC. The principle novelty of the new tube-in-tube membrane microreactor (Figure 2) relies on the continuous, uniform and on-demand dosing of H$_2$O$_2$. This would maximise the use of H$_2$O$_2$ by maintaining a homogeneous distribution and constant concentration of the injected chemical across the whole length of the reactor. Keeping a constant gradient of H$_2$O$_2$ along the reactor length would also reduce treatment times and hence, increase process efficiency significantly considering that treatment time is frequently a limiting factor in water and wastewater treatment processes. Thus, the proposed reactor would maximise the efficiency of H$_2$O$_2$ dosing and therefore, reduce the final H$_2$O$_2$ concentration in the effluent water. This would minimise (or even avoid) the use of subsequent treatment methods needed to remove residual H$_2$O$_2$, as well as the storage capacity and transportation costs of the chemical, maximising the efficient use of H$_2$O$_2$ in real wastewater treatment plants. At the same time, the proposed set up could easily be scale-up by integrating several single membranes into a common bundle. With the proposal of this new reactor, the authors also seek the need of delving into the use of UV-induced advanced oxidation technologies to remove refractory organic compounds from wastewater.

Material and methods

The proposed novel setup (Figure 2) consists of an inner ceramic ultrafiltration membrane ($\gamma$-Al$_2$O$_3$ ultrafiltration membrane from Inopor GmbH) surrounded by a concentric quartz tube that compose the annulus of the reactor (pathlength of 0.4 cm). The tubular membrane is internally fed with H$_2$O$_2$ (30% stock solution from Labbox applying different dilution factors) using a syringe pump (Nexus 6000 from Chemyx Inc.). The oxidant permeates the membrane and is uniformly delivered to the liquid stream to be treated (OTC solution) that flows between the outer membrane wall and inner quartz wall. The OTC solution is injected tangentially to the quartz tube from a jacketed cooling vessel (connected to a F12-MA chiller from Julabo) to the annulus using a gear pump (model BVP-Z from Ismatec). Radiation is provided by four UVC lamps (Puritec HNS 6 W G5 from Osram) located externally to the quartz tube and with a nominal power of 6 W. The setup is surrounded by an aluminium carcase to avoid direct eye contact with UVC lamps, as well as to increase light intensity in the reactor.

In order to measure the potential of the new system in degrading OTC, the following trial was carried out: Using a syringe pump, 0.7 mL/min of 20 times diluted 30% H$_2$O$_2$ stock solution were injected to the inner side of the membrane while a 5-L solution of 2 mg/L of OTC was pumped to the annulus at a rate of 40 L/h (reactor residence time of 4.4 seconds). Due to the injection pressure of the syringe, H$_2$O$_2$ permeates through the membrane to the annulus where the combination

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of UVC and H$_2$O$_2$ degrades OTC. Samples were taken in the outlet of the reactor once the process reached steady state conditions.

Results
Different injection flowrates where analysed for OTC solution (from 10 to 80 L/h), as well as for the dosing of H$_2$O$_2$ (from 0.7 to 3.5 mL/min). Additionally, various dilution factors were applied to the initial H$_2$O$_2$ stock solution and both OTC degradation and residual H$_2$O$_2$ were measured in the outlet of the reactor. Thus, Figure 3 shows OTC degradation percentage for each of the OTC solution flowrates (constant H$_2$O$_2$ injection rate) and compared to the residual H$_2$O$_2$ after the treatment. The highest OTC degradation is achieved with flowrates of 20 and 40 L/h, although a significantly lower residual H$_2$O$_2$ is obtained with the latter. This could be explained by a better mixing obtained in the reactor with 40 L/h, compared to that of 20 L/h. Although increasing solution flowrate up to 80 L/h would improve mixing, residence time would decrease significantly and hence, leading to a reduction in OTC degradation percentage.

Acknowledgements
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References
Acoustic cavitation combined ozonation for real abattoir wastewater treatment

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Introduction

Water shortage is increasing worldwide and becoming a concern not only for the environment, but also for a normal functioning of our society. Advanced oxidation processes (AOPs) are those processes that generate OH radicals (OH) in sufficient quantity to improve water quality by removing organic and inorganic pollutants [1], while the combination of multiple AOPs is commonly used for synergistic effects. Due to the above, there has been an increasing interest in research and implementation in both municipal wastewater plants and industrial facilities for the application of AOPs, ozone and ultrasound being two of the studied subjects.

During ozonation of wastewater, oxidation can occur through direct reaction involving molecular ozone and via an indirect pathway through hydroxyl radicals. Ozone is unstable in water and selectively attacks organic compounds. OH radicals, on the contrary, react non-selectively with many water constituents [2]. By oxidation of the specific cell wall components and subsequent DNA damage by O3 and OH radicals, ozone kills bacteria and disinfects water. Ozone can also increase the biodegradability of organic pollutants converting recalcitrant organic matter into more readily biodegradable one.

On the other hand, ultrasound is an acoustic (mechanical) wave whose frequency is above the upper audible limit of an average person, usually 20,000 Hz. Pre-existing bubble nuclei act as a source for cavitation. When the ultrasound pressure is above the threshold for cavitation, these bubble nuclei grow and coalescence, and once they reach a resonance size, the bubbles undergo violent inertial collapse [3]. During the inertial collapse, the bubble core can reach temperatures of 10,000 K and pressures of up to 1000 atm. Due to the high temperatures reached upon bubble collapse, water vapour inside the bubble dissociates to form reactive radical species such as OH radicals (sonochemistry), as well as the emission of light (sonoluminescence) [4]. Along with chemical effects, mechanical effects also contribute to water treatment as cavitation also promotes the formation of localised microjets (with velocities of up to 120 m/s), formed when bubbles collapse asymmetrically near a surface. Extreme shear forces can tear apart microorganisms and disinfect water.

In regard to wastewater generation, current industrialised livestock agriculture produces more polluted (BOD) wastewaters in comparison to domestic sewage (Table 1), this type of wastewater being characterised by a high organic content and highly variable quality [5]. Additionally, the production of animal products is increasing yearly, leading to the increase in the generation of this type of wastewater. Therefore, suitable wastewater treatment methods are required to ensure the wastewater quality meets regulations before discharge.

Considering the above, these combined ozonation-sonication process could serve as an alternative treatment method with a potential for a higher efficiency and reliability.

Table 1. Characteristics of treated wastewater collected from the abattoir and further treated with a lab scale activated sludge system. Sample variability shown as standard deviation.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Treated wastewater</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg/L)</td>
<td>249 ± 48</td>
</tr>
<tr>
<td>BOD (mg/L)</td>
<td>53 ± 21</td>
</tr>
<tr>
<td>TSS (mg/L)</td>
<td>70 ± 23</td>
</tr>
<tr>
<td>TC (CFU/mL)</td>
<td>4.1×103 ± 4.8×103</td>
</tr>
<tr>
<td>TVC (CFU/mL)</td>
<td>4.1×107 ± 2.2×107</td>
</tr>
<tr>
<td>pH</td>
<td>7.7 ± 0.2</td>
</tr>
</tbody>
</table>

Results

Ultrasound alone was not efficient in reducing organic carbon and inactivating microorganisms when treating real wastewater (abattoir wastewater characteristics shown in Table 1). 300 kHz was the only frequency showing a reduction in organic matter, obtaining 18% reduction in COD and 50% in BOD. No microbial inactivation was measured after 60 min of treatment. Combing ultrasound with ozone, on the contrary, led to a significant increase in COD and BOD removal (43% and 80 %, respectively), as well as achieving a complete inactivation of TC and a 5 log reduction in TVC. A substantial performance improvement was seen in TSS removal using ozonation-sonication. The reduction percentage in TSS was increased from 25% (sonication alone) to 70% (ozonation-sonication), obtaining 18 mg/L with the coupled system. Thus, the combined system was the only treatment method under study (compared to sonication and ozonation alone) reaching...
direct discharge limits for COD, BOD and TSS, as well as meeting drinking water standards for microbial disinfection.

Acknowledgements
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References
Production of clean synthetic fuels using nanocrystalline MFI-based micro/mesoporous zeotypes synthesised via top-down approaches

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The oligomerisation of light olefins is an alternative route to produce synthetic clean fuels and other high added-value products. The oligomerisation of 1-butene was studied under high-pressure (30 bar) and continuous-flow operation, in the presence of micro/mesoporous zeotypes based on the MFI topology, prepared via different destructive top-down strategies, using a commercial ZSM-5 zeolite. The different approaches influenced the material properties and consequently the catalytic performances.

Over the years, the energy consumption has been increasing worldwide. The projections for 2040 indicate that the main sector is the industry (53%), followed by transportation (26%) and buildings (21%) [1]. Regarding the transportation sector, it is fundamental to develop and implement efficient and clean processes for fuel production in order to satisfy the demand and to keep with the strict environmental legislations. Olefin oligomerization is an attractive route to produce clean synthetic fuels, with low content of sulfur and aromatics, using olefins from renewable or non-renewable sources.

Olefin oligomerisation is favoured in the presence of porous solid acid catalysts, especially zeolites possessing significant specific surface areas. An important characteristic of porous materials is their shape-selectivity. In oligomerization it is desirable to use catalysts with appropriate pore sizes to avoid the formation of molecules with too high branching degree and coke. On the other hand, internal diffusion limitations is a critical factor in reaction systems involving the formation of relatively bulky products. Catalysts should possess sufficiently large pores to avoid pore blockage and catalyst deactivation. Therefore, a compromise between mass transport and porosity of catalysts is important to improve product yields.

In recent years, different approaches were used to improve performances of existing catalysts and avoid diffusion limitations. An interesting strategy is to introduce secondary porosity in zeolites by post-synthesis modifications, such as desilication and dealumination. While the micropores may impose shape-selectivity, the mesopores may facilitate internal diffusion, enhancing the overall reaction rate and avoiding severe coking and consequent catalyst deactivation.

In the present work, the oligomerisation of 1-butene was studied under continuous-flow, high-pressure conditions, in the presence of MFI based zeotypes possessing mesoporosity. The zeotypes were prepared via different top-down approaches, using a commercial ZSM-5 as precursor. The catalytic tests were carried out in a fixed-bed reactor, at 200 °C, 30 bar and weight hourly space velocity of 2.2 g\textsubscript{cat} h\textsuperscript{-1}. The products were analysed using a gas chromatograph, assembled on-line with the reactor.

The introduction of mesoporosity by post-synthesis treatments led to MFI-based zeotype catalysts with favourable morphological, textural and acid properties for 1-butene oligomerisation that allowed high conversion of butenes and yield of liquid products, characteristic of diesel cuts. The best-performing zeotype for producing diesel type products was prepared via desilication with NaOH followed by dealumination with HCl.

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References
Há 100 anos a fazer as cores de Portugal.

Muita coisa mudou desde 1917 até hoje. E a CIN assistiu a tudo. Vimos todo um país e também o mundo mudarem. E a CIN também mudou. Em 100 anos passámos de uma fábrica no Porto a líder nacional e ibérica do mercado de tintas e vernizes. E se hoje celebramos um século é graças à sua preferência. Obrigado por dar cor também ao nosso mundo.
Poster Session

ENERGY AND ENVIRONMENT
Multi-electrode window for large-area solar hydrogen production

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One of the main challenges in the field of photoelectrochemical water splitting is the difficulty of preparing high-performing photoelectrodes with a large active area. This work reports a multi-electrode window assembled in the CoolPEC cell for holding up to eight small photoelectrodes connected in parallel. Internal separators placed between electrodes avoid parasitic ionic paths and overpotential losses, as demonstrated with CFD simulations. Each separator is strategically perforated to preserve the main flow path lines inside the cell, avoiding the accumulation of evolved gas bubbles and assuring proper heat dissipation. J-V measurements show that neither the photocurrent density nor the photovoltage are affected properly. The negative effect of an underperforming photoelectrode decreases exponentially with the number of photoelectrodes connected in parallel.

Introduction

Solar energy is the most abundant, clean and renewable source of energy able to supply the human annual energy consumption, ca. 5.67 x 10^8 Tj. Photoelectrochemical (PEC) hydrogen produced from solar water splitting is considered one of the most competitive and environmentally friendly ways to harvest and store the solar energy [1]. Although there are several PEC lab cells reported in the literature, a commercial prototype for continuous production of PEC hydrogen is still missing. In fact, building an efficient and stable upscaled device remains a major challenge in the field and is frequently considered one of the main obstacles for the implementation of this technology [2]. Semiconductor materials for solar water splitting have witnessed great advancements over the last decade; however, the development of efficient PEC devices has been neglected. Record-performing photoelectrodes (PEs) have not been optimized for upscaled cells, and usually have few square centimetres [3]. Also, the high electronic resistance in large-area photoelectrodes is still a primary limitation to the arrival of upscaled PEC cells to the commercial market. The addition of conductive metal lines to TCO-glass substrates is a strategy widely applied in solar cells; however, its integration in PEC cells is more complex. Metal lines need to be protected against corrosion since the photovoltaic electrode is immersed in the electrolyte. Also, a substantial part of the active area can become unusable due to the need of applying a protective coating (usually an epoxy resin) that fully covers the lines [4].

Objectives

This work aimed at developing a multi-electrode window capable of holding multiple photoelectrodes connected in parallel, used as PEC cell window. This solution enables using smaller and highly efficient nanostructured photoelectrodes prepared with state-of-the-art techniques, which can be connected in parallel, fulfilling the large-area requirements [5].

Methods

Hematite photoelectrodes were prepared by spray pyrolysis, as reported elsewhere [6]. First, the glass substrates (120 mm x 70 mm; Solaronix TCO 22-7) heated at 450 °C were hand-sprayed with 20 mL of a TEOS (tetraethyl orthosilicate) solution (99.9 %, Aldrich; 10 % volume in ethanol). Then, the substrates were placed on a heating plate at 475 °C and were sprayed with 10 mM of Fe(acac)₃ (99.9+ %, Aldrich) in EtOH (99 %, Aga), delivered by an automatic syringe pump (Cronus Sigma 2000 Dual Syringe Pump) previously adjusted to 12 ml-min⁻¹, discrete volumes of 1 ml, a time gap of 45 s between sprays and a total volume of 70 ml. The hematite thin films were then annealed for 30 min at 550 °C.

An Autolab/ PGSTAT302N workstation controlled by Nova software package (Nova version 1.11) was used to conduct J-V measurements, performed both in the dark and under simulated sunlight (Sulphur plasma lamp system AS 1300 V 2,0, Plasma International GmbH, 500-2500 W m⁻², 25 °C). The optimization of the multi-electrode support was assisted with CFD (computational fluid dynamics) simulations performed with Ansys® Workbench Academic 18.2; electrochemical reactions, fluid flow and tracer diffusion inside the PEC cell were included in the model. Internal separators were stepwise optimized and strategically perforated to preserve the electrolyte flowpath inside the CoolPEC cell, allowing the collection of evolved gases at the top and assuring proper heat dissipation. The calculation mesh was generated only for the electrolyte volume inside the cell.

Results

The new multi-electrode window can hold up to eight photoelectrodes and was designed to replace one window of the CoolPEC cell, a home-designed PEC cell [7]. This new part is made of acrylic (Perspex®, from Lucite®) and the electrical path between each photoelectrode and the current collector (connected to the potentiostat) is ensured through copper tape and low resistance cables. A highly resistant silicone paste seals the acrylic support to the housing area of the back window. Internal separators are inserted between PEs to minimize parasitic ionic paths and additional overpotential losses. CFD simulations were performed to study the electric potential and current vectors inside the electrolyte reservoir when the cell works at a constant potential. It was observed that when photoelectrodes with different performances are connected in parallel, undesired electric paths are created due to the asymmetric electric field, which creates a non-uniform flow of reactant species (OH⁻), affecting the performance of the cell. Despite these separators being essential to avoid parasitic electric paths, they can disturb the optimized flow path of the CoolPEC cell, generating stagnant zones as well as fluid turbulence, which delay the upward movement of evolved gases. To overcome this limitation, a stepwise simulation approach was...
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implemented to strategically make holes in the separators until reaching the desired flow pattern, as depicted in Figure 1-a.

Figure 1. Tracer test performed with a blue dye and distilled water with a feeding flow rate of 0.5 L min⁻¹ pictured 8 s after the injection of the dye, with the corresponding CFD results for mass fraction of tracer.

The new multi-electrode window allows having a continuous upward flow, avoiding the accumulation of gas bubbles and assuring an efficient heat dissipation, while guaranteeing the desired separation between electrodes. To experimentally assess the CFD results, two sets of tests were conducted. First, a tracer experiment was performed with a feeding flow rate of 0.5 L min⁻¹ – Figure 1-b. Second, two Ni foils and a bare FTO glass window (7 Ω square, 2.2 mm thick) were used as cathode and as anode, respectively, to promote the water electrolysis in 1.0 M KOH; no accumulation of gas bubbles was observed inside the CoolPEC cell. Therefore, good agreement was obtained between CFD and experimental results, since no relevant stagnant areas were identified.

The multi-electrode window comprising eight hematite photoelectrodes connected in parallel was then characterized by performing J-V measurements; two nickel foils were used as counter-electrodes. The number of photoelectrodes connected in parallel was stepwise increased for assessing any negative effect – Figure 2.

The key role of the internal separators to avoid parasitic ionic paths was also experimentally assessed based on the J-V characteristics of the segmented support with and without separators. A substantial decrease in the generated photocurrent was observed without the separators, namely when more photoelectrodes were connected in parallel, the later corresponding to losses of ca. 15%.

Preparing small photoelectrodes with similar performances is another key challenge. When multiple photoelectrodes are connected in parallel, an underperforming one can negatively influence the overall performance of the cell; this was assessed by applying a blocking mask in PE #5 (simulating an underperforming photoelectrode), and then increasing stepwise the number of good-performing photoelectrodes connected to this PE. It was observed that the influence of the underperforming photoelectrode decreases exponentially with the number of photoelectrodes operated in parallel, as it should be expected.

Conclusion

A multi-photoelectrode support was designed, optimized, assembled and tested in the CoolPEC cell. When eight photoelectrodes were operated in parallel, neither the photocurrent density nor the photovoltage were affected, when compared to a single photoelectrode. Internal separators were placed between the photoelectrodes to avoid undesired ionic paths, as shown by CFD results. Experimental results showed that when these separators are not used, losses of ca. 15% are observed in the generated photocurrent. This work also addressed the influence of an underperforming photoelectrode in the overall performance of the cell. It was concluded that its negative effect decreases exponentially with the number of photoelectrodes connected in parallel.

Acknowledgements

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References

Investigation of the viability of converting a leachate from a mechanical biological treatment plant for municipal solid waste into fertilizers

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The main environmental issue associated with the compost production is the production of a liquid leachate. However, the leachate may also be considered as a source of nutrients and used as fertilizer. The main concern is loading the soil with metals that can result in increased metal content of crops. This work intends to determine the physical and chemical properties of a raw leachate from a mechanical biological treatment plant for municipal solid waste, to assess the possibility of converting it into as commercial fertilizer which meets to the specifications required in the proposal of regulation of the European Parliament of 2016, for fertilizers. In a preliminary study, it is concluded that the leachate qualitatively meets the requirements established for the composition of commercial fertilizers and the production cost of leachate as raw material is low.

### Methods

*Chemical Properties of the Leachates Samples*

<table>
<thead>
<tr>
<th>Samples</th>
<th>% TOC</th>
<th>Density (mg/L)</th>
<th>pH</th>
<th>Conductivity (mS/cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EU, 2016</td>
<td>3</td>
<td>0.14</td>
<td>1.01</td>
<td>8.7</td>
</tr>
<tr>
<td>Original</td>
<td>24x</td>
<td>3.48</td>
<td>1.15</td>
<td>36x</td>
</tr>
<tr>
<td></td>
<td>45x</td>
<td>4.69</td>
<td>1.22</td>
<td>45x</td>
</tr>
<tr>
<td></td>
<td>50x</td>
<td>3.54</td>
<td>1.02</td>
<td>90x</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The heavy metals concentration was determined only in the concentrated samples because the original sample did not reach the analysis detection limit. As shown in Figure 1 (top), the content of Pb and Ni fulfill the requirements of the EU legislation while in Figure 1 (bottom), Cd and Cr must be removed to achieve the fertilizer requirements. However, the concentrate leachate still displays viability to produce a commercial fertilizer.

It is observed that the organic carbon content has a strong influence on the final density of the concentrated samples obtained. Therefore, some statistical resources were used to test the correlation, represented in Figure 2, between bulk density of the concentrated samples and TOC.

---

**Introduction**

Disposal of municipal waste is a major environmental problem. Increased urbanization and industrialization, especially in developing countries, requires municipal authorities to handle larger amount of municipal waste than in the past. [1] Considerable attention has been paid to the land application of municipal solid waste composts and sewage sludge worldwide in recent years. [2]

Chemical properties were determined for a raw leachate from a mechanical biological treatment plant for municipal solid waste, to check if it meets the adequate requirements for using as commercial fertilizer according to the proposal of regulation of the European Parliament of 2016, for fertilizers. [3]
Using the test of significance for Spearman's correlation coefficient, it is concluded that there is a significant correlation for significance tests with \( \alpha = 0.05 \) and \( \alpha = 0.01 \).

![Figure 1. Content of Pb and Ni in the leachates samples (top); Content of Cd and Cr in the leachates samples (bottom).](image)

Figure 1. Content of Pb and Ni in the leachates samples (top); Content of Cd and Cr in the leachates samples (bottom).

The procedure for the characterization of organic nitrogen was carried out to obtain parameters for the leachate in the legislation are shown in Table 2. The analysis was proceeded only using the original leachate because with the distillation process, the volatile fraction of nitrogen is lost due to heating.

![Figure 2. Correlation between Density and TOC.](image)

Figure 2. Correlation between Density and TOC.

Table 2. Concentration of nitrogen.

<table>
<thead>
<tr>
<th>Sample</th>
<th>TKN N (% w/w)</th>
<th>Ammoniacal N (% w/w)</th>
<th>Organic N (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.0286</td>
<td>0.0227</td>
<td>0.0059</td>
</tr>
<tr>
<td>2</td>
<td>0.0245</td>
<td>0.0231</td>
<td>0.0014</td>
</tr>
<tr>
<td>3</td>
<td>0.0272</td>
<td>0.0227</td>
<td>0.0045</td>
</tr>
</tbody>
</table>

According to European Union legislation, in order to be considered as a liquid organo mineral fertilizer, the product must contain at least one of the following declared nutrients in the minimum quantities: 2% by weight of total nitrogen of which 0.5% by mass of the fertilizer product must be organic nitrogen or 2% by weight of total phosphorus pentoxide (P₂O₅) or 2% by mass of total potassium oxide (K₂O). Therefore, it is necessary to carry out the phosphorus and potassium analyzes, using the concentrated leachates, to verify the possible content in one of the required nutrients.

For the characterization of total phosphorus, the standard solutions were analyzed to attain the regression curve in order to obtain phosphorus concentration in the samples. The samples were analyzed in sequence, following the same conditions applied to the standards. In Table 3, data referring to absorbances and concentration %w/w of each sample is presented, in addition to the minimum concentrations required by the legislation.

![Table 3. Concentration %w/w of P₂O₅](image)

The values obtained are below the minimum required by the legislation, even in more concentrated samples such as that was concentrated 50 times in relation to the original residue. These values represent a low concentration of P₂O₅ in the sample collected from the supernatant waste, stored in the resting tank.

**Conclusion**

The concentrated leachate shows potential to be used as fertilizer after simple processing. It displays high values of pH and concentration of total organic carbon (TOC) and low levels of heavy metals. Therefore, a few adjustments must be made, especially in pH and heavy metals concentration. Nitrogen and phosphorus presented low concentrations, mainly due to the composition of the sample used, since more than 99% was composed of water. Samples richer in organic content should be analyzed, as well as the samples taken directly at the exit of the plant, instead of the storage open tank where the effluent is collected, before it is sent to the treatment process. These samples may show higher levels of organic carbon and nutrients without requiring an initial concentration process. Seasonal factors also introduce complications for the characterization of the residues, because they induce large variations of composition of the material, which is exposed to external contaminants and weather conditions. This problem could be overcome through the introduction of a mixing previous step for homogenization of the residue, minimizing the need for further correction processes of composition to adapt the liquid waste to fertilizer legislation composition requirements. Though, the potential use of these wastes as fertilizers is still worthy of further studying because it represents the production of a high value-added material and, in addition, the recycling of wastes that are currently only regarded as pollutants which need to be treated.

**Acknowledgments**

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**References**

Terpenes belong to the largest and most diverse class of natural products. Due to the increasing importance of their applications and the emerging perception of their impact on the environment, the available physicochemical characterization is insufficient. In this work the solubility of terpenes in water at six different temperatures was evaluated. Due to the low solubility of these compounds a novel technique was adopted for their measurements and validated using the aqueous solubility data for sparingly soluble aromatic compounds. The experimental information is shown in a two-dimensional chemical space diagram providing indications to their probable distribution in the environment once released. This work contributes and calls for increasing the availability of reliable experimental property data of terpenes, which are also of enormous importance to the improvement, development and test new computational methods aiming for their prediction in such a vast family of compounds.

**Introduction**

Terpenes have been used since the Egyptians and their importance both in nature and for human related applications is enormous. Due to their biological importance and particular properties, these compounds had been studied and widely used in industries as the pharmaceutical, food additives, cosmetics, perfumery, fine chemicals and agriculture.

However, with the advent of the biorefinery, and the increasing importance and volume of terpenes production and applications, their environmental impact needs to be considered. In terms of quantities, biorefineries are on their way to become a new important anthropogenic source of terpenes and thus, envisioning the risk assessment of large scale operations, environmentally relevant physicochemical property data have to be known. Physicochemical parameters such as water solubility, vapor pressures and octanol-water partition coefficients, allow an ample description of the behavior of a substance in the environmental compartments, from multi-compartment analysis to transport and distribution of substances over the compartment boundaries.

Due to the ‘insoluble in water’ label, data on water solubility of terpenes are scarce. Additionally available data are often inconsistent and the temperature dependency largely unknown. Thus, we aim to contribute and call for increasing the availability of reliable experimental water solubility of terpenes. Obtaining accurate water solubilities will relieve the currently unsatisfying data availability and support our understanding of structure-energy relationships of this type of compounds, which are also of enormous importance to the improvement, development and test new computational methods aiming for their prediction in such a vast family of compounds.

In this work the water solubility of geraniol, linalool, DL-citronellol, thymol, eugenol, carvacrol and p-cymene, in the temperature range from (298.15 to 323.15) K, and at atmospheric pressure, was studied. Due the very low solubility values, a special approach has been followed for the solubility measurements; a saturated aqueous solution was generated within a dialysis tubing, followed by careful sampling and dilution in methanol. The process was sped up using an ultrasonic bath with temperature control before the equilibrium step. The main advantage of the present technique is to avoid the presence of solute not dissolved in the sampling phase, a common experimental error which often leads to overestimated solubility values. UV spectroscopy was used for quantitative analysis. Moreover, to determine their fate and behave and assessing their risk, the distribution of these compounds in the different environmental compartments was evaluated.

**Results and Conclusions**

Results show that the applied experimental method provides accurate aqueous solubility data for these sparingly soluble and rather complex molecules.

With the exception of DL-citronellol the solubility of terpenes in water shows a monotonical increase with temperature. Mole fraction solubilities are in the order of $10^{-4}$, confirming the “hydrophobic” label usually attributed to this class of compounds and showing that the dissolved terpenes can be considered at infinite dilution.

The thermodynamic properties of solution were derived from the experimental data at infinite dilution. It is shown that the solubility of terpenes in water is an endothermic process confirming the existence of UCST phase diagrams, and only for carvacrol and eugenol is entropically driven. Computational methods as COSMO-RS, SPARC, UNIFAC and EPI Suite, were tested in order to predict or estimate the solubility of the studied terpenes in water. However, none of them was able to predict satisfactorily the solubility values, nor even the solubility ranking among the different compounds studied.

In general, terpenes are partitioning into the three environmental compartments while toluene, p-xylene and isoprene partition exclusively into air due to their high vapor pressure.

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UID/EQU/50020/2013), both financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. This work is also a result of project “AlProcMat@N2020 - Advanced Industrial Processes and Materials for a Sustainable Northern Region of Portugal 2020”, with the reference NORTE-01-0145-FEDER-000006, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). M.A.R.M acknowledges FCT for her PhD grant (SFRH/BD/87084/2012).
Optimization of soybean oil ethanolysis by response surface methodology

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Biodiesel was successfully produced from soybean oil ethanolysis using a basic heterogeneous CaO catalyst. A green CaO catalyst was obtained by calcination of scallop shells (food waste). The heterogeneous catalyzed ethanolysis reaction conditions were optimized using a RSM methodology. The influence of reaction variables such as ethanol:oil molar ratio (10:1-14:1), catalyst loading (10-15 % based on oil weight) and time reaction (6-10 h) on the FAAE yield were analyzed. Computation was made using Minitab software. The optimal reaction parameters achieved were: 11:1 ethanol:oil molar ratio; 13.8 % catalyst loading and 9.1 h with 99.2 % maximum FAAE yield predicted. The fitted model was verified for the optimal conditions giving 99.0 % of FAAE yield instead of the 99.2 % predicted.

Introduction
In the next decades, the worldwide energy demand will continue to increase. Fossil fuels continue to supply most of the energy consumed in the transportation sector although their availability is limited. Hence, it becomes increasingly important to maximize the use of renewable sources, replacing fossil fuel with more sustainable fuels like biodiesel. Moreover, the use of green fuels is believed to mitigate the anthropogenic carbon emissions.

Biodiesel, a mixture of fatty acids alkyl esters, is mainly produced by catalyzed transesterification of vegetable oils and animal fats with short chain alcohols, such as methanol or ethanol. The mild reaction conditions needed, the fast reaction rate and the easy phase separation combined with its low-cost and industrial availability make the methanol the most used alcohol in biodiesel production [1,2]. However, methanol is mainly obtained from a fossil source, natural gas reforming, so the biofuel produced is not a fully renewable biodiesel. The use of ethanol, instead of methanol, would improve the biodiesel sustainability since ethanol can be obtained from agricultural products such as potatoes, grain, and corn, allowing the production of a completely renewable fuel. Ethanol has also a higher oil dissolving capacity than methanol. Due to the extra carbon atom, fatty acid ethyl ester (FAEE) cloud and pour point are lower than that of fatty acid methyl esters (FAME) [3]. In addition, the combustion heat and the cetane number are higher for FAEE than for FAME. The storage properties of FAEE are also improved [1]. The main drawbacks of ethanolysis for biodiesel production are its lower reactivity, compared with methanol, and the more difficult separation of biodiesel from the coproduced glycerin [4].

Objectives and Methods
In order to contribute to the knowledge on the biodiesel production by ethanolysis, the alcoholysis reaction of soybean oil was carried out at 75 °C, over CaO catalyst obtained by calcination of scallop shells at 900 °C for 3 h. A response surface methodology (RSM) was applied to determine the influence of reaction variables such as ethanol:oil molar ratio, catalyst loading and reaction time on the FFAE yield. The optimization of the reaction conditions was also performed in order to maximize the biodiesel yield. Computation was made using Minitab software.

Results
The as prepared catalyst showed XRD lines ascribable to lime whereas the post reaction catalysts showed XRD lines mainly ascribable to calcium hydroxide. As reported before [5] lime is totally hydrated during the reaction, which seems to point out portlandite as the catalyst active phase.

The experimental values for biodiesel yield, assessed by ATR-FTIR, are given in table 1.

Table 1. FAAE yield (%), assessed by ATR-FTIR.

<table>
<thead>
<tr>
<th>Ethanol:oil</th>
<th>Catalyst Wcat/Wtot (%)</th>
<th>Reaction time (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10:1</td>
<td>10.0</td>
<td>80.6 87.6 66.8</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>87.6 96.2 93.7</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>91.0 95.0 97.1</td>
</tr>
<tr>
<td>12:1</td>
<td>10.0</td>
<td>69.2 84.6 98.2</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>85.1 94.9 93.4</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>63.0 92.3 96.8</td>
</tr>
<tr>
<td>14:1</td>
<td>10.0</td>
<td>68.5 79.8 81.4</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
<td>67.1 86.3 96.9</td>
</tr>
<tr>
<td></td>
<td>15.0</td>
<td>56.8 83.7 93.4</td>
</tr>
</tbody>
</table>

It was applied the RSM to the experimental data of the FAAE yield (Y) using Minitab 18 software. The obtaining polynomial equation was:

\[ Y = -147 + 8.6 \text{ ethanol:oil} + 29.4 \text{ catalyst } - 0.8 \text{ time } - 0.632 \text{ ethanol:oil}^3 - 1.036 \text{ catalyst}^2 - 1.585 \text{ time}^3 - 0.732 \text{ ethanol:oil } \times \text{ catalyst } + 1.685 \text{ ethanol:oil } \times \text{ time } + 0.80 \text{ catalyst } \times \text{ time} \]

The correlation between experimental and predicted FAAE yields was 0.921. Interactions of process variables on FAAE yield are exhibited graphically in response surface and contour plots (Figure 1). Figure 1a shows that independently of the amount of catalyst loading, FAAE yield decreases with the increase of ethanol:oil ratio. On the other hand, an increase in the amount of catalyst increases the FAAE yield until it reaches a maximum value from which the FAAE yield decreases. This trend is not verified for the 10:1 ratio where the FAAE yield does not decrease.

Figure 1b shows that for a lower ethanol:oil ratio the increase of reaction time increases the yield but after reaching the maximum it begins to decrease. However, for a large ethanol:oil ratio the increase in time increases the yield.
In Figure 1c it can be observed that for a small amount of catalyst the increase of reaction time slight increases the yield but for a large catalyst amount the increase of the reaction time increases significantly the FAEE yield.

The optimal reaction parameters achieved, based on the polynomial equation obtained, were: 11:1 ethanol:oil molar ratio; 13.8 % catalyst loading and 9.1 h with 99.2 % maximum FAEE yield predicted. The fitted model (polynomial equation) was verified for the optimal reaction conditions and the average FAEE yield (using 3 replicas) was 99.0% instead of the 99.2% predicted.

**Conclusions**

The optimization of heterogeneous catalyzed soybean oil ethanolysis over CaO catalyst was carried out using a response surface methodology (RSM). For the optimal conditions, a FAEE yield of 99.0% was obtained instead of the 99.2% predicted by the fitted model.

Figure 1. Response surface and contour plot of a) ethanol:oil ratio (A) and catalyst loading (B) interactions impact on FAEE yield with 8 h of reaction time; b) ethanol:oil ratio (A) and time (C) interactions impact on FAEE yield with 12.5 % of catalyst loading; c) catalyst loading (B) and time (C) interactions impact on FAEE yield with 12:1 ethanol:oil ratio.

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**References**


Preliminary analysis of ashes from different agricultural and forestry biomass residues

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Introduction
Lignocellulosic biomass is a resource with a wide utilization in industry for energy purposes. It can be used for combustion, as in boilers or thermoelectric plants, and in pyrolysis and gasification processes. It is mostly composed of cellulose, hemicelluloses, lignin, lipids, proteins, simple sugars, starches, water, hydrocarbon and ash [1]. The ultimate composition of biomass is mainly described in terms of carbon, hydrogen, and oxygen, and Khan et al. [1] referred to percentage weights for C, H, and O of 30 to 60 %, 5 to 6 %, and 30 to 45 % respectively (on a dry basis, db). Additionally, elements like nitrogen, sulphur and chlorine can also be found, but in rather smaller amounts (usually less than 1 % on a dry basis).

Ashes produced from the thermal processing of biomass present a problem in terms of their disposal. They are usually disposed of in landfills, which causes problems for the environment and presents costs for biomass-based power plants. There is a need to arrange solutions for the reuse of these materials in different applications. Demeyer et al. [2] and Renyong et al. [3] investigated the utilization of ashes in soil remediation. Martirena and Monzo [4] reviewed the use of ashes from vegetable wastes as cement substitutes in the production of concrete. Vassilev et al. [5] and Nunes et al. [6] presented reviews on the properties, composition and application of biomass ashes.

The percentage of ashes resulting from the combustion of biomass can vary between 0.5 %, e.g. pulped wood, to 20 % for some cereals (on a dry basis). The ashes are principally composed of SiO₂ and CaO, but also include oxides of Mg, Al, K and P [6].

The objective of this study was to characterize the ashes obtained from the combustion in a muffle furnace at 550 °C (according to standard EN 15148) of different agricultural and forestry residues available in Portugal: hazelnut and almond shells, pine cones, pine bark, olive bagasse and fine high-density cork granules.

Experimental Methods
The different biomasses were characterized in terms of moisture and ash content, following standards EN 14774 and EN 15148.

Results and Discussion
The X-ray fluorescence spectra of the various biomasses under study show traces of Ca, K, Si, Fe, Mn, P, Cl in the samples. Table 1 presents the values of the ash content (on a dry basis) and of the HHV of all biomasses. Hazelnut and almond shells have the lowest ash content (0.7 % and 1.2 %, respectively) and olive bagasse has the highest value (5.8 %). Olive bagasse also has the highest HHV (20.8 MJ/kg) and for the other samples the HHV is in the range of 16.2 to 17.4 MJ/kg. This is an important parameter when the biomass is used in thermochemical processes such as combustion, pyrolysis and gasification.

According to Table 1, almond shell ash presents the highest K and Na content (27.3 % and 26.3 %, respectively), followed by olive bagasse ash (K – 24.6 %; Na – 22.4 %) and hazelnut shell ash (K – 21.5 %, Na – 20.6 %). The greatest Ca concentration was obtained for fine high-density cork granules ash (25.7 %) and pine bark ash had the highest Mg content (2.8 %). FTIR spectra showed the presence of Si in all ashes.
a residue of lignin. Vibration frequencies in the ranges of 1450-1410 cm\(^{-1}\) and 880-800 cm\(^{-1}\) are characteristic of the presence of carbonates [7]. Peaks appearing from 1130 to 1000 cm\(^{-1}\) could be attributed to Si-O-Si asymmetric stretching. The presence of Si in the biomasses under investigation were confirmed by the XRF experiments. The infrared band from 1130 to 1080 cm\(^{-1}\) is characteristic of the inorganic ion SO\(_4^{2-}\) [7].

**Conclusions**

New information on the characteristics of biomass ashes is presented in this study. Knowledge of the composition of biomass and biomass ashes is important if these residues are used in thermochemical processes such as incinerators, boilers or gasifiers. Depending on the composition of the ashes produced they can be reused, for example as a low-cost soil enhancer.

<table>
<thead>
<tr>
<th>Raw material</th>
<th>Ash (% db)</th>
<th>Moisture (%)</th>
<th>HHV (MJ/kg)</th>
<th>K (%)</th>
<th>Na (%)</th>
<th>Ca (%)</th>
<th>Mg (%)</th>
<th>Fe (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hazelnut Shell</td>
<td>0.7</td>
<td>11.0</td>
<td>17.0</td>
<td>21.5</td>
<td>20.6</td>
<td>10.2</td>
<td>1.9</td>
<td>0.1</td>
</tr>
<tr>
<td>Almond Shell</td>
<td>1.2</td>
<td>8.6</td>
<td>17.3</td>
<td>27.3</td>
<td>26.4</td>
<td>3.2</td>
<td>1.0</td>
<td>&lt; LOD*</td>
</tr>
<tr>
<td>Pine cone</td>
<td>1.4</td>
<td>12.3</td>
<td>17.4</td>
<td>10.0</td>
<td>13.1</td>
<td>2.6</td>
<td>1.7</td>
<td>0.9</td>
</tr>
<tr>
<td>Fine high-density cork granules</td>
<td>3.0</td>
<td>13.7</td>
<td>16.2</td>
<td>9.5</td>
<td>16.1</td>
<td>25.7</td>
<td>0.8</td>
<td>0.2</td>
</tr>
<tr>
<td>Pine bark</td>
<td>2.2</td>
<td>13.9</td>
<td>16.8</td>
<td>9.4</td>
<td>9.8</td>
<td>10.7</td>
<td>2.8</td>
<td>0.4</td>
</tr>
<tr>
<td>Olive bagasse</td>
<td>5.8</td>
<td>9.7</td>
<td>20.8</td>
<td>24.6</td>
<td>22.4</td>
<td>6.1</td>
<td>0.8</td>
<td>0.6</td>
</tr>
</tbody>
</table>

*LOD – limit of detection

**Acknowledgements**

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**References**

Thermochemical behaviour of wet blue shavings in an inert atmosphere

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Experimental Methods

Experimental Methods

The main objective of this investigation was to study the thermal decomposition of wet blue shavings using two techniques: TG experiments (with the determination of kinetic parameters) and a slow pyrolysis experiment at a set temperature of 700 °C and at a heating rate of 20 °C.min⁻¹. The residues were characterized in terms of their ultimate and proximate analysis, chromium oxide content and higher heating value. The thermogravimetric experiments show that decomposition of the wet blue shavings occurs into two stages: the first corresponds to the drying of the material, the second is related to the thermal decomposition of the leather waste. The kinetic parameters of the second stage of decomposition were determined by applying the method of Coats-Redfern. For the pyrolysis experiment at a set temperature of 700 °C and HR = 20 °C.min⁻¹, the yield of the biochar obtained was 27.1%. The gas produced contained CO₂, CH₄, CO and H₂.

Introduction

The leather industry, despite using by-products of the meat industry, is considered to be a pollutant because it generates substantial amounts of solid and liquid waste products. According to Buljan et al. [1] for every 1750 kg of cattle hide which enters a leather manufacturer, 195 kg of shoe upper leather, 60 kg of split leather, 400 kg of non-tanned solid waste and 237 kg of tanned waste are produced. From the total chromium oxide utilized in the tanning process, 45% is used in the final leather product, and 30% and 25% respectively are discharged as solid waste and as effluents.

Pyrolysis, a thermochemical degradation process of solid wastes when exposed to high temperatures in an inert atmosphere, could be an alternative to the disposal of leather solid wastes in landfills [2]. The products obtained from pyrolysis (biochar, bio-oil and non-condensable gases, such as CO₂, CO, H₂ and CH₄ etc.) are of interest for their energy content and as a source for producing chemicals [3].

Thermogravimetry (TG) is an experimental technique that can be used to establish the temperatures at which the thermal decomposition of waste materials occurs and to determine the kinetics of the process [3,4].

The main objective of this investigation was to study the thermal decomposition of wet blue shavings using two techniques: TG with the determination of kinetic parameters using the method of Coats-Redfern and a slow pyrolysis experiment at a set temperature of 700 °C and at a heating rate of 20 °C.min⁻¹.

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Experimental Methods

The wet blue residues were characterized in terms of proximate and ultimate analysis. Moisture, volatiles, ash content and fixed carbon were determined using a Netsch STA 449 F3 Jupiter thermogravimetric analyser. During the experiments, nitrogen was fed to the equipment at a flow rate of 50 mL/min and the temperature was varied from ambient to 900°C, at a heating rate of 20 °C.min⁻¹. After reaching this temperature, the system remained at a constant temperature of 900 °C for 7 min. Afterwards, the carrier gas was changed to air, using the same flow rate and maintaining the system at 900 °C, for another 13 min. The ultimate analysis of the wet blue shavings was performed by LNEG (“Laboratório Nacional de Engenharia e Geologia”) according to standard CENT/TS 15104. The chromium oxide (Cr₂O₃) content of the raw material was determined according to standard BS1309-1974. The higher heating value (HHV) was measured using a Parr calorimeter composed of three interconnected units: a 1341 Oxygen Bomb Calorimeter, an 1108 Oxygen Combustion Bomb and a 6772 Calorimetric Thermometer. The wet blue shavings were also qualitatively characterized by X-ray fluorescence (XRF), using an Oxford Instruments model X-SUPREME 8000. After acid digestion, the content of potassium (K) and sodium (Na) in the ashes of the wet blue shavings was determined using a Corning flame photometer.

Pyrolysis of the wet blue shavings was carried out at a set temperature of 700 °C, with a heating rate of 20 °C min⁻¹, using the slow pyrolysis reactor and procedure described in [5]. The mass of sample used was 30 g, and the nitrogen flow rate was set at 2.27x10⁻² kg.s⁻¹. The gas samples collected during the experiment were analyzed in a Dani 1000DPC gas chromatograph.

The TG experiments were conducted in an inert atmosphere, using a nitrogen flow rate of 50 mL min⁻¹, where the temperature changed from ambient to 900 °C. The influence of heating rate was studied for HR = 10 and 20 °C.min⁻¹.

Results and Discussion

The X-ray fluorescence spectra of the wet blue shavings under study shows traces of Cr, Cl, S, Ca, K, Si and Fe in the sample. Table 1 presents the proximate and ultimate analysis and the HHV of the wet blue shavings (13.2 MJ.kg⁻¹) and its Cr₂O₃ content (2.1%). The percentage of K and Na in the ashes were 0.2% and 18.5 % (on a dry basis) respectively.

From the pyrolysis experiment, the biochar obtained at a set temperature of 700°C (which corresponds to a maximum bed temperature of 590°C) had a yield of 27.1% (total basis). During this experiment no data was collected about the bio-oil that formed. Figure 2 shows the evolution of the temperature inside the reactor over time, and that the main compounds in the gas stream that was produced were CO₂, CO, CH₄ and H₂. At this operating temperature, H₂ was the most abundant gas, reaching a maximum of 5.6%, followed by CO with a peak concentration of 2.9%. CO₂ and CH₄ were present at lower concentrations. For these conditions the HHV of the gas produced was 594.9 kJ.(Nm)⁻¹ [6].
The differential weight loss (DTG) curves of the leather shavings at HR of 10 and 20 °C.min⁻¹ are represented in Figure 2. The first peak observed in both curves corresponds to the drying of the material (first stage), where moisture and some volatiles are released. The second stage (second peak of the curves) is related to the thermal decomposition of the leather waste. It is observed that, as the heating rate increases, the peak temperatures are shifted towards higher values. Application of the method of Coats-Redfern [4] to the second stage of the thermal decomposition produced the kinetic parameters shown in Table 2.

**Conclusions**

This work presents results of the thermal degradation of wet blue shavings obtained using a thermal analyser and a slow pyrolysis reactor. The DTG curves show that the decomposition occurs in two stages, with the first stage referring to the release of moisture and some volatiles, and the second corresponding to the degradation of the leather waste. For the pyrolysis experiment at a set temperature of 700 °C and HR = 20 °C.min⁻¹, the yield of the biochar obtained was 27.1%. The gas produced contained CO₂, CH₄, CO and H₂. Further pyrolysis experiments need to be performed using other bed temperatures and the bio-oil that formed should be collected and analysed.

**Table 1. Proximate and ultimate analysis, chromium oxide content and HHV of the wet blue shavings.**

<table>
<thead>
<tr>
<th>Proximate analysis</th>
<th>Ultimate analysis</th>
<th>Chromium oxide</th>
<th>HHV</th>
</tr>
</thead>
<tbody>
<tr>
<td>(total basis -% w/w)</td>
<td>(total basis -% w/w)*</td>
<td>(total basis -% w/w)</td>
<td>(MJ/kg)</td>
</tr>
<tr>
<td>Moisture – 17.9</td>
<td>C – 31.9</td>
<td>2.1</td>
<td>13.2</td>
</tr>
<tr>
<td>Volatiles – 71.7</td>
<td>N – 11.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ash – 9.1</td>
<td>H – 4.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fixed carbon – 1.3</td>
<td>O – 25.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*a*according to [6]

**Table 2. Kinetic parameters derived from the TG experiments by application of the method of Coats-Redfern.**

<table>
<thead>
<tr>
<th>Heating rate (°C.min⁻¹)</th>
<th>Temperature range (°C)</th>
<th>Activation energy (kJ.mol⁻¹)</th>
<th>Pre-exponential factor (min⁻¹)</th>
<th>Order of reaction</th>
<th>Correlation coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>287 - 377</td>
<td>238.8</td>
<td>1.3 x 10²⁴</td>
<td>2.1</td>
<td>0.976</td>
</tr>
<tr>
<td>20</td>
<td>261 - 390</td>
<td>144.9</td>
<td>3.4 x 10¹³</td>
<td>1.6</td>
<td>0.995</td>
</tr>
</tbody>
</table>

**Acknowledgements**

Financial support was provided by the FCT, under the Research Project UID/EQU/00305/2013.

**References**

Introduction and Objectives
The continuous CO₂ atmospheric accumulation, mostly from the use of fossil fuels, is now recognized as a major environmental threat; it is then imperative to mitigate CO₂ emissions. Electrochemical reduction of CO₂ (ERCO₂) is considered a promising approach to produce value-added chemicals, energy storage and carbon recycling [1, 2]. The development of highly efficient electrocatalysts is needed for the practical application of ERCO₂, and it has become a hot scientific topic in recent years. Steering the catalyst selectivity is a major challenge [2]. The hydrogen evolution reaction (HER) takes place as a competitive reaction during CO₂ conversion by the water reduction, leading to H₂ production. However, the presence of H₂ onto the catalyst surface is a requirement for CO₂ activation and further hydrogenation. Thus, an effective ERCO₂-catalyst should be able to suppress the HER so that all the adsorbed H₂ is consumed by the CO₂ reduction [3]. Copper has been pointed out as the best material for ERCO₂. Recent studies have shown that the geometry, morphology, size, shape and electronic properties of Cu catalysts have a great influence on the ERCO₂ catalytic performance [4]. In this work, CuZn catalysts were developed and the influence of the synthesis time on the catalyst’s physicochemical properties and thus catalytic performance studied.

Methods
CuZn catalysts (Cu:Zn atomic ratio of 1.54) were synthesized by a solvothermal route, and electrodes based on the developed CuZn nanoparticles (NPs) prepared by the airbrush deposition of a solution containing the catalyst on the surface of a carbon paper (Sigma 35BC, SGL Group). The prepared electrodes were characterized by several physicochemical techniques and their catalytic activity evaluated by linear sweep voltammetry (LSV). Electrochemical measurements were performed at room temperature and atmospheric pressure in a conventional three-electrodes electrochemical cell. A solution of 0.1 M H₂CO₃ was used as electrolyte. The electrolyte was previously saturated with N₂ or CO₂.

Results
LSV results are presented in Fig. 1. The cathodic currents represented by dashed blue lines are associated to the H₂ formation from water reduction, while the green full lines represent the overall activity (HER and ERCO₂), an estimate of the current fraction originated by the ERCO₂ is given by the orange full lines. It should be emphasized that the onset potential of the hydrogen production and CO₂ reduction is shifted to more negative potentials as the catalyst synthesis time increases. For instance, the onset potential for the ERCO₂ is ca. -0.66 V for CuZn 5 h and -1.03 V for CuZn 25 h, similar to what is observed for the HER reaction. As it can be observed the highest current density (12.34 mA cm⁻²), in CO₂-saturated electrolyte, was observed for CuZn 10 h. However, the contribution of the HER still expressive, which was assessed running the cell with N₂-saturated electrolyte. In fact, the major differences observed in the electrochemical activity as result of the catalyst synthesis conditions are related to the HER, being favored for longer synthesis time.

Conclusions
As suggested by the characterization techniques (TPR, SEM-EDX, XPS and XRD), different synthesis time lead to the formation of CuZn NPs with different morphology, particle size and Cu oxidation state. CuZn 5 h consists on partially amorphous CuZn particles. Increasing synthesis time results initially in a decrease in particle size, CuZn 15 h sample displays the smallest particle size (200 – 300 nm) reported, being then increased to 7 – 12 μm for CuZn 25 h sample. Results also indicates that the degree of crystallization increases, while the fraction of Cu as CuO declines with synthesis time. CuZn 25 h sample presents well defined spherical structures with a Cu metallic core covered by a Cu-Zn mixed-oxide, as shown in the Graphical Abstract.

Hydrogen formation is favored on metal catalysts [3] and small Cu particles [1]. CuZn 5 h and CuZn 25 h samples present a similar particle size, however different Cu oxidation state. According to the electrochemical results herein reported, the Cu valence has a greater influence in the reaction selectivity, explaining the higher HER activity on CuZn 25 h.

Conclusion
The influence of the synthesis time on the physicochemical and catalytic properties of CuZn catalysts was assessed. Special attention was paid to the activity towards the HER reaction. A longer synthesis time, up to 15 h, allows full crystallization of the metal NPs. Increasing the synthesis time above 15 h results in the growth of the produced NPs. Longer synthesis also results in a decrease of Cu as CuO species, explaining the improvement in HER activity.
Figure 1. Linear voltammograms in CO₂- and N₂-saturated 0.1 M KHCO₃ of the prepared electrocatalysts: A – CuZn 5 h, B – CuZn 10 h, C – CuZn 15 h and D – CuZn 25 h.

Acknowledgements
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References
Development of a simple and inexpensive methodology for the determination of estrone and 17α-ethinylestradiol in sludge samples

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Estrogen (E1) and 17α-ethinylestradiol (EE2) are becoming increasingly recognised as important micropolutants to be monitored in wastewater treatment plants (WWTPs). This study describes a method for the simultaneous determination of E1 and EE2 in fresh sludge samples. After spiking fresh digested sludge with E1 and EE2 standards and maintaining contact during 5, 30 and 60 min, the freeze-dried samples were subjected to ultrasonic liquid extraction (ULE), with methanol and acetone, and analysed by high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). The recoveries obtained for E1 and EE2 were 103 ± 3% and 97 ± 4%, respectively. Fresh sludge samples from a WWTP located in Portugal were analysed and only E1 was detected at a concentration in the range of 1.00 - 4.8 μg g⁻¹. The method here developed is fast and simple, reliable and inexpensive, making possible its application for monitoring the contamination of sludge with these estrogens.

Introduction

A group of organic pollutants called endocrine-disrupting chemicals (EDCs) has recently attracted attention due to their disruptive impact in ecosystems, particularly to aquatic organisms [1]. Natural estrogens, estrone (E1) and 17β-estradiol (E2), and the synthetic estrogen 17α-ethinylestradiol (EE2), were reported as the EDCs with higher disrupting potency [2]. Some authors reported that during the sewage treatment process, E2 is readily oxidized to E1 [2]. Estrogens exhibit a high sorption potential and as a consequence, in wastewater treatment plants (WWTPs) a large proportion of estrogens are removed from aqueous medium through adsorption processes onto sludge solid components and only a small proportion is biodegraded during the treatment process [2].

Due to estrogen sorption capacity onto sludge, a robust analytical methodology able to determine the concentration levels of these compounds found in the sludge solid phase after the WWTP treatment is crucial. In the last years, a significant number of studies have described and proposed different analytical methodologies with different extractions processes to detect and/or quantify E1 and EE2 mostly in environmental samples. However, most of these studies focus on the determination of estrogens in water samples, and in most cases, the amount of estrogen retained onto sludge is neglected [1]. In addition, reports of extraction efficiencies considering fresh sludge spiked with standard estrogens are limited and most of them refer that standards were spiked onto freeze-dried sludge. These conditions do not mimic the natural adsorption process that estrogens are subjected in naturally contaminated samples. Thus, the main aim of this work was to develop a method to determine E1 and EE2 in fresh sludge samples from an operating WWTP. The extraction method was evaluated through recovery rates, which were obtained by spiking the standards onto fresh sludge. Furthermore, in the methodology developed the sample preparation was carried out without any clean-up procedure, simplifying the procedure and reducing analysis time and costs. The method was applied to determine the concentration levels of E1 and EE2 in real fresh samples of primary sludge, secondary sludge, anaerobic digested sludge and dehydrated sludge, collected from an operating WWTP located in Portugal.

Material and Methods

Instrumental analysis. E1 and EE2 analyses were performed on a Shimadzu High-Performance Liquid Chromatograph Prominence system equipped with a fluorescence detector (HPLC-FLD). This device consisted of a DGU-20ASR degasser, a LC-30AD pump, a CTO-20AC column oven and a SIL-30AC autosampler. A 150 mm × 4.6 mm i.d. ACE® C18 column-PPF (5 µm particle size) connected to a 4.6 mm i.d. ACE® 5 C18 guard column was used for the separation. The mobile phase used to determine E1 and EE2 consisted of ultrapure water: acetonitrile mixture of 30:50 (v/v) and 55:45 (v/v), respectively. A flow rate of 0.8 mL min⁻¹ and an injection volume of 20 μL were used. Detection was performed using a Shimadzu Prominence RF-20A XS fluorescence detector at an excitation wavelength of 280 nm and an emission wavelength of 310 nm.

Sample treatment and extraction. To determine the recovery efficiency of the ultrasonic liquid extraction (ULE) described, the 50 mL samples of fresh digested sludge were spiked with 100 μg of E1 or 1.1 μg of EE2. Contact time of 5 min, 30 min and 60 min was used to evaluate sorption interactions of the estrogens with the sludge under continuous agitation. The measured individual mass of E1 and EE2 was determined as the sum of the masses quantified in the sludge samples liquid and solid phases.

The 50 mL sludge samples were centrifuged at 4000 rpm for 10 min to separate the liquid from the solid phase. The supernatant liquid phase sample was collected, immediately filtered and analysed. The solid phase was first stored at -80 °C for 24 h and then freeze-dried at -86 °C for 48 h. The resulting dried solid samples were ground, weighed and used within 48 h. The freeze-dried grinded sludge samples were successively extracted using 9.0 and 4.5 mL of methanol and 4.5 mL of acetone per g of dry sample. In each extraction step, the samples were vigorously vortexed (1 min) and ultrasonicated for 1 h. The
slurry was centrifuged at 4000 rpm for 10 min and the supernatant of each extraction was collected. The supernatants from each extraction were filtered and analyzed individually by the HPLC-FLD method, described previously.

Results and Discussion

Extraction performance. The precision and accuracy of the developed ULE method was assessed based on the recovery extraction averages. Results indicated good recovery efficiencies of the overall method, which ranged from 103% to 97% for E1 and EE2, respectively, showing a good accuracy and relatively higher extraction values than the referred in literature [3]; a good precision was also observed with RSD values below 4%. In summary, the ULE method here described was efficient for the extraction of E1 and EE2 adsorbed onto sludge samples. Determination of E1 and EE2 in WWTP sludge. The developed method was applied to the determination of the E1 and EE2 concentration levels in sludge samples (primary sludge, secondary sludge, anaerobic digested sludge and dehydrated sludge) collected from one WWTP in two different periods (September and December 2017). Higher E1 concentrations in solid phase were detected in the primary sludge (above 3.61 μg g⁻¹) followed by digested sludge (1.84 μg g⁻¹) and by dehydrated sludge (1.00 μg g⁻¹). In the other sludge samples analyzed, the values of E1 and EE2 were below the LOD (0.305 μg g⁻¹ for E1 and 0.052 μg g⁻¹ for EE2). The concentrations of E1 detected in the samples collected in December 2017 were higher than those collected in September 2017. Zeng et al. [4] reported that higher temperature improves biodegradation rate of estrogens, thus these results were expected since in December environmental temperature is lower and E1 degradation can be slower. Comparison with other methods. In most of the studies reported in the literature, the recovery rates were obtained in spiked freeze-dried sludge. Braga et al. [5] added E1, E2 and EE2 standards to a 50 mL of fresh sludge samples and achieved satisfactory recovery rates of 75%, 87% and 95%, respectively. However, the clean-up steps adopted and sample derivatization needed for gas chromatography coupled to mass spectrometry detection (GC-MS) analysis require longer handling time [6]. On the other hand, some studies only reported the concentration of estrogens in the solid phase of the sludge, ignoring the estrogen amount in the liquid phase [3,6]. When compared with other methods, ULE-HPLC-FLD presents the following advantages: high extraction recoveries, simplicity, inexpensive, no derivatization required avoiding the additional operation and reducing analysis time, preventing possible biodegradation of estrogens.

Conclusions

This work presents the development of a selective and robust analytical method for simultaneous determination of E1 and EE2 in liquid and solid phases of sludge from WWTP. The methodology is based on ULE followed by high-performance liquid chromatography with fluorescence detection (ULE–HPLC–FLD), providing high extraction recoveries (103% for E1 and 97% for EE2), being at the same time simple and efficient. In comparison with classical analytical methodologies, the main advantages of the proposed method include a good extraction recovery, easy set-up, simplicity, cost-effectiveness and fastness, preventing possible degradation of E1 and EE2. The analytical methodology developed in this study was applied for the monitoring of E1 and EE2 in sludge samples (primary sludge, secondary sludge, anaerobic digested sludge and dehydrated sludge) collected from an operating WWTP. Results indicate that E1 was detected in fresh primary sludge, anaerobic digested sludge and dehydrated sludge, at a concentration in the range of 1.00-4.8 μg g⁻¹. In the other samples analyzed, the concentration values of E1 and EE2 were below the LOD. Overall, the ULE-HPLC-FLD strategy here proposed is a fast and cost-effective methodology to properly quantify E1 and EE2 in WWTPs sludge.

Acknowledgements

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References

Removal of estrone and 17α-ethinylestradiol by digested sludge under different conditions using batch experiments

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The natural estrogen estrone (E1), and the synthetic estrogen 17α-ethinylestradiol (EE2) are of great environmental concern. The adsorption and degradation of E1 and EE2 by activated sludge under anaerobic conditions were investigated by a series of batch experiments. Different treatment conditions were used, including three temperatures (20 °C, 25 °C and 34 °C), three mixed liquor suspended solid (MLSS) (2 g L^{-1}, 3 g L^{-1} and 5 g L^{-1}) and the addition of nitrate. E1 and EE2 were removed from the liquid phase in contact with activated sludge and the rapid adsorption of E1 and EE2 was observed. The E1 was degraded fastest by the bacterial community. On the other hand, EE2 tended to remain longer in sludge solid phase. Temperature seems to be the factor that most influenced the removal of E1 and EE2 from the sludge. In a biological reaction process, both adsorption and degradation processes may occur simultaneously, however, adsorption seems to be the dominant process.

Introduction
The estrogens estrone (E1) and 17α-ethinylestradiol (EE2), excreted into wastewater by humans and mammals mainly through their urine and faeces, can cause serious disturbances in aquatic fauna, such as the feminization of male fish [1]. The purposes of wastewater treatment plants (WWTP) are the removal of organic substances from wastewater and the disinfection of the treated wastewater. However, the conventional and current systems applied in WWTP are not adequate for the efficiently removal of estrogens (61% and 85% for E1 and EE2, respectively) [2]. Presently, no applicable legislation exists about effluent discharge or sludge application standards contaminated with estrogens and/or other organic contaminants.

In WWTP most of the estrogens removed from the effluents are adsorbed to the sludge [3]. Therefore, it is urgent to find efficient methods for the removal of estrogens, from wastewater and from sludge by exploring estrogen removal mechanisms and factors that influence this removal. Biological processes are usually the most cost effective means of removing organic contaminants from wastewater. Aerobic systems often generate persistent metabolites or produce conjugate compounds that can mask the real low estrogen degradation values, which may also suffer deconjugation in the environment, thus recovering their initial environmental toxicity [4]. Some studies suggest that the presence of easily degradable substrates may hinder the estrogen degradation in aerobic systems due mainly to competitive inhibition phenomena [5]. Results from the degradation of estrogens in anaerobic systems are also non conclusive since some authors did not observe biological degradation [6].

In the last decade, important developments were achieved in anaerobic treatment systems, mainly due to the advantages that these processes present comparatively to conventional/ aerobic systems. The success of these systems stems from the improved capacity to develop microbial consortia well adapted to the degradation of a wide variety of substrates. Some studies developed by several authors on estrogen degradation in anaerobic systems indicate that the anaerobic process is capable of degrading estrogens relatively fast [8], with biodegradation efficiencies of 95% [9].

According to recent studies, the degradation of E1 and EE2 strongly depends on the temperature [10], mixed liquor suspended solid (MLSS) contents [10], and the addition of nitrate [9]. Some authors investigated the removal of estrogens by activated sludge under different temperatures and MLSS contents using batch experiments and observed that removal rate efficiencies of estrogens increased when the temperature and initial MLSS contents increased [10]. On the other hand, field surveys have shown that removal efficiencies of estrogens tend to become relatively high in nitrifying activated sludge [9].

The aim of this study was to investigate the influence of different temperatures, MLSS content and the addition of nitrate regarding the adsorption and degradation characteristics of E1 and EE2. For this purpose, we conducted a series of batch mixing experiments using anaerobic digested sludge samples collected from an actual WWTP, and using synthetic wastewater spiked with E1 or EE2.

Material and Methods
A schematic diagram of batch experiments is presented in Error! Reference source not found.. The batch reactors with a working volume of 5 L were inoculated with concentrated digested sludge, synthetic wastewater [11] and E1 or EE2. In all experiments, the initial concentration of E1 or EE2 in the feed was set to an equivalent of 80 μg L^{-1} and 4 μg L^{-1}, respectively. The reactors were prepared under anaerobic conditions with continuous agitation using a magnetic stirrer. Each reactor had a tubing connected to a thermostatic bath allowing the desired temperature to be maintained. In the top layer of each reactor a layer of fiberglass and aluminum foil were applied to prevent heat loss and exposure to sunlight. Samples were collected with a peristaltic pump.
Different conditions were tested as temperature (20 °C, 25 °C and 34 °C), MLSS contents (2 g L\(^{-1}\), 3 g L\(^{-1}\) and 5 g L\(^{-1}\)) and the addition of nitrate [9]. The batch reactors were monitored in terms of E1 and EE2 concentration in the liquid phase and in the sludge solid phase.

**Results and Discussion**

For the different temperatures tested, we observed that after 24 h, E1 was completely removed from the sludge at 25 °C and 34 °C, while at 20 °C only 65% of this compound was removed from the sludge. Moreover, 48 h were required to achieve the complete removal of this compound at 20 °C. For EE2, at the reaction time of 168 h, the highest removal of EE2 was observed at 20 °C (81%). In the experiments with different MLSS contents we found that E1 was completely removed from sludge solid phase after 24 h using 2 g L\(^{-1}\) of MLSS, while at 3 g L\(^{-1}\) and 5 g L\(^{-1}\) MLSS 95% and 91% of this compound was removed from the sludge solid phase, respectively. For EE2, at the reaction time of 264 h, 76%, 79% and 64% of the initial concentration was removed from the solid phase at 2 g L\(^{-1}\), 3 g L\(^{-1}\) and 5 g L\(^{-1}\) MLSS concentration, respectively. For the different contact times analysed, both E1 and EE2 removal presented similar results in the presence and absence of nitrate supplement.

**Conclusions**

In this study, the influence of the different temperatures, mixed liquor suspended solid (MLSS) contents and the addition of nitrate was investigated regarding the removal characteristics of E1 and EE2 from WWTP effluents. The temperature seems to be the factor that mostly influences the removal of E1 and EE2 from the sludge. For the two estrogens studied, EE2 appears to be the most persistent under these conditions. The addition of nitrate does not seem to affect the degradation of both E1 and EE2 from the sludge.

This research may help us to better understand the behavior of E1 and EE2 in WWTPs, which is beneficial for determining the most efficient operational conditions for their degradation, thus preventing their release into the environment.

**Acknowledgements**

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**References**

Solid-liquid extraction for the determination of volatile methylsiloxanes (VMSs) in sewage sludge samples

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Volatile methylsiloxanes (VMSs) present low solubility and high octanol-water partition coefficients, tending to adsorb onto the extracellular polymeric substances (EPS) of the sewage sludge flocs, instead of accumulating in the water phase of the wastewater treatment plants (WWTPs). A method based on solid-liquid extraction followed by gas chromatography-mass spectrometry was successfully applied to detect and quantify seven VMSs (L3, L4, L5 and D3, D4, D5 and D6) on sewage sludge samples. The proposed methodology was validated, providing a good linearity range (5 to 1500 µg/L) and limits of detection (LODs) between 0.0004 (D5) and 0.47 (L3) ng/g dw. Considering this complex matrix, good precision (average relative standard deviation < 15%) and accuracy (recoveries of 69% for D3 to 124% for D5) were achieved. The method was successfully applied to analyze sludge samples collected in different WWTPs. Cyclic siloxanes were the predominant compounds (98%), especially D5.

Introduction

Siloxanes are a subgroup of silicones that consist of Si-O bonds with organic substituents, mostly methyl-groups [1]. Siloxanes are commonly divided in three major classes: volatile methylsiloxanes (VMSs), polydimethylsiloxanes (PDMSs) and functionalized siloxanes [2]. Besides this division, the existence of two basic structural conformations for methylsiloxanes is generally accepted: linear (Ln) and cyclic (Dn), where the subscript refers to the number of silicon atoms.

They are used in several industrial processes and consumer products, such as paper-coatings, textiles, paints, lubricants, plastics, food industry and in cosmetics and personal care products (PCPs). Due to their ubiquity in a wide range of household products and low degradation rates, they reach wastewater treatment plants (WWTPs) and are not completely removed/decomposed, being considered persistent and bioaccumulative [3]. In fact, due to their low solubility and high octanol-water partition coefficients, they tend to adsorb onto the extracellular polymeric substances (EPS) of the sludge flocs, instead of the water phase [4]. The disposal of sludge/compost to agricultural soils as fertilizers, promoting a circular economy concept, may also contribute to the release of VMSs into the environment.

Therefore, the intensive and widespread use of these compounds in numerous applications, combined with their physicochemical properties (e.g. lipophilic nature, low biodegradability, potential to bioaccumulate/biomagnify) makes them interesting targets for research.

Objectives

The aim of this work was to develop and validate a fast and reliable methodology to determine seven VMSs (D3, D4, D5, D6, L3, L4 and L5) in sewage sludge samples. A solid-liquid extraction (SLE) followed by gas chromatography coupled to mass spectrometry (GC–MS) analysis was chosen. The method was optimized and validated based on two other studies found in literature [5, 6].

Methods

Seven volatile methylsiloxanes (four cyclic and three linear) were investigated. Individual linear (L3–L5) and cyclic (D3–D6) volatile siloxanes and also the internal standard used, tetraakis(trimethylsiloxy)silane (M4Q), were purchased from Sigma-Aldrich (St.Louis, MO, USA) with a purity > 97%. Analytical grade n-Hexane (Hex) and ethyl acetate (EtAc) were purchased from VWR (Fontenay-sous-Bois, France). Helium (99.999%), used in the GC–MS system, and nitrogen (99.999%) for solvent evaporation, were supplied by Air Liquide (Maia, Portugal).

For each individual VMS, including the internal standard M4Q, stock solutions were prepared in hexane at 1.0 g/L. From those individual stock solutions, mix stock solutions containing all the target analytes were also prepared in hexane. A diluted M4Q individual stock solution, with a final concentration of 1.25 mg/L, was also prepared in hexane.

Ten calibration standards (5 – 1500 µg/L) were prepared in hexane from the final mix stock solution of siloxanes and M4Q. All solutions were preserved at -22 °C and protected from the light.

Sample Extraction. 0.5 g freeze-dried (64 h) sludge sample was previously homogenized and then weighted into a 50 mL conical tube. 125 ng of internal standard (M4Q) was added and left to equilibrate for 15 min at room temperature. Then, a mixture of 5 mL of Hex:EtAc (1:1 v/v) was added, taken into an ultrasonic bath for 5 min at 420 W and centrifuged at 4000 rpm (2760 g) for 10 min. The extract was transferred to a 12 mL amber glass vial and the extraction procedure was repeated (a total of 2 extractions were performed). The extracts were combined and concentrated in a gentle stream of nitrogen and transferred into an amber-glass microvial that contained 20 µL of isoctane (keeper solvent), through repeated washes with Hex. The nitrogen stream was used again to evaporate almost until dryness. The final extract was reconstituted in 500 µL of Hex and analysed in GC-MS.

Instrumental analysis. The samples were analysed using a Varian Ion Trap GC–MS system. The mass spectrometer was operated in electron ionization (EI) mode (70 eV). Separation was achieved at a constant flow of helium (1.0 mL/min), using a Low-bled DB-5MS ultra-inert column (30 m × 0.25 mm, 0.25 µm). The oven temperature was programmed as follows: 35 °C hold for 5 min, raised at 10 °C/min to 95 °C, then 5 °C/min to 140 °C and then 35 °C/min to 300 °C (hold for 5.5 min) – total...
time of analysis of 30 min. Injection (1 µL) was in split mode, with a split ratio of 100. Temperatures of manifold, ion trap, transfer line and injector were maintained at 50, 200, 250 and 200 °C, respectively. The filament emission current was 50 mA. For quantitative analysis of target compounds, selected ion storage (SIS) mode was applied.

Validation Procedure: Linearity was assessed by the direct injection of 10 standards in hexane containing all the VMSs at concentrations ranging from 5 to 1500 µg/L. Limits of detection (LODs) and quantification (LOQs) were estimated based on a signal-to-noise ratio (S/N) of 3 and 10, respectively. Intra-day precision (repeatability) was evaluated, using the relative standard deviation (%RSD) of three replicates at different spiking levels (100, 500 and 1000 ng/g dw). Inter-day precision (intermediate precision) was evaluated by the relative standard deviation of three determinations of the same sample, by varying the analysis day (3 days). Accuracy was determined by recovery tests, using three replicate spiked samples at the same concentration levels as before.

Results
The calibration curves were constructed correlating the ratio between the mass of the analyte and internal standard injected, with the response factors (R² = Analyte / Internal standard).

A linearity range of 5 to 1500 µg/L was achieved (R² > 0.996). LODs between 0.0004 (D5) and 0.47 (L3) ng/g dw were achieved. The repeatability values (intra-day precision) led to relative standard deviations below 15%, indicating that the method is precise. The obtained value is acceptable for this kind of matrix. Inter-day precision values were slightly higher (3–30%), namely for the lowest spiking level (100 ng/g dw). The obtained recoveries ranged from 69% for D3 to 124% for D5, which is well acceptable for this type of analysis.

Sludge samples (primary, secondary, digested and dewatered sludge) were collected from different WWTPs. Among VMSs, cyclic siloxanes were the predominant compounds (98%), especially D5 and D6, being always present in higher concentrations than linear VMSs. The primary sludge concentration ranged between not detected (nd) for L3 and D3 to 1250 ng/g dw (D5), which are levels higher than those found in literature. In secondary sludge, VMSs concentration varied from nd (L3 and D3) to 6384 ng/g dw for D5. The digested sludge presented values between nd (L3) to 4983 ng/g dw (D5). The final dewatered sludge varied between nd (D3 and L3) to 5703 ng/g dw (D5). Analyzing the total VMSs concentration, from the primary settler (1175 - 2604 ng/g dw) to the final sludge (2493 - 7642 ng/g dw), VMSs concentrations increased, since siloxanes may adsorb to the solids.

Conclusions
The developed method based on solid-liquid extraction followed by GC-MS was successfully applied to detect and quantify seven VMSs on the sludge line of an urban wastewater treatment plant. The proposed methodology was validated, providing a good linearity range (5 to 1500 µg/L) and LODs between 0.0004 (D5) - 0.47 (L3) ng/g dw. Considering this complex matrix, good precision (average RSD < 15%) and accuracy (69% for D3 to 124% for D5) were achieved. The method was successfully applied to analyze sludge samples collected from WWTPs. Cyclic siloxanes were the predominant compounds (98%), especially D5.

The main advantages of the developed method are the decreased number of steps in the procedure, decreasing the sample handling and consequently the risks of external contaminations, use of a lower amount of sample, solvent volumes and extraction times. The obtained LODs and similar to those found in literature, but to the authors’ best knowledge, it has the lowest LOD for D5 (the most detected compound).

Acknowledgements
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References
Electrochemical degradation of Diclofenac on catalysts based on CNT and M/CNT modified electrodes

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Diclofenac (DCF) - a nonsteroidal anti-inflammatory drug - is one of the most frequently detected pharmaceutical compounds in the aquatic environment. Because of its negative effects on public health and environment, this emerging pollutant monitoring and removal from wastewater are of high importance. Electrochemical oxidation has attracted growing interest in the last years, due to its versatility, high efficiency and environment compatibility. Electrochemical oxidation of DCF has been studied using multiwalled carbon nanotubes (CNT) and monometallic catalysts based on carbon nanotubes (M/CNT), at different pH media. The electroreactivity of DCF on modified electrodes and the kinetic parameters of the redox reactions were determined using cyclic voltammetry. Electrooxidation of DCF in aqueous medium was carried out using long-term electrolyses. The products of these electrolyses were identified and quantified by HPLC-MS, GC-MS, HPLC-UV-RID and IC.

Introduction
In the recent years, the intensive use of pharmaceutical products resulting from the growing populations and the interest to promote human health and personal care [1–3], have led to the accumulation of these products into the environment as emerging contaminants. These organic pollutants have been found in many types of water bodies such as: wastewater treatment plant influents and effluents, hospital and pharmaceutical manufacturer wastewater and industrial wastewater [4]. Diclofenac (DCF) (Figure 1) is a synthetic non-steroidal anti-inflammatory drug and among all the anti-inflammatory drugs is the most frequently prescribed for the treatment and protection of different diseases like inflammation, arthritis and other rheumatic diseases [5]. It is considered a persistent toxic pollutant and one of the most relevant compounds in terms of ecotoxicity. Among several technologies studied for the removal of pharmaceutical products in water, electrochemical oxidation has attracted growing interest, by providing efficient conversion in mild conditions, replacing harmful reoxidation agent by electricity [6]. The use of multiwalled carbon nanotubes (CNT) as a heterogeneous catalyst or support material, has attracted growing attention for environmental applications thanks to their peculiar characteristics, like high active surface area and electrical conductivity [7–8].

![Figure 1. Molecular structure of Diclofenac.](image-url)

Objectives
Study of electroreactivity and determination of kinetic parameters of DCF using cyclic voltammetry. Electrocatalytic oxidation of DCF in aqueous medium on CNT and M/CNT modified electrodes. Identification/quantification of the reaction products by chromatographic methods.

Methods
Preparation of CNT and M/CNT modified electrodes. The preparation of the modified electrodes was performed by a previously established procedure described in our published works [7,9] from CNT or M/CNT suspensions in Nafion/water solution. After deposition of the suspension on Carbon Toray (CT, 4 cm²) paper, the electrocatalytic material must dried at room temperature during 24 h. The amounts of Pt or Ru used for the carbon nanotubes supported metal catalysts were always 1 wt%. The cleanness of the surface was tested prior to each experiment by recording voltammograms in the supporting electrolyte medium alone.

Electrochemical Setup. All the he supporting electrolytes were prepared using ultrapure water with analytical grade reagents. The electrochemical studies were performed in a thermostated two-compartment glass cell separated by ion exchange membranes (Nafion 117). The reference and counter electrodes were a saturated calomel electrode and platinum foil (99.95%) respectively. The electrochemical instrumentation consisted of a potentiostat/galvanostat from Amel Instruments coupled to a computer by an AD/DA converter. The Labview software (National Instruments) and a PCI-MIO-16E-4 I/O module were used for generating and applying the potential program as well as acquiring data, such as current intensities.

Results
Voltammetric studies of Diclofenac. The stability of electrocatalyst based on multiwalled carbon nanotubes (CNT and M/CNT) was confirmed by cyclic voltammetric studies. The electroreactivity of DCF, in the concentration range of 1.0 and 2.0 mM at CNT and M/CNT modified electrodes, in carbonate buffer 0.10 M and NaOH 0.50 M media were studied by cyclic voltammetry. Cyclic voltammograms of Pt/CNT and Ru/CNT modified electrodes (in NaOH medium) in presence of DCF are

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given in Figure 2. In the presence of DCF in carbonate buffer and NaOH media, several oxidation/reduction processes were noticed. These results confirm the interaction between DCF and the CNT or M/CNT surface, necessary for further oxidation. High anodic and cathodic current densities were noticed in presence of DCF in both studied media.

Electrolysis of Diclofenac. The electrolysis of DCF (1.0 and 2.0 mM) in carbonate buffer 0.10 M and NaOH 0.50 M media was carried out using CNT, Pt/CNT and Ru/CNT modified electrodes with an applied potential of 1.5 and 2.0 V vs SCE. After 7 h of electrolysis, the decrease of the initial DCF concentration in NaOH medium reaches more than 50% for all used modified electrodes. In carbonate buffer medium, the conversion rate for 8 h of electrolysis reached approximately 90% for Ru/CNT electrode and almost 50% for CNT and Pt/CNT electrodes. A decrease of the conversion rate was noticed due to the decrease of the reaction rate.

Conclusion
The degradation of DCF in aqueous media was carried out by electrochemical methods using CNT and M/CNT modified electrodes. The results show that the modified electrodes were stable in the experimental conditions and it was efficient for the degradation of DCF. Degradation products of DCF such as low molecular weight carboxylic acids and mineralization products was identify by chromatographic techniques.

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References
Fe(III)NaYnano as efficient electrocatalyst for electrodegradation of Congo Red dye

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Textile dyes are one of the most important contaminants of the superficial water resources. Their removal from water bodies constitutes a priority to guaranty water quality. Electrodegradation of Congo Red dye was carried out using modified electrodes prepared by the deposition of iron(III)-zeolite on Carbon Toray. The sample iron(III)-zeolite was prepared by ion-exchange method with a solution of iron(III) using NaYnano as parent zeolite, with small particles, 150 nm. Fe(III)NaYnano was characterized by SEM/EDX and XRD techniques. The introduction of iron by ion exchange method do not modified the morphology of the zeolite but affect the zeolite structure, as prove by the structural characterization results from XRD. However, cyclic voltammetry studies show that iron-zeolite modified electrode is stable in the experimental conditions. The complete degradation of Congo Red dye was achieved by electrochemical route without the use of acid and hydrogen peroxide in reactional medium.

**Introduction**

The discharge of textile dyes into water bodies is an environmental problem due their consequences for water quality. These compounds are usually toxic to aquatic life and they have a negatively impact to the food chain, because they are carcinogenic and mutagenic [1-2]. New emerging technologies have been recently proposed for the degradation of these organic pollutants [3]. Among various technologies, electrochemical oxidation appears as one of the most promising one for the treatment of wastewater containing organic pollutants, considering its environmental compatibility, low temperature and pressure requirements [5-6]. Thus the present work reports the study of the degradation of Congo Red (C₈H₆N₃NaO₂S₃) dye using iron-zeolite modified electrode. The results show that the electrocatalyst was very efficient for the degradation of the dye.

**Methods**

The dye was Congo Red (CR) from Sigma-Aldrich. NaYnano (Nanofau-Y from Nanoscape) was used as parent zeolite for preparing Fe(III)-NaYnano electrocatalyst. The ion-exchange method was achieved with a ratio of iron solution/zeolite weight to 35. So, 3.65 mmol of Fe(III) solution from Fe(NO₃)₃.9H₂O (Sigma-Aldrich) with pH value of 5.0 was added to the zeolite parent at room temperature and mixing during 24 h. The suspension was filtrated off, washed and dried in oven at 80 °C for 24 h, following by a calcination step at 350 °C during 4 h in a static oven. Powder X-ray diffraction patterns were recorded on a Philips Analytical X-ray model PW1710 BASED diffractometer system. Scans were performed at room temperature, using Cu Kα radiation (λ=1.540598 Å) in the 20 range between 5° and 70°. The samples were characterized using a desktop scanning electron microscope (SEM) coupled with energy-dispersive X-ray spectroscopy (EDX) analysis (Phenom ProX with EDX detector (Phenom-World BV, Netherlands)). The preparation of the modified electrodes using Carbon Toray (CT) and the equipment used for electrochemical measurements were described in [6]. The dye concentrations used were 0.036 mM (25 ppm) for the cyclic voltammetry studies and 0.072 mM, 50 ppm for electrolyses in 0.10 M NaCl solution, at room temperature.

**Results**

Preparation and characterization of the samples, Fe(III)NaYnano was obtained by ion-exchange method. This usual method is the best for introduce cations in the zeolite structure, since the charge negative from the aluminum present in the structure need to be compensate with counter ions in order to maintain the neutrality of solid [7].

The presence of iron and the modifications provoked by the ion exchange method and thermal treatment in the sample were examined by different characterization techniques. SEM analysis show that the morphology and the average of the particles were the same as the parent zeolite. However, XRD analysis confirms that NaY was affected by the introduction of iron with a reduction of the crystallinity, 51%. The framework Si/Al ratios obtained are 3.68 for NaY and 1.72 for Fe(III)NaYnano. The differences observed between the total Si/Al ratio (1.86 determined by ICP) and the framework Si/Al ratio for NaY are related to an irregular distribution of silicon and aluminum throughout the zeolite structure, with the presence of extra-framework alumina (EFAL) species. After the ion-exchange treatment, the framework Si/Al ratio of Fe(III)NaYnano decrease (Si/Al = 1.72) suggesting that the EFAL species were removed after the treatments. The presence of the iron also was detected by EDX analysis and it was 1.2 wt%.

**Voltammetric study of Congo Red.** The electrocatalyst, the iron-zeolite modified electrode, was prepared by deposition of Fe(III)NaYnano on Carbon Toray and their stability was confirmed by cyclic voltammetric study. For the range of the potential studied, in NaCl 0.10 M medium, the electrocatalyst was stable. The electroactivity of Congo Red (0.036 mM) at iron-zeolite modified electrode, in NaCl 0.10 M medium was studied by cyclic voltammetry. The cyclic voltammograms of Pt/Fe(III)-NaYnano modified electrode in presence of Congo Red are given in Fig. 1.

The oxidation of CR starts at 0.5 V vs. SCE, after the oxidation of surface iron species. This result shows that the oxidation of CR is mediated by Fe(III). The oxidized CR is reduced during the negative variation of scan potential from -0.2 V vs. SCE. High anodic and cathodic current densities were noticed in presence of Congo Red. During cyclic voltammetric study. For
each measurement successive voltammograms were registered in order to check the absence of surface deactivation.

Electrolysis of Congo Red dye in presence of chloride ions. The electrolysis of CR dye (50 ppm) in 0.10 M NaCl medium was carried out on Fe(III)NaYnano modified electrode with an applied potential of 2 V vs SCE. After 2 h of electrolysis the initial concentration of CR was close to zero, which confirm that the CR dye was effectively degraded on the Fe(III)NaYnano modified electrode.

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References
Hermetic encapsulation of hole transport-free perovskite solar cell
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Perovskite solar cells (PSCs) are the most recent emerging photovoltaic (PV) devices. The power conversion efficiency (PCE) of PSCs has rapidly increased from 3.8 % to 23.3 % within the last 10 years. To date, most of the research efforts were focused on improving the PCE of PSCs while the long-term stability of this class of PV remained an enormous challenge. Conventional PSCs include an electron transport layer (ETL), perovskite light absorber and hole transport material (HTM). Since the perovskite layer transports electrons and holes by itself, PSCs can be also fabricated without HTM. Lower production cost and scalable fabrication of HTM-free PSCs make them favorable for commercialization. Perovskite degrades when exposed to humidity and oxygen. Here, a low temperature hermetic encapsulation for HTM-free PSCs that retained 96 % of its initial performance after 23 days is reported. Moreover, the encapsulated cell showed no loss in performance in water immersing test.

Laser-assisted sealing uses a laser beam to locally heat, melt and bond sealing materials [9]. The most common sealing materials for PSCs are thermoplastics and epoxy resin; these two group of sealants are non-hermetic and not long term stable [10]. In contrast, glass frit sealing is hermetic and long-term stable. Commonly, the melting temperature of glass frits are > 380 °C, but laser-assisted sealing of glass frit can reduce the process temperature to meet the required temperature limit of PSCs. Figure 1 shows the laser-assisted sealing apparatus [9].

Although many studies suggest that CH$_3$NH$_3$PbI$_3$ perovskite is thermally stable up to 130 °C, performance losses of the cells after subjecting them to 115 °C for 75 min during laser-assisted sealing process suggest otherwise. Table 1 and Figure 2 compares the performance of the device before and after glass sealing.

Table 1. Photovoltaic parameters of the cell; initial and after laser sealing.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Initial</th>
<th>After sealing</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{oc}$ / V</td>
<td>0.75</td>
<td>0.65</td>
</tr>
<tr>
<td>$J_{sc}$ / mA cm$^2$</td>
<td>13.78</td>
<td>12.33</td>
</tr>
<tr>
<td>MMP / mW cm$^2$</td>
<td>5.65</td>
<td>3.93</td>
</tr>
<tr>
<td>FF</td>
<td>0.55</td>
<td>0.49</td>
</tr>
<tr>
<td>PCE / %</td>
<td>5.65</td>
<td>3.93</td>
</tr>
</tbody>
</table>

Figure 1. Laser-assisted sealing apparatus. Reprinted with permission from reference 9.

PSCs are considered as one the most attractive candidates for competing with silicon PV cells. Unlike Si solar cells, the fabrication process of perovskite solar cells is simple and then cost is low. PSC was first reported in 2009 [1] and since then their PCE has reached 23.3 % [2]. Apart from efficiency, long term stability is the most importance feature for commercialization. A recent study on levelized cost of electricity (LCOE) of PSCs suggests 3.0 - 4.2 € cents/kWh for a device of 12 % PCE and 15 years of lifetime [3].

There are three major factors that can affect the stability of the PSCs; temperature, humidity and oxygen [4]. Temperature can degrade both perovskite crystals and HTM which results in vast performance loss. While the perovskite is stable up to 130 °C, common HTM materials such as spiro-OMeTAD (2,2′,7,7′-tetraakis(N,N-di-p-methoxyphenyl-amine)9,9′-spirobifluorene and PTAA (poly(triarylamine)) degrade at ca. 85 °C [5]. Studies confirmed that for long time exposure (< 60 h) irreversible decomposition of the perovskite occurs [6]:

\[
\text{CH}_3\text{NH}_3\text{PbI}_3(s) \rightleftharpoons \text{PbI}_2(s) + \text{CH}_3\text{NH}_2\text{I} \quad \text{(aq)} \quad \text{Eq. (1)}
\]

\[
\text{CH}_3\text{NH}_2\text{I} \quad \text{(aq)} \rightleftharpoons \text{CH}_3\text{NH}_2\text{I} \quad \text{(aq)} + \text{HI} \quad \text{(aq)} \quad \text{Eq. (2)}
\]

\[
\text{HI (aq)} + \text{O}_2(g) \rightleftharpoons 2 \text{I}_2(s) + 2\text{H}_2\text{O} \quad \text{Eq. (3)}
\]

Though low oxygen concentrations may contribute for improving charge mobility at the perovskite layer, a hermetic encapsulation prevents humidity and excess oxygen exposure of perovskite and therefore increases the stability of PSCs. To avoid possible temperature stability issues of PSCs, HTM-free devices are more suitable to pass the requirements for the standard PV tests [7].

HTM-free devices display relatively lower PCE (i.e. 12.8 % [8]) compared to HTM-based cells. However, the fabrication method of HTM-free cells are based on screen-printing which is low cost and fully scalable. In contrast, HTM-based are commonly fabricated with spin-coating which is only suitable for lab-scale processes. Here, we are reporting a low temperature laser-assisted hermetic encapsulation method for fully screen-printed HTM-free PSC.

![Hermetic encapsulation diagram](image-url)
As shown in Table 1, all the PV parameters had major losses after sealing. The open-circuit potential had ca. 13 % loss which is the evidence of partial thermal degradation of the perovskite. The $J_{sc}$ had ca. 10 % decrease which is also due to the damage in the perovskite that causes poor charge transport. Nevertheless, the main goal of the present work is to achieve long-term stable sealed PSC device.

Figure 2. J-V curves under AM 1.5 solar radiation.

The stability of the sealed device was then monitored during 23 days while the device was stored under normal atmospheric conditions (in the dark). The PV parameters of the device was measured under AM 1.5 during the stability test and shown in Figure 3. There was less than 5 % decrease in the performance of the cells. Moreover, at day 23, the encapsulated cell was immersed in water for 150 min for extreme humidity test on the sealing quality. The performance of the sealed cell remained unchanged after the water immersion test, confirming the hermetic sealing.

Figure 3. History of the PV parameters of the sealed PSC.

Conclusions
In conclusion, HTM-free PSCs glass-sealed at 115 °C display superior stability. A detailed study on the thermal stability of the perovskite material is now envisioned. In addition, other perovskite crystal such as $\text{Cs}_0.06\text{FA}_{0.94}\text{Pb}(\text{I}_{0.83}\text{Br}_{0.17})_3$ with higher thermal stability will be assessed [5].

Acknowledgements
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References
Electrochemical characterization of commercial activated carbons

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This work deals with the electrochemical characterization of two commercial activated carbons, with distinct textural properties, through cyclic voltammetry, using modified electrodes made by the deposition of activated carbons’ samples on a glassy carbon electrode surface. The obtained electroactive surface areas are confronted with previously determined gas and liquid phase adsorption parameters as well as with their surface chemistry by point of zero charge (pHexp) determination. The results obtained show that despite having very distinct textural parameters, the electroactive surface areas of the two carbon samples are quite similar suggesting that the electrocatalytic properties of carbon-based materials depend on the interaction of electroactive species with the surface’s chemical groups of this type of materials.

Introduction

Activated carbons are a class of materials with a vast number of applications namely in adsorption and separation processes. They are also highly attractive candidates as electrocatalysts or electrocatalytic supports for energy storage and conversion devices as an alternative to Pt-based electrocatalysts, aiming to reduce production cost of fuel cells [1]. The high surface area and well-developed pore structure of activated carbon materials are favorable conditions for a high electrocatalytic activity [2]. However, the adsorptive and physicochemical properties of activated carbons are determined not only by its porous structure but also by the chemical surface groups present, namely oxygen surface groups that depend on the source of each activated carbon [3].

In this work the electrochemical characterization of two commercial activated carbons was performed, in order to determine their electroactive surface areas. The results were confronted with previously determined adsorption and surface chemistry parameters of these materials, with the purpose of discussing the influence of those parameters on the available electrochemical surface area and possibly disclose their applicability as metal free electrocatalysts.

Methods

Two samples of commercial activated carbons from Panreac (Lot 0000115091) and Fagron (Lot 11090097) were used as received. The textural properties of activated carbons samples were determined in a previous study from N2 adsorption isotherms obtained from an automatic gas adsorption apparatus (Micromeritics ASAP2010). The adsorption capacity in liquid phase medium was also previously quantified using Mordant Blue-9 dye as adsorbent, as well as the point of zero charge (pHexp) using the procedures described in [4]. The electrochemical characterization of the materials was made through cyclic voltammetry using an Autolab PGSTAT10 potentiostat (EcoChimie B.V.), controlled by GPES software. The assays were performed using the redox probe K3[Fe(CN)6] with a 1.0×10⁻³ M solution in KCl 1.0 M, prepared using ultra-pure water. A conventional three electrode compartment cell was used, with a 3 mm diameter glassy carbon electrode (GCE) as working electrode, a platinum wire as auxiliary electrode and a “red rod” (Ag/AgCl) as reference electrode. All electrodes were acquired from Radiometer Analytical. Prior to the essays the solution was deaerated with N2 for 30 min and then the cell was enclosed in a grounded Faraday cage where the assays were performed at room temperature.

For the modified electrodes carbon samples (1 mg) were dispersed in 1 mL N,N-Dimethylanilime (Sigma Aldrich, 99%) and sonicated for about 20 min (Elma, Transsonic TS540). Then, a 3 µL drop of each dispersed sample was deposited onto the GCE surface followed by solvent evaporation under air flux. Prior to modifications, the GCE electrode was conditioned by a polishing cleaning procedure, using a 200 Å alumina suspension (PRESI) on a microcloth polishing pad after which the electrode was rinsed with ultrapure water.

The potential was cycled between 0.60 and -0.10 V with scan rates ranging from 0.010 to 0.500 V s⁻¹. Each experiment was performed in triplicate to confirm results’ reproducibility. The electroactive surface areas were determined using the Randles-Sevcik equation (Eq. 1) under the assumption that the electrochemical process is diffusion controlled,

\[ I_p = 2.69 \times 10^5 n^{3/2} A D^{1/2} v^{1/2} \]  

(Eq. 1)

where \( n \) refers to the number of electrons transferred during the reaction (1 for this particular reaction), \( A \) is the electrode surface area (cm²), \( D \) is the diffusion coefficient (6.30 × 10⁻⁵ cm² s⁻¹ for [Fe(CN)₆]³⁻⁴⁻), \( C \) is the concentration of the species (mol cm⁻³), \( v \) is the scan rate (V s⁻¹) and \( I_p \) is the current intensity peak (A).

Results

The textural parameters of the two activated carbon samples are displayed in Table 1. As can be observed, the two samples have distinct textural properties, with the highest mesoporous volume (\( V_{meso} \)) and external surface area (\( A_{eΘ} \)) belonging to the Fagron material (almost twice of those of the Panreac carbon [4]). However, the data related with the liquid phase adsorption of MB-9 dye, also quoted in Table 1, show that the adsorption capacity (\( q_0 \)) of the two samples is of the same order of magnitude, denoting that differences found on textural parameters are not reflected on the adsorption capacity of the activated carbon samples, pointing to other characteristics, such as surface chemistry groups, playing an active role. In
fact, the values of pH\textsubscript{pzc}, although not very different for the two carbon samples, indicate some differences on their surface chemistry. Cyclic voltammograms (CV) of Panreac and Fagron modified electrodes as well as for the reference Glassy Carbon Electrode (GCE) are presented on Figure 1. CV profiles for GCE and modified electrodes have a similar shape, with a cathodic peak \( E_{pc} \approx 0.24 \text{ V} \) and an anodic peak \( E_{pa} \approx 0.32 \text{ V} \), assignable to Fe\textsuperscript{3+}/Fe\textsuperscript{2+} electron transfer.

Electroactive surface areas (A), calculated using Eq. 1, are quoted in Table 1. As can be observed, areas determined for both modified electrodes are significantly higher (two-fold) than that of the bare GCE electrode. However, when comparing Fagron and Panreac modified electrodes, their electroactive areas are quite similar, resembling the behavior found for the adsorption of MB-9 dye in aqueous media by the same electrodes [4]. These results suggest that, despite the important role of textural parameters in allowing access and diffusion of electroactive species, their interaction with the surface chemical groups seems to be distinct for each activated carbon sample and thus, also relevant.

**Conclusions**

In this work the electrochemical characterization of two commercial activated carbons was performed by determining their electroactive surface through cyclic voltammetry experiments using modified glassy carbon electrodes made from Panreac and Fagron carbon samples. The results obtained in this preliminary study show that despite large differences in the textural parameters of both activated carbon samples, electroactive surface areas are very similar, being both significantly higher than that of the unmodified glassy carbon electrode. These results points to surface chemistry of carbon samples playing an effective role on the electrocatalytic properties of the materials and therefore this fact should also be taken into consideration.

Table 1. Textural parameters: microporous volume (\( V_{\text{micro.}} \)), mesoporous volume (\( V_{\text{meso.}} \)) and external surface area (\( A_{\text{ext.}} \)), adsorption capacity of MB-9 dye in aqueous media (\( q_{m} \)), point of zero charge (pH\textsubscript{pzc}) and electroactive surface areas (\( A_{\text{electroactive}} \))

<table>
<thead>
<tr>
<th>Sample</th>
<th>( V_{\text{micro.}} ) ( (\text{cm}^{3}/\text{g}) )</th>
<th>( V_{\text{meso.}} ) ( (\text{cm}^{3}/\text{g}) )</th>
<th>( A_{\text{ext.}} ) ( (\text{m}^{2}/\text{g}) )</th>
<th>( q_{m}\text{-MB-9} ) ( (\text{mg}/\text{g}) )</th>
<th>pH\textsubscript{pzc} ( .^{*} )</th>
<th>( A_{\text{electroactive}} ) ( (\text{A/cm}^{2}) )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glassy Carbon</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>6.9</td>
<td>0.027</td>
</tr>
<tr>
<td>Fagron</td>
<td>0.33</td>
<td>0.66</td>
<td>514</td>
<td>221</td>
<td>6.9</td>
<td>0.063</td>
</tr>
<tr>
<td>Panreac</td>
<td>0.19</td>
<td>0.35</td>
<td>296</td>
<td>213</td>
<td>6.7</td>
<td>0.060</td>
</tr>
</tbody>
</table>

* Previously determined values quoted from [4].

**Acknowledgements**

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**References**

Preparation of carbon-based electrodes to be used as back-contact in perovskite solar cells

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Perovskite solar cells (PSC) are an emerging photovoltaic technology with a great potential to revolutionize the photovoltaic field with a very rapid growth from 3.8% power conversion efficiency to 23.3% in just 10 years. PSCs present low-cost, easy fabrication processes and display special features for building-integrated photovoltaics. However, there are still limitations hindering its commercialization such as the complete cell encapsulation and the expensive back-contact made of gold. Carbon papers are currently used in proton-exchanged membrane fuel cells; this study presents a pioneer work using carbon papers as back-contact in solar cells. In this approach, different adhesion layers were tested to enhance the interfacial contact between the carbon paper and the hole transport material of the PSC and two different carbon papers were studied to perceive the influence of the paper characteristics. A maximum power conversion efficiency of ~7% was reached with the best configuration.

Achieving high-efficient, cost effective, easily to process and versatile solar cells has always been a challenge for the scientific community. Perovskite solar cells (PSC) technology is a very attractive candidate to fulfill all these requirements and is marching ahead in the emerging photovoltaic (PV) energy conversion efficiency race. In the last five years, organic/inorganic lead halide PSCs in solid-state device configuration have leapt from ca. 10% at the beginning of 2013 [1] to a certified value of 22.1% in 2016 [2]. Very recently a value of 23.3% was reported in the NREL chart of photovoltaic efficiencies [2]. This astonishing performance evolution undoubtedly attracted the scientific community attention. The benefits of this technology are overwhelming: foreseeing costs lower than the state-of-the-art silicon PV, lower CAPEX which makes their production in Europe competitive, very high energy conversion efficiency (> 23%), already comparable to silicon-based technology and, not least important, they are solid-state devices that simplifies their manufacture [3].

Typical PSC configuration consists of a transparent conducting oxide (TCO) glass substrate coated with a TiO$_2$ compact layer deposited by an aerosol spray pyrolysis. A 350-nm-thick mesoporous TiO$_2$ layer composed of 20-nm-sized particles is then deposited by spin-coating. After drying, the films are impregnated with the perovskite material solution. The hole transport material (HTM) is deposited by spin-coating. A thin layer of gold (ca. 80 nm) is then thermally evaporated on top of the device to form the back-contact [4]. Upon light absorption the perovskite injects an electron into the conduction band of the TiO$_2$ semiconductor and a hole into the HTM. The TiO$_2$: conduction-band electrons, as well as the holes in the HTM, are transported by electron conduction to the gold contact electrodes [5]. The astonishing efficiency values reached by this technology are consequence of very high electronic and hole conductivity of the organic-inorganic lead halide perovskites used and very low energy losses at the photoelectrode/perovskite and perovskite/HTM interfaces, related to the efficient energy edges match.

Despite the tremendously successful effort for obtaining PSC devices with high-energy conversion efficiencies, there are two main environmental concerns regarding PSC: i) it contains soluble lead (II) salts that are toxic and strictly regulated and ii) the use of gold and high temperature processes during the manufacturing of the devices [6]. There is though a consensus in many reports that the lead content is not critical in terms of environmental impact since with perovskite layers of around 300–400 nm thickness would amount to less than 1 g/m$^2$ of lead, considerably lower than other lead pollution sources as lead paints or lead batteries [7, 8]. In what concerns gold back-contact, gold is a scarce and economically highly valuable metal, which allied to the use of high temperature processes will result in a significant environmental impact and increased costs [9, 10]. Among all the back-contact materials, carbon based materials display several advantages [11]. Their properties, such as abundant sources, low-cost, high electrical conductivity, chemical stability and tunable work function (ca. 5.0 eV, which is near the 5.1 eV from Au fermi level), make them an ideal replacement for the currently used Au [12].

A carbon material that is commonly used in fuel cells and electrolyzers applications, but also potential for solar cells, is carbon paper, which consists in carbon fibers (with $\approx$ 7 µm diameter) held together by a carbon matrix. Its manufacturing comprehends two heat treatments, carbonization and graphitization, which result in a product with graphite like properties. The features that makes it an interesting candidate for perovskite’s back-contact are: low electrical resistivity, high flexural/tensile strength, high chemical and mechanical stability, improved thickness uniformity, improved durability and low cost [13]. To the best of the authors’ knowledge, the use of these materials in solar cells has never been reported in previous studies.

This study presents a pioneer work using carbon papers as back-contact in perovskite solar cells. In this approach, different adhesion layers (SpiroOMeTAD and PEDOT:PSS) were tested to enhance the interfacial contact between the carbon paper and the hole transport material of the perovskite solar cell and two different carbon papers (39AA and 28BC from Sigracet®) were studied to perceive the influence of the paper characteristics. A maximum power conversion efficiency of about 7% was reached with the best configuration; there was a loss of 1% in the fill factor and an increase of 4% in the open-circuit voltage for the best-performing cells when compared with gold reference device – Figure 1.

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Two types of carbon papers were used for studying their structural and electrical properties influence on the solar cell performance. It was observed that an improved overall power conversion efficiency of the solar cell is achieved for the 28 BC carbon paper that presents smaller pore diameter, as it results into an increased interfacial contact (where holes and electrons from the external circuit recombine) with a consequent interfacial resistivity reduction.

Analyzing the effect of the different adhesion layers used, it was observed that spiroOMeTAD has less affinity to both carbon papers and so lower photovoltaic performance results were obtained (maximum efficiency of 3.30 %). In what concerns PEDOT:PSS, two different additives were considered for improving its properties and the deposition uniformity but none resulted in enhanced photovoltaic performance of the final devices: best efficiency of 6.91 % for bare PEDOT:PSS, 5.55 % for PEDOT:PSS with D-sorbitol and 6.25 % for PEDOT:PSS with isopropanol. D-sorbitol, despite improving the interface bonding between the adhesion layer and the HTM, failed at improving conductivity and stability of the solution, but it is believed that this can be further improved by optimizing its concentration and cure procedure. Adding isopropanol for allowing the deposition of PEDOT:PSS by spin-coating showed several advantages, such as ensuring greater uniformity of the capping layer of the adhesion layer (because it allows spincoating deposition) and greater probability of total filling of pores (because it makes the solution more hydrophobic). However, due to PEDOT:PSS dilution, its properties may be compromised, diminishing the photovoltaic performance of the final device.

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References
Thermally and chemically activated biochar obtained in mechanical biological treatment plants for carbon dioxide adsorption

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In this study, based on the scopes of CCS strategy and municipal solid waste management, a novel Integrated Environment Management (IEM) strategy has been proposed. In this way, the obtained compost in the mechanical biological treatment from municipal solid wastes has been considered as a source of adsorbents for CO₂ capture. In this way, the maturated compost waste was modified by liquid phase treatment with sulfuric acid and thermal treatment at 800 °C. Then, the prevalent operational conditions of post-combustion processes have been considered to find the best prepared samples for CO₂ capture.

Introduction
In the recent years, synthesis, preparation and development of valuable carbon materials have received much interest in the view of energy efficiency and sustainability for various applications in CO₂ capture, wastewater treatment and gas storage studies [1, 2]. On the other hand, based on European legislation to management of solid wastes and limiting the utilization of fertilizers from waste as well as finding approaches to manage these materials, novel approaches are required [3]. In this study, obtained compost by mechanical biological treatment plant from municipal solid waste has been considered as a source of activated carbons. Then, by using sulfuric acid (H₂SO₄) and treating at 800 °C, chemically and thermally activations, respectively, were implemented on the prepared samples.

Objectives
In this work, based on the scopes of CCS technique and municipal solid waste management a novel strategy has been proposed. In this way, the obtained compost in the mechanical biological treatment from municipal solid wastes has been considered as a source of adsorbents for CO₂ capture.

Experimental
The employed compost was obtained in mechanical biological treatment plants for municipal solid waste, supplied by the company “Resíduos do Nordeste, EIM”. In order to homogenise and remove the soluble compounds and suspended solids, the compost was first mixed with water and washed. Then, two different materials were prepared by carbonization at 400 (C-400) and 800 °C (C-800). In addition, following the procedure previously described [4], two materials were prepared with H₂SO₄ before and after the carbonization at 800 °C (C-S-800 and C-800-S, respectively). Then, breakthrough measurements of CO₂ carried by He were conducted at post-combustion conditions (1 bar and 40-100 °C).

Results and Discussion
The equilibrium data for CO₂ adsorption were collected using breakthrough method in a fixed bed reactor at the post-combustion operational conditions. Figure 1 shows the uptake capacity of preared samples at 40 °C. The results show the prepared sample by the subsequent treatments with acid sulfuric and thermal calcination has the higher uptake capacity than other ones and literature reports; which it can derived from several factors. First, better textural properties of proposed sample, including: higher external surface area (Sext), microporous surface area (Smicrop) and external surface area (Sext). It can be also ascribed for desorption of weak superficial groups as consequence of the thermal treatment at 800 °C.

Conclusions
In this study, the potential of municipal solid wastes as a source of adsorbents for CO₂ capture were investigated at the post-combustion operational conditions. Then, the breakthrough measurements in the fixed bed adsorption column were performed. The equilibrium adsorption capacity of the considered samples revealed that the adsorption capacity of the sample which has been treated with the subsequent treatments with acid sulphuric and thermal calcination is the best one. Finally, the results proved the proposed strategy is a proper tactic for two of main current challenges of mankind, including: CO₂ capture to reduce the global warming also to control the accumulation of waste on landfills.
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References
Metal-free carbon nanotubes as catalysts for wet air oxidation of oxalic acid: The role of S, B and P heteroatoms

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The surface chemistry of carbon nanotubes (CNTs) was modified using a solvent-free methodology involving mechanical and thermal treatments in the presence of precursors of different heteroatoms (S, B, P), promoting significant changes in the CNT textural and chemical properties. The effect of the different heteroatoms on the catalytic performance of the materials was evaluated in the oxidation of oxalic acid by Catalytic Wet Air Oxidation (CWAO), as an alternative to the noble metal and rare earth oxide catalysts traditionally used in this process. Improved catalytic activities were obtained using the P-, B-doped CNTs in oxalic acid oxidation, while S-doped CNTs underperform compared to the pristine material.

Wet air oxidation (WAO) is an attractive process to treat effluents with a pollutant too high for biological treatments and simultaneously too low for incineration. The non-catalytic process requires high temperatures (200-320 °C) and pressures (20-200 bar) to ensure solubility of oxygen and to promote fast mineralization and degradation rates. In the last decades, efforts have been made to decrease the severity of the operating conditions by the addition of homogeneous and heterogeneous catalyst, mostly based in the use of noble metals and metal oxides. Carbon materials can replace these traditional catalysts, avoiding the common problems associated with metal leaching to the liquid phase. CNTs have already proved to be good candidates for CWAO; moreover, their catalytic performance can be enhanced by doping or by incorporation of surface functional groups. Ball milling is a promising method for modifying CNTs, by adjusting tube lengths, opening closed end caps or doping with heteroatoms.

In the present work, we extend a recently developed solvent-free post-doping methodology involving a mechanical treatment under ball-milling followed by a thermal treatment under inert atmosphere to the incorporation of S, P and B onto CNT surfaces, using different precursors (Graphical Abstract). Sodium thiosulfate was used as S-precursor (CNT-TS) while sodium dihydrogen phosphate and boric acid were tested as P (CNT-P) and B precursors (CNT-B), respectively. The catalytic performance of the prepared materials was evaluated in the oxidation of oxalic acid (OxAc), which is a typical refractory compound that appears as end-product in oxidation processes.

Nitrogen adsorption at -196 °C was used to assess the changes in the textural properties induced by the applied treatments, while the nature and amounts of the surface groups incorporated on the CNT structures were determined by suitable methods, such as X-ray photoelectron spectroscopy (XPS) and elemental analysis. Additionally, thermogravimetric analyses were also performed.

Each precursor promoted significant changes on the textural properties of the carbon samples (Table 1), decreasing the surface area ($S_{BET}$) and the free-space between the tubes (evaluated by the total pore volume, $V_p$). With respect to the surface composition, large amounts of S (5.2 %), mainly as -C-S-C- groups were identified, while sulphyl/sulphone groups were incorporated. Phosphorus was introduced as phosphate and phosphonate groups (4.5 %), while the boric acid treated sample revealed essentially B-O bonds (18.7 %).

Table 1. Textural characterization of CNT samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>$S_{BET}$ (m² g⁻¹)</th>
<th>$V_p$ (cm⁴ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT-O</td>
<td>291</td>
<td>1.100</td>
</tr>
<tr>
<td>CNT-TS</td>
<td>172</td>
<td>0.356</td>
</tr>
<tr>
<td>CNT-P</td>
<td>93</td>
<td>0.230</td>
</tr>
<tr>
<td>CNT-B</td>
<td>45</td>
<td>0.077</td>
</tr>
</tbody>
</table>

The catalytic performance of the prepared materials was evaluated in the oxidation of oxalic acid by CWAO, performed in a 160 mL 316-stainless steel high pressure reactor. Experiments were performed at 140 °C, 40 bar of total pressure (7 bar of oxygen) with an initial concentration of 1000 mg L⁻¹ of oxalic acid and 0.2 g of catalyst. Oxalic acid is poorly oxidized under the operating conditions employed in the absence of a catalyst (curve WAO, Figure 1), while the CNT materials show high catalytic activity with almost complete degradation of oxalic acid after 120 min of reaction, regardless of the sample tested (pristine, CNT-O, and treated samples). However, oxalic acid oxidation significantly increased in the presence of the P- and B-doped CNTs, which can be assigned to the high electron-donating ability of these heteroatoms. CNT-TS, despite the higher $S_{BET}$, showed a lower activity.

The surface chemistry of carbon nanotubes was modified using a solvent-free methodology involving mechanical and thermal treatments in the presence of precursors of different heteroatoms (S, B, P). Although the methodology allows to incorporate different surface functionalities, in some cases promotes significant changes on the textural properties. Further optimization is required in order to preserve the textural properties. Oxidation of oxalic acid by wet air oxidation shown to be influenced by the surface chemistry of the CNTs and faster using the P- and B-doped CNTs due to their to the higher electron-donating ability.
Figure 1. Dimensionless concentration of oxalic acid during CWAO experiments.

Acknowledgements
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References
Synthesis of high surface area host-guest hematite photoelectrodes for photoelectrochemical cells

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Hematite is a promising semiconductor material for photoelectrochemical devices, due to its abundance, low cost, excellent stability and suitable bandgap (ca. 2.1 eV). However, the low mobility of minority carriers (holes) and the fast electron-hole recombination kinetics compared to the light extinguish coefficient, are challenges to overcome; nanostructuring and coating the surface of hematite were the strategies used in the present study for enhancing the performance of this photoelectrode. A thin mesoporous SiO$_2$ host template (ca. 193 nm) was synthesized and optimized, using particles with a surface area of ca. 141.6 m$^2$·g$^{-1}$, and coated with an intermediate conductive layer of TiO$_2$ by atomic layer deposition. Finally, a hematite guest thin film was deposited by spray pyrolysis on the prepared host structure, and after coating a FeNiOOH co-catalyst a photocurrent-density of ca. 0.98 mA·cm$^{-2}$ at 1.45 V RHE was obtained, showing a lower onset potential of ca. 0.70 V RHE.

Introduction

The current energy production dependence on fossil fuels have negative impacts both on the economy and environmental health of our planet that cannot be ignored. In 2015, the global CO$_2$ emissions from the combustion of fossil fuels reached 32.3 Gt of CO$_2$, whereof 45 % is allotted to coal and 34 % to oil.[1, 2] Decarbonization of our energy supply routes based on the exploitation of efficient and clean energy technological solutions plays a very central role in future energy policies, making it a major concern.

The sun is the biggest renewable energy resource available in our planet. However, sunlight-based systems can only operate during daytime and with favorable atmospheric conditions. To make the generated energy dispatchable, one approach is to store solar energy in the form of a chemical fuel. Solar hydrogen (H$_2$) is an attractive clean energy-dense fuel, when produced via solar water splitting, due to its potential applications: it is storable, transportable, it can be converted into electricity for mobile applications and used as feedstock.[3]

A promising route to produce H$_2$ from solar water splitting is through photoelectrochemical (PEC) cells: a device composed of two externally connected electrodes, with at least one of them being a photoelectrode, immersed in an aqueous of a supporting electrolyte, which allows the transport of ionic species. In a PEC cell, the photoelectrode is the component that converts incident photons to electron-hole (e$^-$/h$^+$) pairs, provided with enough energy to promote the necessary redox reactions to create chemical fuels. Hematite (α-Fe$_2$O$_3$) emerged as one of the most studied and promising semiconductors in the PEC water splitting field due to its abundance, good light absorption and long-term stability.[4] Despite these encouraging characteristics, the highest solar-to-H$_2$ efficiency of α-Fe$_2$O$_3$ achieved so far was 3.1 %.[5] Research efforts with α-Fe$_2$O$_3$ photoelectrodes aimed at: i) improving the generated photocurrent density through the optimization of morphology; and ii) reducing the required overpotential by employing various surface treatments, which also enhances the generated photopotential and decreases the e$^-$/h$^+$ recombination. Therefore, to reach the performance of an ideal hematite photoelectrode, these two strategies should be combined - Figure 1.[6]

The motivation and challenge for this work is the design of a nanostructured host-guest approach based on SiO$_2$ nanoparticles host scaffold supporting a thin guest layer of α-Fe$_2$O$_3$, which allows preserving a very thin layer of α-Fe$_2$O$_3$ to minimize recombination problems whilst highly increasing surface area and light absorption. An Earth-abundant and efficient co-catalyst of FeNiOOH was also deposited on top of the optimized host-guest approach for improving the water oxidation kinetics.

Methods and Results

SiO$_2$ mesoporous template coated with a TiO$_2$ thin layer. SiO$_2$ mesoporous templates were prepared by spin coating a paste on top of the FTO glass, containing 8 g of SiO$_2$ nanoparticles in a mixture of water, acetic acid, ethyl cellulose, terpineol and ethanol (ratios of 1:1:3:18:136 respectively) followed by a sintering treatment. Two different types of nanoparticles were used (particles A: 87.8 m$^2$·g$^{-1}$ and particles B: 141.6 m$^2$·g$^{-1}$). Thickness was optimized for various paste dilutions with different terpineol volumes, according to Table 1.
### Table 1. Optimization conditions for SiO$_2$ mesoporous film.

<table>
<thead>
<tr>
<th>Type of particles</th>
<th>Paste conditions</th>
<th>Dilutions with terpineol (mL)</th>
<th>Deposition times (s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Non-evaporated</td>
<td>4, 8, 16</td>
<td>90, 180, 240</td>
</tr>
<tr>
<td>B</td>
<td>and evaporated</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

From cross-section SEM images, it was possible to determine the film thickness as a function of terpineol dilutions - Figure 2. The template thickness of ca. 193 nm was selected.

![Figure 2. Left: SiO$_2$ mesoporous film thickness in function of volume of terpineol added in all paste conditions; right: optimum thickness value, checking SEM cross-section image.](image)

Afterwards, a thin film (ca. 3 nm) of TiO$_2$ was deposited by atomic layer deposition over the SiO$_2$ mesoporous film to collect electric charges. This layer presented better performances (30% higher photocurrent) as a $\alpha$-Fe$_2$O$_3$ underlayer than the standard tetraethyl orthosilicate (TEOS) underlayer – Figure 3.

**$\alpha$-Fe$_2$O$_3$ film optimization**

Hematite film was deposited by spray pyrolysis from an iron (III) acetylacetonate precursor, as reported elsewhere.[4] Various volumes of precursor solution were tested in different samples over SiO$_2$ mesoporous film coated with TiO$_2$: underlayer (15 mL, 30 mL, 42 mL, 60 mL). The best performing results were obtained for the samples with 30 mL of precursor solution (reaching 0.81 mA·cm$^{-2}$). This result was improved to 0.98 mA·cm$^{-2}$ after coating with an abundant and stable co-catalyst (FeNiOOH), representing a photocurrent improvement of 17%. Comparing with a sample without SiO$_2$ nanostructure, these optimization tests represent an improvement of 85% (Figure 3). Impedance spectroscopy was used to correlate the improved performances with the photoelectrode configuration. The novel host-guest photoelectrode allowed to improve the charge transfer kinetics and minimize the recombination phenomena in the bulk and surface of hematite, which resulted in an enhanced water oxidation efficiency.

**Conclusions**

A SiO$_2$ mesoporous film was optimized to a thickness of ca. 193 nm using nanoparticles with a surface area of 141.6 m$^2$·g$^{-1}$. The fabricated film was highly homogeneous and crack-free. To transport charges to the underlying FTO, a TiO$_2$ thin conductive layer was tested, presenting better performances than standard underlayer of TEOS. A thin film $\alpha$-Fe$_2$O$_3$ was then deposited by spray pyrolysis on top of the mesoporous film and the deposition conditions optimized, obtaining a photocurrent of ca. 0.81 mA·cm$^{-2}$ at 1.45 V$_{RHE}$, which is higher than the one obtained for a hematite thin film on top of a flat FTO glass coated with TiO$_2$. To combine the nanostructuring approach with a surface treatment, a FeNiOOH co-catalyst was electrodeposited and the onset potential was shifted to a lower potential of ca. 0.70 V$_{RHE}$ and a final photocurrent of ca. 0.98 mA·cm$^{-2}$ at 1.45 V$_{RHE}$ was obtained (185% compared to hematite thin films deposited onto TEOS-pre-treated FTO glasses).

**Acknowledgements**

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**References**

Increasing cyclone performance with an optimized Post-Cyclone recirculator: ReCyclone® MHV

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Cyclones are gas/solid separator devices, highly used for the recovery of raw materials and to comply with particle emission limits. Previous works show that tangential inlet, reverse-flow cyclones can be coupled with straight-through concentrators (MR) to increase overall efficiency. The complete setup is named ReCyclone®. On the other hand, pressure drop also increases, negatively affecting the energy efficiency of the system. Unlike mechanical recirculation, post-cyclone systems take advantage of the remaining swirl on the cyclone vortex finder. Post-cyclone systems are mechanical devices designed to increase overall efficiency and energy recovery, by partially recirculating the outlet vortex flow. The results showed an improvement of the cyclone performance when coupled with post-cyclone devices as compared to the MR or to the single cyclone setups. The use of recirculation shows better efficiencies than the single cyclone, at equivalent cyclone inlet velocities.

Introduction

Cyclones are gas/solid separator devices, commonly used for the recovery of raw materials and/or to comply with particle emission limits. Previous works show that tangential inlet, reverse-flow cyclones can be coupled with straight-through recirculators (MR) [1-3] to increase overall efficiency. On the other hand, pressure drop also increases, negatively affecting energy efficiency of the system.

Unlike mechanical recirculation, post-cyclone systems (PoC) [4-6] take advantage of the remaining swirl on the cyclone vortex finder duct. PoC systems are mechanical devices designed to increase overall efficiency and energy recovery, by partially recirculating the outlet vortex flow.

Methodology and Experimental work

The experiments were performed in a pilot test facility, which was composed by a dust feeder, a high efficiency Hurricane® cyclone [7], and two fans (the recirculation one placed upstream and the i.d. fan placed downstream of the cyclone, see Graphical Abstract). Also, pressure and sampling points for isokinetic testing were located at the inlet and outlet of the cyclone. All the tests were done with process flow rates between 130 and 1275 Nm/h, and recirculation, defined as the ratio of recirculated flow to total flow, of 0, 25, 35 and 67%.

The velocity field was previously mapped, and so it was well known. Industrial sampling automatically changes the volume flow rate of the sampled flow, to maintain isokineticity. However at the pilot test facility, the sampling flow rates were manually adjusted, using constant volume samplers (Techora Bravo). The isokinetic performance accepted at industrial scale is 100 ± 10%, while the isokinetic performance obtained at the pilot scale was much better (100.0 ± 0.3%).

The Graphical Abstract shows the pilot scale system. Dust enters the system using a TOPAS 410G dust feeder with adjustable feeding rates. The inlet velocity inside the cyclone was controlled by the i.d. fan (cyclone alone, HR), and in case of recirculation, by both fans.

The annular post-cyclone [4-6] device that was tested (PoC), was designed to allow a 360° capture of particulate matter around the vortex pipe, where three different ring openings were tested (13, 19 and 24 mm). The best results were obtained with a 24 mm ring opening and are shown in this work.

Global and fractional efficiencies were calculated from offline sampling mass concentrations and particle size distribution (PSD) measurements. The PSD were determined by a Beckman Coulter LS230 laser spectrometer using ultrasounds and ethanol as dispersant media.

Results

All the tests reported in this work were made at ambient temperature (20 ± 3 °C) using fly ash (Figure 1), at concentrations between 281 ± 33 mg/Nm³ for the PoC setup, and 312 ± 36 mg/Nm³ for the MR setup. Comparison was made with the well-established MR solution [1-3]. A single test was also performed with a coarser dust (talc, see Fig. 1) to compare the three different setups (HR, MR and PoC), at similar concentrations as used for the fly ash.

Figure 1. Fly ash and talc experimental particle size distributions.
The PoC setup showed an increase in global efficiency (emissions dropped from 40 mg/Nm³ at the cyclone outlet to 27 mg/Nm³ using fly ash and from 9 mg/Nm³ to 5 mg/Nm³ using the talc powder, Table 1), albeit with a negative tradeoff, since this improvement was reached at higher pressure drop.

The mapped inlet velocities and recirculation fractions are shown in figures 2 and 3, with an interpolation surface.

Figure 4 shows typical grade efficiency curves obtained for the PoC and MR systems for the fly ash sample, at 25 m/s and 25% recirculation. It is clear that higher efficiencies are obtained with PoC in the range 0.2-3μm.

Conclusions
The major conclusions of this study are given below, taking into account that the complete set of information will be presented in detail afterwards:

- The maximum efficiencies were obtained for both recirculation systems at an inlet velocity of 20 m/s and 25% recirculation.
- The PoC setup showed a greater sensitivity to non-ideal effects and this can be inferred from the grade efficiency curves (not shown here).
- The PoC setup showed higher efficiencies in the range [0.2, 3.0] μm particle diameter, as compared to the MR setup.
- The major single parameter to contribute to the maximum overall efficiency is the cyclone inlet velocity, provided that non-ideal effects are minimized.
- Both recirculating systems showed higher efficiencies than the single cyclone.

Future work will be to develop a new PoC that increases the energy efficiency over the current one, while maintaining the increased particle removal efficiency, when compared to the standard MR recirculator.

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References
The negative effects of excessive use of fossil fuels are visible today more than ever and the search for alternative and clean energy sources is essential for the future of the planet. Hydrogen can be that alternative. One way to produce and store hydrogen is to use sodium borohydride (NaBH₄). It has a high hydrogen content (10.8 wt. %) and, when hydrolyzed, releases it at a moderate pressure and temperature. However, a by-product, sodium metaborate, is formed. Due to the excessive cost of NaBH₄, its regeneration is essential to be used as a viable method for hydrogen production and storage. In this study, the influence of additive sodium carboxymethyl cellulose was reported. It was observed the formation of NaB(OH)₄, Na₂CO₃·H₂O and Na₂B₄O₇(OH)₄·(H₂O)·δNaB(OH)₄ is a regeneration of the sodium metaborate molecules and Na₂B₄O₇(OH)₄·(H₂O)·δNaB(OH)₄ is tincalconite, a less hydrated form of borax. These are promising results for the regeneration of NaBH₄.

**Introduction**

Hydrogen is the most promising alternative to fossil fuels, given its gravimetric energy content (142 MJ/kg, at gaseous state [1]). However, it is not found in its molecular form and it is flammable and denser than air, which hinders its storage in safe conditions. It is mainly stored in liquid or gaseous state at high pressure and temperature [2]. An alternative to this method, is to store in chemical hydrides, for example sodium borohydride (NaBH₄). This compound has one of the highest hydrogen content (10.8 wt. %) [3] and, when hydrolyzed, releases hydrogen at moderate pressure and temperature, as shown in Equation 1,

\[
\text{NaBH}_4 + (2+x) \text{H}_2\text{O} \rightarrow \text{NaBO}_2 \cdot x \text{H}_2\text{O} + 4 \text{H}_2 + \text{heat} \quad (1)
\]

where \(x\) is the hydration factor. As observed in Equation 1, a by-product of reaction, sodium metaborate (NaBO₂ \(\cdot\) \(x\) H₂O) is formed. This compound retains typically 2 to 4 molecules of water, which is the main reason to increase the hydration factor. Increasing this parameter decreases the hydrogen released. Nevertheless, other parameters, such as the yield and hydrogen generation rate, are significantly increased [4].

This reaction, although exothermic, requires a catalyst to release hydrogen in an acceptable rate. Different ones have been tested and one of the best hydrogen generation rates was obtained by Pinto et al. [5] with a Ni-Ru based catalyst. Although expensive, this catalyst can be easily recycled and reused up to three hundred times until significantly loses its activity. The regeneration of NaBH₄ from the by-product is essential to the success of its use as a source for hydrogen production and storage. The main method to recycle sodium metaborate is the Brown-Schlesinger process. However, this process requires the use of high pressure and temperature and waste products are formed. As alternative, the ball milling and electrochemical processes are simpler and cleaner ways to regenerate NaBH₄. However, in ball milling by-product must be dried and in the electrochemical process electric energy is required. Additives can be used in hydrolysis, not only to increase the reaction’s parameters, but to influence the by-product formed. Ferreira et al. [6] reported a rearrangement of the sodium metaborate molecules into sodium boron hydroxide (NaB(OH)₄) with the addition of 0.25 wt. % carboxymethyl cellulose (CMC), a derivative of cellulose.

**Objectives**

The objective of this study was to replicate the result obtained by Ferreira et al. [6]. Thus, it was studied the effect of the concentration of CMC on the hydrolysis of NaBH₄, i.e., the effect on the yield, hydrogen generation rate and gravimetric and volumetric hydrogen storage capacities. Moreover, the by-product of reaction was analyzed by different methods.

**Methods**

Catalytic hydrolysis of NaBH₄ was performed on a 229 cm³ cylindrical reactor with conical bottom. 20 mL solutions were prepared with the mass percentages of reactants displaced in Table 1. Sodium hydroxide (NaOH) was added to the solution to inhibit the spontaneous reaction and a Ni-Ru catalyst with a catalyst to NaBH₄ mass ratio of 0.4 g/g was previously inserted in the reactor. It was synthesized and provided by the National Laboratory of Energy and Geology. 5 mL of each solution were injected after closure of the reactor.

<table>
<thead>
<tr>
<th>Reactant</th>
<th>Mass Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaBH₄</td>
<td>3.0 - 7.0</td>
</tr>
<tr>
<td>H₂O</td>
<td>83 - 82.75</td>
</tr>
<tr>
<td>NaOH</td>
<td>7 - 7</td>
</tr>
<tr>
<td>CMC</td>
<td>0 - 0.25</td>
</tr>
</tbody>
</table>

After reaction, the by-product was extracted from the reactor, separated from the catalyst by sedimentation and dried in a hoven at room temperature, assuming a crystalline form. Then, by-products’ crystals were milled and a small quantity was analyzed by X-ray photoelectron spectroscopy (XPS) and scanning electron microscopy (SEM) in all experiments, and X-ray diffraction (XRD) in experiments 3 to 7. The catalyst separated was washed several times and dried in a hoven at 80 – 120 ºC during 30 – 60 minutes until full evaporation of the water.
Results

Figure 1 presents the variation of pressure inside the reactor with time during the experiments. As observed, the use of additive CMC did not influence the hydrogen quantity produced, i.e., the yield and the gravimetric and volumetric hydrogen storage capacities did not change with its use. However, the hydrogen generation rate decreased with the increase of CMC concentration from 0.25 % (exp. 3 and 4) to 1 % (exp. 5 to 7).

![Figure 1. Pressure inside the reactor vs time in experiments.](image)

The main observation of the SEM and XPS analysis of the by-product was the fact that it is very heterogenic, as detailed by the element’s percentage in each experiment, presented in Table 2.

Table 2. Element’s percentage in by-product.

<table>
<thead>
<tr>
<th>Element</th>
<th>Experiment 1 - 2</th>
<th>3</th>
<th>4</th>
<th>5 - 6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>B 1s</td>
<td>15.0</td>
<td>13.3</td>
<td>1.3</td>
<td>4.0</td>
<td>16.0</td>
</tr>
<tr>
<td>C 1s</td>
<td>22.5</td>
<td>26.9</td>
<td>33.7</td>
<td>42.7</td>
<td>20.2</td>
</tr>
<tr>
<td>O 1s</td>
<td>44.9</td>
<td>42.9</td>
<td>39.1</td>
<td>35.8</td>
<td>45.6</td>
</tr>
<tr>
<td>Na 1s</td>
<td>17.5</td>
<td>16.9</td>
<td>25.9</td>
<td>17.6</td>
<td>18.2</td>
</tr>
</tbody>
</table>

The XRD results confirmed the rearrangement of the sodium metaborate molecules into sodium boron hydroxide. This compound is similar to NaBO₃, however, it does not retain water. This observation is very important, given the necessity to evaporate the water molecules retained by the by-product, to successfully regenerate NaBH₄. This way, a high-energy consuming step is eliminated during the hydrogen production. However, the by-product was composed with other compounds. Hydrolysis with 0.25 % CMC produced also sodium carbonate hydrate (Na₂CO₃•H₂O) and the by-product of hydrolysis with 1 % additive was composed by those compounds and sodium borate hydroxide hydrate (Na₂B₂O₅(OH)₂•(H₂O)ₓ)ₜ, also referred as tincalconite, which is a less hydrated form of borax. The presence of these compounds can be related with the exposure to carbon dioxide, since the reactor was not isolated from the air prior to the reaction neither the extraction nor the treatment of the by-products from it [7]. The percentage of each compound on the by-product of hydrolysis of NaBH₄ with different concentration of CMC is shown in the Table 3 and in the graphical abstract. The significant variation of the percentage of each compound between experiments with the same CMC concentration can be due to the different time of exposure to carbon dioxide. Nevertheless, the formation of these compounds, mainly tincalconite, is a step forward to the regeneration of NaBH₄, given their resemblance.

Table 3. Composition of by-product.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Experiment 3</th>
<th>4</th>
<th>5 - 6</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaB(OH)₄</td>
<td>62</td>
<td>72</td>
<td>21</td>
<td>29</td>
</tr>
<tr>
<td>Na₂CO₃•H₂O</td>
<td>38</td>
<td>28</td>
<td>42</td>
<td>53</td>
</tr>
<tr>
<td>Tincalconite</td>
<td>-</td>
<td>-</td>
<td>37</td>
<td>18</td>
</tr>
</tbody>
</table>

Conclusions

The influence of the concentration of additive sodium carboxymethyl cellulose on the hydrolysis of NaBH₄ for hydrogen production and storage was studied in this work and it was based on the results of Pinto et al. [6]. It can be concluded that the additive CMC does not influence the yield and the gravimetric and volumetric hydrogen storage capacities but the hydrogen generation rate decreased with the increase in the additive’s concentration. Moreover, the SEM and XPS analysis of the by-product of reaction allowed only to conclude that it is very heterogenic. However, the presence of sodium boron hydroxide, and consequent rearrangement of sodium metaborate molecules were confirmed and other compounds were detected in the XRD analysis. By-product of hydrolysis of NaBH₄ with 0.25 % CMC contained sodium boron hydroxide and sodium carbonate hydrate and the by-product of hydrolysis with 1 % CMC was composed of sodium boron hydroxide, sodium carbonate hydrate and tincalconite. These are promising results for the regeneration of NaBH₄ and its use for hydrogen production and storage.

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References


Monitoring of emerging micropollutants in hydric media in Bragança district

A. Nemoto1,2, M. Gonçalves2, A. Ribeiro1, ..., Diclofenac Sodium, Naproxen Sodium, Azithromycin, Sulfamethoxazole, Carbamazepine

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This work aims to develop and validate a method for the quantification and monitoring of emerging micropollutants belonging to the class of pharmaceuticals and personal care products (PPCP’s) in several hydric media from Bragança district, Portugal. Six pharmaceutical drugs were selected for this study, namely, ibuprofen, diclofenac, naproxen, azithromycin, sulfamethoxazole and carbamazepine and belongs to three different subclasses that were worldwide prescribed: three anti-inflammatory, two antibiotics and one antiepileptic, respectively. These compounds were chosen after searching which compounds could be more frequently medical prescribed in Bragança, based on related studies associated with regions with similar socioeconomic data. The next step will be the selection of an extraction and an instrumental method of analysis, as well as the selection of the samples collection points in Bragança and to perform some preliminary monitoring of the referred emerging micropollutants.

Introduction

The water is an extremely important substance for life. It is used in distinct metabolic functions of the body and even to produce energy in hydroelectric stations [1]. The ingestion and contact with contaminated water may cause harmful effects to living beings and are related to most of the main diseases that affect global population [2]. It is well known that with the increase of the human action and the industrial activities, the quality of this resource has been strongly affected [1].

The recent advances of the analytical techniques allowed the identification of new compounds in the environment. The emerging pollutants are substances found in different media and that are not considered as pollutants until now. When found in very small concentrations, as micrograms or nanograms per liter, these substances are named as emerging micropollutants [3]. These contaminants may cause damaging effects even in low quantities, but as this fact was recently found, some of them are not yet legislated. For this reason, toxicity, bioaccumulation and health effects studies are important to indicate whether it is necessary or not to legislate about these substances [4].

In the European Union, for example, the Directive 2008/105/CE presents a list of 33 priority substances and their maximum concentrations allowed in nature. These substances are pollutants known for decades. The Directive 2013/9/EU included other 12 compounds in this list and created a vigilance list with 10 substances that could indicate risk to the aquatic matrix and that did not have enough data to their monitoring [5].

Emerging micropollutants have been found in different groups of substances, as pharmaceutical drugs, beauty and cleaning products and illegal drugs. Due to the lack of legislation, there is no specific classification for these substances, but the most common classification found in literature divides them among: persistent organic pollutants (POP’s), endocrine disrupting compounds (EDC’s) and pharmaceuticals and personal care products (PPCP’s) [4].

The PPCP’s are known as pseudo-persistent pollutants, because despite not being totally resistant to degradation, such as POP’s, they are continually reintroduced to the environment, keeping significant concentrations in hydric media [6].

Despite of their importance to society avoiding and treating diseases, medical drugs may cause undesired effects to health when consumed in wrong quantities or ways. It is believed that the main cause of wrong ingestion of these products is self-medication, but the contact with contaminated water is another relevant cause supported during the previous decades by the recent advances of the analytical techniques [7].

Another important concerning about PPCP’s is the fact that they are logically developed with a high pharmacological potency, which means that they can cause the desired effects in a significant way, even at low concentrations while retaining their physicochemical properties long enough to serve the medical purpose developed [3]. Because of these and other factors, PPCP’s are substances excreted in relevant quantities by the human body and that are not effectively removed from water in sewage treatment plants [4].

Objectives

Because these are compounds present in very low concentrations, their analysis and study are not easy tasks. Sophisticated instrumental methods of analysis are needed to measure substances concentrations at the micrograms and nanograms orders of magnitude. Portugal, like many other countries from the European Union, does not have legislation for PPCP's micropollutants yet.

Therefore, it is necessary to develop effective methods for quantification and consequent maintain a serious monitoring of these substances in different hydric media.

The aim of this study is to develop a method of analysis to quantify and to monitor the concentration of several PPCP’s in aqueous media. These compounds are present in pharmaceutical drugs, such as the main active principles, of different classes that are believed to be found in the hydric media from the Bragança district.

Methods

In order to select what substances will be analyzed in this study, it was necessary to investigate which, among several types of drugs, would be found most frequently in hydric media from Bragança.

The first step was to search for works with a similar objective performed in the north region of Portugal and the second step
was to search for studies carried out in places like the region of interest, considering socioeconomic data. Among the characteristics taking into account to select these studies were: to be a small or medium city, maximum 180000 habitants per wastewater treatment plant (WWTP), with a WWTP that receives predominantly domestic discharges, which can include sewage from a hospital. These locations may have agricultural and service activities, but not industrial activities in significant quantities.

Results and Conclusions
After a preliminary screening, six pharmaceutical drugs were selected: ibuprofen, diclofenac, naproxen, azithromycin, sulfamethoxazole and carbamazepine. The first three are anti-inflammatory, followed by two antibiotics and one antiepileptic, respectively. Table 1 presents the results of some studies that quantified these substances in different hydric media. Concentrations for the same place are expected to be higher in the raw sewage, followed by the influent, effluent and groundwater. In the table, this correlation does not happen because the samples are from different aqueous matrices and different locations, which explains the significant variability of the data showed. Moreover, the results presented are average values for each one of the collection sites.

The next step will be to select an extraction/concentration technique and the instrumental method of analysis. The main alternatives for extraction/concentration considered in this study are Solid Phase Extraction (SPE) and Solid Phase Micro Extraction (SPME). The SPE uses cartridges filled with sorbents, which retains the analytes according to the polarity and consequent affinity to their molecules. SPME is a technique which uses a silica fiber covered by a thin layer of a polymer or a solid adsorbent, chosen according to the desired purpose. The coated fiber is wrapped inside the needle of a microsphere inserted into the sample for extraction of the analytes [4].

For the methods of instrumental analysis, the possibilities that are considered are High Performance Liquid Chromatography coupled with mass spectrometry (HPLC-MS) or Gas Chromatography coupled with mass spectrometry (GC-MS). After the development and validation of the method with real samples, it will be possible to detect and quantify this type of pollutants in some of the hydric media in the district of Bragança.

Table 1. Pharmaceutical drugs quantified in some published studies.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Average concentration (ng/L)</th>
<th>Hydric media</th>
<th>Method of analysis</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ibuprofen</td>
<td>110</td>
<td>Effluent</td>
<td>SPE-GC-MS</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td>87.1</td>
<td>Groundwater</td>
<td>SPE-LC-MS</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>84000</td>
<td>Influent</td>
<td>SPE-GC-MS</td>
<td>[9]</td>
</tr>
<tr>
<td>Diclofenac</td>
<td>100</td>
<td>Raw sewage</td>
<td>SPE-HPLC-MS</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>290</td>
<td>Effluent</td>
<td>SPE-GC-MS</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td>75.3</td>
<td>Groundwater</td>
<td>SPE-LC-MS</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>1500</td>
<td>Influent</td>
<td>SPE-GC-MS</td>
<td>[9]</td>
</tr>
<tr>
<td>Naproxen</td>
<td>410</td>
<td>Effluent</td>
<td>SPE-GC-MS</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td>0.43</td>
<td>Groundwater</td>
<td>SPE-LC-MS</td>
<td>[8]</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>96.3</td>
<td>Groundwater</td>
<td>SPE-LC-MS</td>
<td>[8]</td>
</tr>
<tr>
<td>Sulfamethoxazole</td>
<td>13</td>
<td>Raw sewage</td>
<td>SPE-HPLC-MS</td>
<td>[4]</td>
</tr>
<tr>
<td></td>
<td>15.3</td>
<td>Groundwater</td>
<td>SPE-LC-MS</td>
<td>[8]</td>
</tr>
<tr>
<td>Carbamazepine</td>
<td>440</td>
<td>Effluent</td>
<td>SPE-LC-MS</td>
<td>[7]</td>
</tr>
<tr>
<td></td>
<td>44.32</td>
<td>Groundwater</td>
<td>SPE-LC-MS</td>
<td>[8]</td>
</tr>
<tr>
<td></td>
<td>350</td>
<td>Influent</td>
<td>SPE-GC-MS</td>
<td>[9]</td>
</tr>
</tbody>
</table>

References
Numerical simulation of the membrane chemical degradation in a PEM fuel cell

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The durability of proton exchange membrane (PEM) fuel cells is a key aspect that still hampers the generalized commercialization of these devices. In particular, the chemical degradation of the membrane is one of the most common failure modes of a stack. In this work, a numerical model is presented that predicts the membrane degradation and its effect on the cell electrical output, as well as the locations along the active area where the membrane degrades most. 

Semi-empirical laws developed and validated based on experimental results are used to create a membrane degradation sub-model that is incorporated into a commercial PEM fuel cell model. Simulations were conducted at various operating conditions and their effects on the membrane degradation and cell performance are discussed. It is also shown the spatial variation of the membrane thickness after degradation (see Graphical Abstract on the left) and its relation with the local operating conditions.

Introduction

Proton exchange membrane (PEM) fuel cells are a promising technology for clean and efficient power generation for various applications. Nevertheless, their durability still stands as a key issue for their widespread commercialization [1-3]. Within this respect, the chemical degradation of the membrane appears as a critical aspect. It leads to membrane thinning that decreases the cell performance and, eventually, to pinhole formation that is a common cause of stack failure [3]. Mathematical models have been very useful to gain a better understanding of the underlying mechanisms of this complex phenomenon. However, they are not able to capture the heterogeneities in degradation over the active area of the cell [3]. In this work, a degradation model was developed with the aim of not only predicting the overall membrane degradation and the correspondent performance decay over time, but also of identifying the location(s) where the membrane degrades most and analyze the effects of the local operating conditions.

Numerical Model

The present model is built upon the PEMFC Model add-on module from the ANSYS Fluent software (version 18.2) [4] by compiling a customized source code that contains a sub-model for the chemical degradation of the membrane. The degradation sub-model is centered on the following semi-empirical equations provided by Chandesris et al. [3]:

\[ v_{F^-} = A_1 \frac{\Delta P_{O_2}}{T_0} \frac{e_M^0}{e_M} \left( \frac{a_{NaF}}{\gamma_{NaF}} \right) e^{-\frac{E_a}{RT}} \left( \frac{1}{\gamma} \right) \] (1)

\[ \frac{d e_M}{d t} = A_2 v_{F^-} v_{NaFion} \] (2)

Eq. (1) describes the fluoride release rate \((v_{F^-})\) as a function of \(O_2\) crossover (represented in a non-dimensional way with constant permeability), the cell voltage \((U)\) and temperature \((T)\). \(\Delta P_{O_2}\) is the differential \(O_2\) partial pressure, \(e_M^0\) is the initial membrane thickness, \(e_M\) is the membrane thickness, \(a_{NaF}\) is the equivalent transfer coefficient, \(F\) is the Faraday’s constant, \(R\) is the ideal gas constant, \(E_a\) is the activation energy, \(P_0\) and \(T_0\) are the reference pressure and temperature fixed arbitrarily to 1 bar and 95 °C, respectively, and \(A_1\) is an adjustable constant. In Eq. (2), the temporal evolution of \(e_M\) is assumed to be proportional to \(v_{F^-}\), where \(v_{NaFion}\) is the Nafion volume for 1 g of fluoride and \(A_2\) is another constant. Eq. (1) considers that the degradation of the membrane occurs mostly on the anode, with \(O_2\) crossing from the cathode side. Once in the anode, \(O_2\) can combine with \(H_2\) generating \(H_2O_2\) that decomposes into radicals in the presence of metal ions (especially iron). These radicals then react with the membrane, leading to its thinning (thickness decrease) and the release of fluoride.

The effect of the membrane thinning is accounted for in the calculation of the crossover current \((i_{crossover})\):

\[ i_{crossover} = \frac{P_{H_2}K_{H_2}2F}{e_M} \] (3)

where \(P_{H_2}\) is the \(H_2\) partial pressure and \(K_{H_2}\) is the \(H_2\) permeability. The latter was computed as a function of temperature and the membrane humidification, based on detailed measurements of Nafion membranes permeability [5]. The \(i_{crossover}\) in turn affects the open circuit voltage \((OCV)\):

\[ \Delta OCV = -RT \frac{\alpha}{\lambda} \ln \left( \frac{i_{crossover}}{i_0} \right) \] (4)

where \(\alpha\) is the transfer coefficient and \(i_0\) is the exchange current density.

Simulations were conducted for a single cell with an active area of 2.4 cm², having a single-serpentine flow field and a 25.4 µm thick Nafion membrane (NR211). A base case was considered where \(U = 0.70 \, V\), \(T = 80 \, ^°C\), \(P = 2.0 \, bar\), \(RH_{H_2/air} = 50/100 \%\) and \(\lambda_{H_2/air} = 4/4\). RH stands for relative humidity and \(\lambda\) for reactant stoichiometry. The effects of voltage, temperature, pressure and \(RH_{air}\) were investigated. A stationary operation was simulated (i.e., all conditions were kept constant during time). An important aspect is about the simulation of time, because compute hundreds or thousands of hours, which is the time scale of this degradation phenomenon, is unfeasible with such CFD model. This issue is overcome by setting a time-step in Fluent (10⁻⁵ s) to correspond to several
that is, the degradation is stronger, close to the air inlet where the O\textsubscript{2} partial pressure is higher. Because of the small size of the cell and the conditions simulated, there were almost no differences in temperature along the cell, but they will occur for larger cells or stacks, and it is expected that such differences will influence the local degradation of the membrane. Therefore, the simulation of cells with larger active areas will be one the topics for future work.

Table 1. Effects of operating conditions on the membrane degradation after 4000 hours of operation.

<table>
<thead>
<tr>
<th>Voltage (V)</th>
<th>Membrane thickness(^a) (µm)</th>
<th>Current density (% of decrease)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5</td>
<td>25.36</td>
<td>0.04</td>
</tr>
<tr>
<td>0.7</td>
<td>22.16</td>
<td>6.02</td>
</tr>
<tr>
<td>0.8</td>
<td>20.00</td>
<td>100</td>
</tr>
<tr>
<td>0.9</td>
<td>20.00</td>
<td>100</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>25.08</td>
<td>0.33</td>
</tr>
<tr>
<td>50</td>
<td>24.33</td>
<td>1.51</td>
</tr>
<tr>
<td>80</td>
<td>22.16</td>
<td>6.02</td>
</tr>
<tr>
<td>Pressure (bar)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>24.29</td>
<td>1.89</td>
</tr>
<tr>
<td>2</td>
<td>22.16</td>
<td>6.02</td>
</tr>
<tr>
<td>3</td>
<td>19.83</td>
<td>11.6</td>
</tr>
<tr>
<td>RH\textsubscript{air} (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>21.51</td>
<td>5.65</td>
</tr>
<tr>
<td>50</td>
<td>21.75</td>
<td>5.64</td>
</tr>
<tr>
<td>100</td>
<td>22.16</td>
<td>6.02</td>
</tr>
</tbody>
</table>

\(^a\)Initial thickness = 25.4 µm

Figure 1. Temporal evolution of current density for different operating voltages.

Acknowledgements
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References
The anaerobic digestion of the crude glycerol from the production of biodiesel is an alternative for methane production. The high organic load of crude glycerol can cause an inhibition of microorganisms. Ultrasound promotes the breakdown of the cell wall and membrane, releasing intracellular material that favors biodegradation. In addition, such microorganisms can produce lipases capable of degrading other impurities contained in the residual glycerol, such as long chain fatty acids. The aim of this study is to improve the anaerobic digestion of glycerol through physical (ultrasound) and biological (Aspergillus niger and Escherichia coli) pretreatments. The results indicate that the use of ultrasound improved methane generation by 23% for a lower concentration of glycerol (2%). There were also improvements from the use of A. niger in 1.7% glycerol. For the concentration of 3.2% glycerol and the use of E.coli in all experiments caused inhibition of the methanogenic microorganisms.

Introduction

The production of biodiesel generates crude glycerol a by-product, which can be used in a wide range of industrial processes. It has purity ranging from 23% to 87% [1, 2], so depending on its use it is necessary to remove impurities, which increases the price and may become the application economically unviable [3, 4]. In addition, increased industrial production of biodiesel has been led to a surplus of glycerol, which causes a commercial devaluation and an impact on the price of biodiesel. In this context, other forms of glycerol valorization become interesting [4].

One means of valorization of the crude glycerol is its biotransformation, using microorganisms, in compounds with greater added economic value. Although glycerol is used for several industrial purposes, the impurity content of the residual glycerol limits its processing. The anaerobic digestion of glycerol to produce energy through the generation of methane is an alternative to its valorization. The high organic load of crude glycerol (1800 g\(^{-1}\) COD.L\(^{-1}\)) can cause kinetic stress that leads to the inhibition of methanogenic microorganisms [5, 6].

The use of pretreatment can improve the anaerobic digestion. Ultrasounds are used as physical pretreatment to improve the efficiency of the digestion process [7, 8]. The ultrasound helps to deagglomerate biological flakes and transforms large organic particles into smaller ones [9]. In addition, biological pretreatment using specific microorganisms such as Aspergillus niger, Escherichia coli and Yarrowia lipolytica [2, 10, 11] can help glycerol to follow IST metabolic pathways to produce hydrogen, ethanol, citric acid, 1,3-propanediol, among others [12, 13].

Objectives

This study had as main objective to test alternative pretreatments of the reactor feed with ultrasound and microorganisms, namely, Aspergillus niger and Escherichia coli prior to the anaerobic digestion of glycerol.

Methods

In a preliminary phase, the quality of the sludge from two different sources i.e. from the anaerobic digester of the Vila Real Wastewater Treatment Plant (AD-VR) and the anaerobic digester of the Vila do Conde WWTP (AD-VC), was evaluated for the anaerobic digestion of glycerol concentrations of 0.2%, 1.7% and 3.2%. Posteriorly the effect of pre-treatments by sonication for different times (3 to 15 h) and by the previous degradation with either A. niger or E. coli microorganisms was assessed.

Tests were performed using a continuous stirred-tank reactor in a batch operation (500 mL). The experiments were carried out with temperature control in the mesophilic regime, between 35ºC and 40ºC. A total volume of 300 mL (sludge + substrate) was employed. According to the characteristics of the glycerol, some compounds were added: potassium nitrate (C:N of 25:1), disodium phosphate (C:P of 120:1) and sodium bicarbonate (2 g.L\(^{-1}\)). The daily biogas production (% CH\(_4\) and volume), COD (initial, after 15 days and final, after 30 days), ST and SV (after 15 days and final) were evaluated.

Results

Sludge Type. Sludge samples from two anaerobic reactors, one from the AD-VR WWTP and another from the AD-VC WWTP, were tested. Higher SV/ST values correspond to a greater stabilization of the sludge [14]. Tawfik and Klapwijk [15] and Al-Jamal and Mahamoud [16] indicate values of 0.66 and 0.67 suitable for the good performance of the reactor. The SV/ST ratio was 0.75 for the AD-VC slurry and 0.65 for the AD-VR one, being the later in agreement with the literature reference values. This was evidenced by COD values. AD-VC sludge (with higher SV / ST ratio) yielded COD=0.5 g.L\(^{-1}\), whereas AD-VR sludge (with lower SV/ST ratio) presented a COD=12.8 g.L\(^{-1}\). According to the results shown in Figure 1, the experiments performed with 1.7% and 3.2% of glycerol originated biogas yields of 200 to 900 mL (A), however, the quality of the gas was not relevant with a variation of methane volume between 0 and 15 mL (B). In the case of the experiment with a concentration of 0.2% of glycerol, a better biogas production was obtained: the sludge from AD-VR and AD-VC allowed to obtain a methane volume of 575 mL and 294 mL, respectively (B). This indicates that the best results were obtained with the AD-VR sludge.

Pretreatment. Pretreatments provided better methane generation. The pretreatment with ultrasound (15h) for 0.2% glycerol led to an increase of about 23% of biogas with a methane content of...
about 77% (Figure 2-A). The exposure of microbial cells to ultrasound energy breaks the cell wall and the membrane releases intracellular material, which favors digestion [9]. For a concentration of 1.7% glycerol, the best performance was obtained after pretreatment of glycerol by the A. niger fungus (117 L of CH₄ kg⁻¹ glycerol, 76% increase) (Figure 2-B). A. niger produces lipase capable of catalyzing and synthesizing various dicarboxylic acids and diols, in addition, there are studies showing that the fungus can optimize the components of the biofuel synthesis of glycerol carbonate by 12% [17]. Figure 2-C shows the lowest volume of biogas generated with the lowest methane content (8.7%). Using 3.2% of glycerol leads to inhibition of methanogenic microorganisms in all the experiments. Athanasoulia et al. [18] obtained desalting results, proving the inhibition of the anaerobic digestion process when using 4% glycerol. In addition, the E. coli microorganism impaired the anaerobic digestion at all concentrations of glycerol.

Figure 1. Volume of biogas (A) and methane (B) in the batch reactor for AD-VR and AD-VC sludges. 0.2-VR; 1.7-VR; 3.2-VR; 0.2-VC; 1.7-VC; 3.2-VC.

Conclusion

Among the two types of sludge tested (AD-VR and AD-VC from the anaerobic digesters of Vila Real and Vila do Conde WWTPs), the AD-VR sludge allowed, in general, to obtain better results feeding the reactor with 0.2 % glycerol, i.e. a production of 937 L CH₄ kg⁻¹ glycerol containing 75% methane and the SV/ST=0.65. For the batch reactor tests, the most satisfactory results were achieved with 0.2% glycerol concentration and pretreatment by ultrasounds during a period of 15 h; the obtained biogas had 77% methane, and the volume generated reached 1153 L CH₄ kg⁻¹ glycerol. For a concentration of 1.7% glycerol and pretreatment by A. niger, the content CH₄ in the resulting biogas was 65% and the production was 117 L CH₄ kg⁻¹ glycerol. Feeding with 3.2% glycerol resulted in an inhibition in all experimental trials.

Figure 2. Volume of methane in the batch reactor for (A) 0.2% of glycerol, (B) 1.7% of glycerol and (C) 3.2% of glycerol. L CH₄ kg⁻¹ DOC; L CH₄ kg⁻¹ SV; L CH₄ kg⁻¹ glycerol.

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References

Introduction

Dye sensitized solar cells (DSSCs) are a technological approach for direct photoelectrochemical conversion of solar light energy to electricity [1] suitable for building- or appliances-integrated photovoltaic [2]. One of the obstacles for upscaling DSSCs is the low conductivity of TCO-substrates, that produces ohmic losses in large area devices. One approach to overcome this limitation consists in improving the charge collection by adding a grid of current collectors [3-6]. These collectors are usually applied on top of TCO, restraining the height of the metal line to the space between the electrodes, which limits the cross-section area and thus the conductance. When used in the photoanode side, the current collector may promote recombination reaction of collected electrons with electrolyte, muffling their benefits, or corrode [7,8]. Therefore, the use of a stable and corrosion resistant metal [6] is a popular solution for this application. This work demonstrates a new rote for applying current collectors on transparent conducting glasses. The grid was formed underneath the TCO-coated glass, which allows significantly increase of cross-sectional area of the current collectors and opens the possibility to utilize corrosion resistant metals. Chromium current collectors were embedded into FTO-coated glasses rendering a device, which for the first time ever shows full stability and recombination suppressing behaviors.

Experimental

The TCO-coated glass substrates were masked with a photoresist layer and a photolithography pattern was generated. Samples were then submitted to FTO electrochemical etching and glass chemical etching to craft a grid of grooves [9]. After magnetron sputtering of Ti/Cr seed layer and previous photolithography masking, Cr was electrodeposited into etched grooves using standard aqueous plating bath [10]. Graphical procedure can be observed in Figure 1.

Three sizes with square-shaped photoanodes were assembled, as seen in the graphical abstract from bottom to top: Small (S, 0.25 cm²), Medium (M, 6.25 cm²) and Large (L, 42.25 cm²). Photoanodes were applied on blank FTO-glass and on FTO-glass with embedded chromium current collectors (terminology “Cr”) by sequential screen printing of transparent mesoporous layer and opaque atop layer. After sintering, sensitization was achieved dipping the substrates in a fresh solution of N719 dye (0.5 mM) for 24 h. FTO-glass substrates with printed Pt nanoparticles and FTO-glass with sputtered Pt film (terminology “Pt”) were used as counter electrodes. The two components were sandwiched and sealed using thermoplastic film. The assembly was completed with electrolyte injection through, previously, drilled holes on counter-electrode substrate. The holes were then closed with lamella glass using an interlayer of thermoplastic film.

Results

Chromium collectors were chosen because the metal exhibits resistance to corrosion attack when in contact with the electrolyte, is thermally stable in air up to 500 °C (sintering temperature of TiO₂) and prevents triiodide ions reduction. Titanium was applied first because of its good adhesion to the substrate, which dissipates interfacial and thermal stresses between glass and chromium lines. Electrochemical impedance spectroscopy (EIS) results on M- and L-cells (Figure 2) show that replacing the FTO glass on counter electrode side with sputtered Pt film decreased the series resistance and that using Cr-embedded FTO glass as the photoanode substrate has lowered significantly the electron transport resistance in the working electrode, revealing little barrier to electron transport and effective current collection in this component.

Graphical abstract plots show photocurrent-voltage (I-V) curves of large (M-, L-series) and small area DSSCs recorded 96 h after encapsulation. The key parameters of photovoltaic performance were extracted, normalized to photoanode’s active
area and summarized in Table 1. In both series, increasing the substrates conductivity improves the FF: the shape of the curves approaches the typical photodiode. There is also an increase of \( J_{SC} \) in \( L \)-devices. The outcome results in a PCE rise.

**Table 1. Photovoltaic metrics of the DSSCs produced.**

<table>
<thead>
<tr>
<th>Cell</th>
<th>( V_{OC}, ) V</th>
<th>( J_{SC}, ) mA cm(^{-2} )</th>
<th>FF</th>
<th>PCE, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>( S )</td>
<td>0.72</td>
<td>15.70</td>
<td>0.67</td>
<td>7.59</td>
</tr>
<tr>
<td>( M )</td>
<td>0.70</td>
<td>11.18</td>
<td>0.33</td>
<td>2.59</td>
</tr>
<tr>
<td>( M-Pt )</td>
<td>0.70</td>
<td>11.51</td>
<td>0.46</td>
<td>3.74</td>
</tr>
<tr>
<td>( M-Cr-Pt )</td>
<td>0.71</td>
<td>11.67</td>
<td>0.64</td>
<td>5.34</td>
</tr>
<tr>
<td>( L )</td>
<td>0.66</td>
<td>2.28</td>
<td>0.25</td>
<td>0.39</td>
</tr>
<tr>
<td>( L-Pt )</td>
<td>0.69</td>
<td>4.41</td>
<td>0.27</td>
<td>1.77</td>
</tr>
<tr>
<td>( L-Cr-Pt )</td>
<td>0.70</td>
<td>12.54</td>
<td>0.40</td>
<td>3.60</td>
</tr>
</tbody>
</table>

Figure 3 exhibits chromium collector cells’ PCE history under natural ageing. Within the first 100 h, the efficiency gradually increases and after 200 h stabilizes remaining practically constant until the end of the test (3000 h), pointing to perfect compatibility of embedded chromium current collectors with DSSCs.

**Conclusions**

Transparent conducting substrates with enhanced conductivity for efficient charge collection in photoanodes of large area DSSCs were produced. It was achieved by electrochemical embedding of chromium current collecting grid into FTO-glasses. Additional to corrosion resistance in contact with the iodine electrolyte, Cr is electrochemically passive for the reduction of iodine/triiodite ions. Metal lines occupy only 2% of photoanode area but rise efficiency 2 times for 6.25 cm\(^2\) cells and 9 times for 42.25 cm\(^2\) cells. Improvement of PCE is attributed to drastic decrease of series resistances and the resistance of electron transport reaction on photoanode when current collectors are used. DSSCs assembled on Cr-embedded glasses show stable PCE under natural ageing during 3000 h.

**Acknowledgements**

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**References**

Inhibition effect of heavy metals in microalgal growth and nutrient uptake in wastewaters: an experimental and mathematical approach

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The freshwater availability has been diminishing as a consequence of the discharge of numerous effluents with heavy loads of nitrogen, phosphorus, and other toxic compounds (e.g. heavy metals) [1]. The classic wastewater treatment requires physical-chemical techniques that may be expensive or inefficient to treat effluents from mining, metallurgical and electroplating industries (high levels of heavy metals) [2,3]. Thus, a more economical and environmentally friendly treatment of effluents with heavy metals is required. Microalgae have a wide range of applications, namely for wastewater treatment [4,5]. The use of microalgae to remove nitrogen and phosphorus from wastewaters has been extensively studied; however, the presence of inhibitory compounds, such heavy metals, can influence the microalgal growth and nutrients removal. For small heavy metals concentrations, the microalgae can contribute effectively to the removal of heavy metal due to their large surface and high affinity [6]. Previous studies have proved the microalgae’s ability to remove heavy metals but in order to integrate the microalgal cultures in the wastewater treatment further studies are necessary [3,7]. The impact of the metals presence on the nutrient removal and microalgal growth are almost non-existent, as well as the kinetic and equilibrium models to enable the scale-up of this technology. Therefore, this study aims to evaluate the inhibitory effect of the heavy metal (Cd, Ni and Zn) in the cultivation of *Chlorella vulgaris* in simulated wastewater, evaluating the nutrient and metal removal and the specific objectives are: (i) to assess the isolated and combined effect of the three metal on microalgal growth; (ii) to determine the inhibitory effect on the growth; (iii) to determine the kinetic (for microalgal growth and heavy metal concentration in both liquid and solid phases) and the equilibrium (between heavy metal concentrations in liquid and solid phases – Langmuir isotherm) models; and (iv) to evaluate the nutrient and metal removal by microalgae. The highest growth rate were obtained for cultures with Zn for all the tested concentrations. Cd cultures obtained similar growth rates, which indicates that Cd and Zn have an identical effect on the *C. vulgaris* growth. The specific maximum growth rate of 0.34 d⁻¹ was observed in both cultures with Cd and with Zn. The culture with Cd obtained the maximum biomass productivity (98.1 mg·L⁻¹·d⁻¹) and higher removal efficiencies of nitrogen (90.6%) and phosphorus (98.7%) were achieved. Ni causes the highest inhibition percentages (>88%). Regarding metals removal, the best efficiency was obtained in Zn culture (85.4%). Kinetic and the equilibrium were described by a Monod model and Langmuir isotherm, respectively. This work presents relevant results for the integration of microalgae culture in the wastewater treatment containing heavy metals, improving this process in different sectors of economic activity.
containing heavy metals. High nutrient and metal removals were obtained in some cultures tested as well as biomass productivity in the presence of the metals. This integration can improve the sustainability of the wastewater treatment process in different sectors of economic activity.

Acknowledgements
This work was financially supported by the projects POCI-01-0145-FEDER-031736 – PIV4Algae - Process Intensification for microalgal production and Valorisation, and by projects POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013) and POCI-01-0145-FEDER-006984 (Associate Laboratory LSRE-LCM), financed be the European Regional Development Fund (ERDF), through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by FCT/MCTES through national funds (PIDDAC) as well as by projects “LEPABE-2-ECO-INNOVATION” – NORTE-01-0145-FEDER-000005 and AlProcMat@N2020 - NORTE-01-0145-FEDER-000006, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). J.C.M. Pires acknowledges the FCT Investigator 2015 Programme (IF/01341/2015). V.J.P. Vilar acknowledges the FCT Investigator 2013 Programme (IF/00273/2013).

References
Impact of environmental conditions in perovskite solar cells: temperature, oxygen and moisture

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Perovskite solar cells technology have emerged, in the last few years, as an attractive candidate for photovoltaic applications due to their exceptional optoelectronic properties. However, the devices still suffering of a lack in stability at high temperatures and in contact with oxygen and moisture, what is delaying their commercialization. This work aims at to help understanding the impact of different environmental elements in the preparation and operation of the perovskite solar cells, reporting not only the impact of oxygen and humidity during the fabrication of devices, but also the impact of temperature during their operation. The results led us to conclude that while the temperature affects mostly the hole extraction layer due to additives evaporation, the humidity and oxygen affect mainly the morphology of the perovskite layer and its stability during time.

Introduction

In only few years, perovskite solar cells (PSC) have emerged as a new family of photovoltaic devices presenting a surprising power conversion efficiency (PCE) evolution from 3.8 % in 2009 to 23.3 % this year [1]. This type of solar cells is a very promising alternative to conventional photovoltaic technology that use harmful chemicals and complex purification processes [2]. Although the astonishing PCE evolution, these cells suffer of serious stability issues when submitted to high temperatures, oxygen and moisture [3]. It is important to understand the impact of these factors, since in real operation conditions the device is normally exposed to relative humidities between 50-80 % and can easily reach temperatures up to 85 ºC under direct sunlight; even in countries with mild climate like Portugal [4].

More recently, a great effort is being made to prepare more efficient and stable perovskite devices by engineering their composition and structure [5,6]. Normally, a perovskite solar cell is composed by five main layers: 1) transparent conductive oxide (TCO) glass substrate; 2) a semiconductor compact layer (typically called blocking layer); 3) a mesoporous semiconductor film (scaffold); 4) a perovskite absorbing material; 5) a hole extraction layer (HEL); and 6) an electrical conductive back contact. This work focuses on the impact of different environmental conditions (temperature, oxygen and humidity) in the perovskite solar cells, not only during their preparation but also during their operation. This impact is evaluated by assessing the effect of each factor in the photovoltaic parameters of the cells ($V_{oc}$ - open circuit voltage; $J_{sc}$ - short circuit current; $FF$ - fill factor; and $\eta$ - efficiency), but also on its morphology and crystallinity using scanning electron microscopy (SEM) and X-ray diffraction (XRD).

Methods

Triple cation perovskites were prepared by anti-solvent method as described elsewhere [7], using spiro-OMeTAD and TiO$_2$ as hole extraction layer (HEL) and electron extraction layer (EEL), respectively. For the temperature stress tests, cells were sealed by double side Kapton® tape as main sealing and hig h temperature epoxy resin (JB Weld®) on the edges to avoid the entry of humidity and oxygen. To mimic as close as possible real operating conditions in terms of temperature, the cells were characterized in an in-house assembled setup – Figure 1 – and submitted to thermal stress from low to high temperatures (-5 ºC to 80 ºC).

In the humidity tests, three cases were studied: water content in the solvents used for preparing the devices, different levels of ambient humidity during preparation of devices and effect of ambient moisture over time (stability). The variation of humidity percentage during the preparation of the perovskite layer was possible due to an in-house assembled glove box that allows controlling ambient humidity and oxygen concentration. $I-V$ curves were obtained in a setup equipped with a 150 W xenon light source (Oriel class A simulator, Newport USA) irradiating 100 mW·cm$^{-2}$ with an air mass filter of 1.5 (Newport, USA); applying an external potential bias and measuring the generated photocurrent with an Autolab electrochemical station.

Results

Thermal-stress measurements were conducted in PSC devices collected from different batches of cells prepared and characterized in different days. At temperatures below 22 ºC the performance of the cells showed to be unaffected. Nevertheless, at higher temperatures ($T > 40$ ºC), the performance of the tested cells decreased irreversibly with temperature. For devices tested at 80 ºC, they decreased an average of (36 ± 5.5) % of the initial PCE and this value was not fully recovered when the cell was exposed again to room temperature (22 ºC). The efficiency recovery after being brought back to room temperature is only of (14.4 ± 2.3) % (average in reference to the cell tested at 80 ºC). The most affected photovoltaic parameters were $V_{oc}$ and $FF$. Scanning electron microscopy images of the cells exposed to 80 ºC showed voids on top of the spiro-OMeTAD layer, that can be attributed to the evaporation of additives used to enhance its conductivity. This conclusion was corroborated by the characterization of the devices, since the photovoltaic parameters of spiro-OMeTAD based cells with additives was very similar to the photovoltaic parameters of spiro-OMeTAD based cells without additives - Table 1.

Table 1. Photovoltaic parameters of Spiro-OMeTAD based cells, with and without additives (TBP + Li-TFSI + FK209) at 22 ºC after thermal-stress test at 80 ºC.
**Photovaltaic parameters**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Spiro-OMeTAD with additives</th>
<th>Spiro-OMeTAD without additives</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_{oc}$ (mv)</td>
<td>940</td>
<td>955</td>
</tr>
<tr>
<td>$J_{sc}$ (mA·cm²)</td>
<td>16.5</td>
<td>15.5</td>
</tr>
<tr>
<td>$FF$</td>
<td>0.551</td>
<td>0.583</td>
</tr>
<tr>
<td>$\eta$ (%)</td>
<td>9.64</td>
<td>9.61</td>
</tr>
</tbody>
</table>

The effect of humidity was studied in three different ways, as described above. The use of humidified solvents to prepare the precursor solutions of perovskite did not significantly affect the efficiency of the cell; the photovoltaic parameters of the devices did not present high fluctuations between humidity values of 0 – 60 % RH. However, when the cells were prepared in a humidified environment, the efficiency of the cells decreased with humidity concentration – Figure 2 – mainly due to the loss of $V_{oc}$ and $J_{sc}$. At 10 % RH the efficiency of the cells starts to drop and at 30 % RH it was half of the value of the cells prepared in a dry nitrogen environment. Since, only the perovskite layer was prepared inside the humidified glove box, and the other layers were prepared in a dry nitrogen glove box, it is possible to conclude that the humidity is affecting the crystallization step of the perovskite. By SEM images was possible to observe a formation of a non-uniform layer of perovskite that is allowing the direct contact between spiro-OMeTAD and the TiO₂ semiconductor inducing small short-circuits and decreasing the performance of the cells. This relative humidity is also affecting the stability of the devices over time when they are exposed to ambient conditions of oxygen and humidity.

**Acknowledgements**

I. Mesquita is grateful to FCT (Fundação para a Ciência e a Tecnologia) for her Ph.D. fellow (ref.: PD/PB/105985/2014). L. Andrade also acknowledges FCT for funding (IF/01331/2015). The research leading to these results has received funding from: European Union’s Horizon 2020 Programme through a FET Open research and innovation action under Grant agreement no. 687008; Project WinPSC (POCI-01-0247-FEDER-017796) co-funded by the European Regional Development Fund (ERDF), through the Operational Programme for Competitiveness and Internationalisation (COMPETE 2020), under PORTUGAL 2020 Partnership Agreement; POCI-01-0145-FEDER-006939 (LEPABE - UID/EQU/00511/2013), funded by the ERDF, through COMPETE 2020 and by nationals funds through FCT; and NORTE-01-0145-FEDER-000005 LEPABE-2-ECO-INNOVATION, supported by North Portugal Regional Operational Programme (Norte 2020), under the Portugal2020 Partnership Agreement, through the ERDF.

**References**

Degradation of benzodiazepines and carbamazepine by electrochemical oxidation using boron doped diamond electrode and effects on neuronal toxicity

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Currently, wastewater discharge has become a serious concern due to the potential risk for the ecosystem. Continued exposure to contaminated water, even at low concentrations, can cause negative effects on human health and environment. Some pharmaceutical substances are not fully eliminated under conventional wastewater treatment. Therefore, new technologies are required to remove these compounds, such as advanced oxidative processes. Here, the electrooxidation process was used to degrade five pharmaceutical compounds: alprazolam (ALP), clonazepam (CZP), diazepam (DZP), lorazepam (LZP) and carbamazepine (CBZ). The oxidation ability of the system was evaluated varying the pH, current density and aqueous matrix. The best results were obtained with 0.3 A, 1.5 g L⁻¹ NaCl, pH 7 and ultrapure water, leading to complete degradation of ALP, CZP, DZP, LZP, and CBZ. Toxicity tests showed a decrease in ROS (Reactive Oxygen Species) production.

Introduction

For many years, the indiscriminate use of industrially produced substances was treated irreparably. However, a few decades has arisen concern with the effects of these compounds can cause to the environment, living beings and mainly humans [1]. Population growth has led to an increase in wastewater production, such as domestic and industrial effluents. At present most of the effluent passes only by biological treatment, where many substances with a recalcitrant characteristic are not degraded and, as a consequence, they are carried to rivers, lakes and oceans. There are many studies on the effect caused by these substances on aquatic animals and plants, it is difficult to know the damage caused by long-term [2, 3] An example of substances that are not degraded in conventional effluent treatment are the benzodiazepines and carbamazepine compounds, that are detected in rivers and lakes around the world. These medications are widely used in the treatment of insomnia and stress, especially in large metropolises [4]. Therefore, it is important to investigate technologies to eliminate these recalcitrant compounds. An alternative is the recourse to advanced oxidative processes, which have the ability to degrade this kind of substances through strongly oxidizing radicals, such as the electrooxidation process [5, 6]. In this work, the electrooxidation (EO) process was used to investigate the degradation of the drugs ALP, CZP, DZP, LZP and CBZ. The influence of pH, current intensity and aqueous matrices were evaluated. In addition, the effect of the selected compounds on neuronal activity of rat brain slices was verified before and after EO treatment.

Material and Methods

The drugs ALP, CZP, DZP, LZP and CBZ were spiked in ultrapure water (Interlab Direct-Pure system) at the concentration of 100 µg L⁻¹. The EO reactor had a volume of 500 mL and a magnetic stirring bar. The electrodes were composed of BDD (boron doped diamond). The concentration of the pharmaceutical compounds was determined by HPLC (Shimadzu) containing a diode-array detector and a C18 column, and samples run in an isocratic method at 25 °C and flow rate of 1 mL min⁻¹. The initial mobile phase proportion was 70:30 of acetonitrile:water. Sodium chloride (NaCl) from Absolve was used as electrolyte. The EO process was evaluated at different pH values (3, 7 and 10), current densities (0.3, 0.1 and 0.05 A), electrolyte concentrations (1.5 and 0.5 g L⁻¹) and two different aqueous matrices: water from Rio Mondego (Portugal) and from wastewater treatment plant (WWTP). In neuronal studies, female pregnant Wistar rats (31 weeks old) were sacrificed by cervical dislocation, the brains were quickly removed and immersed for dissection into ice cold oxygenated (95 % O₂ and 5 % CO₂) standard artificial cerebrospinal fluid (ACSF), and then hippocampal slices (400 µm) were obtained. Afterwards the slices were incubated in ACSF solution containing 20 µM of ROS indicator H₂DCFDA. After one hour, the slices were transferred to the experimental chamber and they were perfused with ACFS at 30-32 °C. A fluorescence microscope (Zeiss Axioskop) equipped with a water immersion objective (40x, N.A. 0.75, 1.6 mm working distance) was used for the detection of the optical signals [7].

Results and Discussion

Figure 1A shows the removal of ALP, CZP, DZP, LZP and CBZ in different current intensity (0.1 and 0.05 A) after 5 min. According to the results, the current intensity affects directly the degradation of the compounds, and higher current intensity led to an increase in drug removals, likely due to greater availability of electrons. When applying 0.05 A, the removals were similar for all compounds, however when a current of 0.1 A was applied, the LZP and CBZ removals were 1.8-fold and 2.2-fold higher comparing with 0.05 A. In the experiments with 0.3 A (data not shown) all the compounds were degraded in less than 2 minutes. No significant effects in drugs degradation were observed in terms of pH (Figure 1B). In all three pH values, the degradations varied from 30% to 60%. CBZ presented the higher degradation profile, with 58%, 48% and 53% at acid, neutral and basic medium, respectively. The lower drug degradation was observed
for DZP at acid (36%) and neutral (26%) medium. At basic solution, the CZP showed the lower degradation (31%). The results using wastewater and river water are shown in Figure 1C.

The lower efficiency of the drugs degradation in WWTP can be explained by the presence of a greater amount of complex organic compounds. In river water, the higher degradation was found to LZP and CBZ, 87% and 84%, respectively. The lower efficiency was observed for DZP with a removal of 65%. In WWTP degradation of the drugs has the same behavior, but due to the large amount of organic matter the efficiency was lower. And the degradation were 25, 24, 14, 39 and 35 % for ALP, CZP, DZP, LZP and CBZ, respectively.

The effluent containing ALP, CZP, DZP, LZP and CBZ (100 µg L⁻¹) and treated by electrooxidation was used to evaluate the effect on neuronal metabolism. For this reason, fluorescence ROS signals were detected from hippocampal slices incubated with the ROS indicator H₂DCFDA (Figure 2). According to Fig. 2, when only ACSF medium was inserted (first 10 min) the signals remained stable. When the treated effluent was perfused, the ROS signal decreased 20%. Returning to the control medium (ACSF) the signals increased towards to the baseline. These results indicate that in the presence of the treated effluent there is less ROS production.

**Conclusions**

The current intensity directly influenced the degradation of BDZ and CBZ compounds, increasing for large current values. The pH had a negligible effect on the degradation of the compounds. Carbamazepine showed the better degradation efficiency in all three pH values. The presence of organic matter in real matrices influenced the degradation of the studied compounds and reduced the efficiency of drugs removal. The decrease in ROS signals indicates that the treated effluent caused a negative effect on ROS formation, which is a positive result.

**Acknowledgements**
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**References**


Drying sewage sludge with coal fly ash for producing a soil amendment

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The high quantities of sewage sludge (SS) and coal fly ash (CFA) produced require an adequate management for protecting human health and the environment. This study aims to investigate the possibility of using CFA as SS drying adjuvant for producing a soil amendment. The study involved the characterization of SS and CFA at a physical and chemical level. The drying process of small cylinders, for several combinations of SS:CFA (100:0 - control; 95:5; 90:10 and 85:15) at different temperatures (40, 70, 100 and 130 °C) was investigated. The dynamic model of the drying process was based on the Fick law and an infinite length was assumed for the small cylinders. The kinetics analysis showed that as the quantity of CFA increases the rate of drying is faster. The effective diffusion coefficient and other relevant parameters were determined for each thermal drying condition. The results showed that the drying process of SS with small quantities of CFA may be a route for valorizing these wastes in agricultural soil.

Introduction

Coal fly ash (CFA) is produced in large quantities in thermoelectric power plants, that burn coal. CFA is a fine particulate material, composed mainly by ferroaluminosilicates and other minor elements, whose quantities depend on the characteristics of the coal, the operating conditions of the furnace and the sampling point [1]. In general, CFA contain significant amounts of Si, Al, Fe, Ca, K and Na. The recycling of the CFA can be an excellent alternative to manage this waste, allowing significant economic and environmental benefits. Utilization has been encouraged in several countries, and the quantities valorised are about 90% in the European Union, 50% in USA, 60% in India and 70% in China [2].

Regarding sewage sludge (SS) production in Portugal, data from the literature shows figures around 300 kt year⁻¹ in dry base [3]. The management of this waste is expensive due to the high moisture content (greater than 80%), which leads to significant costs associated with transportation, storage and final disposal. The main constituents of SS are water, biomass (microorganisms), inorganic solids and fats. Moreover, important macronutrients (N, P, K), Ca, Mg and Fe are also present in SS, while some potentially toxic metals (PTM) may be found [4]. The application of SS in agriculture soil is an attractive option due to the presence macronutrients and organic matter (OM) [5]. The combination of these two wastes (SS + CFA) may be an excellent strategy to produce a soil conditioner, for correction of the pH and organic matter, which is important for the case of Portuguese soil (acidic pH<5.5 and poor in OM - around 2%) [4, 6].

Objectives

This work aims at assessing the recovery of two wastes (SS and CFA) as soil amendment, where CFA is employed as an adjuvant in the drying process of SS. The production of a soil amendment to agricultural application, is in line with the circular economy adopted in Portugal and in other European Countries as well.

Methods

The CFA sample used in this work was collected by a composed sampling strategy, by collecting 10 subsamples at depth 0.5 – 1 m in a landfill of a power plant in the centre region of Portugal. The CFA sample was dried at 105 °C for 24 hours, disintegrated by hand and sieved to obtain particles with a diameter of 100 - 200 mesh. Oxides were analyzed by X-ray fluorescence (XRF) using a Nex CG Rigaku spectrometer [4]. SS sample was collected also in the Centre Region of Portugal, in a wastewater treatment plant (WTP) with primary and secondary treatments, after mechanical dewatering by centrifugation. pH and electric conductivity (EC) in CFA and SS samples were measured in a 1:10 (mass:volume) suspension [7]. Initial SS moisture was close to 80%. For the drying studies, different percentages of SS:CFA were mixed (Control, 95.5, 90:10 and 85:15). The mixtures of SS and CFA were extruded as shown in Fig. 1.

Results

Table 1 shows some properties of SS and CFA samples. The initial moisture of SS was around 78%. The use of SS from WTP in the soil has been encouraged by the European regulations as an
alternative to the management of this waste, mainly due to the presence of nutrients such as P (4.3% - P2O5) and K (1% - K2O). The pH of both wastes is alkaline. SS is rich in OM (about 64%), whereas CFA is an inorganic material.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>SS</th>
<th>CFA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moisture (%)</td>
<td>78</td>
<td>0.07</td>
</tr>
<tr>
<td>pH</td>
<td>7.7</td>
<td>8.6</td>
</tr>
<tr>
<td>EC (µS cm⁻¹)</td>
<td>1 723</td>
<td>487</td>
</tr>
<tr>
<td>OM (% TS)</td>
<td>63.7</td>
<td>-</td>
</tr>
<tr>
<td>Ash (% db)</td>
<td>36.3</td>
<td>100</td>
</tr>
<tr>
<td>P (P₂O₅) (% TS)</td>
<td>4.3</td>
<td>0.2</td>
</tr>
<tr>
<td>K (K₂O) (% TS)</td>
<td>1.08</td>
<td>2.4</td>
</tr>
<tr>
<td>MgO (% TS)</td>
<td>4.8</td>
<td>1.5</td>
</tr>
<tr>
<td>CaO (% TS)</td>
<td>12</td>
<td>1.4</td>
</tr>
<tr>
<td>BaO (% TS)</td>
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<td>0.1</td>
</tr>
<tr>
<td>TiO₂ (% TS)</td>
<td>0.6</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table 1. Properties of SS and CFA used in this study.

Table 2 reveals the concentration of six metals analyzed for SS and CFA samples. It is also possible to confirm that the values found for SS are below the limits established by the Portuguese Law (Decreto-Lei N°. 276/2009) for use in agriculture soil. For agronomic applications, the inactivation of pathogenic microorganisms is mandatory by law. In this case, thermals processes may be effective to attain that objective, and thus, in this study the drying process was considered. As an example, Figure 2 presents MR as a function of time for the experiments at 100 °C, for each formulation SS:CFA (control, 5, 10, and 15:15). The results show that the adjuvant of drying has a positive effect, since the loss of moisture is faster as the quantity of CFA increase. This positive effect was also observed for 40, 70 and 130 °C. The convective diffusion coefficient at 40 °C ranged from 4.99 × 10⁻⁹ m² s⁻¹ (5% CFA) to 7.81 × 10⁻⁹ m² s⁻¹ (15% CFA). At 130°C, these values were 8.95 × 10⁻⁹ m² s⁻¹ (5% CFA) to 1.21 × 10⁻⁸ m² s⁻¹ (15% CFA). The convective mass transfer coefficient varied slightly with the increase of the adjuvant percentage and the temperatures studied. For example, at 40 °C and 5% of CFA, hm is equal to 8.61 × 10⁻⁷ m s⁻¹ and for 15% CFA this parameter is 8.46 × 10⁻⁷ m s⁻¹. The values of D吸 and hm are found to be close to those reported in the literature [10].

**Conclusion**

In general, the addition of CFA as an adjuvant has a positive effect on the drying process. This encouraging effect may be observed through the effective diffusion coefficients. The product obtained may be further used as soil amendment, which enables to carried OM and nutrients to poor agriculture soil. The potentially toxic metals do not present constraints to agronomic applications in Portugal.

Table 2. Potentially toxic metals (PTM) concentration in this study to SS and CFA and limits to the soil at Portuguese laws.

<table>
<thead>
<tr>
<th>PTM (mg kg⁻¹)</th>
<th>This work</th>
<th>Literature</th>
<th>Decreto-Lei N°. 276/2009</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SS</td>
<td>CFA</td>
<td>SS (b)</td>
</tr>
<tr>
<td>Cd</td>
<td>&lt; LD (a)</td>
<td>0.19</td>
<td>1.3-30</td>
</tr>
<tr>
<td>Cr</td>
<td>129.08</td>
<td>12.82</td>
<td>102-200</td>
</tr>
<tr>
<td>Cu</td>
<td>297.81</td>
<td>14.48</td>
<td>400-625</td>
</tr>
<tr>
<td>Pb</td>
<td>23.56</td>
<td>4.00</td>
<td>109-158</td>
</tr>
<tr>
<td>Ni</td>
<td>61.88</td>
<td>14.48</td>
<td>49.6-50</td>
</tr>
<tr>
<td>Zn</td>
<td>331.24</td>
<td>13.26</td>
<td>1 300-1 850</td>
</tr>
</tbody>
</table>

(a) LD = < 0.07; (b) From [9]; (c) CFA generated in electrostatic precipitator (coal F-grade with 40% coal ash) [2]; (d) Soil limits depends on pH of the soil.

**Acknowledgements**

The authors gratefully acknowledge Federal Institute of Education, Science, and Technology of Brasília - IFB, Campus Ceilândia, for authorizing the Ph.D studies of L.A. Gomes.

**References**

Biodiesel production using nanocatalyst from calcium waste materials

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* samuelpantos@tecnico.ulisboa.pt.

Considering heterogeneous catalysts for transesterification reaction, calcium oxide (CaO) is a widely-used catalyst due to being cheap, non-corrosive, economically benign, easy to handle and having a high basicity compared to homogeneous base catalysts [1]. It can be obtained from natural sources through the calcination of waste egg and oyster shells (95% CaCO3) at 850°C for 3 hours, exhibiting high activity for the transesterification of soybean oil due to its superior basic strength [2][3].

However, heterogeneous catalysts, are currently, somewhat time consuming, need more reaction time to achieve high FAME (Fatty Acids Methyl Esters) yields and presents some mass transfer limitations.

Using CaO nanocatalysts, it is possible to overcome some of these drawbacks, as they present higher surface area and catalytic activity, thus allowing to achieve a significant improvement on transesterification efficiency. In the present work, CaO from natural sources was micronized using the SAS (Supercritical Anti-Solvent) technique with Supercritical CO2. The obtained nanocatalysts were then analysed by SEM and DLS. The transesterification of soybean oil into biodiesel using these catalysts was tested at 60°C with methanol reflux. Reaction conditions such as reaction time, methanol/oil molar ratio and catalyst loading amount were studied as well as its effect on the yield of the triglyceride conversion into FAME.

Introduction

Considering heterogeneous catalysts for transesterification reaction, calcium oxide (CaO) is a widely-used catalyst due to being cheap, non-corrosive, economically benign, easy to handle and having a high basicity compared to homogeneous base catalysts [1]. It can be obtained from natural sources through the calcination of waste egg and oyster shells (95% CaCO3) at 850°C for 3 hours, exhibiting high activity for the transesterification of soybean oil due to its superior basic strength [2][3].

However, heterogeneous catalysts, are currently, somewhat time consuming, need more reaction time to achieve high FAME (Fatty Acids Methyl Esters) yields and presents some mass transfer limitations.

Using CaO nanocatalysts, it is possible to overcome some of these drawbacks, as they present higher surface area and catalytic activity, thus allowing to achieve a significant improvement on transesterification efficiency, resulting into faster reactions i.e., shorter reaction times, low reaction temperatures and lower catalyst concentration.

In the present work, the transesterification of soybean oil into biodiesel using micronized CaO from natural sources by the SAS (Supercritical Anti-Solvent) technique with Supercritical CO2 was tested at 60°C with methanol reflux. Reaction conditions such as reaction time, methanol/oil molar ratio and catalyst loading amount were studied as well as its effect on the yield of the triglyceride conversion into FAME.

Materials and Methods

Catalyst Preparation. The catalyst was prepared from egg shells. The calcium carbonate present in these shells was dissolved onto an acetic acid solution. The shells organic matter, insoluble in the acetic acid solution, was filtered. Calcium acetate is formed and then it was dissolved in an acetic acid/DMSO (50% v/v) solution to be able to proceed to its micronization into the nanoscale.

The SAS (Supercritical Anti-Solvent) technique uses supercritical carbon dioxide (CO2) to remove the solvent and allow the controlled calcium acetate precipitation. This technique allows to study and optimize the particles size by fine tuning the conditions used in the supercritical unit. After the micronization, the precipitated powder is collected, and its calcination takes place. Table 1 describes the calcination steps performed to obtain calcium oxide.

The first calcination step for the calcium acetate was performed to allow the complete removal of the formed acetone (C3H6O) as show in the following reaction equation:

\[
\Delta \text{Ca(CH}_3\text{COO)}_2 \rightarrow \text{CaCO}_3 + \text{C}_3\text{H}_6\text{O}
\]

After the first step is concluded, the second step took place. The calcium carbonate formed from the first calcination step was, then, transformed into calcium oxide.

Table 1. Calcination steps

<table>
<thead>
<tr>
<th>Step</th>
<th>Calcination Temperature (ºC)</th>
<th>Time (h)</th>
<th>Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>400</td>
<td>3</td>
<td>Ca(CH3COO)2 → CaCO3</td>
</tr>
<tr>
<td>2</td>
<td>850</td>
<td>3</td>
<td>CaCO3 → CaO</td>
</tr>
</tbody>
</table>

The obtained calcium oxides were analysed by Scanning Electron Microscopy (SEM) to characterize its particle size.

Sojbean oil transesterification. The transesterification of soybean oil was performed using as catalysts, the micronized CaO using the SAS method and CaO obtained from grinding of egg shell and posterior calcination.

For both types of catalysts, the transesterification reaction took place in a 25ml flask. 5g of soybean oil were heated in a water bath to achieve the reaction temperature of methanol reflux (60°C). 2.24g of methanol were weighted and placed inside the reaction flask. The amount of methanol used is correspondent to a methanol/oil molar ratio of 12:1. 250mg of the catalyst (catalyst loading of 5 wt%) were added to the methanol, and the mixture was stirred at high rpm. The soybean oil was added to the previous mixture slowly.

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Tests with different reaction times were also performed, ranging from 1 to 5 hours. Then, the mixture was filtered and placed inside a separation funnel to allow the separation of the FAME phase from the glycerol phase.

**Results**

**Catalyst preparation.** The calcium oxide obtained by SAS micronization and the grinding of egg shells were analysed by SEM. Figure 1 shows the particle size and proportion for both analysed catalysts.

![SEM analysis of both types of catalyst](image)

Figure 1. SEM analysis of both types of catalyst: a) Calcium oxide using SAS. b) CaO obtained from egg shell grinding.

It is possible to conclude that the CaO obtained from the micronized calcium acetate has, when compared with the one originated from the grinding and calcination of egg shells, a more homogeneous distribution regarding the size and shape of the particle and smaller particles sizes.

**Acknowledgements**

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Samuel Santos acknowledges financial support from FCT by PhD grant, PD/BD/128450/2017.

**References**


**Soybean oil transesterification.** Firstly, it was established a benchmark that allows the comparison between the transesterification performed by CaO from egg shell and the ones using micronized CaO.

In this case, CaO from egg shell grinding will be said benchmark. A series of reactions using this catalyst at the conditions mentioned before was performed. The following reactions at these conditions with micronized CaO will be compared with the benchmark. Reaction conditions will also be studied and so will their effect on the transesterification, i.e., yield, methyl ester content, glycerol content, etc.

**Conclusion**

The transesterification of soybean oil using solid-base heterogeneous catalysts from calcium waste materials at a nanometric scale is the main case study in the present work. Instead of using calcium oxide directly obtained from the calcination of egg shells, the use of calcium oxide from the micronization of calcium acetate by the SAS technique and posterior calcination is expected to improve not only both the conversion yield and content, but also the efficiency of the process, by, for example, lowering the catalyst loading and reducing the reaction time, due to the catalyst having smaller particles.
Preliminary tests on the use of additives to decrease fine particles emission in biomass combustion

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This work describes the results of the effect of additives on PM (Particulate Matter) emission in pinewood biomass combustion. Thus, preliminary combustion tests of pinewood biomass with and without additives were carried out to evaluate the effectiveness of these additives on the reduction of fine particles emission. The additives tested were CaO, TiO2 and Montmorillonite (Na0.2Ca0.2Al3Si4O10(OH)2(H2O)10). The additives concentrations studied were 1.5%, 3% and 4.5% (w:w) and an apparatus was designed to perform these preliminary tests. PM concentration was measured using a DustTrak DRX Aerosol Monitor 8533 (TSI, Inc., Shoreview, Minn.). Particulate Matter (PM) emissions in a drop tube furnace specially built for this purpose in Torbel (http://www.torbel-systems.com/). Particulate Matter (PM) concentration was measured using a DustTrak DRX Aerosol Monitor 8533 (TSI, Inc., Shoreview, Minn.). This equipment

Introduction

One of the major environmental and health hazard is air pollution. The air quality guidelines provided by World Health Organization (WHO) stipulates the health hazards of several air pollutants. The WHO estimated that in 2014, 92% of the world population was living in places that do not meet the WHO air quality guideline limits. It also estimated that air pollution causes 3 million premature deaths worldwide, every year, solely due to particles smaller than 10 microns. The directive 2008/50/EC of the European Parliament and of the council of 21 May 2008 on ambient air quality and cleaner air for Europe gives a target limit value of 20 μg/m3 for particles smaller than 2.5 microns (PM2.5) in the atmosphere, by 1st of January 2020. Fine particles emissions from combustion of biomass are controlled using several strategies, among which is the use of Class II additives, i.e. combustion additives that improve combustion and reduce several pollutants. Most biomasses have high Potassium and Chlorine content. Potassium content leads to lower ash melting temperatures and Chlorine content favours the formation of fly ash [1].

Despite the complex nature of the reactions between additives and ash components and the seemingly impossible task of controlling their behaviour, there are several studies on the reaction mechanisms of additives with ash from different types of coals, biomasses and oils. In summary, PM emissions can be controlled by preventing the reaction of KCl with other ash components and/or capture the fine ash particles before their elutriation. The compounds resulting from the reactions between additive and ash should have a high melting point so as not to create slagging and agglomeration problems during blow down operation. Höfer et al. [2] concluded from their studies on additives during wood and straw combustion that the additives Al2O3, a blend of 46% Al2O3, 44% CaCO3, 10% CaHPO4, and another blend of 46% Al2O3, 44% MgCO3, 10% MgHPO4 help to bind problematic species and reduce particulate matter (PM) emissions. Fournel et al. [3] studied combustion of reed canary grass blended with 50 wt. % wood and 3 wt. % fuel additives such as aluminium silicates (sewage sludge), calcium (limestone) and sulfur (lignosulfonate) based additives and found that combustion of these blends resulted in 17%–29% decrease of PM concentrations compared to pure reed canary grass. A report by Boman et al. indicates that kaolin has a good effect in decreasing PM emissions from combustion of many different types of biomass [4]. Ninomiya et al. concluded that the use of Ca or Mg-based additives to coal combustion could result in the decrease of PM2.5 and PM1 emissions by improving the coalescence of fine particles [5]. The effects of similar additives on PM from biomass combustion could be studied. There are more combustion additives than the common Al, Al-Si, Ca and P-based additives. For instance, Wiinikka et al. concluded from their study on straw combustion that the addition of an optimum amount of TiO2 as additive reduced the vaporization of K by approximately 40 to 50% indicating its great potential in reduction of PM emissions [6]. Besides their effect on combustion, additives are chosen having in mind criteria such as absence of increased environmental toxicity due to adding it to combustion, stability of resultant compounds at high temperature and overhead cost of using the additives.

Objectives

This work describes the results of the preliminary combustion tests of pinewood biomass with and without additives with the aim of testing the effectiveness of these additives in the reduction of fine particles emission during combustion. The pre-selected additives were CaO, TiO2 and Montmorillonite (a silicate with the formula Na0.2Ca0.2Al3Si4O10(OH)2(H2O)10). After these screening tests, the additives will be further tested in a drop tube furnace.

Methods

To test these additives, the biomass and the additives were blended in a ball-mill for 15 minutes at 400 rpm. In these tests, biomass and biomass-additive blends (with 1.5%, 3% and 4.5% weight with respect to total biomass) were burned in the furnace specially built for this purpose in Torbel (http://www.torbel-systems.com/). Particulate Matter (PM) concentration was measured using a DustTrak DRX Aerosol Monitor 8533 (TSI, Inc., Shoreview, Minn.). This equipment
allows the continuous measurement of PM$_1$, PM$_{2.5}$, PM$_4$ and PM$_{10}$ µm particles. The suction line was placed in the stack of the test equipment in the same position for all tests. The suction flow rate was 3L/min and the measurements were recorded for 5 min maximum or just before the saturation of the equipment that occurs when the concentration reaches 150 mg/m$^3$. Thus, 5g of pinewood, referred as the bed, was distributed on the grate and ignited with a lighter. After having a stable flame, 2.5g of the biomass to be tested was carefully added to the bed and the PM measuring equipment was turned on to begin the measurement. The aspiration of the flue gas was stopped before the detector saturation that happened when the amount of smoke was too high because of the flame extinction.

**Results**

Figures 1 and 2 present the main results. Figure 1 shows that, despite the error bars, PM$_1$ emissions were lower in the combustion of the samples containing the additives. On the other hand, the results obtained with the three additives were similar and concentrations of 1.5%, 3 or 4.5% seem to be good values to test in the drop tube furnace. Instead of the mean values, Figure 2 illustrates the change of PM$_1$ concentration with time for the combustion of the as-received (solid lines, average of three measurements) and additives blended biomass samples. It is worth noting that PM$_1$ was the most abundant fraction, usually higher than 99% of the measured particles.

As shown in Figure 2, biomass combustion was stable for around 3 to 4 min. After that time, it is observable a fast increase of the particles concentration due to the formation of a large amount of smoke that tends to saturate the detector. This happens since, after 3 to 4 minutes, the optimum conditions for combustion are no longer present in the experimental setup used. This observation is specific for the setup used and for the amount of biomass used. In a bigger scale, the combustion would proceed without the saturation of the detector.

**Conclusions**

The preliminary results of pinewood biomass combustion before and after the addition of CaO, TiO$_2$ and Montmorillonite (1.5 %, 3 % and 4.5% weight) indicate that these compounds contribute to the decrease of PM emissions. Therefore, these additives will be further tested in a drop tube furnace.
Co-combustion of residual forest biomass derived from *Eucalyptus* with sludge from wastewater treatment in the pulp and paper industry: NO and chlorine emissions

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Residual forest biomass mixed with different mass percentages of primary and secondary sludge from the pulp and paper industry was studied as fuel for combustion in a pilot-scale bubbling fluidized bed. The main objective was the determination of sludge addition influence on the overall process and on the composition of the exhaust gases, with emphasis on chlorine emissions, namely present in the solid phase (fly ashes) and in the gaseous phase (HCl), and NOx emissions. The co-combustion process of residual forest biomass with primary sludge (up to 5%wt) and secondary sludge (up to 10%wt) was successfully demonstrated as a valid energy valorization option for the sludges. Except specific cases, no significant emissions increase of NOx, CO or HCl were found with the addition of sludge. In fact, HCl emissions decreased due to an increase in the chlorine retention in ashes caused by the high inorganic content present in the sludges.

### Introduction

During biomass combustion processes, dependent on the process conditions, chlorine present in the fuel can be released with the exhaust gases. Consequently, diverse problems of deposition and corrosion can occur in combustion systems.

In the literature, it is commonly suggested that adding sludges to the combustion process of Cl-rich fuels may reduce chlorine deposition and bed agglomeration, while increasing NOx, due to the sludges high nitrogen content, and chlorine gaseous emissions (HCl) [1]. It is suggested that the presence of sulfur in sludges modifies the chemistry involved due to the reaction between KCl and sulfur. Theoretically, a total removal of chlorine from the fly ashes is possible if sulfur exists in sufficient quantity to react with all KCl.

Thus, with sludge addition it can be expected a decrease of Cl emissions in the solid phase and an increase of Cl emissions in the gaseous phase [1]. Nonetheless, if the sludges are rich in calcium, chlorine retention on ashes might increase while HCl concentration in the exhaust gases decrease. These aspects need to be further clarified.

In this work, the co-combustion process of residual forest biomass derived from eucalyptus with primary sludge (up to 5%wt) and secondary sludge (up to 10%wt) was characterized. The influence of the addition of sludge on the overall process and on the exhaust gases composition, with emphasis on chlorine emissions, namely present in the solid phase (fly ashes) and in the gaseous phase (HCl), and NOx emissions, was evaluated. The objective is to develop valid and attractive energy valorization solutions for the sludges resulting from the wastewater treatment in the pulp and paper industry.

### Experimental work

The experimental infrastructure used in this work was described in detail in [2] and includes a bubbling fluidized bed reactor with a combustion chamber of 0.25m diameter and 2.3m height made of AISI 310SS. The operating conditions of the reactor were characterized, namely the biomass fuel feed rate, air feed rate, temperature and pressure along the reactor and gas composition at the exhaust. Table 1 shows information regarding the operating conditions used in this work.

The characterization of the experiments was performed according to the following procedures:

1. Characterization of the exhaust gases:
   - O₂ concentration determination in a paramagnetic analyzer (ADC);
   - H₂O, O₂, CO, CO₂, CH₄, NO, SO₂ and HCl sampling and analysis (heated sampling and analysis system (180ºC); analysis system using FTIR (CEM II, Gasmet);
   - Total particles concentration determination through isokinetic sampling in quartz filters and chemical characterization (Cl, Na, K and Ca). The content of Cl, K, Na and Ca in the particles present in the exhaust gases was determined in terms of the content of soluble inorganic ions (Cl⁻, K⁺, Ca²⁺ and Na⁺) by ion chromatography in liquid phase according to the procedure described by Calvo et al., in [3].

2. Characterization of bottom bed and fly ash deposited in different locations of the combustion system:
   - Determination of chemical elements content (Cl, Na, K, Ca, Mg, Al, Mn and P).

### Results and discussion

The monitoring of the operating parameters during the co-combustion process of the different mixtures of residual forest biomass and sludges, such as temperature and exhaust gas composition, along time and in different locals of the fluidized bed reactor, shows that the process was under steady-state conditions of operation. Regarding the composition of the exhaust gases, HCl concentration profile presented a distinct behavior from the other gases. In the initial phase of the combustion process, HCl concentration showed a trend to increase until attaining a maximum value. Afterwards, with the increase in operation time, HCl concentration decreased until stabilizing in a lower value (see Graphical Abstract). This behavior can be related to the chlorine retention promoted by the alkaline elements present in the biomass, e.g. Ca and K. Addition of sludges in the fuel mixture caused a decrease in the concentration of HCl in the exhaust gases, which might be justified by the high content of alkaline elements in the sludges (e.g. Ca) retaining CI in the solid phase (ashes). Accordingly, Cl concentration in the solid phase, e.g., in the fly ashes present in the exhaust gas, was found to be much higher than on the gaseous phase (HCl).

Regarding NO concentration in the exhaust gases, it was observed that the type of residual forest biomass used as feedstock has significant influence on this gas. It was observed that the addition of sludges to the residual forest biomass with...
low nitrogen content (e.g. eucalyptus wood chips) caused a significant increase in NO concentration. On the other hand, addition of sludges to residual forest biomass with high nitrogen content (eucalyptus bark or eucalyptus foliage) did not seem to promote any increase in the NO concentration value. Nonetheless, the observed NO concentration values in the exhaust gases, expressed as NO₂, meet the emission limit value for biomass furnaces in the Portuguese legislation (Decreto-Lei nº127/2013 [4]), however, fail to meet the emission limit value referred in Best Available Technologies reference document for Large Combustion Plants [5] (see Figure 1).

In terms of CO concentration, it was observed that biomass feeding fluctuations associated to the heterogeneous physical characteristics of the biomass mixtures used as fuel, promoted significant fluctuations in the concentration of CO in the exhaust gases. Nonetheless, sludges introduction in the fuel mixture did not seem to cause an increase in CO concentration. Furthermore, an adequate control of the feeding conditions and the usage of an appropriate stoichiometric ratio, allowed to obtain relatively low values of CO concentration, which met the stipulated emission limit value for biomass furnaces in the Portuguese legislation (Decreto-Lei nº127/2013 [4]).

Regarding the thinner fly ashes, which are emitted with the exhaust gases, it was observed that the major chemical elements present in its composition are Cl and K. It is reasoned that these two elements might be in the form of KCl, due to the observed linear relation between Cl and K with a mass ratio K:Cl close to 1:0.91.

In terms of the characterization of the bottom bed ashes and fly ashes emitted along the combustion system, considering the chemical composition, it was found that independently of the location of the sampling point, the average concentration of chemical elements on the sampled ashes is, by decreasing order of abundance: Ca>K>Cl>Mg>P>Na>Al>Mn. Thus, these ashes have distinct characteristics than the thinner fly ashes emitted in the exhaust gas. This knowledge is relevant due to the potential negative effects that the composition of the ashes can cause upon the equipment, such as corrosion, and upon the environment after release of the exhaust gases to the atmosphere.

Conclusions
This work demonstrates the potential of the co-combustion process of residual forest biomass from eucalyptus with primary (up to 5%wt) or secondary (up to 10%wt) sludges in bubbling fluidized beds. Except specific cases, no significant emission increases in terms of NO, CO or HCl were found. Furthermore, HCl concentration decrease was observed with the addition of sludges, most probably due to Cl retention in ashes. Nonetheless, the high inorganic content present in the sludges can originate a significant increase in ashes production. The accumulation of these ashes along the combustion system, e.g. pipes and heat exchangers, can promote operation problems, such as decrease of the process efficiency, increase of maintenance operation needs and equipment damage.

![Figure 1. NO concentration profile along time for the different combustion experiments performed and comparison with the limit value referred on the Best Available Technologies reference document for Large Combustion Plants [5]. Experiments reference according to Table 1.](image_url)

### Table 1. Combustion experiments main operating parameters.

<table>
<thead>
<tr>
<th>Experiment reference</th>
<th>Biomass [%wt]</th>
<th>Type of eucalyptus</th>
<th>Average bed temperature [°C]</th>
<th>O₂ [%v, dry gas]</th>
</tr>
</thead>
<tbody>
<tr>
<td>EL-0</td>
<td>100% eucalyptus</td>
<td>Eucalyptus RFB type A</td>
<td>804</td>
<td>7.0</td>
</tr>
<tr>
<td>EL-0 #2</td>
<td>100% eucalyptus</td>
<td>Diverse pellets</td>
<td>821</td>
<td>8.1</td>
</tr>
<tr>
<td>EL-5</td>
<td>5% secondary sludge + 95% eucalyptus</td>
<td>Eucalyptus RFB type A</td>
<td>815</td>
<td>7.0</td>
</tr>
<tr>
<td>EL-10</td>
<td>10% secondary sludge + 90% eucalyptus</td>
<td>Eucalyptus RFB type A</td>
<td>810</td>
<td>7.0</td>
</tr>
<tr>
<td>EL-10 #2</td>
<td>10% secondary sludge + 90% eucalyptus</td>
<td>Eucalyptus RFB type B</td>
<td>825</td>
<td>7.4</td>
</tr>
<tr>
<td>EL-10 #3</td>
<td>10% secondary sludge + 90% eucalyptus</td>
<td>Pellets A</td>
<td>837</td>
<td>7.1</td>
</tr>
<tr>
<td>EL-10 #4</td>
<td>10% secondary sludge + 90% eucalyptus</td>
<td>Pellets B</td>
<td>848</td>
<td>7.2</td>
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<tr>
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<td>Pellets C</td>
<td>839</td>
<td>6.9</td>
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<tr>
<td>EL-10 #6</td>
<td>10% secondary sludge + 90% eucalyptus</td>
<td>Pellets A*</td>
<td>830</td>
<td>5.9</td>
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<tr>
<td>EL-10 #7</td>
<td>10% secondary sludge + 90% eucalyptus</td>
<td>Pellets E</td>
<td>828</td>
<td>6.0</td>
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<tr>
<td>EL-10 #8</td>
<td>10% secondary sludge + 90% eucalyptus</td>
<td>Pellets A*</td>
<td>836</td>
<td>4.5</td>
</tr>
<tr>
<td>ELP-5</td>
<td>5% primary sludge + 95% eucalyptus</td>
<td>Pellets D</td>
<td>837</td>
<td>8.0</td>
</tr>
<tr>
<td>ELP-5 #2</td>
<td>5% primary sludge + 95% eucalyptus</td>
<td>Pellets E</td>
<td>819</td>
<td>6.0</td>
</tr>
<tr>
<td>ELP-5 #3</td>
<td>5% primary sludge + 95% eucalyptus</td>
<td>Pellets A*</td>
<td>838</td>
<td>5.5</td>
</tr>
<tr>
<td>ELP-5 #4</td>
<td>5% primary sludge + 95% eucalyptus</td>
<td>Pellets E</td>
<td>820</td>
<td>7.9</td>
</tr>
</tbody>
</table>

### Acknowledgements
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### References
Optimization of Fenton/photo-Fenton processes for AOX removal from real pulp and paper bleaching wastewater

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Introduction and Objectives
Wastewaters from the pulp and paper (PP) industry contain a variety of compounds, the most concerning ones being the adsorbable organic halides (AOX), a family of environmentally persistent and bioaccumulative chemicals produced when halogens react with organic matter. The Fenton process is a fast and cost-effective method to remove AOX from wastewaters based on the catalytic (Fe²⁺) dissociation of the oxidant H₂O₂ into hydroxyl radicals OH*, capable of oxidizing strongly and non-selectively organic pollutants [1-4]. The main drawback of this process is perhaps the formation of an iron sludge requiring additional treatment/management costs [1,5,6]. The photo-Fenton process offers a way to improve regeneration of Fe²⁺ by photo-reduction of Fe³⁺ thus increasing the production of OH* by addition of UV light to the treatment [2,4,6]. The efficiency of the Fenton processes is mainly ruled by the operation conditions, namely the oxidant and catalyst concentrations, pH, temperature, and reaction time [3]. On the other hand, the best experimental conditions to treat any wastewater depend mainly on the characteristics of that wastewater [4].

This work aimed to optimize the conventional Fenton and photo-Fenton processes for AOX removal from a real PP wastewater. Since AOX appears essentially in pulp bleaching operations, this work focused on AOX removal from that stream only, before its mixing with other wastewater streams. Despite focusing on AOX removal, “side-effects” from Fenton/photo-Fenton treatment were assessed in terms of chemical oxygen demand (COD) and colour removal.

Methods
Bleaching wastewater samples were provided by a Portuguese PP industry producing bleached eucalyptus Kraft pulp in a 4-stage bleaching process – D₀EpD₁D₂. The wastewater was collected directly from the industrial plant, after the D₀ bleaching stage. Central composite experimental design (CCED) was used to plan the experiments, which allows assessing the conditions that will yield the most desirable response without performing unnecessary experiments, thus minimizing resource consumption and costs [4]. In this work, the response under study was AOX removal from the D₀ bleaching stream. The Fenton and photo-Fenton operational conditions optimized were [H₂O₂], [Fe²⁺] and treatment time. The pH and temperature were fixed at the original values of D₀ wastewater (pH = 2-3 and T = 60°C). Experiments were performed in a quartz reactor equipped with a 150W UV lamp (real irradiance of 142 W.m⁻²) using 0.5 L of D₀ wastewater in each experiment. After reaching the desired temperature, H₂O₂ and Fe²⁺ were added to the wastewater to initiate Fenton process. In the photo-Fenton experiments, the UV lamp was turned on immediately after reagents addition. Samples were taken from the reactor at pre-established reaction times for AOX (EN 16166:2012, ISO 9562:2004 and EPA Method 1650C), COD (Standard Methods for Examination of Water and Wastewater Protocol) and colour determination (ISO 7887:2015).

Results and Discussion
The effect of the Fenton process variables on AOX removal from D₀ wastewater is depicted in Figure 1. Maximum AOX removal around 90% was reached for [H₂O₂] ≥ 140 mM. It seems that [Fe²⁺] only influenced AOX removal for [H₂O₂] ≤ 70-80 mM. Above that value, AOX removal increased mainly with increasing [H₂O₂], which was in fact the only statistically significant variable (p<0.01). This is not an unprecedented result for different substrates degraded by this method [1,2]. Reaction time showed little effect on AOX removal for values above 10 minutes (p≥0.45). Moreover, Figure 1(b) shows that for reaction times below 30 min, increasing [H₂O₂] clearly promoted higher AOX removal, from values below 30% to maximum around 80-85% for [Fe²⁺] of 4 mM. Figure 1(c) shows the effect of [Fe²⁺] on AOX removal at a fixed [H₂O₂] of 137.5 mM; although it seems that [Fe²⁺] between 6 and 12 mM contributed to improve AOX removal, this variable was not statistically significant (p=0.10). Thus, the effect of [Fe²⁺] on AOX removal from D₀ wastewater by Fenton process was somehow inconclusive.

COD removal varied between 15 and 50% for all the experimental conditions tested. This means that any solution adopted for AOX removal by the Fenton process would also potentiate COD removal, which is a positive feature of this method. Maximum removal (~50%) was reached for [H₂O₂] around 140-150 mM (p=0.02). The highest colour intensities were recorded for lower [H₂O₂]/[Fe²⁺] pairs, regardless of reaction time. In this range, corresponding to AOX removal ≤
50%, the colour of D0 wastewater treated would be more than three times worse (i.e. higher) than before treatment. On the other hand, pairs of high [H₂O₂] and low [Fe²⁺] produced the lowest colour intensities: below 500 mg Pt.L⁻¹, corresponding to more than 60% colour removal.

The effect of the photo-Fenton process variables on AOX removal from D0 wastewater process is shown in Figure 2.

AOX removal by photo-Fenton process was affected mainly by [H₂O₂] (p<0.0001), with higher oxidant concentrations leading to higher AOX removal from D0 wastewater. Maximum AOX removal (≈ 90%) was obtained for [H₂O₂] ≥ 100 mM, regardless of [Fe²⁺], which means that similar AOX removal may be attained by the photo-Fenton process with lower amount of added chemicals, when comparing to the Fenton process: e.g. for ≈ 90% AOX removal, [H₂O₂] ≥ 140 mM; [Fe³⁺] ≥ 4 mM (see Figure 1).

The removal of COD observed in these experiments increased with increasing [H₂O₂] (p<0.01), for all conditions of [Fe²⁺] and reaction times tested. Maximum removal was about 60%, recorded for: (a) [H₂O₂] between 140 and 200 mM and [Fe³⁺] ≤ 8 mM, for t=10 min; and (b) [H₂O₂] between 140 and 200 mM [Fe³⁺] = 4 mM for t ≥ 10 min. In both cases AOX removal was above 85%. Ensuring a minimum reaction time of 10 minutes was enough for satisfactory colour removal from the bleaching wastewater. At the optimum operational condition for AOX removal, colour reduction of at least 40% could be attained.

Conclusions
Both Fenton and photo-Fenton processes proved suitable for AOX removal from PP D0 bleaching wastewater. Removal efficiency, depending mainly on oxidant concentration ([H₂O₂] was the only statistically significant variable at 95% confidence level). For the Fenton process maximum AOX removal (85%) was attained for [H₂O₂] = 177 mM, [Fe³⁺] = 8.5 mM and t=10 min, at pH=2-3 and T = 60 °C. At these conditions, COD and colour removal ranged 30-40% and 10-20% respectively. The photo-Fenton process was more efficient than Fenton, achieving higher AOX removal efficiencies with lower chemicals' consumption. For this method, maximum AOX removal registered was 95%, for [H₂O₂] = 178 mM, [Fe³⁺] = 2.0 mM and t=10 min, for UV irradiance of 142 W.m⁻², pH=2-3 and T = 60 °C. The corresponding COD and colour removal were ≤ 50% and 40-60%, respectively.

Acknowledgements
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References
Photocatalytic reduction of bromate in fresh waters using a static mixer as photocatalyst support

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The current study focuses on the photocatalytic reduction of bromate (BrO\textsuperscript{3-}) in fresh waters using a tubular photoreactor containing a Kenics\textsuperscript{®} static mixer (SM) coated with a TiO\textsubscript{2}-P25 thin film. Two fresh water samples from a drinking water treatment plant (DWTTP) were used: one collected downstream ozonation process (DWO) and the other collected downstream ozonation followed by flocculation and flotation/filtration (DWF). BrO\textsuperscript{3-} reduction rates were assessed for both samples and further compared with a synthetic water prepared with ultrapure water (UPW) at different pH values. Furthermore, the effect of humic acids (HAs) on BrO\textsuperscript{3-} reduction was evaluated. Overall, reaction rates using DWF were higher than the ones obtained for DWO and UPW only. Carbonate species present in higher contents enhance BrO\textsuperscript{3-} reduction. Addition of organic matter in the form of HAs proved to improve the photocatalytic reduction of BrO\textsuperscript{3-}. The highest BrO\textsuperscript{3-} reduction rate was attained for UPW spiked with HAs.

Introduction

Bromate (BrO\textsuperscript{3-}) is a by-product compound that commonly occurs as a result of oxidation processes applied to the treatment of waters containing bromide (Br\textsuperscript{-}), such as ozonation. As a result, it can be present in drinking waters (DWS) and, because it is considered possibly carcinogenic to humans, a maximum value of 10 µg L\textsuperscript{-1} in these waters has been set by various entities [1-2]. Therefore, advanced methods to reduce BrO\textsuperscript{3-} levels in DWS are required.

The photocatalytic reduction of BrO\textsuperscript{3-} to Br\textsuperscript{-} is one way to reduce its levels. It is initiated with the illumination of the photocatalyst (PCT) with photons with energy equal or greater than its band-gap energy, which leads to the excitation of electrons (e\textsuperscript{-}) that migrate from the filled valence band (VB) to the empty conduction band (CB), creating a positive hole (h\textsuperscript{+}) in the VB. This generates an electron/hole (e\textsuperscript{-}/h\textsuperscript{+}) pair that migrates to the surface of the PCT and undergoes redox reactions with substrates [3].

In a previous work reported by our research group, BrO\textsuperscript{3-} reduction was successfully achieved for a synthetic water (200 µg BrO\textsuperscript{3-} L\textsuperscript{-1} solution prepared with ultrapure water, UPW) using a tubular photoreactor containing a Kenics\textsuperscript{®} static mixer (SM) coated with a TiO\textsubscript{2}-P25 thin film. However, the photocatalytic process may be affected by the water matrix, such as inorganic constituents (sulfates, nitrates, etc.), natural inorganic (carbonate species – CO\textsubscript{3}\textsuperscript{2-}, HCO\textsubscript{3}-) and organic matter (humic and fulvic acids), pH, among others [4,5].

Objectives

This study focuses on the photocatalytic reduction of BrO\textsuperscript{3-} in fresh waters (200 µg L\textsuperscript{-1} = 1.56 µM) from a drinking water treatment plant (DWTTP) using a tubular photoreactor containing a Kenics\textsuperscript{®} SM coated with a TiO\textsubscript{2}-P25 thin film. Two fresh water samples from the DWTTP were applied: one collected downstream ozonation process (DWO) and the other collected downstream ozonation followed by flocculation and flotation/filtration (DWF). BrO\textsuperscript{3-} reduction rates were evaluated at different pH values using the two fresh waters and a synthetic solution prepared with UPW. Moreover, the effect of humic acids (HAs) on BrO\textsuperscript{3-} reduction rate was evaluated using the synthetic water.

Methods

Water samples were collected from a DWTTP located near Porto, Portugal, in July 2018. The DWTTP included the following treatment line: filtration (pre-treatment), ozonation (pre-oxidation), flocculation, flotation/filtration, and chlorination (final disinfection). UPW was obtained from a Millipore\textsuperscript{®} Direct-Q system (18.2 MΩ cm resistivity at 25 °C). Total dissolved carbon (TDC) and dissolved inorganic carbon (DIC) were determined in a Shimadzu TOC-VCSN analyzer and the dissolved organic carbon (DOC) was calculated by the difference between these two parameters.

Commercial HAs from Sigma-Aldrich were added to UPW at a set concentration of 3.8 mg L\textsuperscript{-1} (average value for the collected DW).

The Kenics\textsuperscript{®} SM was made of plain polished 304 stainless steel, composed of 66-74% of iron, 18-20% of chromium, 8-10% of nickel, among other minor constituents. The experimental unit was mainly comprised by: (i) a jets photoreactor of 0.271 L containing the SM, (ii) a 1.6 L capacity recirculation cylindrical glass vessel thermostatically controlled and magnetically stirred, (iii) a solar radiation simulator (Suntest XLS from Atlas) equipped with a 1700 W air cooled xenon arc lamp at the top, and (iv) a compound parabolic collector (CPC) placed below the jets photoreactor. This unit is detailed described in Dicz et al. [6].

Before TiO\textsubscript{2} deposition, the SM was subjected to: (i) sandblast abrasion, (ii) impurities removal, and (iii) thermal treatment. The thermal treatment was performed in a furnace using a temperature ramp rate of 2 °C min\textsuperscript{-1} until it reached 500 °C and this temperature was maintained for 4 h. Afterwards, the furnace was cooled down up to reach room temperature. 6 layers of TiO\textsubscript{2} were deposited by dip coating using a TiO\textsubscript{2}-P25 aqueous suspension of 2% (w/v). An automatic dip coating unit was employed using a speed of insertion and drawing of 50 mm min\textsuperscript{-1} and a dipping time of 30 s. After each dip, the SM was dried at room temperature for ca. 3 min and at 60 °C for 30 min.

BrO\textsuperscript{3-} and Br\textsuperscript{-} were quantified by ion chromatography. Under the operational conditions used, almost all the added BrO\textsuperscript{3-} was converted to Br\textsuperscript{-} with no identification of intermediary species.

Results and Discussion

The influence of solution pH (5.5, 6.5 and natural water pH, ca. 7.5) on BrO\textsuperscript{3-} reduction was assessed for DWF, DWO and UPW (Figure 1). Higher BrO\textsuperscript{3-} reduction rates were achieved for more acidic pH values. This can be mainly ascribed to the TiO\textsubscript{2}-P25 surface charge at the different pH values – the point of zero charge (PZC) of TiO\textsubscript{2}-P25 is 6.2 [7]. The catalyst surface is negatively charged at pH 6.5 and natural pH.
occurring an electrostatic repulsion between the TiO$_2$-P25 nanoparticles and BrO$_3^-$, affecting negatively the reaction rate.

At pH 5.5, the catalyst surface was positively charged, favoring the adsorption of BrO$_3^-$ on the catalyst surface and enhancing the reaction rates.

Lower BrO$_3^-$ photocatalytic reduction rates were observed for DW at natural water pH when compared with UPW. This can be explained by the presence in DW of other compounds that compete for the PCT adsorption sites, decreasing the reaction kinetics [4]. Similar findings were attained at pH 5.5, for which BrO$_3^-$ reduction presented higher rates in UPW than in DWO. Higher BrO$_3^-$ reductions were attained for DWF at pH 6.5 and 5.5 when compared with DWO and UPW. This can be associated with the higher amount of DIC present in DWF. Bicarbonates can react with hydroxyl radicals (HO*) or h$^+$, generating species with a redox potential negative enough to reduce BrO$_3^-$ to Br$^-$ [8], enhancing the photocatalytic reduction rate.

Adding HAs at pH 5.5 positively influenced BrO$_3^-$ reduction as a result of its action as organic sacrificial agent. HAs reactive functional groups could react with h$^+$ or HO*, improving the photocatalytic process by reducing e$^-$/h$^+$ recombination and avoiding Br$^-$ oxidation to BrO$_3^-$ [9]. It is also important to notice that the presence of HAs alone improves BrO$_3^-$ reduction reaction more favourably than its occurrence with fresh water matrices. This is possibly due to the presence of other species in DW that compete with BrO$_3^-$ for the adsorption sites on the catalyst surface.

Conclusions
A Kenics® SM was successfully applied as a TiO$_2$-P25 support for the photocatalytic reduction of BrO$_3^-$ in DWs. More alkaline pH values affected negatively the photocatalytic process. Higher amounts of inorganic carbon, mainly bicarbonates, enhanced BrO$_3^-$ reduction rates. The addition of organic matter in the form of HAs proved to enhance the photocatalytic process, for which the highest BrO$_3^-$ reduction was attained.

Figure 1. Influence of water matrix on BrO$_3^-$ photocatalytic reduction rate for various pH values: natural pH (a), pH 6.5 (b), pH 5.5 (c). DWF (■), DWO (○), UPW (▲), HAs (▲). Conditions: [BrO$_3^-$]: 1.56 μM; SM pre-treatment: thermal; SM position during dip coating: vertical; Number of TiO$_2$-P25 layers: 6; T: 25 °C; [DO]: 212-239 μM; Q: 50 L h$^{-1}$.

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References
**The effect of Ni-Ru supported catalyst in the sodium borohydride catalytic hydrolysis for hydrogen generation.**


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Climate changes and fossil fuel depletion are the main reasons leading to hydrogen technology. Since early 2000’s the chemical hydrides, in particular sodium borohydride (NaBH₄), hydrolysis reaction attracted much attention as a hydrogen carrier due to its high hydrogen storage capacity (10.8%), safe handling and fast kinetics of H₂ release. Despite the many efforts dedicated to understanding and increase the hydrolysis reaction efficiency through the research and development of a suitable catalyst, there are some issues that need to be addressed. The performance of Ni-Ru supported catalysts in the NaBH₄ hydrolysis was studied both in stabilized hydrolysis (x = 16) and alkali-free hydrolysis (x = 2) reactions. The results present high hydrogen generation rates, at uncontrolled room temperature, that compete directly with the rates previously obtained with a Ni-Ru unsupported catalyst, which are advantages to portable applications.

**Introduction**

Climate change is becoming more remarkable each day and it is the major environmental threat of the 21st century. The increase of CO₂ and other greenhouse gases in the atmosphere, due to the exponential use of fossil resources together with a massive exploitation of fossil fuels led to a demand for clean and abundant energy.[1] Therefore, as an alternative energy carrier, hydrogen can be applied in fuel cell systems for powerful portable supplies. The chemical energy per mass of hydrogen (142 MJ kg⁻¹) is at least three times higher than other chemical fuels. Although hydrogen is the most abundant element on Earth, only less than 1% is presented as molecular hydrogen gas.[2] Nevertheless there are many processes for hydrogen production from both conventional and alternative energy resources. Bearing in mind a hydrogen releasing on demand, chemical hydrides are pointed as the chosen hydrogen carrier since they exhibit an impressive hydrogen content and fast kinetics of hydrogen release. Sodium borohydride (NaBH₄), with a hydrogen content of 10.8 wt%, is the finest example. Through a simple hydrolysis reaction it is possible to produce pure molecular hydrogen adding stoichiometric amount of water to NaBH₄, as shown in Eq.1, where x represents the hydration factor.[3]

\[
\text{NaBH}_4 + (2 + x)\text{H}_2\text{O} \rightarrow \text{NaBO}_2 \cdot x\text{H}_2\text{O} + 4\text{H}_2 + \text{heat} \quad \text{Eq.1}
\]

The hydrolysis of NaBH₄ is a spontaneous, exothermic reaction (-219 kJ mol⁻¹) that can be accelerated due to a well-chose metal based catalyst.[4] Despite the many efforts dedicated to understand and increase the hydrolysis reaction efficiency through the research and development of a suitable catalyst, the kinetics of NaBH₄ hydrolysis reaction is not fully understood. Ruthenium based catalysts appears to be relevant to achieve highest hydrogen generation rates and gravimetric storage capacities in NaBH₄ hydrolysis. Moreover, catalysts with ruthenium present a high reuse property, which is economically significant.[5]

Previously, the group reported for the first time an innovator portable batch mini-reactor with an ovoid geometry to generate and storage hydrogen through the catalytic hydrolysis of NaBH₄ using a Ni-Ru unsupported catalyst. The obtained results gave emphasis to the aptitude of the catalyst to be reused during successive loads of fuel without any treatment and minor loss of activity.[3] Thus, three Ni-Ru foams, with different ruthenium concentrations, were developed to study the influence of Ni-Ru supported catalysts in the catalytic hydrolysis of NaBH₄. Several conditions were studied, and the results obtained – reaction yield, hydrogen generation rate and storage capacities – were compared with the results previously obtained using a Ni-Ru unsupported catalyst.

**Objectives**

The main goal of the work is to study the influence of Ni-Ru supported catalysts in the catalytic hydrolysis of NaBH₄ using a mini-reactor with an ovoid geometry. Three different Ni-Ru supported catalysts, with different ruthenium concentrations, were developed and several experiments were carried out, at moderate pressure and room temperature, and aimed to assess the hydrogen storage capacities (gravimetric and volumetric), the reaction yield and the hydrogen generation rate.

**Methods**

Three Ni-Ru supported catalysts, with the specifications presented in Table 1, were synthetized by the National Laboratory of Energy and Geology (LNEG). These catalysts were used to performed experiments of NaBH₄ stabilized hydrolysis in the presence of an inhibitor (x = 16) and alkali-free hydrolysis with stoichiometric amount of water (x = 2). The experiments were carried out in an ovoid mini-reactor (9 cm³) with a catalyst/NaBH₄ mass ratio range from 0.01 to 0.4 g/g at uncontrolled room temperature. Furthermore, the results were compared with the results previously obtained for NaBH₄ catalytic hydrolysis using a Ni-Ru unsupported catalyst.

**Results**

The hydrogen generation curves for the hydrogen generation rates in function of time for the experiments with x = 16 and x = 2 are presented in Figure 1. The results reveal that Ni-Ru supported catalysts showed a great performance. When compared to the results obtained with the unsupported catalyst, for the stabilized hydrolysis studies, the supported catalysts
present not only similar reaction yields but also hydrogen generation rates in the same order of magnitude of the results previously obtained with a Ni-Ru unsupported catalyst at 50°C. These results stand out since the issue related with energy consumption may be overcome. Moreover, it is noteworthy that the experiments with ESPRU 3, which presented higher hydrogen generation rates (5.33 L min⁻¹ gcat⁻¹ for x = 16 and 0.36 L min⁻¹ gcat⁻¹ for x = 2), were performed with a catalyst/NaBH₄ mass ratio 8 times lower than the experiments carried out with the unsupported catalyst. Nevertheless, it is important to study a method to increase the durability of the supported catalysts since, after 3 experiments, a quick deterioration of the coating in the supported catalysts was observed.

Table 1. Ni-Ru supported catalysts properties.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Support</th>
<th>Amount of catalyst (mg)</th>
<th>Volume (cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ESPRU 1</td>
<td>Nickel foam</td>
<td>2.04</td>
<td>0.21</td>
</tr>
<tr>
<td>ESPRU 2</td>
<td>Nickel foam</td>
<td>0.95</td>
<td>0.22</td>
</tr>
<tr>
<td>ESPRU 3</td>
<td>Nickel foam</td>
<td>0.44</td>
<td>0.22</td>
</tr>
</tbody>
</table>

Conclusions
According with the results of this study, supported catalysts may be the key to improve the hydrogen generation rates in the NaBH₄ hydrolysis reaction.

Acknowledgements
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References
Assignment of hazardous characteristics to wastes: HP14 ecotoxicity

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The distinction between hazardous and non-hazardous wastes is essential for its proper management. In this context, it is crucial to define if a waste displays the hazard property HP 14 ("ecotoxic"), i.e., if it is likely to pose a hazard to the environment. The assessment of HP 14 has been mainly based on the wastes composition. This is due to the lack of guidance for practical approaches that are representative of its behavior in the environment [1]. Currently, it is important to determine which experimental tests should be performed.

In the European countries, coal fly ash (CFA) is the main waste produced in the coal-fired power plants [2] and green liquor dregs (GLD) is one of the main produced in the pulp and paper industry [3]. Moreover, both wastes show typically high pH range: 9-11 for CFA [4] and 10-12 for GLD [5]. In this sense, it seems interesting to assess the hazardousness of these wastes regarding ecotoxicity.

The aim of this study was to assess the suitability of a practical and reliable methodology to evaluate the hazard property HP 14 (ecotoxicity) of wastes. For this purpose, it was intended to evaluate a methodology including not only the chemical analysis but also focusing on a battery of biological tests (biotests) involving different trophic levels.

To perform the chemical analysis, the total elemental composition of the wastes was determined by using XRF and by collecting data from the literature. Each elemental composition was entered in the software ClassifyMyWaste™ (www.ecn.nl/classifymywaste) of ECN (Energy research Centre of the Netherlands). The number of simulations performed was 34 for CFA and 24 for GLD. Finally, a report of each simulation was obtained.

In the case of the biological analysis, five different organisms were used: the bacteria Aliivibrio fischeri, the microalgae Raphidocelis subcapitata, the macrophyte Lemna minor, the microcrustacean Daphnia magna and the plant Lepidium sativum L. In order to test the wastes in the aquatic environment, the eluates were prepared following the European standard EN 12457-2, but the wastes were tested for different liquid to solid (L/S) ratios. In the tests with Lepidium sativum L, the following L/S ratios were tested for both wastes: 4, 5, 10, 25, 50, 100, 200 and 500 L/kg. For the remaining tests the L/S ratios tested are shown in Table 1.

<table>
<thead>
<tr>
<th>Waste</th>
<th>L/S ratios (L/kg) tested in biotests*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFA</td>
<td>4, 5, 12, 64, 8 and 10</td>
</tr>
<tr>
<td>GLD</td>
<td>10, 20, 40, 80, 160 and 320</td>
</tr>
</tbody>
</table>

*excluding Lepidium sativum L.

All the prepared solutions were tested without pH adjustment (around pH 9 for CFA and pH 10 for GLD) and with pH adjustment to around 7 (using HNO₃).

The ecotoxicity assessment using Lepidium sativum L. was based on phytotoxicity tests, using the methodology described by Pinho et al. [6].

For Aliivibrio fischeri, the bioluminescence inhibition was measured. The luminescent bacteria test LCK 480 was performed following the international standard ISO 11348-2. Dr Lange equipment and LUMIStox 300 software was used. In the biotest using Raphidocelis subcapitata the parameters measured were the inhibition in yield and the growth rate inhibition. The adopted methodology was based on the OECD guideline 201 (2006) adapted to 24-well microplates [7], with a duration of 96 h.

In the case of the Lemna minor test, the parameters measured were the inhibition in yield and the growth rate inhibition. The methodology used was the one defined by the OECD guideline 221 (2006) adapted to 6-well microplates [8], with a duration of 7 days.

Finally, in the test using Daphnia magna the parameter observed was its immobilization. This test followed a methodology based on the OECD guideline 202 (2006), with the duration of 48 h. The results obtained through chemical analysis are shown in Figure 1. For CFA, in 76% of the simulations performed in ClassifyMyWaste™ there was “No Hazard” (NH) associated with the waste in relation to HP 14, while 24% of the simulations indicated that there is a “Possible Hazard” (H) in this context.

On the other hand, for GLD 25% of the simulations indicated “No Hazard” regarding ecotoxicity, but in 75% indicated a “Possible Hazard”.

It should be noted that the chemical analysis is carried out considering the “worst case scenario”, which means that the elemental content is in the worst chemical form in the waste.
Therefore, when the "NH" indication appears, there is no need for an additional assessment, while an "H" indication will require a further in-depth assessment of the waste to determine its potential ecotoxicity.

Regarding GLD without pH adjustment, only a L/S ratio of 20 showed inhibition in yield above 50% (57%) in the test using *R. subcapitata*. Growth rate inhibition was under 14%. In the *Lemna minor* test, for the frond number parameter, the inhibition in yield was above 71% and the growth rate inhibition was above 65%. For the dry weight, relatively large inhibition was observed in yield (>81%) and in growth rate (>65%) for L/S ratios of 10, 20 and 40. In the *Daphnia magna* test, significant immobilization was observed for L/S ratios of 10, 20, 40 and 80, ranging from 95% to 100%. Regarding the *Lepidium sativum* test, only in L/S ratios of 300 and 200 germination indexes higher than 60% were obtained, with the remaining ranging from 0% to 22%. In the *Alivibrio fischeri* test, inhibitions were above 50%, excepting for L/S ratios of 40 and 80. Finally, for GLD with pH adjustment, only L/S ratio of 10 promoted inhibition in yield above 50% (60%) in the tests with *R. subcapitata*. Growth rate inhibitions were lower than 16%. In the *Lemna minor* test, in the case of the frond number, yield inhibition was reduced but only L/S ratio of 320 promoted an inhibition lower than 50%. Likewise, there was a reduction in growth rate inhibition with L/S ratios of 320 and 160 resulting in inhibitions lower than 50%. In the case of the dry weight parameter, only L/S ratio of 10 resulted in an inhibition in yield above 50% (59%). Growth rate inhibitions did not exceed 40%. In the *Daphnia magna* test, only the L/S ratios of 10 and 20 induced immobilization values of 100%. In the *Lepidium sativum* test, all germination indexes were below 40%. Regarding *Alivibrio fischeri* test, inhibition was below 50%, except for the ones resulting from L/S ratios of 40 (51% and 49%, at 15 min and 30 min) and 320 (48% and 51%, at 15 min and 30 min).

Even though higher L/S ratios were tested for GLD, this waste exhibited higher inhibition in the tested endpoints, i.e. higher toxicity. This agrees with the results obtained in the chemical analysis. In general, pH adjustment reduces inhibition in the parameters tested. The *A. fischeri* test presented high variability of results. *D. magna* seems to be the most sensitive organism and *R. subcapitata* seems to be the least sensitive one. In general, the battery of biotests chosen may be a good choice to represent aquatic ecosystems to be used in the context of HP 14.

**Acknowledgements**

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**References**


Thermochemical characterization of tars produced in the context of biomass gasification

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Gasification is a well-developed process for the production of energy and fuels but most of the gasifiers are run on coal as their feedstock. In the context of CO2 emissions reduction the direct gasification of biomass is gaining increasing interest but there are still several issues that must be solved for this process to gain wider applicability. One of the major problems in biomass gasification is the production of tars that occurs mainly in the pyrolysis stage inside the gasifier. This work is aimed at analyzing the tars that are formed during the pyrolysis stage of biomass feedstock by thermochemical analysis using simultaneous TG/DSC analysis. The results, albeit preliminary, indicate that the TG/DSC analysis can be used to characterize the tars produced, in particular in what concerns the thermochemical reactivity of the heavier components.

Introduction

Tar formation is a very important issue in biomass gasification and it is important to understand their behavior under gasification conditions. This is a huge concern for a successful application of biomass-derived producer gas, since tar condensates at low temperatures and it can lead to clogging or blockage in fuel lines, and equipment [1].

Tars are considered as the condensable fraction of the gasification product, which includes mostly benzene and high molecular weight aromatic hydrocarbons. These compounds have special characteristics, including their chemical refractory properties which makes difficult to remove them by thermal, catalytic and physical processes. Its formation and composition are highly dependent on operation conditions [2].

Two main concerns about tar content in the final product are their properties and composition, which are related with the condensation behavior and component. Dew point is one of the most the critical parameters since when it is reduced to levels below the lowest expected temperature, fouling related problems by condensation or tar aerosols are solved [3].

This study is aimed at studying the thermochemical conversion of tars derived from thermochemical conversion of biomass. The thermochemical analysis will allow us to develop knowledge about the reactivity of tar compounds, under the reaction conditions to support the optimization of the all process.

Methods

The tars samples used in this work were collected from biological sewage sludge pyrolysis experiments performed in a bench-scale pyrolysis facility equipped with a fixed bed reactor, located at the Department of Environment and Planning at Aveiro’s University (see Table 1 and 2 for the characteristics of biological sludge and for the biomass pyrolysis experiments conditions, respectively). The tars in the product gas from the pyrolysis process were collected in a set of three impingers immersed in cold water bath at 0ºC.

Table 1. Characteristics of biological sludge used in the pyrolysis experiments.

<table>
<thead>
<tr>
<th>Biological Sludge</th>
<th>Humidity (% m/m, bg)</th>
<th>Ashes</th>
<th>C</th>
<th>H</th>
<th>N</th>
<th>S</th>
<th>O (by difference)</th>
<th>Cl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20.0</td>
<td>26.5</td>
<td>36.7</td>
<td>5.0</td>
<td>2.2</td>
<td>0.4</td>
<td>29.0</td>
<td>0.19</td>
</tr>
</tbody>
</table>

The tars collected were analyzed in a TG/DSC apparatus, using a quartz pan. The thermogravimetric analysis were carried out at a heating rate of 10°C/min in nitrogen atmosphere, at a constant flow rate of 54.3 mL/min. The experiments were performed in an SDT 2960 simultaneous DSC/TGA. TA Instruments. The samples were heated up to 800°C and hold for 30 minutes at this temperature. At the end of each run, the oven was cooled.

Results and Discussion

The thermal degradation profile was similar in all the experiments (see Figure 1). At low temperature (from 40ºC to 150ºC) the devolatilization of water and light hydrocarbons is observed, with a weight loss around 70-75%. At higher temperature (from 150ºC to 400ºC) the degradation occurs with almost complete loss of weight. (see Figure 1).

To study samples variation over time, in the sense of its maturation during storage, pyrolysis experiments were performed on sample 3, for different periods since collection. The degradation profile for sample 3 (see Figure 2) have the same shape in all the experiments and much of is weight loss occurs in the first region, about 50-60%.

A detailed analysis of the DSC profile (see Graphic Abstract) shows that DSC peak occurs at 113-121ºC, which corresponds to the first region on the degradation profile (Figure 2). The second region is not associated with a clear DSC peak, since the weight loss is too low (comparing with the first). After 400ºC, no significant changes are observed.

Figure 1. Normalized weight curves for pyrolysis of samples 1 and 2.

It is also observed that as the storage time increases, a larger amount of heat is required to volatilize the material, which indicates the possible presence of heavier compounds.

The results were also analyzed using a dynamic kinetic model. This model allows to estimate the kinetic parameters, pre-
exponential factor and activation energy for the different processes. A pseudo-component model with two components was used with a first order mass decay [4]. The fitting results (see Figure 3) shown that the models describe well the experimental results.

![Figure 2. Normalized weight curves for pyrolysis of sample 3 with distinct times of maturation.](image)

**Conclusions**

The thermogravimetric analysis conducted in this study can provide valuable information on the tar thermochemical behavior. From the TG curves, two different processes were observed: i) devolatilization of water and light hydrocarbons (from 40°C to 150°C); ii) pyrolysis associated with the decomposition of heavier hydrocarbons (from 150°C to 400°C). The study of the aging of the samples’ behavior over time showed that the longer the time, the less the amount of volatile matter and the higher the fraction of heavier compounds. This denotes a possible higher degree of polymerization and, also, an increased maturation of the compounds.

![Figure 3. Comparison of normalized weight curves of experimental and modelling values for sample 1.](image)

<table>
<thead>
<tr>
<th>Table 2. Characteristics of biomass pyrolysis experiments.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Experiments Reference (samples)</strong></td>
</tr>
<tr>
<td>-----------------------------------</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Sample</strong></th>
<th><strong>1st stage weight loss (%)</strong></th>
<th><strong>2nd stage weight loss (%)</strong></th>
<th><strong>Residue (%)</strong></th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>63.58</td>
<td>29.11</td>
<td>1.21</td>
</tr>
<tr>
<td>2</td>
<td>63.57</td>
<td>29.27</td>
<td>2.37</td>
</tr>
<tr>
<td>3 (First experiment)</td>
<td>58.10</td>
<td>41.00</td>
<td>0.42</td>
</tr>
<tr>
<td>3 (After 1 week)</td>
<td>56.15</td>
<td>38.49</td>
<td>1.21</td>
</tr>
<tr>
<td>3 (After 4 weeks)</td>
<td>52.38</td>
<td>40.84</td>
<td>---</td>
</tr>
<tr>
<td>3 (After 20 weeks)</td>
<td>47.28</td>
<td>41.83</td>
<td>3.62</td>
</tr>
</tbody>
</table>

**Table 3. Tar (bio-oil) composition, in terms of percentage of the pseudo-components in samples’ pyrolysis**

References

Performance of a passive and semi-passive direct alcohol fuel cell

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The direct alcohol fuel cells (DAFCs) are a promising technology to replace the conventional batteries in portable applications, since they allow an easy handling, high energy density and are more environmentally friendly. The DAFCs can be categorized in passive, semi-passive and active, according to the reactants feeding system. Passive systems have potential to be implemented in portable electronic devices since they do not require additional power consumption and moving parts. However, active fuel cells offer better performances. Semi-passive DAFCs can combine the best characteristics of both active and passive fuel cells. In this work, the effect of some operational parameters on the performance of a passive and a semi-passive DAFC was evaluated, towards its optimization. The methods used for this purpose were the polarization and electrochemical impedance spectroscopy (EIS) measurements. The polarization measurements allow the identification of the different losses that negatively affect the DAFC performance, while the EIS ones its quantification.

Introduction

The negative environmental impacts of fossil fuel power sources have received considerable attention in the last decades. Fuel cells appear as an alternative and promising power solution due to their efficiency, simplicity, low environmental impacts and quick refueling. Among them, the direct alcohol fuel cells (DAFCs) convert the chemical energy of an alcohol directly into electrical energy and are easy to handle and refueling [1,2]. The disadvantages of using DAFCs are the slow anode kinetics, resulting in higher anodic overpotentials and the fuel crossover, which not only lowers the fuel utilization but also degrades the cathode performance and generates extra heat. Up to a few years ago, methanol was the most used fuel and much progress was made in the development and optimization of DAFCs feed with methanol. However, methanol is toxic for human beings and is nonrenewable. Among the other alcohols available, ethanol appears as an attractive and promising fuel since it is non-toxic, has a high power density and is easily produced in large quantity via fermentation of biomass. Ethanol is a green, sustainable and carbon-neutral fuel. However, the performance of a DAFC is better with methanol, based on the slow electrochemical ethanol oxidation reaction, due to the difficulty in breaking the C-C bond that is presented on its structure.

Concerning the different alcohol and oxidant delivery systems, the DAFCs can be classified as active, semi-passive and passive. In active systems, the reactants are supplied using pumps and fans and so, they require energy to operate and additional components. The transport of fuel and oxidant in passive systems occurs by diffusion and natural convection. Therefore, a passive DAFC has a simple structure and is more suitable for portable applications. In a semi-passive DAFC, which is a combination of active and passive modes, one side operates actively and the other passively [3,4].

In the present work, the effect of different operating parameters on the performance of a passive and semi-passive DAFC was studied. In the semi-passive cell two situations were analyzed: alcohol actively supplied to the anode, while air is passively fed to the cathode and active supply of air to the cathode, and a passive supply of fuel, through an alcohol reservoir build on the anode side. The cell performance was evaluated by the polarization curves and the electrochemical impedance spectroscopy (EIS) data.

EIS is a complementary diagnostic tool that is used to analyze the fuel cell performance since it allows to identify and quantify the individual contributions of each component and process that occur in a working fuel cell. More specifically, it allows the quantification of the three major losses that negatively affect the DAFC performance (activation, charge and mass transfer losses) [4].

Methods

The passive and semi-passive DAFC, with an active area of 25 cm², were operated at ambient conditions (ambient pressure and temperature). The passive DAFC has two acrylic end plates, two isolating rubber plates, two connector plates (with 36 holes with a diameter of 6 mm), two diffusion layers (carbon cloth with a thickness of 0.400 mm), two catalyst layers (Pt-Ru at the anode and Pt at the cathode) and a Nafion 117 membrane. The semi-passive DAFC has a similar structure than the passive cell, however, the active side has an aluminum end plate and a monopolar graphite plate with a serpentine flow field. The serpentine flow field was selected following the generally accepted idea that this design results in a better cell performance, since it facilitates the removal of reaction products, such as carbon dioxide on the anode and water on the cathode side, enhancing the two-phase mass transport.

Different alcohol concentrations were tested on the passive DAFC in order to study its effect on the cell performance, towards its optimization. The influence of the feed rate on both anode and cathode sides was evaluated on the semi-passive DAFC.

The experimental tests were performed with a commercial fuel cell test station (Zahner Elektrik GmbH &Co. KG). The polarization curves were performed galvanostatically and the EIS measurements were carried out potentiostatically with an amplitude of 10 mV and a frequency range of 0.01 Hz to 100 kHz. The EIS experimental results were fitted to an equivalent electric circuit (Figure 1), that consists on an inductance (L1) linked in series with a resistance (R1) and with three other circuits, each one involving a resistance (R2 to R4) in parallel with a constant phase element (CPE1 to CPE3).

Figure 1. Equivalent electric circuit.
The inductive behavior is due to the adsorption of different species at the catalyst surface and the CPEs are associated with a non-uniform distribution of the current and a non-homogeneity of the reaction rates at the electrode surface. In Figure 1, R1 represents the ohmic resistance, R2, R3 and R4 the anode and cathode resistances.

Acknowledgements
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References
Gasohol direct production for energy-efficient bioethanol downstream processing

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This work addresses the production of gasohol using gasoline as a separation agent, corresponding to an alternative approach to processes that recover and dehydrate ethanol before this is mixed with gasoline. Experimental data was used to build the thermodynamic modeling framework to adequately describe phase equilibrium and predict thermophysical properties. Two configurations for the direct manufacture of E10 gasohol were designed and analyzed. Process 1 is extraction-based whereas Process 2 is extractive distillation-based. Energy requirements were compared against each other and with the most commonly used commercial process where ethanol dehydration is achieved with molecular sieves (Process MS).

The results show that Process 1 is energetically more favorable, consuming on average less 12% than Process MS, for ethanol concentrations in vinasse between 2 and 10 wt%. On average Process 2 also spends less energy than the commercial Process MS, but now only just 2%.

Introduction

The global economy's dependency on petroleum-based fossil fuels and the environmental consequences thereof have stimulated technological developments regarding the manufacture of biofuels, bioproducts and energy. Gasohol, a gasoline and ethanol blend, is an alternative drop-in fuel marketed for light road transport vehicles. Ethanol is mainly produced via fermentation of carbohydrates from any sugar-, starch- or cellulose-based biomass within some hierarchical structure of processes (biorefinery), whereas gasoline is obtained from petroleum fractionation in a conventional oil refinery.

The conventional downstream processing of ethanol includes the removal of the solid fraction and secondary products in the vinasse (spent broth), a pre-concentration step, and its dehydrogenation to prevent phase separation in fuel systems, known to cause engine malfunctions. This is an energy intensive process, complicated by the ethanol-water azeotrope that limits the process' economic performance. Various configurations for ethanol recovery and dehydrogenation appear described in the literature, and are reviewed elsewhere [1,2]. A double distillation column arrangement for the pre-concentration and a molecular sieve system for ethanol dehydration (Process MS) corresponds to the state-of-art process for ethanol downstream processing [1].

However, the majority of the existing technology is designed to produce anhydrous ethanol, which may not be the best solution, especially if gasoline may be blended on-site, or if the integration with a petrochemical plant is possible. Consequently, it is important to explore configurations where gasoline is incorporated as a separating agent, leaving the process already as gasohol, not requiring gasoline recovery and potentially saving energy compared with other distillation-based methods. Some works describe the direct use of gasoline in ethanol downstream processing. Leeper and Wankat [3] proposed a design here denoted as Process 1, (Figure 1) with a pre-concentration step up to near azeotropic concentration (~90 wt%), followed by an extraction column, with the gasohol leaving the extract and the raffinate is sent to a gasoline recovery distillation column.

Black [4] suggested a process configuration, Process 2, where the extraction column is replaced by an extractive distillation, where the product leaves in the bottoms and the distillate is decanted (Figure 3). The aqueous phase enters a recovery column, to remove water and recycle the ethanol and gasoline. Since the organic phase that leaves the decanter is essentially gasoline and ethanol (stream 111), is mixed with the product.

Figure 1. Simplified process flowsheet diagram of Process 1.

Objectives

This work addresses the direct production of gasohol employing gasoline as a solvent. Experimental data was collected and fitted into thermodynamic models to adequately describe the phase equilibrium. A model validation was done by comparing the simulation results with others available in the literature. Finally, the optimal operating conditions for Processes 1 and 2 were determined and pinch analysis was used to design the respective heat exchanger networks. Both processes were compared against each other and with Process MS in terms of energy consumption.

Scope and Methodology

The scope of analysis was the downstream processing of ethanol after the fermentation assuming a simplified vinasse composition, i.e., with the secondary products and solids previously removed. Therefore, the components involved are water, ethanol, and gasoline. The latter is modeled as a pseudo-component with the physical-chemical properties estimated from a distillation-curve of reference. Phase equilibrium is modeled considering the vapor as an ideal gas and the liquid phase is modeled with NRTL model. VLE and enthalpy of excess data of the binary ethanol-gasoline, and water-ethanol-
gasoline LLE ternary data from various works were regressed simultaneously using a similar approach to the one described in [5]. Finally, a continuous processing plant with an annual capacity of 230 kton of ethanol (dry mass) was considered.

Results
The Graphical Abstract shows the ternary phase diagram of the water-ethanol-gasoline system (in weight percentage), where experimental tie-lines are represented by black lines with empty circles and the predicted ones are represented by orange lines with filled disks. A good agreement between the experimental and model predicted tie-lines is observed, with an ARD in the compounds’ mass fractions of 0.7%. For the total vapor pressure and the enthalpy of excess of the binary ethanol-gasoline system, the ARDs were approximately 10% and 34%, respectively. To validate the model, the flowsheets described in [3,4,6] were simulated and the results were reconciled with the original results, using Aspen Plus V10. Generally, a good agreement between our simulations and those reported for similar processes was observed; relative deviations in the mass fractions and mass flows are typically around 5%, as shown in Table 1.

Table 1. Model validation to the extraction column from [3].

<table>
<thead>
<tr>
<th>Compound</th>
<th>Extract (mass fractions)</th>
<th>Raffinate stream compositions</th>
<th>Reported RD (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Ethanol</td>
<td>H2O</td>
<td>0.105 0.116 9.5</td>
</tr>
<tr>
<td></td>
<td>Gasoline</td>
<td></td>
<td>0.887 0.874 1.5</td>
</tr>
<tr>
<td></td>
<td>Ethanol</td>
<td>Water</td>
<td>0.659 0.630 4.4</td>
</tr>
<tr>
<td></td>
<td>Water</td>
<td></td>
<td>0.181 0.190 4.9</td>
</tr>
<tr>
<td></td>
<td>Gasoline</td>
<td></td>
<td>0.161 0.170 5.4</td>
</tr>
</tbody>
</table>

Figure 2 shows that a double distillation column in the pre-concentration step is more favorable than a single column, since a higher reflux ratio can be used in the second column to achieve the desired final concentration with a lower boilup ratio. It is also observed that Process 1 requires on average 0.9 MJ/kg-ethanol to produce an E10 gasohol from concentrated ethanol, while the Process 2 energy needs are about 1.6 MJ/kg-ethanol. This corresponds to a total reduction of energy of 12% and 2% relatively to Process MS, which is the current benchmark for ethanol recovery and dehydration. This is possible due to heat integration between the pre-concentration distillation columns and the gasoline recovery / extractive distillation columns.

Conclusions
This contribution addressed the direct production of gasohol, using gasoline as a solvent. It was demonstrated that these alternative processes can be competitive when gasoline can blended on-site. Relatively to the reference process solution, the extra capital costs are also limited, since the retrofit of existing distillation facilities requires very few modifications.

Acknowledgements
The authors acknowledge financial support provided for the post-doctoral grant of José F.O. Granjo through the project POCI-01-0247-FEDER-021874, and the research project POCI-01-0145-FEDER-016403 for Ângelo Dinis.

References
Improved sorbents for Calcium looping CO$_2$ capture in the cement industry: Increasing sintering resistance using waste resources and steam

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Introduction
The cement industry is one of the world’s largest industrial sources of CO$_2$ emissions, accounting for 5-6% of world’s total anthropogenic CO$_2$ emissions [1]. Among the technologies developed for CO$_2$ capture, Ca-looping is one of the most promising processes for CO$_2$ capture based on the reversible chemical reaction between CaO-based sorbents and CO$_2$ to form CaCO$_3$. Relevant advantages of the Ca-looping technology integration in the cement industry is the use of cement raw matter (CaCO$_3$) to capture CO$_2$ before its incorporation in the clinker, reducing costs and avoiding the production of wastes associated to the carbon capture and storage (CCS), and also the possibility of recycling the exhausted spent sorbents as raw matter for the clinker, fulfilling the circular economy concept. However, avoiding the sorbents deactivation along the carbonation-calcination cycles is still a challenge. The use of synthetic sorbents [2] with high and stable capture capacity along the cycles has been investigated, as well as, the use of marble powder wastes rich in CaCO$_3$ [3]. One alternative approach to delay the sorbent loss of reactivity and increase the stability, consists on the addition of inert materials that will act as structural supports and create a physical barrier that prevents sintering and aggregation of the CaO nanoparticles. With the aim of incorporating the spent sorbents in the clinker production, the use of wastes with a high silica and alumina content as the spent FCC and coal fly ash [4] wastes is an interesting option. Another interesting option to increase the CaO conversion could be the use of steam during the Ca-looping. The steam modifies the morphology of sorbents and the pore structure appears to become more stable. When only CO$_2$ is used during the calcination step, a pure stream of CO$_2$ is obtained (relevant for CO$_2$ applications), however the sorbent deactivation increases due to the use of higher calcination temperatures (>900ºC).

Objectives
The main goal of this work is the enhancement of sintering resistance of CaO-based sorbents using different CaO precursors (commercial CaCO$_3$, natural CaCO$_3$ and Ca(NO$_3$)$_2$·4H$_2$O) supported on two different wastes: coal fly ash (CFA) and spent FCC (SFCC). The second objective is identifying the optimal sorbent/support ratios. The effects of the steam addition and the use of a pure CO$_2$ stream during the calcination step for natural CaCO$_3$ are also evaluated.

Methods
The CaO-precursors were physically dry mixed with different ratios of the support (90/10, 75/25 and 60/40) during 30 min in a ball mill. The CaO conversion along the carbonation-calcination cycles was evaluated in a thermogravimetric analyzer (TGA) for 10 cycles. Firstly, the sorbents were pre-activated at 900ºC under air atmosphere, then the carbonation was carried out at 700ºC (60 min, with 25% of CO$_2$ balanced in air) and calcination was carried out at 800ºC (10 min, air atmosphere). The samples mass changes during the carbonation and calcination steps are due to the CO$_2$ that is captured and released along the cycles. The CaO conversion is estimated by equation 1, where $m$ and $M$ are respectively the mass (g) and the molecular weight (g/mol).

\[
\text{CaO conversion} = \frac{m_{\text{CO}_2} \times M_{\text{CaO}}}{m_{\text{CaO}} \times M_{\text{CaO}} + M_{\text{CO}_2} \times 100} \%
\]  

The reactivity and stability of the commercial and natural CaCO$_3$ and of some selected mixed sorbent samples were also tested in a fixed bed reactor unit. The sorbents were pre-activated in the reactor at 900ºC under air atmosphere until the CO$_2$ release stopped. After this step, the reactor temperature was cooled down to 700ºC and the carbonation step was conducted with a flow of 25% of CO$_2$ balanced in air, until the CO$_2$ output stream composition stabilizes. After the carbonation step the CO$_2$ flow was stopped and the sorbent heated until 800ºC (or 900ºC for a pure CO$_2$ stream) until the CO$_2$ release stopped, to ensure the complete conversion of CaCO$_3$ to CaO. The same procedure was carried out when using 5% of steam. The BET surface area of pre-activated sorbents and after 20 carbonation-calcination cycles was assessed by N$_2$ sorption technique. First the samples were outgassed under vacuum at 90 ºC for 1 h and then at 350 ºC for 3 h. The pore volume (Vp) was calculated...
from the adsorbed volume of nitrogen for a relative pressure (P/P0) of 0.97. The morphology of the sorbents was examined by scanning electron microscopy (SEM) using a JEOL Model 7001 emission gun microscope.

Results
The CaO conversion along the carbonation-calcination cycles was tested for all the sorbents and mixtures on the TGA. As shown on the Graphical Abstract, after 10 cycles, the commercial CaCO3 is the sorbent with higher CaO conversion, followed by the Ca(NO3)2.4H2O and the natural CaCO3. Figure 1 shows comparatively with the natural CaCO3 (b) the commercial CaCO3 (a) presents a more branched and filamentous structure, which can explain the higher CaO conversion. The addition of different proportions of CFA and SFCC increased the CaO conversion of all the sorbents. For the case of Ca(NO3)2.4H2O and of commercial CaCO3 sorbents, the higher improvement was observed for the mixture with 10% of SFCC, but in the case of natural CaCO3 the best results were obtained for the mixture with 25% of CFA.

![SEM images](image)

Figure 1. SEM images: in 1μm scale, (a) fresh commercial CaCO3 and (b) natural CaCO3, in 10μm scale, (c) coal fly ash (CFA) and (d) 75% CaO (natural CaCO3 precursor) + 2% CFA (20 cycles).

As the natural CaCO3 is the most suitable sorbent for the cement industry, it was selected and tested in a fixed bed reactor. As in case of the TGA experiments, the higher CaO conversion was observed for the mixture with 75% CaO + 25 % CFA (Figure 2). The steam addition reduces the CaO conversions on the first three cycles; however, the sorbent is more stable along the cycles. Table 1 presents the S_Bet and V_p of the sorbents after pre-activation and after 20 cycles. The sorbents tested with steam present a higher S_Bet after the 20 cycles, which evidences that the steam reduces the particles sintering leading to a higher stability. On the other hand, the calcination with CO2 at higher temperature contributes for a higher sintering, i.e., the CaO conversion and the S_Bet are lower. The CFA particles apparently do not react with the sorbent (Figure 1.d) and should act as a physical barrier that prevents the sintering.

![Graph](image)

Figure 2. CaO conversion of natural CaCO3 sorbent during the carbonation-calcination cycles carried out in a fixed bed unit

Conclusions
The work presented shows that the use of waste resources is a promising approach to increase the reactivity and the sintering resistance of CaO-based sorbents for Ca-looping CO2 capture, besides it has economic and environmental advantages. Synergies between the mixed sorbents, steam and CO2 calcination atmosphere should be evaluated in future work.

Table 1. Specific surface area (S_Bet, g/m²) and total pore volume (V_p, cm³/g) of natural CaCO3 sorbent tested in the fixed bed unit after the pre-activation (0 cy) and 20 carbonation-calcination cycles (20 cy)

<table>
<thead>
<tr>
<th></th>
<th>CaCO3 natural</th>
<th>90% CaO+10% CFA</th>
<th>75% CaO+25% CFA</th>
<th>5% steam CO2 calcination</th>
<th>Coal Fly Ash</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>S_Bet</td>
<td>20 cy</td>
<td>0 cy</td>
<td>20 cy</td>
<td>0 cy</td>
</tr>
<tr>
<td></td>
<td>31</td>
<td>10</td>
<td>21</td>
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<td>3</td>
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<tr>
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<td>0.06</td>
<td>0.09</td>
<td>0.05</td>
<td>0.07</td>
</tr>
</tbody>
</table>

Acknowledgements
The authors gratefully acknowledge the financial support by the Portuguese Foundation for Science and Technology through Research Project Carbon Emissions Reduction in the Cement Industry (PTDC/AAG-MAA/6195/2014) and the postdoctoral fellowship (SFRH/BPD/91397/2012).

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On the efficacy of single and catalytic ozonation using volcanic rock in mitigating the ecotoxicity of a parabens mixture

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Parabens are widely used as antimicrobial and preservative compounds in pharmaceutical and personal care products. Some studies relate these compounds with the onset of human breast cancer [1] and their estrogenic activity, although weak, was already shown [2]. In fact, parabens were already detected in human urine, milk and serum [3]. In this context, these substances have also been quantified at the micro- and nanogram per liter range in wastewater treatment plant and surface waters worldwide.

On the one hand, the traditional municipal wastewater treatments are not totally effective on the removal of these biorefractory compounds, which makes their discharge into natural waterbodies more likely to occur, threatening aquatic ecosystems. On the other hand, drinking water treatment facilities using contaminated waters may not totally deplete these substances. Bearing in mind that freshwater scarcity is nowadays more present than ever, the seek for alternative water sources such as recycled water increases. Even though the uses for recycled water can be restricted, it has the potential to supply e.g. agriculture, which highlights the significance of improving treatment to ensure a non-toxic profile of this resource.

The positive relationship between alkyl chain length and the aquatic toxicity of parabens has been generally confirmed in the previous studies. These studies addressed the single toxicity of parabens, but it is worth stressing out that these compounds are usually used as mixtures to increase their preservative spectrum, thus analysis of the toxicity of parabens blends is necessary. Accounting to the increasing presence of these contaminants in natural waterbodies and their hazardous potential, research has been focusing on the development of methodologies for their removal from wastewater matrices before discharge. Ozone is a powerful oxidant often used to promote the degradation of nucleophilic and high electronic density species such as parabens Normally, heterogeneous catalysts can be used to enhance ozonation. The selection of the catalyst must be carried out by looking at the cost production, the stability and performance. Low cost materials such as Volcanic rock were already proven efficient for parabens degradation [4].

Still, most of the studies addressing the development of treatment processes targeting parabens degradation do not analyze the mitigation in the ecotoxicity of the water through the degradation process, although in some cases toxicity levels are analyzed at the end of the treatment process. An appraisal on the ecotoxicity of the treated samples through the treatment time would make it possible to more accurately select the earliest point where the degradation was long enough to significantly reduce toxicity to negligible levels, thus reducing treatment length and costs.

The aim of the present study was to compare single and catalytic (using Volcanic rock) ozonation as to their effectiveness in reducing the ecotoxicity of a mixture of 5 parabens (Methyl, Ethyl, Propyl, Butyl and Benzylparaben). We aimed assessing the levels of toxicity throughout the degradation process, thus providing information about the toxicity of the parent compounds and byproducts formed along the reaction. The applied ecotoxicological battery was designed to properly reflect potential impacts over aquatic ecosystems, thus included species representing different functional levels: the green microalga Raphidocelis subcapitata, the macrophyte Lemna minor, the cress Lepidium sativum (plant) the cladoceran zooplankter Daphnia magna, the benthic bivalve Corbicula fluminea.

A mixture of these parabens (10 mg/L each) was treated by single and catalytic ozonation using different transferred ozone doses. Treated solutions, comprising different concentrations of parabens and by-products were chemically analysed and underwent ecotoxicity assessment. As an attempt to isolate the effects of treatment by-products, the ecotoxicity of these treated solutions was compared with that of several direct geometric dilutions from the initial mixture of parabens (10, 7.5, 5, 2.5, 1.25, 0.625 mg/L each paraben).

A 2-L reactor magnetically stirred at 700 rpm was used in the single and catalytic ozonation experiments, under controlled temperature conditions (25 ± 1°C) achieved with a thermostatic bath. The volcanic rock used as catalyst was collected in São Miguel (Azores, Portugal). A comprehensive characterization
of this catalyst can be found in a previous study [4], with the presence of silica and aluminium as being the main features, augite and diopside as main minerals, and a specific surface area of 28.3 m²/g. The adsorption of parabens to the volcanic rocks surface was previously found irrelevant (Gomes et al., 2018). The amount of catalyst used for different experiments was fixed at 0.5 g/L.

The concentrations of the 5 parabens and degradation intermediates (4-HBA, 2,4 and 3,4-di HBA, 3,4-diMeBA, hydroquinone and 1,4-benzoquinone) were analytically determined for different untreated and treated solutions by HPLC. Chemical oxygen demand (COD) and Total organic carbon (TOC) were analysed.

The samples treated with single ozonation were obtained for TOD values of 6, 18, 78, 123, 170 mg/L. On the other hand, the samples used on ecotoxicity tests from catalytic ozonation were obtained for TOD values of 3, 9, 29, 42, 55 mg/L. These TOD values correspond in both cases respectively to 5, 15, 60, 90, 120 minutes of reaction time. The pH of all samples was adjusted to values within the range 6.5 - 7.5 to mitigate the interference of different pH levels in the tests outcome.

The results achieved show (Figure 1 and Table 1) that the interaction between degradation by-products and initial parabens produces different effects over the wide range of the species tested. The toxicity observed for the treated samples was related with the intermediates produced and, according to literature, the most problematic among the identified ones are hydroquinone and 1,4- benzoquinone. In fact, the treated samples for highest TOD value for both processes (after total parabens removal) present higher toxicity over D. magna (just for catalytic ozonation), R. subcapitata, L. Sativum than a solution containing a parabens mixture with a concentration of 625 µg/L. This means that the by-products generated even at very low concentrations should be more harmful for these species than the original parabens mixture. Meanwhile, no acute toxicity was verified for C. fluminea and L. minor when these species contacted with the final treated samples for both oxidation processes, showing that the sensitivity to pollutants differs from species to species.

![Figure 1.](image)

**Table 1.** D. magna immobilization for single and catalytic ozonation.

<table>
<thead>
<tr>
<th>TOD (mg/L)</th>
<th>D. magna Immobilization (%) (± SD)</th>
<th>Catalytic ozonation TOD (mg/L)</th>
<th>D. magna Immobilization (%) (± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>100 (± 0)</td>
<td>3</td>
<td>100 (± 0)</td>
</tr>
<tr>
<td>18</td>
<td>100 (± 0)</td>
<td>9</td>
<td>100 (± 0)</td>
</tr>
<tr>
<td>78</td>
<td>100 (± 0)</td>
<td>29</td>
<td>100 (± 0)</td>
</tr>
<tr>
<td>123</td>
<td>100 (± 0)</td>
<td>42</td>
<td>100 (± 0)</td>
</tr>
<tr>
<td>170</td>
<td>0 (± 0)</td>
<td>55</td>
<td>85 (± 7.5)</td>
</tr>
</tbody>
</table>

Acknowledgements
J. Gomes and R. C. Martins gratefully acknowledge Fundação para a Ciência e Tecnologia by the financial support under IFCT2014 programme (IF/00215/2014) with financing from the European Social Fund and the Human Potential Operational Programme. Thanks are due for the financial support to CESAM (UID/AMB/50017 - POCI-01-0145-FEDER-007638), to FCT/MCTES through national funds (PIDDAC), and the co-funding by the FEDER, within the PT2020 Partnership Agreement and Compete 2020. J. L. Pereira is recipient of an individual post-doctoral grant by FCT (SFRH/BPD/101971/2014).

References
Bromate removal by heterogeneous photocatalysis in a drinking water treatment plant


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The main goal of this study was to evaluate the removal of bromates from drinking water by heterogeneous TiO$_2$ photocatalysis using a multi-photoreactor based on the NETmix technology. The channels and chambers of the multi-photoreactor were coated with a TiO$_2$ thin film by a spray deposition technique. The catalyst surface was illuminated by UVA-LEDs. Water samples from a water treatment plant (WTP), after ozonation (OW) and after ozonation+flocculation+filtration (FW), were used as reaction matrices. Bromate reduction rates were assessed for both water samples at different pH values and then compared with a synthetic water (BrO$_3^-$ solution prepared using UPW). Overall, reaction rates using the OW were higher than FW, except at pH 5.5 where they were similar. Higher [HCO$_3^-$] and [DOC] for OW samples at pH 6.5 and ~7.5, when compared with FW, showed a positive effect on the reduction rates. Still, bromate reduction rates were higher for the synthetic water than for the waters from the WTP.

Introduction

Bromate ions (BrO$_3^-$) are inorganic contaminants that can be generated during the treatment of natural waters containing bromide ions (Br$^-$). Although Br$^-$ may be naturally present in the majority of water bodies within a range of 15-200 µg L$^{-1}$, BrO$_3^-$ is not usually found in surface or groundwater used for drinking water production. However, when ozonation or other oxidation processes are used in water treatment, BrO$_3^-$ concentrations can range from 0.4-60 µg L$^{-1}$ [1]. Since this contaminant has been classified by the International Agency for Research on Cancer (IARC) as possibly carcinogenic to humans (group 2B), a maximum value of 10 µg L$^{-1}$ in drinking water has been established by the United States (US) [2] and the European Union (EU) [3]. Therefore, advanced treatment processes for BrO$_3^-$ removal from waters intended for human consumption are required.

The acceleration of a photoinduced reaction by the presence of a catalyst is called heterogeneous photocatalysis. Titanium dioxide (TiO$_2$) has been established as the most photoactive catalyst [4]. In photocatalysis, TiO$_2$ absorbs a photon with energy equal to or greater than its bandgap energy, and photogenerated electrons and holes migrate to the surface of the catalyst, reacting with substances adsorbed or close to the surface, promoting oxidation and reduction reactions. The redox potential of $e_{CB}$ generated on the semiconductor conduction band (CB) is negative enough to reduce BrO$_3^-$ to Br$^-$. In a previous work reported by our research group, bromate reduction was successfully achieved for a synthetic water (SW) (200 µg BrO$_3^-$ L$^{-1}$ solution prepared with ultrapure water (UPW)) using the NETmix multi-photoreactor irradiated by UVA-LEDs. When dealing with fresh water matrices, it is necessary to consider some characteristics that might affect the photocatalytic reduction reaction, such as: i) inorganic constituents (sulfates, nitrates, etc.); ii) natural inorganic (CO$_2^-$, HCO$_3^-$) and organic matter (humic and fulvic acids); iii) pH; among others [5].

The main goal of this study was to evaluate the removal of bromates from BrO$_3^-$-spiked drinking water by heterogeneous TiO$_2$ photocatalysis using the same multi-photoreactor. Samples of treated water from a WTP after the ozonation and ozonation+flocculation+filtration steps were used as reaction matrices. Bromate reduction rates were evaluated for both fresh waters at different pH values (5.5, 6.5 and at the natural pH) and further compared with a bromate aqueous solution.

Materials and Methods

The NETmix multi-photoreactor has already been fully described elsewhere [6]. Basically, it consists on a back stainless-steel slab (SSS) imprinted with a network of channels and chambers and a front borosilicate slab (BS), sealed together by mechanical compression and O-rings. All the surfaces of channels and chambers were coated with a thin film of TiO$_2$-P25 suspension (15 mL of a 2% wt.) using a spray technique, resulting in a catalyst surface area per reactor volume of 989 m$^2$ m$^{-3}$. The radiation source, placed on top of the BS, consisted of 9 UVA-LEDs (270 mW, $\lambda_{peak}$ = 365 nm). Before the catalyst deposition, SSS was washed with alkaline detergent and UPW and dried in an oven. During the spray deposition process, SSS was placed over a heating plate ($T$ = 150°C).

Two water samples were collected from a Water Treatment Plant (WTP) located near Porto, Portugal. Both samples: OW (after ozonation, $[Br^-] = 24.2$ µg L$^{-1}$; $[BrO_3^-] < 3.7$ µg L$^{-1}$; pH 7.3); Dissolved Organic Carbon: $[DOC]$ = 3.8 mg L$^{-1}$; Dissolved inorganic carbon: $[DIC]$ = 17.8 mg L$^{-1}$; conductivity = 277 µS cm$^{-1}$) and FW (after a sequential ozonation-flocculation-filtration process, $[Br^-] = 24.2$ µg L$^{-1}$; $[BrO_3^-] < 3.7$ µg L$^{-1}$; pH 7.4; $[DOC]$ = 3.5 mg L$^{-1}$; $[DIC]$ = 16.0 mg L$^{-1}$; 277 µS cm$^{-1}$) were spiked with bromate to obtain a concentration of 200 µg L$^{-1}$ and used to evaluate the matrix effect in bromate reduction kinetics. The experimental procedure started by: i) adding 1.5 L of the surface water spiked with BrO$_3^-$ (200 µg L$^{-1}$) to a jacketed glass vessel, connected to a thermostatic bath set to maintain the solution at the intended value (25 °C); ii) adjusting the solution pH to the desired value (5.5, 6.5), when lower values from the natural waters pH were required; and iii) switching on the recirculation pump (at 75 L min$^{-1}$). Then, the initial sample was collected immediately after the reaction was initiated by turning on the UVA-LEDs. Samples were collected at predefined times, during a 2-hour reaction period, to quantify BrO$_3^-$ and Br$^-$. 
Results and Discussion

Preliminary tests had already been performed with BrO₃⁻ synthetic solutions (200 μg L⁻¹ in UPW) at different pH values (5.5, 6.5 and 7.5). As expected, higher reduction rates were observed at lower pH values (Figure 1) since TiO₂–P25 point of zero charge (PZC) is around 6.4 and, below this value, the positive charges are predominant in TiO₂ surface, favoring the adsorption of negatively charged species such as BrO₃⁻ [7]. When compared with synthetic water, both drinking water samples (OW and FW) showed lower BrO₃⁻ reduction rates for all the pH values tested (Figure 1). Natural organic matter (NOM) present in the drinking water samples can act as sacrificial agent of reactive species, such as holes or hydroxyl radicals (HO·), avoiding the re-oxidation of bromides into bromates and minimizing the electron/hole recombination. However, NOM in the solution or adsorbed on the catalyst surface can block light, decreasing the number of photons absorbed by the photoactive sites [8]. In this case, NOM is most likely the main contributor for the observed slower reaction rates using drinking water samples when compared to synthetic water.

Both ozonated (OW) and filtrated water (FW) had the same [DOC] at pH 5.5 and no difference between the reduction rates was observed, which evidences that the organic matter has the same effect on both samples. Nonetheless, with the pH increase to 6.5 and ~7.5, the differences between the kinetic profiles obtained for the two drinking water samples are visible (Figure 1). The [DOC] in pre-ozonated water increased 1.3 times at pH 6.5 and 1.5 times at pH 7.3/7.4, when compared to filtered water. This is in good agreement with the 1.3-fold and 1.5-fold increase on BrO₃⁻ reductions rates for pre-ozonated water at pH 6.5 and 7.3/7.4, respectively. Therefore, the presence of NOM as a scavenger of oxidizing species improves the reaction rates for pH values of 6.5 and ~7.5, since there are fewer active sites to reduce BrO₃⁻ when approaching TiO₂ PZC. The effect of carbonates and bicarbonates in solution has also been reported as an important factor in photocatalytic reactions, since they can act as h⁺/HO· scavengers, generating species with a redox potential negative enough to reduce BrO₃⁻ to Br⁻ [9]. By analyzing DIC in both samples, the following HCO₃⁻ concentrations were obtained: i) 0.07, 0.54 and 1.15 mM at pH 5.5, 6.5 and 7.3, respectively, for the pre-ozonated water; and ii) 0.04, 0.46 and 1.02 mM at pH 5.5, 6.5 and 7.4, respectively, for the filtered water. This indicates that the higher bromate reduction rates observed for OW at pH values of 6.5 and 7.5 can be also related to the higher amounts of HCO₃⁻ observed for OW when compared to FW. For OW and FW samples the bromates removal efficiency was, respectively, around 93-94 % at pH 5.5, 73-64 % at pH 6.5 and 47-38 % at pH 7.3/7.4. Higher reductions rates were obtained at lower pH values, which is in concordance with that observed for synthetic water.

![Figure 1. Comparison of BrO₃⁻ photoreduction using SW (closed symbols), OW (open symbols) and FW (half closed symbols) for different pH values: (■) 5.5, (○) 6.5 and (●) 7.5, for synthetic water; (□) 5.5, (△) 6.5 and (◇) 7.3 (average), for OW; (◆) 5.5, (◆) 6.5 and (◇) 7.4 (average), for FW.](image)

Conclusions

Heterogeneous TiO₂ photocatalysis using the NETmix mini-photoreactor showed promising results for the reduction of bromates from fresh water matrices. According to the water characteristics, mainly inorganic and organic matter, bromates removal step should be incorporated immediately after the ozonation step. A pH correction step may be also needed to enhance the bromate reduction rates.

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References

Comparative studies between homogeneous and heterogeneous Fenton’s process – low-cost materials experimentation and alternatives to the classic process

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The efficiency of three ceramic catalysts (Fe-Ce-O, Fe-Cu-O and Fe-Co-O), three low cost materials (zero valent iron, ZVI, from both iron bars and iron cutting shavings and Fenton’s sludge) and the homogeneous Fenton’s process are put side to side and compared regarding TPh and COD removal from an Olive Mill synthetic wastewater. The best conditions are indicated for each and the most interesting points are highlighted. Being the center of this discussion, the Fenton’s oxidative capacities on the remediation of a phenolic synthetic wastewater is exposed. A different approach of the classical Fenton’s process is analyzed as an attempt to overcome some of its disadvantages. Several peroxide injections enhanced the procedure’s efficiency in organic charge depletion and reduced the major disadvantage of the Fenton’s reaction: sludge formation.

Introduction
This is a comparison study carried out in four kinds of Fenton’s reaction: ceramic catalysts, low-cost catalysts, classical homogeneous reaction and a new approach using multiple hydrogen peroxide injections. After analyzing each batch of experiment amongst the others of the same kind, the best results are put together for a new debate.

In a global context based on environmental sustainability, the possibility of applying solid wastes to the treatment of liquid wastes is explored. As a final goal, the process costs reduction by using non-expensive materials, would mutually target solid wastes valorization by using non-wanted leftovers.

Materials and Methods
All ceramic catalysts were produced through co-precipitation (Imamura et al., 1985). This procedure consists on the precipitation of a solution containing metal nitrates at the desired molar proportion. The selected catalysts for this comparison and the used salts to produce them were: Fe-Ce-O at 70/30 proportion, Fe-Cu-O at 50/50 and Fe-Co-O at 30/70. A fourth element was added to the ceramic catalysts comparison: Fe-Ce-O at 70/30 produced with iron extracted from Fenton’s sludge. The iron used in the production of this catalyst was extracted by precipitation of a solution containing metal nitrates at the desired molar proportion. The selected catalysts for this comparison and the used salts to produce them were: Fe-Ce-O at 70/30 produced with iron extracted from Fenton’s sludge.

The iron used in the production of this catalyst was extracted by baking iron-rich mud in HCl before filtration and dilution and, afterwards, used to substitute the iron nitrate on the co-precipitation methodology. At the discussion below, this solid will be presented as Fe-Ce-O 70/30*.

The first low cost material considered as Fenton’s catalyst was sludge from an industrial homogeneous Fenton’s reactor used for detergent wastewater treatment. This mud was dried and crushed into small particles. Calcination at 1000 °C presented the highest activity regarding organic charge removal (Rossi et al., 2013). A homogeneous Fenton’s process was inserted in this evaluation due to the high leaching values presented by the studied solids. When multiple peroxide injections are performed, each injection takes place with a 20 minutes interval. The ZVI process is carried out differently: sample cups containing the synthetic effluent are used as reactors. ZVI and H2O2 are added to the reactors and the system’s mixture is induced by an orbital stirrer. The sample withdrawal process is the same described above.

Analytical Techniques
All system’s efficiencies are compared through the abatements of chemical oxygen demand (COD) and total phenolic content (TPh). COD was measured throughout the closed reflux standard method 5220D (Greenberg et al., 1985) using a thermostator and a photometer. Total phenolic content is assessed with a spectrophotometer following the standard procedure presented by Folin and Ciocalteu (1927).
Ceramic Catalysts Performance

Table 1 displays the performances of each experiment regarding TPh and COD removals at equal reaction conditions. Higher efficiency is presented by the cerium-containing solids, followed by Fe-Cu-O 50/50 – which is also capable of completely removing the phenolic compounds from the simulated wastewater. Fe-Co-O 30/70, on the other hand, presented poor results regarding Fenton’s activity. The pattern is maintained regarding COD depletion: Fe-Ce-O 70/30 and Fe-Ce-O 70/30* > Fe-Cu-O 50/50 > Fe-Co-O 30/70, highlighting the cerium as the best metal inside our screening to be coupled with iron to produce Fenton’s ceramic catalysts. Moreover, it is important to stress out that the resources for ceramic catalysts production does not have to employ metal nitrates, since both TPh and COD removals for Fe-Ce-O 70/30 and Fe-Ce-O 70/30* are similar.

Table 1. Ceramic catalysts performance.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>TPh removal</th>
<th>COD removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe-Ce-O 70/30</td>
<td>100 %</td>
<td>49 %</td>
</tr>
<tr>
<td>Fe-Cu-O 50/50</td>
<td>100 %</td>
<td>36 %</td>
</tr>
<tr>
<td>Fe-Co-O 30/70</td>
<td>58 %</td>
<td>32 %</td>
</tr>
<tr>
<td>Fe-Ce-O 70/30*</td>
<td>94 %</td>
<td>51 %</td>
</tr>
</tbody>
</table>

Low-cost materials

The first solid is Fenton’s sludge dried and calcinated at 1000 °C. Since high iron leaching is expected when this kind of solid is exposed to low pH solutions, a homogeneous comparison using Fe³⁺ as catalyst at the same concentration found as leached iron from Fenton’s sludge is performed, and this experiment is also included in the discussion. The third solid is ZVI gathered from iron bars in the shape of shavings. Table 2 displays the organic charge depletions of the three experiments.

Table 2. Low-cost catalysts performance.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>TPh removal</th>
<th>COD removal</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fenton’s sludge</td>
<td>99 %</td>
<td>51 %</td>
</tr>
<tr>
<td>Fe³⁺</td>
<td>Non-Calculated</td>
<td>50 %</td>
</tr>
<tr>
<td>ZVI</td>
<td>98 %</td>
<td>65 %</td>
</tr>
</tbody>
</table>

TPh and COD removals, due to the close value presented for both Fenton’s sludge and Fe³⁺, lead us to believe that all sludge’s activity is from the leached iron, indicating homogeneous process. Although ZVI presents slightly lesser TPh removal than Fenton’s sludge, the H₂O₂ concentration must be considered: 35 mM against 244 mM. Even using this much lower peroxide concentration, ZVI is able to deplete the COD value in 65 %, a better result compared to Fenton’s sludge. It can be concluded that ZVI is a promising low-cost material due to its high catalytic activity and H₂O₂ yield.

Best results and homogeneous process consideration

The last comparison encompasses Fe-Ce-O 70/30 (ceramic catalyst), ZVI (low-cost material) and two approaches of the Fenton’s reaction: classical versus several hydrogen peroxide injections. Information regarding these experiments’ conditions are displayed in Table 3, along with their organic charge depletions.

The homogeneous sets can be pointed out as more efficient in this oxidative process. Regarding COD abatement, ZVI presents a better result than Fe-Ce-O 70/30’s. However, ZVI cannot reach the homogeneous process’ achievements.

The classical Fenton’s process reaches the best COD abatement. Nevertheless, the alternative method using multiple injections of hydrogen peroxide present close COD removals and best TPh depletion using lesser reactant amounts. Even when the three peroxide injections are considered, the oxidant agent dose is 27 % lesser than that of the classical Fenton’s.

Conclusions

Amongst all ceramic catalysts, Fe-Ce-O 70/30 presents the best results as Fenton’s catalyst. The higher surface area and porosity presented by this solid might explain its greater efficiency. The broader valence range of the secondary metal, cerium, compared to the other two secondary metals (copper and cobalt), would allow further electron exchanges with the ferric ions, enhancing the hydroxyl radical formation and leading to higher oxidative power. Moreover, the solid produced using iron extracted from Fenton’s sludge presents similar TPh and COD profiles, showing that it is possible to recover metal from the mud and produce a catalyst as active as the one produced using iron nitrate. Regarding low-cost materials, ZVI can be pointed out as the best. The comparison between the homogeneous and heterogeneous processes shows that the solid catalysts evaluated in our facilities are not as active as dissolved Fe³⁺. Even presenting the closest results, ZVI’s COD removal is still lesser than the homogeneous process for the classical Fenton’s operation. However, the use of several peroxide injections reached organic charge abatements close to the best ones, but using much lesser reactants concentrations – which would overcome some of the process’ disadvantages, mainly those associated to final unwanted iron sludge production.

Table 3. Reaction conditions and performances for low cost catalysts.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>[Catalyst] (g.L⁻¹)</th>
<th>[H₂O₂] (mM)</th>
<th>TPh removal (%)</th>
<th>COD removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe-Ce-O 70/30</td>
<td>1.0</td>
<td>244</td>
<td>100</td>
<td>49</td>
</tr>
<tr>
<td>ZVI</td>
<td>20.0</td>
<td>35</td>
<td>98</td>
<td>65</td>
</tr>
<tr>
<td>Classic Fenton</td>
<td>0.5</td>
<td>122</td>
<td>96</td>
<td>78</td>
</tr>
<tr>
<td>Several Injections</td>
<td>0.1</td>
<td>24.4 (x3)</td>
<td>100</td>
<td>70</td>
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</table>

References

Renewable energy sources are often not fully dispatchable making critical the development of energy storage systems. Among the various options for storing energy easily convertible in electricity, batteries are seen as one of the most promising approaches.

In this work, a life cycle assessment of a prototype 5 kW and 18 kWh vanadium redox flow battery is performed on a cradle-to-gate approach. Primary data from the prototype was used to build the inventory, complemented when needed with information and data from the literature and the EcoInvent V.34 life cycle inventory database. The ILCD 2011 Midpoint+ method was used to estimate the potential environmental impacts. The results show that the environmental hotspots lie mostly in the battery’s structural components and materials, in particular the vanadium electrolytes, evidencing the need for alternative or recycled materials preferably produced locally. Moreover, within a cradle-to-gate perspective, there is a need to develop electrolytes with higher energy density and lower usage of sulphuric acid.

Introduction

Energy storage systems are accompanying the investment growth on technologies to convert energy from renewable sources, mostly because these energy sources are often not dispatchable. It is estimated that by 2050 the investment on batteries will reach 548 billion dollars, adding more 1.291 GW to the currently installed world battery capacity [1]. Among the various options, redox flow batteries are considered a reliable, modular and inexpensive approach due to their long charge-discharge cycles, independent of power and energy rating, with low storage losses and high efficiencies. Vanadium Redox Flow Batteries (VRFB) are one of the most promising technologies for stationary energy storage applications due to the use of the same element in the anolyte and catholyte, which avoids cross-contamination and reduces maintenance, besides being an abundant element.

Due to the growing demand for energy storage solutions to move to a future of zero-carbon energy supply, it is necessary to choose which technologies are the best options, taking into account not only the economic viability but also the environmental and societal dimensions of sustainability. Thus, this study aims to contribute to the current state of the art by evaluating the environmental impacts associated with production of a VRFB prototype using a life cycle approach.

Study goals and methodology

A life cycle assessment (LCA) of a 5 kW vanadium redox flow battery (VRFB) with a storage capacity of 18 kWh prototype is conducted on a cradle-to-gate approach. The goal of this study is to identify key eco-design opportunities for improvement. The study will also allow a better understanding of the factors responsible for the main environmental impacts during the battery’s production, helping define and implement solutions for improving its environmental performance.

The LCA methodology, as described in ISO 14040 and 14044 standards [2,3], is used. It consists of a holistic analysis of a system or a product’s flow of material and energy requirements throughout its life cycle, accounting for the environmental inputs and outputs generated by the transactions that occur in the creation, use and final disposal of the product.
The life cycle inventory (LCI) includes primary data from the prototype VRFB design and development, complemented with secondary data from literature, in particular the Ecoinvent database V3.4 available in the software SimaPro™ V8.5.2.0. Since the system under analysis is still at an early stage of its design and development, some technical aspects are still not completely defined. Therefore, assumptions were made regarding the battery storage capacity and the monitoring equipment. For the background data, European conditions were considered whenever possible. A cut-off criteria was implemented to exclude all energy and material flows that correspond to less than 1% of the total energy and mass inventory. The chosen characterization methodology for the environmental impacts was the International Reference Life Cycle Data System (ILCD) 2011 Midpoint+ V.1.10 [4], with the following impact categories: climate change (CC), ozone depletion (OD), photochemical ozone formation (POF), acidification potential (AP), freshwater eutrophication (FE) and mineral, fossil & renewable resource depletion (MFRRD).

Results
The calculated environmental impacts per 1 kWh of stored energy are presented in Figure 2.

![Figure 2. Total environmental impacts [%] during the production and assembly phase of the battery prototype per unit of kWh stored energy, using ILCD 2011 Midpoint+ V.1.10. Impact categories: CC – climate change, OD – ozone depletion, POF – photochemical ozone formation, AP – acidification potential, FE – freshwater eutrophication, MFRRD – mineral, fossil & renewable resource depletion.](image)

Calculations were done with SimaPro™ V8.5.2.0. Results show that the battery structure (corresponding to the cell stack components) and the vanadium electrolytes have the largest share of the potential environmental impacts. The battery structure is more relevant in the freshwater eutrophication (55%), climate change (38%) and photochemical ozone formation (37%) categories, due to the copper and plastic components used in the battery. The structure has a high potential for improvement in a cradle-to-grave analysis since these components can be easily recycled or reused after proper treatment and conditioning. The vanadium electrolytes contribute 75% to MFRRD and 65% to acidification, mainly due to sulphuric acid present on its composition. The electrolytes also account for 32% of the POF impacts and 24% of ozone depletion. These results confirm the need for the development of future vanadium electrolytes with better technical and environmental performance, especially in terms of energy density, in agreement with the literature [5,6].

The transportation of the battery’s structural components and electrolyte generates 16% of the impacts in the climate change category, followed by the structure of the storage tanks. Regarding the balance of system (BOS in Figure 1) components, they have higher contributions in the freshwater eutrophication (26%) due to the monitoring equipment components, and MFRRD (11%). Waste has a 42% contribution to ozone depletion due to the single use polymer materials.

Conclusions
This study analysed the life cycle of a vanadium redox flow battery, concluding that the cell stack components and the vanadium electrolytes are the largest contributors to the potential environmental impacts in the production phase. The impacts can be significantly reduced by using recycled and local materials or components. The vanadium electrolytes contribute 65% to acidification and 75% to the mineral, fossil and renewable resource depletion, mainly due to production of the sulphuric acid present in the electrolytes. Future work will involve assessing the environmental performance of the battery as an integrating part of an energy production and supply system. A cradle-to-grave LCA and an Economic and Social-LCA for a full Sustainability performance evaluation will also be performed.

Acknowledgements
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References
Nitrogen and COD removal enhancement in a SBR: Influence of using step-feed and indirect parameters monitoring, in a real-time control perspective

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The objective of this work was to investigate a real-time control (RTC) strategy using indirect parameter probes (ORP, pH and DO) in a SBR treating winery effluent. The aim was enhancing N and COD removal and reduce aeration costs in an industrial wastewater treatment plant. The test variables were the duration of each aerobic/anoxic phase and the use of a step-feed approach. Results revealed that increasing the duration of the aerobic and anoxic phases allows a more efficient nitrogen removal, but the use of step-feed decreased both TN and COD removal, obtaining effluents inadequate for discharge. DO breakthrough control point was successfully found both in DO and ORP profiles. Only 3 N control points were found in the study, invalidating the possibility for RTC on N removal, but effluent’s nitrogen fulfilled discharge parameters. The results showed strong evidences that RTC based on COD removal is possible by tracking DO breakthrough point, reducing global aeration time in 56%.

Introduction

The wine industry is a very prevalent sector in Portuguese economy. The production of 1 L of wine can generate between 0.1 to 2.4 L of wastewater [1], which must be treated before disposal in the environment. Winery wastewaters are seasonal with highly variable composition. They are rich in COD, have high C/N, with an acidic pH, and are rich in organic acids, alcohols, sugars, esters and phenolic compounds. Like its composition, the amount of wastewater produced is variable throughout the year, depending on the stage of wine production. With these characteristics, batch treatment systems are adequate, since reaction time is adaptable to wastewater characteristics. This work focuses a specific treatment process: the sequential batch reactor. This reactor operates in 5 stages in the same equipment: feed-react-decant-draw-idle. The stages are time-controlled, usually. Due to this configuration, SBR requires much less area when compared with conventional activated sludge processes, and supports load variations easier than those systems. SBR also allow modifications on the reaction phase, such as aerobic and anoxic periods, which are greatly useful when the goal is to remove COD and nitrogen [2]. Nitrogen removal through biological nitrification/denitrification processes requires aerobic and anoxic stages; nitrification happens only in the presence of oxygen (aerobic bacteria) and denitrification in the absence of it (facultative heterotrophic bacteria) [2]. The versatility of SBR technology allows not only the existence of different phases but also the possibility of varying the time dedicated to each one of them, and the number of alternating phases, in the case of multiple aerobic/anoxic periods. During biological nitrification/denitrification, 3 steps are verified. In aerobic phases, ammonification (Eq. (3)) and nitrification (Eq. (4)) take place, converting ammonia ($NH_4^+$) to nitrates ($NO_3^-$) and, after that, nitrates ($NO_3^-$), with the release of hydroxides ions ($OH^-$). During denitrification, nitrates are reduced to molecular nitrogen ($N_2$), in the presence of organic matter for reduction purposes. In the aerobic organic matter degradation (aerobic digestion – Eqs. (4) and (5)), the ceasing of oxygen consumption marks the end for degradation.

Equations (1), (2), (3), (4) and (5) represent these biological processes [2].

\[
\begin{align*}
2NH_4^+ + 3O_2 & \rightarrow 2NO_2^- + 4H^+ + 2H_2O \\
2NO_2^- + O_2 & \rightarrow 2NO_3^- \\
5CH_3CO_2H + 6NO_3^- & \rightarrow 3N_2 + 5CO_2 + 7H_2O + 6OH^- \\
CHONS + O_2 & \rightarrow H_2O + CO_2 + NH_3 \\
C_2H_5O_2 + 5O_2 & \rightarrow NH_4^+ + 5CO_2 + 2H_2O \\
\end{align*}
\]

Integrating the knowledge of these reactions, several control strategies were developed to adjust the SBR cycle time. Some of the strategies are based on zero discharge parameters, namely oxidation-reduction potential (ORP), pH and DO probes, to mark the ceasing of oxygen consumption marks the end for degradation.

Objectives

The objective of the present work was to follow an SBR system performance with ORP, pH and DO probes in a perspective of real time control, to enhance nitrogen and organic matter removal. For this study, the reaction cycle (aerobic and anoxic stages duration) was varied and step-
feeding was applied. Wastewater composition was also modified throughout the study.

Methods
2 SBR pilot reactors (30 L) were used, one for control and the other with modifications and probes. The wastewaters used in the studies were sampled from a winery of Douro Valley, Portugal. 7 samplings were collected at different times of the year and used in the system. The SBR cycle was composed of feeding (30 min), reaction time (21 h), settling (2 h) and discharge (30 min). The study had 4 stages. The reaction time was composed of 30 minutes aerobic treatment followed by 30 minutes anoxic in loop, and after it was changed to 1/1 hour. The HRT was of 10 days, and during most of the study the feeding was at the beginning of the cycle; it was then changed to a step feeding process by adding the 3L in a sequential way with 1 L at hour 0 of reaction, 1 L at the 7th hour and 1 L at the 14th hour. Wastewaters were analysed for TSS, TS, COD, TN, BOD₅ and pH. Influent and effluent were analysed daily for COD, TN and ammonia, and for BOD₅ weakly. Nitrate, COD and TN analyses were conducted in the beginning of some aerobic and anoxic stages. Reactors sludges was analysed for volume and solids weakly. Methods used to COD, TN, Ammonia and Nitrates analysed with reagent kits according to methods USEPA-410.4 (COD), Chromotropic Acid Method, (TN and nitrate) and ASTM D1426-92 (ammonia). Other analyses were conducted according to SMEWW 1985.

Results
ORP, pH and DO profiles, shown in the Graphical Abstract, revealed to be characteristic in what concerns to DO BP, shown both in DO and in ORP, and this point was proved to correspond to the degradation of readily biodegradable COD. After this point, little or no further degradation was achieved. In matters of total nitrogen degradation, no significant control points were identified, possibly due to the lack of nitrogen in the influent (in the majority of days, nitrogen legislation criteria was fulfilled only with dilution in the pilot reactors). Wastewater characteristics revealed to have a strong impact in both COD and nitrogen removal, as well as in the pH, ORP and DO profiles. Figure 1 shows the differences in profiles using wastewater with COD of 3000 mgO₂/L, C/N of 180 mgCOD/mgTN, and COD of 1300 (C/N of 37) (right – also shown in Graphical Abstract) and COD of 7650 (C/N of 80) – left -. Nitrogen removal was also enhanced with the use of wastewaters with higher COD values. Table 1 shows total nitrogen, COD and ammonia removals in reactor 2 based on wastewater COD. The values show that, although ammonia removal stays mostly the same, nitrogen removal is strongly increased by enhanced WW COD, as so is the COD removal.

As for reaction cycle, results show that the use of longer aerobic/anoxic phases could lead to better nitrogen removals, though the differences are not very significant when compared with control reactor results. Even though, nitrogen removals were significantly increased. Results on this matter are shown in Table 2.

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References
Kinetic modelling of the pyrolysis and combustion of woody biomass

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Thermochemical conversion of biomass into energy and feedstock chemical is gaining increasing importance in the current context. Gaining insight on the thermochemical conversion of biomass is very important and constitutes the objective of many research works. In this work, the thermochemical transformation of wooden chips (alder) sample provided by Institute of Coal Chemical Processing in Zabrze (Poland) were studied by, thermoanalytical methods in particular, using Differential Scanning Calorimetry (DSC/TG) technique. Experiments were conducted both under pyrolysis and combustion conditions, at different heating rates (10, 20 and 50 °C/min), in a temperature range from 40 to 800°C. The information was analyzed by means of pseudo-component kinetic models where, as a first approach, the reactions were assumed to be first order.

Introduction

The thermochemical conversion of biomass, by processes such as pyrolysis, gasification and combustion, is a very complex multicomponent problem. The interactions between chemical kinetics and mass transfer processes involved in the thermal degradation of biomass makes the mathematical modelling difficult. Nevertheless, an understanding of these processes is crucial for the development and design of more efficient processes and equipment. The pyrolytic behavior of biomass is general modeled as the weight sum of individual reference components products such as cellulose, hemicellulose, lignin and extractive components [1, 2].

Pyrolysis is one of the most important methods to convert woody biomass into charcoal, liquid oils and gases and a possible general source of chemicals. Moreover, it is an effective way to reduce bulky biomass into an energy dense, uniform and easily transportable fuel. [3, 4]

Objectives

The main goal of this work consisted in develop a kinetic model for pyrolysis and combustion to describe the processes of thermochemical transformation of a specific woody biomass material.

Methods

The experiments were performed in TA Instruments SDT 2960 Simultaneous DSC/TG device at different heating rates. Approximately 10 mg of sample were weighted and placed in an alumina crucible. In the case of pyrolysis and combustion, a flow of nitrogen (80 mL/min) and air (70 mL/min) was used, respectively. A set of additional experiments was carried in the same conditions but using a mixture of air and nitrogen so as to obtain different partial pressures of oxygen in the reaction atmosphere.

Calculations regarding kinetics and thermal analysis were carried out in Excel.

Model

The model was developed using a pseudo-component approach were 5 pseudo-components were assumed. These can be generally assigned to the different types of materials in the biomass, such as water, cellulose, hemicellulose, lignin and ash. As a first approach all the components, except for the ash, were assumed to be converted by a first order kinetic rate and were considered to be independent of the others.

The relevant differential equations, for each of the components, we integrated using the Euler method using the actual temperature profile measured by the apparatus and the weight obtained from the model was fitted to the experimental data using a least-square approach using the Solver tool.

The fitting procedure was initiated by an individual fitting of each curve to obtain starting values for the final fitting that was carried-out simultaneously for all the heating rates that we used using the pre-exponential factors, activation energies and component fractions as the fitting parameters.

Five pseudo-components were distinguished based on changes in slopes of curves. The decomposition rates of the components for each time were estimated using the Arrhenius equation, written as

\[
\frac{dx_i}{dt} = -k_{ref} \cdot e^{-\frac{Ea}{RT}} \cdot \left(1 - \frac{1}{Ea} \cdot \frac{1}{RT}ight) \cdot x_i
\]

where the reference temperature was considered as 573.15 K.

The model was developed both for pyrolysis and combustion and then combined to describe the series of results where the partial pressure of oxygen was varied.

Results and Conclusions

Figure 1 illustrates the behavior observed under pyrolysis conditions of the sample at a heating rate of 10°C/min. Except water evaporation peak, there is only one observable peak at a temperature of around 630 K, that can be attributed to cellulose and hemicellulose decomposition; In fact, this peak is highly asymmetric and can be viewed as the overlap of two different processes. Apart from these sharp mass losses which correspond to clear DTG peaks, a slower mass loss is also observed, going well to the highest temperatures, which is likely to be the degradation of lignin.

In case of combustion mode (Figure 2) it was possible observe the peak of water evaporation as well as three other DTG peaks at 578, 683 and 743 K, also attributable to the combustion of the lignocellulosic content: cellulose, hemicellulose and lignin.

For pyrolysis (Figure 3), apart from the endothermal peak related to moisture loss all the other processes, related to volatile matter loss are also endothermal. On the other hand, when combustion occurs (Figure 4) there are two peaks present (apart from first one associated with moisture loss) which are strongly exothermal.
The description obtained by the kinetic model was very good, as it can be seen in the example of the fitting, obtained for pyrolysis, shown in Figure 5.

Figures 1 and 2 show the TG and DTG curves for pyrolysis and combustion modes, respectively.

Figures 3 and 4 show the heat flow signal for both experiments.

In the experiments with lower oxygen partial pressures the behavior is intermediate as combustion is incomplete at lower temperatures and pyrolysis also occurs.

In the current communication we discuss the use of a combined model for the description of pyrolysis and combustion that will, eventually, be applicable to the description of the behavior of biomass in a gasifier.

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References
Energy optimization of sludge anaerobic digestion

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In Portugal the optimization of anaerobic digestion is desirable, however, the sludge amount does not present a great growth potential in volume (or mass), since the country's infrastructure is consolidated. However, there is an opportunity to identify potentials for optimization and valorization of anaerobic digestion stages in the WWTP either by process changes or by incorporation of other substrates. The present work main issue is to analyze the installed capacity and efficiency of the anaerobic digestion process carried out by the WWTP and the possibility of performing an anaerobic co-digestion with other substrates. Also, to determine the potential for energy manufacture by biogas production. Therefore, a survey of existing WWTP was done but due to the high number of WWTP, it was necessary to select some regions, namely center and south of Portugal. As a result it was found that, the energy produced from biogas represents in average from 10 % to 20 % of WWTP of energy needs.

Introduction

Nowadays, wastewater treatment plants (WWTP) produce a significant amount of sludge due to the need to treat wastewater as a consequence of population and water consumption growth. Usually, WWTP sludge stabilization is made by anaerobic digestion with biogas valorization by heat and electricity production [1].

In Portugal there was around 2,743 WWTP in 2016 [2], but only the medium and large capacity have sludge stabilization, usually by anaerobic digestion. In the last years, however, the sludge quantity did not present a great growth potential in volume (or mass), since the country's infrastructure was already consolidated.

Several studies concerning anaerobic digestion of sludge with other organic substrates were conduct with a significant biogas production. The organic fraction of municipal solid waste (MSW) is one of the most used substrate [3]. In Portugal, the MSW amount was 4,660,000 ton with an organic fraction of around 51 % in 2016 [4]. With anaerobic co-digestion it’s possible to contribute to the management of waste from different origins and at the same time produce renewal energy like biogas.

Objectives

In the present study it is intended to carry out a survey on the installed capacity and efficiency of the anaerobic digestion process carried out by WWTPs in Portugal. Also, the possibility of performing an anaerobic co-digestion with other substrates that are not being incorporated will be evaluated, as well as being aware of the potential for biogas production.

Methodology

In the present study, an extensive literature review was carried out on national and European official websites, articles, thesis and reports of the wastewater management entities in Portugal in order to collect data concerning WWTP, sludge amount, anaerobic reactors, biogas, methane and energy production. Also, surveys with specific data were sent to the wastewater management entities of the WWTP previously selected. Due to the high number of WWTP it was necessary to select some regions of Portugal (e.g. center and south).

In the present study main issue is to analyze WWTP and the possibility of performing an anaerobic co-digestion with other substrates. Also, to determine the potential for energy manufacture by biogas production. Therefore, a survey of existing WWTP was done but due to the high number of WWTP, it was necessary to select some regions, namely center and south of Portugal. As a result it was found that, the energy produced from biogas represents in average from 10 % to 20 % of WWTP of energy needs.

Results and Conclusion

To carry out the present work it was necessary to gather data concerning the WWTP treatment scheme and with more detail the sludge amount and characteristics and anaerobic digestion process. In Table 1 the data from two WWTP from different region of Portugal are presented. Both WWTP have the same treatment level but with different wastewater flow, WWTP B received almost half flow.

Table 1. WWTP data.

<table>
<thead>
<tr>
<th>WWTP</th>
<th>Region</th>
<th>Population (p.e.)</th>
<th>WW Flow (m³/d)</th>
<th>Treatment level</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>South</td>
<td>138,000</td>
<td>41,800</td>
<td>Tertiary</td>
</tr>
<tr>
<td>B</td>
<td>Centre</td>
<td>96,000</td>
<td>21,082</td>
<td>Tertiary</td>
</tr>
</tbody>
</table>

The anaerobic process from that WWTP are described in Table 2. The WWTP B is the smallest but has 2 anaerobic reactor that operate in batch mode.

Table 2. Anaerobic digestion process.

<table>
<thead>
<tr>
<th>WWTP</th>
<th>Reactor number</th>
<th>Volume (m³)</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1</td>
<td>n.a.</td>
<td>Mesophilic</td>
</tr>
<tr>
<td>B</td>
<td>2</td>
<td>1.200</td>
<td>Mesophilic</td>
</tr>
</tbody>
</table>

In both WWTP the biogas is measured and characterized in continuous way. In Table 3 the biogas characterization is presented in terms of CO₂, CH₄ e H₂S. The methane percentage is more than 63%, which permits the energy production. The WWTP B has an average value of CH₄ (67 %) higher than WWTP A. In WWTP A it can be observed a higher H₂S variation from 2 to 4.039 ppm.

From the surveys sent to the management entities the energy production has evaluated from 2015 to 2018, as presented Figure 1.

The WWTP A, has experienced a growing fluctuation in their energy production due to the oscillation of the sludge amount received in the last years. Being on of largest WWTP in the southern area the quantity of energy consumed is considerably high, therefore the production of their own energy is an important issue. Nowadays, the energy production, of more than 25,000,000 kWh (Figure 1), does not meet more than 10...
% of their monthly needs. Since the process is done mainly with sludge, the incorporation of other substrates would be significantly helpful and in the WWTP region there are several organic wastes available, such as MSW.

The WWTP B had a slight step back on the anaerobic reactor during 11 days in June 2015 and at the last 5 months of 2016, due to technical difficulties. However, the amount of biogas produced in the last year has been able to meet part of their energy needs, making up for more than 20% of their monthly consumption. Moreover, this WWTP not only incorporates sludge but also substrates that come from nearby cheese shops and industrial septic tanks. Nevertheless, the shortage of activities such as the alkalinity measurement and biogas enhancement and purification, puts a barrier in their efficiency.

Table 3. Biogas composition.

<table>
<thead>
<tr>
<th>Biogas component</th>
<th>WWTP A</th>
<th>WWTP B</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂ (%)</td>
<td>33.67</td>
<td>40.65</td>
</tr>
<tr>
<td>CH₄ (%)</td>
<td>60.02</td>
<td>63.33</td>
</tr>
<tr>
<td>H₂S (ppm)</td>
<td>2</td>
<td>380</td>
</tr>
</tbody>
</table>

Figure 1. Energy production from WWTP A and B.

Conclusion

It is perceptible that there is a large window of opportunity regarding energy production through biogas in Portugal, although significant effort was being done in sludge anaerobic digestion, there is space to improvement. The collected data from the surveys was considerably helpful but some WWTP have incomplete data concerning all sludge anaerobic process, namely the operational reactor conditions. The present work is still under development.

Acknowledgements

The authors thank all wastewater management entities from Portugal for the data provided.

References

Mercury is one of the most hazardous toxic metals due to its ability of accumulation into the living organism tissues and bioamplification along the food chain. In line with the concept of circular economy, agricultural wastes and industrial by-products have been investigated in order to provide eco-friendly sorbents for water treatment applications. Banana and potato peels, egg shells, Eucalyptus globulus bark and coffee wastes were used to remove mercury from tap water solutions. The ability of these biosorbents was evaluated through batch experiments under room temperature and Hg(II) concentration of 50 µg/L. The best performance was achieved by banana peels, which showed removals of mercury(II) in solution superior than 90 %. The mechanism of sorption was studied applying well known kinetic reaction- and diffusion-based models.

Introduction
Water is an essential resource for the human beings and its preservation and reuse is a worldwide concern. Among the relevant contaminants of aqueous streams, mercury is classified by the Agency for Toxic Substances & Disease Registry (ATSDR) as the third most dangerous substance based on a combination of its frequency, toxicity, and potential for human exposure [1]. Furthermore, under Directive 2000/60/CE the European Union imposes a progressive reduction of pollutants and ceasing or phasing out emissions by 2020. Therefore, the removal of trace levels of mercury from water and wastewaters is a required action. A variety of processes is available for the uptake of toxic metals from aqueous waste streams, such as chemical precipitation, membrane processes, ion exchange and adsorption [2]. Comparatively, sorption processes are largely used in water treatment because of their low operational and maintenance costs, design simplicity and efficiency to treat water with realistic low metal concentrations [3]. Agricultural wastes and industrial by-products are economic and eco-friendly due to their abundant availability and renewable nature that make them excellent options in water treatment processes. They are mainly composed of lignin and cellulose and may also include other polar functional groups such as alcohols, aldehydes, ketones, carboxylic, phenolic, and ether groups. These groups are capable to establish binds with the pollutants through different mechanisms [3].

The objectives of this work are: (i) evaluating the ability of five biowastes (banana and potato peels, egg shells, Eucalyptus globulus bark and coffee waste) in order to determine their uptake performances to remove Hg\(^{2+}\) from low concentrated tap water solutions, and (ii) the study and interpretation of the mechanisms involved in the sorption process.

Materials and Methods
Batch experiments were carried out with Hg\(^{2+}\) initial concentration of 50 µg L\(^{-1}\) in tap water, at 22 °C±1, pH = 6.0, and magnetically stirred at 650 rpm. The biosorbent mass of 0.5 g L\(^{-1}\) was added into the solutions and samples were taken at increasing times, filtered through a Millipore membrane of 0.45 µm, adjusted to pH < 2 with HNO\(_3\) and immediately analysed afterwards. The mercury(II) quantification was accomplished by cold vapo atomic fluorescence spectroscopy (CV-AFS) Eucalyptus globulus bark was real industrial waste provided by a paper and pulp industry. The coffee wastes used in this study were a mixture of coffee, rye, chicory and barley, and they were granted by a coffee industry. The remained biomasses were obtained from a local market and all the materials were applied without any thermo or chemical pre-treatments.

Results and Discussion
The removal curves of Hg\(^{2+}\) for all the materials studied are shown in Figure 1 in terms of metal concentration (C\(_{eq}\)) versus time. The trends found exhibit two distinct sections. The first one is characterized by a fast initial mercury elimination due to the large driving force between the mercury solution and the biomass particles, which are initially free of metal. Then, as time increases, the active sorption sites become occupied with the sorbed metal, and the system tends to equilibrium and reaches an inferior plateau.

As can be observed, the behavior of egg shells is clearly different of the other sorbents, being characterized for its slowest kinetics. The potato peels presented a fastest removal, and reached the equilibrium after only six hours. However, this biomass exhibits a low mercury elimination. The removal efficiencies are presented in Figure 2. For the same conditions, the banana peels were able to remove 90.5 % of the mercury from tap water solution. Despite the kinetics of the coffee wastes was not as fast as banana peels kinetics, the percentage removal was of 85.2 %. The other sorbents presented also good mercury concentration decreasing, 81.3 % for egg shells and 78.8 % for Eucalyptus globulus bark. The potato peels were able to reduce only 50.9 % of the metal in solution.
Normally, sorption can be described by three steps: (i) the external mass transfer of the sorbate from the bulk solution to the surface of the sorbent, (ii) diffusion into the pores of the particle, and (iii) the sorption on the active sites. The reaction-based models for sorption processes assume that sorption is the limiting step, and the diffusion models consider that film diffusion and/or intraparticle diffusion are the slower steps [4]. The reaction-based models of pseudo-first order, pseudo-second order and Elovich, and the diffusion-based models of Boyd et al. and Webber have been fitted to the experimental data. The different kinetic behaviour is confirmed by the distinct adjustments of the models. The pseudo-first order is better to describe E. globulus bark experimental points, while pseudo-second order offered the best fit to egg shells and potato peels. The Elovich model achieved the best description of the banana peels and coffee waste data. Concerning the results obtained from the diffusion-based models, the Hg$^{2+}$ sorption by egg shells and E. globulus bark is controlled by intraparticle diffusion, while for the biosorbents banana peels and coffee waste the trends found can be explained by an initial period of a film diffusion, followed by a slower stage of intraparticle diffusion towards equilibrium. In the case of potato peels, the sorption is controlled by film diffusion.

Conclusions
The major goal of this study was to find value to the biosorbents under analysis, taking into account attractive features like their sorption capacity and high ability for mercury(II) removal. Such biowastes have no costs and, what is even more relevant, they can present industrial disposal expenses. Furthermore, biosorbents offer notable advantages over currently available expensive commercial sorbents for water remediation since they are available in large quantities, they do not require any pretreatment and can contribute to an overall waste minimization approach.

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References
Adsorption heat pumps (AHPs) have been widely studied as potential replacers for vapour compression systems (VCSs), since they can use natural refrigerants (e.g. water) instead of fluorocarbon-based ones, and may be powered by solar energy or waste heat instead of electricity [1]. The operation cycle of an AHP consists of four stages: isobaric adsorption, isobaric heating, isobaric desorption and isosteric cooling [1]. The efficiency and cost of AHPs are strongly influenced by the performance of the adsorbent/adsorbate pairs. Binder-based granules of zeolites 4A and 13X have been considered, since they are cheap and widely available, albeit the binder may reduce the adsorption capacity and kinetics [2]. Aiming at improved performances, binderless zeolites have emerged in the market.

The optimization of the design and operation of AHPs is challenging due to the high complexity of the process and the need to fit variable thermal demands [3]; it has been mainly performed using phenomenological models and/or prototypes [4], although statistical tools like design of experiments (DoE) and response surface methodology (RSM) may aid to accomplish optimizations much more easily and efficiently. In this work, the performance of commercial binderless and binder-containing 13X and NaY zeolites for water AHPs was compared, considering a DoE with four factors (time of adsorption and desorption; heat source temperature; condensation temperature; bed thickness) and three levels [1]. A set of 25 simulations per adsorbent was accomplished, and the performance of the AHPs was evaluated by means of the coefficient of performance (COP) and the specific heating power (SHP). The results suggested that the presence of the binder in the formulation of 13X does not penalize the zeolite performance significantly, and that the binderless NaY is the most promising material. For the latter adsorbent, statistical outcomes were analyzed and insights about their usefulness to optimize the design and operation of AHPs were provided: Pareto charts displaying the impact ranking of the factors upon COP and SHP are discussed, and simple equations for the expeditious estimation of both indicators are provided. Such models were used to map the system performance and to select optimal geometric/operating parameters that meet specific performance requisites.

Acknowledgements
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References
Copper foam coated with CPO-27(Ni) metal-organic framework for adsorption heat pump:
Simulation study using OpenFOAM

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The performance of the metal-organic framework (MOF) CPO-27(Ni) for adsorption heat pumps (AHPs) using water as adsorbate was investigated in this work through modeling and CFD simulations. A customized solver and methodology for simulating adsorption cycles were developed in OpenFOAM and then validated with literature data. Due to the potential of adsorbent coatings and metal fibers and foams for improving AHPs performance, a tube surrounded by a composite of copper foam and CPO-27(Ni) coating was considered. The AHP performance was evaluated through the coefficient of performance (COP) and the specific heating power (SHP). The obtained COPs and SHPs for the composite coating CPO-27(Ni)/copper foam were 1.16-1.39 and 1922-5130 W kg⁻¹. Under similar conditions, the MOF performance was outperformed by the well-known adsorbent AQSOATM FAM-Z02, essentially due to the faster intraparticle mass transfer kinetics of the benchmark material.

Adsortion heat pumps (AHPs) have been widely investigated as potential replacers for vapour compression systems (VCSS), since they can be powered by solar energy or waste heat instead of electricity, and can use natural refrigerants like water instead of fluorocarbon-based ones [1]. The cyclic operation of an AHP encompasses four stages: isobaric adsorption, isosteric heating, isobaric desorption and isosteric cooling [1]. So far the market penetration of AHPs has been small, essentially due to the high costs and significant dimensions of the appliances, along with low thermal efficiency [2]. Among the identified R&D priorities for AHPs are the development of compact and high surface area adsorbent heat exchangers (AHExs), where the adsorbent is applied in coating configurations to obtain enhanced heating power and compact designs [2]. The use of directly coated metal fibers or foams [3] enables high surface area, good vapor permeability, and improved bed thermal conductivity.

Regarding adsorbents for AHPs, activated carbons, silica gels and zeolites are among those applied nowadays, with emphasis on AQSOATM FAM-Z02 (a SAPO-34 developed by Mitsubishi Chemical), which is considered very promising for such applications [2]. Metal-organic frameworks (MOFs) are receiving increasing scientific attention in the field of AHPs, since they are promising materials in terms of energy efficiency and cost improvements [4]. The MOF CPO-27(Ni) is claimed to be particularly promising for AHPs operating in cold climate [5].

In this work, the performance of tubular AHEx units possessing composites of adsorbent coating/copper foam were investigated through rigorous modeling and CFD simulations. A customized solver and methodology for simulating adsorption heating cycles was developed in OpenFOAM and validated previously using literature data. Taking into account flow and heat transfer resistances within the composite bed, and mass transfer resistances in the adsorbent coating, the cyclic process was simulated considering typical AHP operating conditions in cold climate. The MOF CPO-27(Ni) was benchmarked against the commercial zeotype adsorbent AQSOATM FAM-Z02. The obtained COPs and SHPs for the composite coating CPO-27(Ni)/copper foam were in the range 1.16-1.39 and 1922-5130 W kg⁻¹, respectively. These performances are inferior to those obtained for the AQSOATM FAM-Z02 under similar conditions, which is mainly attributed to the lower adsorbate loading swing and to the slower intraparticle mass transfer kinetics of the MOF. The benchmark material also showed to enable more compact AHEx designs. In the whole, CPO-27(Ni), which is part of a set of very promising claimed materials for which huge scientific investigation is ongoing, seems currently unable to surpass the performance of a benchmark material for specific AHP applications.

Acknowledgements

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PQH, QREN, BOSCH).

References

Mercury is one of the most hazardous water contaminants, since its presence in water, even at very low concentrations, causes severe harmful effects to biota, being for that reason classified as a priority pollutant by the European Union [1]. It is one of the top 3 hazardous substances established by the Agency for Toxic Substances and Disease Registry [2]. Sorbent materials like ion exchangers are potential candidates for the removal of low levels of contamination, considering their high capacity and selectivity. In particular, synthetic microporous materials like titanosilicates and zirconosilicates have been successfully studied as ion exchangers [3-6]. ETS-4 (Engelhard Titanium Silicates No. 4), with chemical composition [Na2Ti5Si12O38(OH)4·H2O], presents a great ion exchange capacity (theoretical exchange capacity of 6.39 eq kg⁻¹), which makes it an excellent ion exchanger [7].

Previous studies with ETS-4 revealed that Hg²⁺ removal increases with the solution pH until it attains a maximum value at pH 4-6, and with decreasing temperature [4]. To adjust the pH, NaOH is commonly added to the contaminated solution which may influence the mercury removal efficiency since the excessive concentration of Na⁺(aq) will compete with the target ions (Hg²⁺) during the ion exchange process. To avoid this competition effect, in this work a tetrapropylammonium hydroxide solution (TPAOH) is tested for pH adjustment, as the tetrapropylammonium cation (TPA⁺) is large enough and cannot diffuse into the ETS-4 pores. Accordingly, this essay focuses the experimental study and modelling of equilibrium and kinetics of the Hg²⁺/Na⁺/ETS-4 system, using TPAOH solution as pH adjuster. The mass action law, rigorously expressed in terms of activities, was used to model equilibrium, and the Ioannidis et al. method (IO) was adopted to optimize noncorrelated parameters [8]. The Debye–Hückel (DH) and Wilson (WL) models were used to calculate the corresponding activity coefficients of cations in the liquid and exchanger phases, respectively [8]. With respect to the ion exchange kinetics, the Maxwell–Stefan (MS) flux equations were adopted [8].

Materials and methods

All reagents used in this work were of analytical grade and used without further purification. The ion exchange studies and the analytical procedures are described elsewhere [5]. The initial concentration of Hg²⁺ was fixed in all assays (ca. 5 × 10⁻³ mol m⁻³) and the solutions were prepared by diluting a mercury stock solution in ultrapure water. The pH was adjusted to 6 [4], using NaOH or TPAOH aqueous solutions, and the experiments were carried out at room temperature under batch conditions. For the kinetic studies, rigorous masses of pre-washed ETS-4 were added to the metal solution.

Results and discussion

Figure 1 displays the isotherm for the Hg²⁺/Na⁺/ETS-4 system, which is approximately linear over the range of studied concentrations. Concerning modelling, it is possible to observe the goodness of the fit achieved by mass action law expressed in activities in opposition to the biased ideal line.

![Figure 1. Equilibrium data (symbols) and modelling results (lines) for the Hg²⁺/Na⁺/ETS-4 system. (Model acronyms: IO=Ioannidis et al., DH=Debye-Hückel, WL=Wilson).][1]

The experimental data for normalized Hg²⁺ concentrations in the fluid along time are plotted in Figure 2 together with the MS modelling results. The two sets of data were measured for similar conditions of sorbent dose, initial concentration of Hg²⁺ in solution, stirring speed and temperature, differing only in the solution used for pH adjustment (TPAOH and NaOH aq.).

The results suggest that, independently of the pH adjusting solution, a few milligrams of ETS-4 per liter give rise to

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[P-EE50] Influence of distinct cations in solution on the equilibrium and kinetics of mercury removal using titanosilicate ETS-4

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significant decrease of the Hg$^{2+}$ concentration in solution. However, the equilibrium is reached much faster when using TPAOH instead of NaOH. This is due to the higher concentration of Na$^+$ in solution (when NaOH is used for pH adjustment) which reduces significantly the driving force for Na$^+$ transport, from the particle to the solution, and thus the ion exchange becomes slower in comparison with the experiment carried out with TPAOH.

Concerning the results achieved with the MS model (curves in Figure 2) a good agreement is observed between modelling and measurements. The calculated MS diffusion coefficients of the counter ions are in the order of $10^{-16} – 10^{-11}$ m$^2$ s$^{-1}$, being similar to those reported in the literature for ETS-4 and other systems [6].

![Figure 2. Evolution of Hg$^{2+}$ normalized concentration in the fluid versus time (Experimental data – symbols; Maxwell–Stefan (MS) results – lines).](image)

**Conclusions**

The removal of Hg$^{2+}$ from aqueous solution using titanosilicate ETS-4 was carried out under isothermal batch conditions, at pH 6.0 room temperature. TPAOH and NaOH aqueous solutions were used to adjust the pH, in order to compare the influence of the counter ion (TPA$^+$ and Na$^+$) on the process. The results show that ETS-4 is highly effective for Hg$^{2+}$ removal and that the pH adjustment solution has no impact on the equilibrium but influences the kinetic behavior of the ion exchange process. In fact, the equilibrium was reached much faster when TPAOH was used instead of NaOH. The equilibrium was accurately modeled by the mass action law expressed in terms of activities (Debye–Hückel model for solution and Wilson model for the solid exchanger phase). The Maxwell-Stefan (MS) approach was adopted to model the ion exchange kinetics, being able to describe accurately the Hg$^{2+}$ removal curves along time.

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**References**


Poster Session
INNOVATIVE MATERIALS AND APPLICATIONS
Green and sustainable strategy to produce plastic antibodies for highly-specific pharma separation processes

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Pharmaceutical industry spends between 50 to 80 % of production costs in API (Active Pharmaceutical Ingredients) purification processes. To address this worldwide demand, several highly-specific separation processes/materials have been proposed, such as plastic antibodies. The use of the Green Chemistry principles has changed the way materials are produced. Green technologies applied on the development of plastic antibodies [1] are appearing not only due to environmental issues but also by the improved features of the final product and process itself. Plastic antibodies have been developed in our lab using supercritical carbon dioxide (scCO2) technology for a wide range of applications, such as API purification [2], enrichment of natural products [3], removal of contaminants from diesel [4] and from water resources [5]. The plastic antibodies are typically obtained as solvent-free, ready-to-use and easy-to-handle dry-powders with narrow particle size distribution.

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References
Design of RAFT synthesized amphiphilic and stimuli-responsive block copolymers for encapsulation of polyphenols in polymersomes

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This research is devoted to the design and synthesis of amphiphilic/stimuli-responsive block copolymers for encapsulation of polyphenols in polymersomes. The Reversible Addition- Fragmentation Chain Transfer (RAFT) polymerization technique is used to get tailored amphiphilic block copolymers. Multiple combinations between hydrophilic/hydrophobic monomers, RAFT agents and initial compositions are the main design variables of the synthesis runs. Experimental studies are guided by modeling tools to aid in the design of the materials (e.g. to specify the sizes of the two blocks, pH sensitivity). Isolated polyphenols (e.g. resveratrol, quercetin) and more complex extracts from different kinds of plants are afterwards encapsulated in polymersomes, aiming at the development of carriers for protection and controlled release of these bioactive compounds. It is shown that the produced aggregates have a long colloidal stability and are promising vehicles for the stimulated-release of polyphenols.

Introduction

The beneficial effects of polyphenols on human health, namely due to their antioxidant/anti-inflammatory activities and protective actions on cardiovascular system (e.g.) are widely acknowledged by different scientific communities and observed in common populations. However, these bioactive compounds are prone to fast degradation processes (e.g. due to the effect of temperature, pH or light), have a low solubility in biological fluids and their fast metabolism and secretion rates are also commonly observed. Thereafter, the development of vehicles for polyphenols stabilization, protection from degradation and sustained release is a key point to improve their bioavailability and the efficient application in pharmaceutical, biomedical, cosmetic or food industries [1].

Different encapsulation approaches are being considered as possible routes to develop carriers for polyphenols and mitigate the above mentioned issues, namely physical methods (e.g. involving spray drying with a coating agent), physicochemical methods (e.g. based on ionic or hydrophobic interactions, resulting in micelles or liposomes formation) and chemical methods (e.g. in-situ polymerization/crosslinking systems) [1].

In the present research, we explore the Reversible Addition-Fragmentation Chain Transfer (RAFT) polymerization technique [2] to design amphiphilic and stimuli-responsive block copolymers for the encapsulation of polyphenols in tailored artificial vesicles (polymersomes [3]). Indeed, among other controlled polymerization techniques, RAFT polymerization offers the possibility to improve the precision concerning the design of special molecular architectures, namely the synthesis of amphiphilic block polymers with segments of tailored size and/or the incorporation of functional groups in polymer structure allowing stimulation (e.g. due to temperature/pH changes).

The work here presented comprises the RAFT synthesis of different kinds of amphiphilic and stimuli-responsive block copolymers, the characterization of the products and the testing of their self-assembly as polymersomes for polyphenols encapsulation. Isolated polyphenols (e.g. resveratrol, rutin, quercetin, etc) and more complex extracts from different kinds of plants (e.g. from chestnut, cherry or olive trees, obtained by hydro-alcoholic or supercritical extraction processes) are considered in these encapsulation studies. The RAFT polymerization synthesis work is accompanied by the development of polymer reaction engineering modeling tools, useful to aid with the design of the block copolymers.

Materials and Methods

The monomers acrylic acid (AA), methacrylic acid (MAA), 2-(dimethylamino)ethyl methacrylate (DMAEMA) and 2-hydroxyethyl methacrylate (HEMA) were used as hydrophilic monomers, leading to the formation of polymer blocks susceptible for stimulation in aqueous environments, namely due to pH changes. Styrene (S) and methyl methacrylate (MMA) were considered to generate hydrophobic moieties in the block copolymers. Azobisisobutyronitrile (AIBN) and 4,4-azobis(4-cyanovaleric acid) (ACVA) were used as thermal initiators. 2-cyano-2-propyl dodecyl trithiocarbonate (CPDPT), 4-cyano-4-[(dodecylsulfanylthiocarbonylsulfanyl)pentanoic acid (CDTPA), 4-cyano-4-(phenylcarbonylthio)pentanoic acid (CPA) and 2-(dodecylthiocarbonylthioyl)2-methylpropionic acid (DDMAT) were used as RAFT polymerization agents. The RAFT synthesis of the block copolymers was performed in two steps, starting with the formation of the first selected homopolymer in a solution (using dimethylformamide (DMF) or dimethyl sulfoxide (DMSO) as possible solvents) containing the selected amounts of monomer, initiator and RAFT agent. The obtained polymers were purified and dried after stopping the reactions (24 to 48 h are typical reaction times) and the second polymerization step was performed in a homogeneous solution containing the RAFT-macro chain (the first block), the second monomer and a proper amount of initiator (AIBN or ACVA). The block copolymers were obtained after the purification of the products of this second reaction step. The polymerization runs were typically performed at a temperature in the range 60 to 70 °C.

The different homopolymers and block copolymers synthesized were characterized using techniques allowing getting information concerning their composition (e.g. FTIR) and the
size of the homo/copolymer chains. SEC with refractive index and multi-angle laser light scattering detection was used with the later purpose, considering tetrahydrofuran (THF) or DMF as eluents. Note, however, that the SEC analysis of such kinds of polymers is cumbersome due to solubility limitations and multiple interactions between the different domains of the chains, the stationary phases of the columns and the eluent. In spite of the use of salts (e.g. LiBr) non-ideal SEC is often observed with possible erroneous determination of the block sizes. Batch static light scattering was thus also considered in the analysis of the homo/copolymers synthesized in this work. Polymersomes loaded/non-loaded with polyphenols were prepared through the dissolution of the selected block copolymer in THF (or DMF) in the presence/absence of the bioactive molecule (or a more complex plant extract). Afterwards, water (or other aqueous media) was dropwise added, under stirring, to that solution (e.g. at ~ 0.02 mL/min) up to achieve the final desired composition in the two solvents (e.g. THF/water 50/50).

Results

The self-assembly process of amphiphilic block copolymers in polymersomes and the properties of the resulting aggregates (size, shape, etc) is strongly dependent on the molecular architecture of these polymers, namely on the sizes of the hydrophilic and hydrophobic domains. Thus, our research includes the development of modeling tools (see [4] and references therein) to describe and carry out the design the structural features of the self-assemblies (e.g. radius of gyration and shape from the form factor $P(q)$) and images of the polymersomes were obtained using TEM.

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References

Adsorbent based on ZIF-8 for chiral separations in liquid chromatography

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Introduction

The biologic systems are constituted by chiral moieties that distinguishes easily the enantiomers of a chiral drug or metabolics thus producing distinct pharmacologic responses [1]. In this way the enantiomeric purity is a fundamental variable in the research and development of new synthetic drugs.

In the last years the pharmaceutical industry has shown a special interest for the studies of new solid stationary phases regarding separation of enantiomers. Concerning this issue, High Performance Liquid Chromatography (HPLC) allows a relatively fast resolution for racemic mixtures [2,3].

Troger basis (Figure 1) has been extensively used as model systems in chiral separation chromatography. This substance is an diamine that exhibit chirality due the presence of two stereogenic nitrogen atoms, forming two enantiomeric forms (5S, 11S)-(+) e (5R, 11R)-(−) as described by [4] and [5].

Figure 1. Troger basis enantiomers. Adapted from Zhang, Q. et al., 2017 (Ref. 4).

Chiral stationary phases (CSPs) are responsible for the enantiomeric discrimination [6]. Among the most used CSPs are those based on polysaccharides supported in silica. These (CSPs) exhibit mainly good mechanic stability and efficiency and well-defined structures based on cellulose e amyllose e which are optical active polymers easily found in nature [7].

In this context a new emergent class of coordination polymers named Metal Organic Frameworks (MOFs) appears as an alternative as a support for stationary phases. These materials are a combination of inorganic parts (metal ions) and organic parts (ligands) to form tri-dimensional crystalline structures with covalent bonds. (Figure 2). MOFs exhibit a high flexibility to form structures with several chemical properties mainly due to the benefit of the combination of different construction units and the possibility of chemical modification before and after the synthesis. By using a rational design, we can adjust chemical and physical properties of MOFs to generate materials with superficial areas as high as 7000 m²/g. The particular characteristics as high porosities, selectivity and forms as well as multiple active sites makes MOFs a very promising support which attracts the interest in the use in new high performance stationary phases for liquid chromatography. Several applications are being considered as gas storage, catalysis and drug delivery [8,9].

A sub-class of MOFs named zeolite imidazole frameworks (ZIFs). One example is the ZIF-8 formed by the metal zinc (Zn+2) linked to 2-metlimidazole (2-MeIM). This structure is one of the most known ZIF because of his high for polar and nonpolar solvents chemical stability and high mechanic strength, being considered for an extensive range of applications [10].

Only a few works in the literature applies MOFs as supports for chiral stationary phases. A recent revision concerning the synthesis of a chiral selector points out the experimental planning aiming at aminoacids separation [3].

Strategies to obtain effective chiral MOFs aim at the porosity maintenance to assure high adsorption capacities without selectivity loss. The preparation methods use both the direct coordination between enantiopure ligands and metallic ions or chiral matrices inducing chiral structures. An example of the of chiral ligands applications uses D-POST-1 in enantioselective separation [13].
Figure 2. Scheme for the process to build MOFs structure from basic units to structures of variable dimensions. Adapted from [11].

Objective
The main objective of the work is the development and characterization of a new stationary phase based on ZIF-8 and polysaccharides for enantiomeric separations in high performance liquid chromatography (HPLC).

Methodology

References
Sequential photocatalysis-electro-Fenton process mediated with magnetic Fe-TiO$_2$ beads for the treatment of polluted effluents

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Introduction

Pesticides are worldwide applied in order to enhance the agricultural efficiency reducing the percentage of product attacked by plagues. However, these pesticides can reach the environment after the washing of the agricultural products, the containers and due to run off because of rain and irrigation. Among them, Chlordimeform is a formamidine pesticide with an extremely high biological activity, being a potent toxin which main metabolite (4-chloro-o-toluidine) is highly carcinogenic [1].

Advanced Oxidation Processes (AOPs) are hereby proposed as alternative treatments for wastewater polluted with this stable compounds. Thus, the generated hydroxyl radicals (HO·) which are extremely powerful molecules (2.6 V vs SHE) would be able to attack the pollutant. Among AOPs, the Fenton process generates the radicals by the reaction between H$_2$O$_2$ and Fe$^{2+}$ (Eq. 1) [2]

$$\text{H}_2\text{O}_2 + \text{Fe}^{2+} \rightarrow \text{HO}^\cdot + \text{HO}^\cdot + \text{Fe}^{3+}$$ (Eq 1)

As it can be seen, H$_2$O$_2$ and Fe$^{2+}$ concentrations diminish as the reaction takes place, therefore, and in order to continue the process, more reagents should be added. What is more, the generation of Fe complexes with HO· diminish the availability of both of them, reducing the potential of the process [2].

For avoiding that, other AOPs can be coupled and thus, the electrolysis (electro-Fenton) can generate the H$_2$O$_2$ in situ due to O$_2$ reduction and regenerate Fe$^{3+}$ by the reduction of the spent Fe$^{3+}$. Moreover, coupling a photolysis process (photo-Fenton), the generated Fe complexes can be broken, favouring the availability of both Fe$^{3+}$ and HO· [3].

However, applying the UV radiation is usually the most costly step on this aforementioned photo-electro-Fenton process. Therefore, and in order to leverage more the radiation application, a photocatalyst can be added (photocatalysis-electro-Fenton process), so the radiation not only enhances the Fenton process but also starts a series of oxidation and reduction reactions due to the activation of a semiconductor compound. TiO$_2$ has been reported as an efficient photocatalyst due to its wide band gap (3.2 eV) [4], its stability and its inexpensive price.

Objectives

At this point, the main objective of the work was to obtain a heterogeneous bifunctional Fe-TiO$_2$ catalyst in order to avoid a secondary pollution on the effluent. In addition, this catalyst should be easily removed from the treated effluent in order to avoid an increase on the operational cost through typical options such a filtration, coagulation-flocculation, etc. The whole process should be useful for the remediation of a real wastewater spiked with Chlordimeform.

Methods

Thus, magnetic particles immobilized in chitosan beads where synthetized with the aim of facilitating the posterior magnetic separation [5]. There, the mixture between Fe and TiO$_2$: was accomplished in order to favour the photocatalysis-electro-Fenton process. The homogeneous distribution of Fe and TiO$_2$: into the chitosan beads was evaluated by SEM, EDS, XRD and FTIR tests. Moreover the high magnetization of the catalysts was quantified by measuring their saturation magnetization. This process was carried out in a cyclic process as shown in the Graphical Abstract, thus a two-reactors configuration was used, which was able to treat 400 mL of a 25 mg/L Chlordimeform solution. On the first reactor the electrochemical field was applied (50 mA) which was supplied with air (0.75 mL/min) in order to generate H$_2$O$_2$. On the second one, the photo radiation was applied (low consumption LED lamp, 40 W). The catalyst was added on this second photoreactor to favour its activation and to avoid its electrochemical degradation. A recirculation flow (240 mL/min) was used to connect both reactors.

Several variables affect this novel procedure such as the ratio Fe-TiO$_2$ or the catalyst concentration on the reactor. Thus, the optimization of the process was followed by Chlordimeform removal (HPLC) and mineralization (reduction on Total Organic Carbon: TOC).
Results
To begin with, the synergistic effect of the Fe and TiO$_2$ on the photo-electro-Fenton process was evaluated. For that the comparison between the catalyst with only Fe and with different Fe:TiO$_2$ ratios was accomplished. Additionally, a control test without catalyst was done.

As it can be seen, the TiO$_2$ addition caused an enhancement on the Chlordimeform removal when adding it the proper ratio Fe:TiO$_2$. The best performance of the ratio 1:2 can be clearly noticed (Table 1), as the removal was quicker and higher, harmonising with the 60 % mineralization obtained in 4 h (Table 1).

Then, the optimization of the catalyst dosage was accomplished in a range of 0 to 1.5 g/L. Regarding the pollutant elimination, the optimal concentration was 1 g/L (Figure 1) and above or under this value the performance of the process was reduced. At lower concentrations, the amount of active sites on TiO$_2$ may not be enough as well as the Fe quantity for reducing the available H$_2$O$_2$ to HO·. However, at high catalyst concentrations, not only scavenger reactions may happen but also the light scattering due to the presence of too many beads [6]. Nevertheless, the differences on mineralization are not so noticeable between 1 and 1.5 g/L; therefore, the lower concentration was selected in order to avoid extra costs.

Finally, the validation of the results was accomplished by degrading a secondary real wastewater effluent picked with 25 mg/L of Chlordimeform and by testing the reutilization of the utilized catalyst. The real wastewater was obtained of a treatment plant which had 35 mg O$_2$/L (chemical oxygen demand), 53 mg/L (TOC) and 2 mg/L (biological oxygen demand). Hopefully, the catalyst was reused 3 times without detriment on the real effluent remediation and without any structural modification. Complete mineralization was attained in 8 h.

![Figure 1. Chlordimeform removal at different catalyst concentrations after 30 min treatment.](image)

Conclusions
The two-reactors configuration of the photocatalytic-electro-Fenton process favors good catalyst activation and reutilization. This results could be easily applied to real scenarios because of the utilization of a reusable magnetic catalysts which provide promising results for the treatment of real effluents.

Table 1. Degradation and mineralization of Chlordimeform achieved after 30 min and 4 h, respectively, operating under the different conditions evaluated in the developed photocatalytic-electro-Fenton process.

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Catalyst (g/L)</th>
<th>Removal (%)</th>
<th>Mineralization (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without catalyst</td>
<td>0</td>
<td>41.95</td>
<td>8.24</td>
</tr>
<tr>
<td>Only Fe</td>
<td>1</td>
<td>45.52</td>
<td>23.93</td>
</tr>
<tr>
<td>Fe:TiO$_2$ (1:1)</td>
<td>1</td>
<td>62.08</td>
<td>28.48</td>
</tr>
<tr>
<td>Fe:TiO$_2$ (1:2)</td>
<td>1</td>
<td>81.23</td>
<td>59.42</td>
</tr>
<tr>
<td>Fe:TiO$_2$ (1:3)</td>
<td>1</td>
<td>75.99</td>
<td>34.73</td>
</tr>
<tr>
<td>Fe:TiO$_2$ (1:2)</td>
<td>0.25</td>
<td>51.39</td>
<td>29.83</td>
</tr>
<tr>
<td>Fe:TiO$_2$ (1:2)</td>
<td>0.5</td>
<td>56.59</td>
<td>49.82</td>
</tr>
<tr>
<td>Fe:TiO$_2$ (1:2)</td>
<td>1.5</td>
<td>58.71</td>
<td>59.97</td>
</tr>
</tbody>
</table>

Acknowledgements
This work was supported by Xunta de Galicia [ED431C 2017/47] and the Spanish Ministry of Economy and Competitiveness [Grant N° CTM2017-87326-R] funding the researcher Aida Maria Díez Sarabia (FPI program).

References
Calix[4]arenes are fascinating cage macrocycles, widely investigated due to their valuable properties, namely their ability to recognize and differentiate neutral and ionic guests of several origins.

Our recent work in the field has been focused on the synthesis and application of several calix[4]arene architectures as chemosensors, either as single-standing molecules or incorporated in conjugated polymer systems, having nitroaromatic explosives, explosive taggants and pollutants as target analytes. [1-5]

In this communication we describe the synthesis and characterization of new bicyclic calix[4]arene-based polymers, integrating phenylene-ethynylene-carbazole units as fluorescent segments, and their sensing ability as solid state sensors for several NAs in vapor phase.

Highly fluorescent polymers (CALIX-OCP-PPE-CBZs) were synthesized by Sonogashira-Hagihara cross-coupling polymerizations in good yields using 1,3-oxacyclophane tethered calix[4]arene derivatives integrating carbazole segments as fluorescent signaling moieties. The new materials were characterized by GPC, FT-IR and \(^1\)H NMR and their photophysical properties studied by UV-Vis and fluorescence spectroscopies. These new conjugated polymers were used as solid-state fluorescent chemical sensors for the detection of nitroanilines (NAs). Using fluorescence as the transduction technique, experiments have shown that films obtained by drop-coating are selective for \(o\)-nitroaniline (\(o\)-NA) as compared with isomeric NAs (\(m\)-nitroaniline (\(m\)-NA) and \(p\)-nitroaniline (\(p\)-NA)).

![Cross-coupling polymerization of Calix-OCP-di-I (3 and 4) and ethynyl carbazole derivatives.](image)

Figure 1 - Cross-coupling polymerization of Calix-OCP-di-I (3 and 4) and ethynyl carbazole derivatives.

The narrow rim 1,3-oxacyclophane tethered calix[4]arene derivatives (Calix-OCP-di-I; 3 and 4) were prepared from Calix-di-Br (1 and 2) which were in turn obtained from the parent calix[4]arene-tetraol. [1] After cross-coupling with reporter diethynyl carbazole monomers [2] Calix-OCP-PPE-CBZs (5-8; Scheme 1) were obtained as yellow brownish solids in good yields. Characterization of Calix-OCP-PPE-CBZs were performed by FTIR and \(^1\)H NMR, which confirm their proposed structures. Gel permeation chromatography (GPC) was used to estimate the polymers’ molecular weights. Figure 2 depicts the polymer traces thus obtained (3.2x10\(^3\) g mol\(^{-1}\) < \(M_n\) < 1.6x10\(^4\) g mol\(^{-1}\)). The ground-state absorption and emission spectra of synthesized polymers are presented in Table 1.

![Gel permeation chromatography traces of isolated Calix-OCP-PPE-CBZs against monodisperse polystyrene standards.](image)

Figure 2 - GPC traces of isolated Calix-OCP-PPE-CBZs against monodisperse polystyrene standards.

The films showed good photochemical stability as demonstrated by the fact that the intensity and the profile of the emission barely changed under the same conditions of the quenching experiments. The fluorescence response of the \(^\text{Bu}\)-Calix-OCP-PPE-2,7-CBZ (5) in the solid state upon exposure to \(o\)-NA, \(m\)-NA and \(p\)-NA vapors were evaluated through steady-state fluorescence quenching experiments with the materials as neat films. The \(^\text{Bu}\)-Calix-OCP-3,6 CBZ (6) polymer and debutylated polymers (7 and 8) did not afford good films for sensory applications. Figure 3 highlights the absorption and emission spectra of polymers 5 and 6.

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Calix[4]arene-carbazole-containing polymers synthesis, properties and thin film as molecular sensors

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Table 1 - Photophysical properties of Calix-OCP-PPE-CBZs.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$\lambda_{\text{max, abs}}/\text{nm}$ (CHCl$_3$)</th>
<th>$\lambda_{\text{max, em}}/\text{nm}$ (CHCl$_3$)</th>
<th>$\phi^a$ (CHCl$_3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>423</td>
<td>439</td>
<td>0.59</td>
</tr>
<tr>
<td>6</td>
<td>383</td>
<td>417</td>
<td>0.44</td>
</tr>
<tr>
<td>7</td>
<td>423</td>
<td>438</td>
<td>0.65</td>
</tr>
<tr>
<td>8</td>
<td>383</td>
<td>416</td>
<td>0.44</td>
</tr>
</tbody>
</table>

$^a$ Fluorescence quantum yields were determined using 9,10-DPA ($\phi=0.72$ in EtOH) as reference standard in air-equilibrated conditions (excitation at 380 nm, right angle geometry).

Figure 3. Absorption (solid line) and emission (dashed line) spectra of tBu-Calix-OCP-PPE-CBZs 5 and 6 in CHCl$_3$.

Figure 4 depicts the fluorescence response of the tBu-Calix-OCP-PPE-2,7-CBZ (5) film to o-NA in vapor phase.

It was found that tBu-Calix-OCP-PPE-2,7-CBZ (5) showed a remarkable sensitivity for o-NA vapors as compared to the other isomeric NAs. After 60 s of exposure to equilibrium vapor of o-NA more than 30% fluorescence quenching was observed, against 14% and 2% for m-NA and p-NA, respectively (Figure 5).

In conclusion, using isomeric NAs in vapor phase as analytes, it was pleasantly found that tBu-Calix-OCP-PPE-2,7-CBZ (5) shows an excellent behavior as a solid state chemosensor for o-NA even using non-optimized film thicknesses, as assessed by the time-dependent fluorescence quenching efficiencies. The vapor pressures of analytes ($o$-NA > $m$-NA > $p$-NA) may explain, in part, the observed selectivity.

Figure 5. Time-dependent fluorescence quenching efficiencies of tBu-Calix-OCP-PPE-2,7-CBZ (5), after being exposed to NAs vapors at rt (excitation at 380 nm).

Acknowledgements
We thank IPL (Project IPL/2016/NoSeMeTox/ISEL) and FCT/MCTES (UID/QUI/00616/2013) for financial support.

References
Over the last two decades, investigations in the field of conjugated polymers (CPs) have attracted significant attention due to their unique optical and electrochemical properties, in particular, their application in sensing devices. Sensory signal amplification (compared to small-molecules), versatile structures that can be easily adapted to adjust solubility, absorption/emission wavelengths, etc. are some of the features that have made CPs a fluorescence sensing platform of choice in the recent years. [1] The incorporation of calixarenes, a class of synthetic molecular receptors possessing intramolecular cavities capable of selectively interact and establish specific host–guest complexes with neutral and ionic molecular guests in solution and solid-state [2], in CPs allows the construction of systems with enhanced sensitive and selective capabilities. Concerning the development of these type of sensory materials for sensing explosives, contaminants, and biological materials, some fluorescent calix[4]arene-based polymers incorporating calixarene receptors into their constitution have been synthesized by us [3] and evaluated as fluid and solid-state sensors for the detection of explosives (nitroaromatic compounds (NACs) and nitroaliphatic compounds) and nitroaromatics (NAs). [4]

In this communication we report the results of the chemosensing ability of newly designed bicyclic calix[4]arene-carbazole-polymers (Calix-OCP-PPE-CBZs) towards the detection of NAs and NACs in liquid phase. The new materials possess either different dimensions and electronic inputs in the upper rim of calixarene cup or distinguished carbazole linkages in the polymer main chain (chief transduction site), prompting for enhanced molecular interactions with envisaged analytes. The Calix-OCP-PPE-CBZs (Figure 1) were prepared by Sonogashira polycondensation following our reported method. All polymers were obtained in good isolated yields, having $M_n$ ranging from 3200 – 16000 gmol$^{-1}$. Detailed results are available in another poster communication at this conference. In order to evaluate the polymers’ photostabilities under the same conditions of the quenching experiments, photobleaching studies in solution were performed by continuous UV irradiation.

Fluorescence titration experiments were carried out with diluted solutions of the fluorophores ($6\times10^{-7}$ M in CHCl$_3$) and known amounts of isomeric nitroanilines ($o$-NA, $m$-NA and $p$-NA) and nitroaromatic explosives (TNT and PA), under 380 nm excitation.

The potential of new fluorescent polymers (CALIX-OCP-PPE-CBZs) toward the selective detection of isomeric nitroanilines (NAs) and explosives, namely 2,4,6-trinitrotoluene (TNT) and picric acid (PA) was evaluated in fluid phase. All polymers showed a considerable photoluminescence (quantum yields ranging from 0.44 to 0.65 in CHCl$_3$) and a great stability toward photobleaching. A Stern-Volmer analysis of steady-state fluorescence data in solution showed that all polymers display a better response and a differentiated sensitivity for NAs than for explosives. In particular, a remarkable response for $o$-NA was retrieved, as could be evaluated from the corrected Stern-Volmer constants ($K_S$ up to $1.3\times10^4$ M$^{-1}$ in CHCl$_3$).

The extent of the developed interaction between the sensors and the analytes were quantified by the Stern-Volmer approach. In general, it was found that all polymers displayed better sensitivity toward NAs detection in solution when compared with NACs (Table 1). A greater selectivity to $o$-NA (Figure 2) was witnessed, particularly when 3,6-CBZ polymers 2 and 4 were used as sensors (cf. Figure shown in the Graphical Abstract for polymer 4 with $o$-NA). The corrected (at both excitation and emission wavelengths) Stern-Volmer constants are presented in Table 1. It was verified that the quenching efficiencies of $o$-NA > $p$-NA > $m$-NA do not correlate with the analytes’ electron affinities, $m$-NA ($E_{LUMO}=-2.24 \text{ eV}$) > $o$-NA ($E_{LUMO}=-2.17 \text{ eV}$) > $p$-NA ($E_{LUMO}=-1.96 \text{ eV}$) meaning that photoinduced electron transfer as the mechanism for emission reduction does not hold. Formation of a dark ground-state complex between the fluorophores and the most polarizable analytes ($o$- and $p$-NA) and/or energy transfer between the donor fluorophores and analytes (acceptors) may explain the observed results. The complex formation between $^1$H-Calix-OCP-PPE-3,6-CBZ (4) and $o$-NA was confirmed by the continuous
variation method (Job plot; Figure 3), evidencing the formation of a 1:1 complex.

![Figure 2. Stern-Volmer plots after correction for NAs absorption with polymer 4.](image)

The results obtained for $\text{pH}$-Calix-OCP-PPE-3,6-CBZ (4) in the detection of nitroanilines clearly point to future applications of this polymer as a sensitive and selective sensor for $\text{o-NA}$.

Table 1. Corrected Stern-Volmer constants ($K_{sv}/M^{-1}$) found for Calix-OCP-PPE-CBZs/analytes pairs.

<table>
<thead>
<tr>
<th>Polymer</th>
<th>$\text{o-NA}$</th>
<th>$\text{m-NA}$</th>
<th>$\text{p-NA}$</th>
<th>TNT</th>
<th>PA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5923</td>
<td>2793</td>
<td>3733</td>
<td>229</td>
<td>609</td>
</tr>
<tr>
<td>2</td>
<td>7153</td>
<td>1191</td>
<td>2676</td>
<td>247</td>
<td>1751</td>
</tr>
<tr>
<td>3</td>
<td>5808</td>
<td>882</td>
<td>2924</td>
<td>357</td>
<td>1065</td>
</tr>
<tr>
<td>4</td>
<td>13289</td>
<td>985</td>
<td>2565</td>
<td>209</td>
<td>714</td>
</tr>
</tbody>
</table>

![Figure 3. Job plot for 4:o-NA complexation in CHCl$_3$ (1.0x10$^{-6}$ M).](image)

Acknowledgements
We thank IPL (Project IPL/2016/NoSeMeTox/ISEL) and FCT/MCTES (UID/QUI/00616/2013) for financial support.

References
Introduction

Due to its durability and versatile properties polyurethane foams (PUFs) have been used in domestic and industrial applications as well as in space travel and medicine.[1] Unfortunately, the PUFs industry is still heavily petroleum dependent, so extensive research has been concentrating on the use of polyols from renewable sources. Besides the use of bio-polyols, the use of industrial by-products, namely crude glycerol (CG) has been explored as polyol to produce rigid PUFs which has the potential to reduce their cost and environmental impact.[2]

The versatility of PUFs application arises from the fact that a wide range of properties can be achieved by small modifications on the formulation used. In that sense, it is common to adjust the type and content of the polyol, isocyanate, catalyst, surfactant, blowing agent and additives in order to control the properties of the ensuing foams.[3]

Rigid PUFs are traditional used as thermal insulation materials and if heat storage materials are incorporated into PUFs, the heat gain/loss, from/to the surroundings might be reduced and the energy saving can be more efficient.[4] In the last years, this concept has found growing interest as a result of the rise of the phase change materials (PCMs). However, due to the low thermal conductivity of rigid PUFs, to enhance the efficiency of PCMs it is required the use of conductive heat transfer agents such as expanded graphite (EG). In turn, PUFs as well as most organic materials burn very easily. Although for many years the fire performances of PUFs were suitable, nowadays the fire performances must be improved due to the greater attention paid to fire safety. EG is widely used as flame retardant in PUFs. Once exposed to heat, it forms a low density “worm” like structure on the surface of the PUFs that prevents heat and oxygen transfer, providing a good fire retardant performance.[5]

The versatile use of PUFs as construction materials is not just associated with their thermal insulation properties, but also with their sound insulation properties. An easy way to design a PUF with enhanced sound performance is by tuning its formulation or use different reactants in order to achieve the appropriate cellular structure.[6]

Finally, due to the variety of applications of PUFs, their production has increased in the past decades, leading to an increase of PUF waste, as well as an increase of the concern about its disposal. In that sense, PUFs wastes must be effectively recycled, aiming the environment protection.

Methods

The polyol components and the corresponding amounts of catalyst, surfactant and blowing agent, were placed in a polypropylene cup and homogenized using a mechanical stirrer. Next, if used, the fillers were added and the mixture blended again. Finally, the appropriate amount of isocyanate was added, the mixture homogenized again and the foams were obtained. PUFs specimens (10x10x10 mm³) were cut and weighed to determine the density. A KD2 Pro (Decagon Devices) was used to measure the thermal conductivity of the PUFs after introducing the thermal conductivity sensor inside the foams. An Instron 5966 universal mechanical test analyzer was used to measure the compressive strength of the foams, according to the ASTM D 695.

Results

Ecofriendly PUFs have been produced using CG samples with different compositions and the results obtained indicate that the methyl esters and fatty acids present in CG affect the foaming process, the crosslinking density and can have some plasticizing effect. As a result, the mechanical properties of the ensuing foams are lower than those obtained for PUFs derived from refined CG. Nonetheless, the thermal stability and thermal conductivity are not dependent on the CG composition. The rationale to fine tune formulations of PUFs derived from unrefined CG in order to modulate their physical properties has been established using statistical analysis. The results obtained (see Figure 1) proved that both density and thermal conductivity are governed by the blowing agent and surfactant contents. In turn, the mechanical properties are essentially determined by the content of isocyanate and catalyst.

Figure 1 - Relative effect sizes (Eta squared) of the formulation components on the properties of the foams [3]
To increase the heat storage capacity, the foams were filled with PCMs and to enhance their action, EG was added. From the numerical simulations, it was observed that the foams presented similar peak temperatures and amplitude, since the addition of EG and PCMs results in an increase of thermal conductivity and density, but similar enthalpy. In other words, the foams filled with PCM have higher thermal conductivity and density, which balances out the expected PCM effect needed for improvement. EG was also used to enhance the reaction to fire of PUFs. The presence of EG increased the CO/CO\text{2} ratio of the smoke released and the residual mass of the foams after burned. Also due to the physical barrier effect of EG, the heat release rate (HRR), the effective heat of combustion (EHC), the mass loss ratio and the specific extinction area (SEA) decreased. More importantly, EG acted like a flame extinguisher (see Figure 2).

A polyol obtained from the liquefaction of coffee ground was used to improve the sound insulation properties of CG derived PUFs. From Figure 3, the NRC values (noise reduction coefficient) it can be observed that the foams prepared using polyols derived from renewable resources are generally similar to those reported for other materials used as sound absorbers. In fact, in some cases they are even higher, indicating that these rigid foams have potential as sound absorptive materials. Furthermore, judicious blending of these polyols and formulations allows broadening the range of frequencies where rigid PUFs can be efficiently used as sound absorbers.

![Figure 2 - Time dependence of the maximum temperature of all the samples during combustion](image)

A polyol obtained from the liquefaction of coffee ground was used to improve the sound insulation properties of CG derived PUFs. From Figure 3, the NRC values (noise reduction coefficient) it can be observed that the foams prepared using polyols derived from renewable resources are generally similar to those reported for other materials used as sound absorbers. In fact, in some cases they are even higher, indicating that these rigid foams have potential as sound absorptive materials. Furthermore, judicious blending of these polyols and formulations allows broadening the range of frequencies where rigid PUFs can be efficiently used as sound absorbers.

**Figure 3 - Noise reduction coefficient (NRC) CG and/or liquefied coffee grounds PUFs [5]**

Finally, from the perspective of the circular economy, PUFs residues were recycled. Despite of several chemical recycling methods reported in literature, a new chemical method, the acidolysis is emerging. This involves the conversion of PUFs wastes into a reactant, which can be used in the production of new PUFs, according to scheme 1.

**Scheme 1 – Reaction scheme of acidolysis**

To test the effectiveness of this method, CG derived PUFs wastes were milled and fed to a reactor. Also to the reactor was added a dicarboxylic acid, an antioxidant and CG and the reaction was carried out under controlled temperature for a reasonably short period. The reaction product obtained was used up to 25% (w/w) of substitution of CG in the production of foams. Since the resulting foams presented similar properties, comparing with the original ones, the suitability of this recycling method was demonstrated.

**Conclusions**

To contribute to the sustainability of the PUF industry an eco-friendly polyol (the CG) was used to produce foams. The CG was used directly, without any pre-treatment or purification step and the ensuing foams presented excellent properties, meaning that CG is a suitable feedstock for the production of PUFs. Furthermore, to increase the added value of these materials, their reaction to fire and their sound absorption properties were enhanced successfully. Regarding the enhancement of thermal comfort, it was observed that foams filled with PCM have higher thermal conductivity and density, which balances out the expected PCM effect needed improvement. Finally, a recycling process was developed for this type of foams which helps to improve even more the sustainability of the PUFs industry.

**Acknowledgements**

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**References**

Incorporation of IL@ZIF-8 composites into Matrimid® 5218 to produce mixed-matrix membranes for gas separation

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Abstract

Low-energy demanding, environmentally friendly and cost-effective strategies to mitigate greenhouse gas (GHG) emission to the atmosphere are vital and expected. Metal-Organic Frameworks (MOFs) and Ionic Liquids (ILs) stand out as promising alternatives to more traditional materials for gas separation, mainly due to their high CO₂ sorption and their physicochemical properties. Their combination into IL@MOF composites have potential to improve gas uptake or/and selectivity in adsorption-based processes. Also, the use of mixed-matrix membranes (MMMs) combining a polymeric matrix with a filler is a potential alternative process for CO₂ separation. High CO₂ permeability, chemical and thermal resistances can arise when using MOFs fillers. This solves the trade-off problem of polymeric membranes by combining the sieving effect of MOFs with the properties of polymeric matrixes. Moreover, the use of IL@MOF composites as fillers in MMMs have shown advantages on their mechanical properties and gas permeability and/or selectivity. In this work, IL@MOF composites, previously developed, were used as fillers in a Matrimid® 5218 matrix. The performance study of the MMMs produced is ongoing to assess their CO₂/CH₄ separation ability.

Introduction

The atmospheric concentration of CO₂, the main greenhouse gas (GHG), has surpassed the 400 ppm threshold [1]. The emission of GHGs has been identified as the main contributor to global warming and, consequently, to climate change. Mitigating GHGs emissions is arguably the most urgent societal challenge of this century. Thus, it is of vital importance to develop new strategies, processes and materials for such a task. Membrane-based processes have been designed for gas separation due to their simplicity, cost and energy-efficiency, and relative easiness to scale up. Common membrane properties, useful for CO₂ capture/ separation, include high CO₂ permeability, chemical and thermal resistances, aging resistance and ability to be cheaply produced [2]. Mixed matrix membranes (MMMs) combine the best features of both polymeric and inorganic membranes. They are composed by an inorganic filler (discrete phase) incorporated into a polymeric matrix (continuous phase) [2]. Metal-organic frameworks (MOFs) are topical adsorbent materials consisting of metal atoms or small metallic clusters linked by organic functional groups. These materials have been used as filler of MMMs because they could solve the trade-off problem of polymeric membranes, combining the sieving effect of MOFs with the properties of polymeric matrixes [3].

Recently, some publications used MOFs impregnated with ionic liquids (ILs), resulting in new materials designated as IL@MOF, as fillers for MMMs [4]. ILs are salts with low melting point (below 373 K) and consist of organic, asymmetric cations and organic or inorganic anions. The incorporation of ILs into the structure of the MOF is expected to have a positive impact on the gas permeability and/or the selectivity of the membrane. Therefore, previously produced IL@MOF composite materials were used as fillers for MMMs based on Matrimid® 5218 polymeric matrix.

Objectives

The main purpose of this work is to study the performance of produced MMMs with distinct IL@ZIF-8 composites for the separation of CO₂/CH₄ in terms of gas permeability and CO₂/CH₄ selectivity. Another aspect of this study is the role of the IL impregnated into the MOF structure on gas permeation.

Materials and Methods

The commercial MOF used in this work is ZIF-8 (2-methylimidazole zinc salt), chosen due to its physical and chemical properties such as microporosity, high thermal stability and high specific surface area [5]. Three imidazolium-based ILs were chosen to impregnate ZIF-8 due to their properties and structure: 1-hexyl-3-methylimidazolium bis[(trifluoromethyl)sulfonyl]imide ([C₆MIM][NTf₂]), 1-hexyl-3-methylimidazolium tetracyanoborate ([C₆MIM][B(CN)₄]) and 1-ethyl-3-methylimidazolium acetate ([C₃MIM][Ac]).

The composites used as fillers were fully characterized previously, through several techniques: Helium Pycnometry, N₂ Adsorption-Desorption at 77 K, Fourier Transform Infrared Spectroscopy (FT-IR), Powder X-Ray Diffraction (PXRD), Scanning Electron Microscopy (SEM), and adsorption-desorption equilibria measurements of CO₂ and CH₄ in the range of 0-16 bar of pressure and at the temperature of 303 K. In this study, the produced MMMs are characterized by Scanning Electron Microscopy-Energy Dispersive X-ray Spectroscopy (SEM-EDS), Thermogravimetric Analysis (TGA), contact angles and mechanical resistance essays.

The MMMs with pristine ZIF-8 or IL@MOF loadings ranging between 10-30 wt.% were prepared, through solvent evaporation method. Gas permeations experiments are being performed at 303 K to determine the permeability of the prepared membranes for CO₂ and CH₄, using a gas permeation set-up previously reported [6,7]. The generated driving force between the feed and permeate compartments was about 0.7 bar of relative pressure.
Results and Conclusions
An ongoing study of the incorporation of previously prepared IL@ZIF-8 composites as fillers for the preparation of MMMs using Matrimid® 5218 as the polymeric matrix is presented. The MMMs are being fully characterized to assess their performance towards the permeabilities of CO₂ and CH₄ and CO₂/CH₄ selectivity. The MMMs prepared with IL@ZIF-8 in the MMMs are being compared with the MMM filled with pristine ZIF-8 to assess the role of the IL impregnated into the MOF structure on gas permeation. Figure 1 illustrates some results obtained from the gas permeation experiments and its comparison with Matrimid® 5218/ZIF-8 MMMs retrieved form the literature, as well as the reference Robeson upper bound correlation for CO₂/CH₄ separation [8,9]. The results obtained suggest that the use of IL@MOFs as fillers in the MMMs can be a promising strategy to improve their gas separation performance. The incorporation of ILs into the structure of ZIF-8 predominantly increases the CO₂ permeability of the membrane. However, some of the MMMs tested show high performance and surpasses the Robeson’s limit, relative to the performance of the regular polymeric and Matrimid®/ZIF-8 MMMs. Tuning the IL structure of the MOF filler will be of critical importance, as it influences the selectivity/permeability of the produced MMMs.

Acknowledgements
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References
Aiming to stabilize Ibuprofen (Ibu) in the amorphous form, it was loaded by capillarity in MCM-41 and modified MCM-41$_{\text{sil}}$. The obtained composites were characterized by ATR-FTIR, ssNMR, DSC and TGA. The thermal decomposition occurs at a higher temperature relative to Ibu, indicating an increase of thermal resistance of the drug in both matrices; the decomposition occurs in a two-step profile, pointing to two different populations (bulk-like and another which interact with the pore surface). The calorimetric detection of a glass transition and the absence of melting confirmed the amorphization of Ibu in the composites. Moreover, the DSC thermograms evidence a bimodal heat flux discontinuity and a shift of the glass transition temperature to higher temperatures more significant in MCM-41$_{\text{sil}}$ due to stronger interactions of the drug with the host surface. The drug release studies were carried out in a PBS buffer (pH=6.8), analyzed with UV-VIS and simulated with several kinetic models.

This work aims to rationalize the drug release profile from mesoporous matrices, in terms of guest-host interactions influenced by the surface chemical composition. The incorporation of pharmaceutical drugs in a matrix was extensively studied as a strategy to stabilize the amorphous form and simultaneously enhance the solubility of currently commercial drugs [1, 2]. Ibuprofen (Ibu), a pharmaceutical drug with antipyretic, analgesic and anti-inflammatory properties [3], was loaded in unmodified mesoporous inorganic silica MCM-41 and modified by silylation (MCM-41$_{\text{sil}}$) with functional organosilane (methyltrimethoxysilane); nitrogen adsorption-desorption analysis provided a narrow pore size distribution, characteristic of this type of silica, being centered at 3.5 nm and 3.3 nm, respectively for MCM-41 and MCM-41$_{\text{sil}}$. The comparative spectroscopic study of the unloaded matrices by attenuated total reflectance Fourier transform infrared (ATR-FTIR) and by $^{29}$Si MAS solid state nuclear magnetic resonance (ssNMR) together with $^{13}$C CP/MAS ssNMR, confirmed a successful silylation (shown in the Graphical Abstract for the unmodified silica). The decrease of surface area and pore volume as observed by nitrogen adsorption-desorption analysis, reinforced the success of surface modification.

The drug loading, carried after vacuum heat treatment at 3 mbar and 150 °C for 8h to remove water and impurities, was performed by capillarity under vacuum from an ibuprofen solution using chloroform as solvent. The obtained composites were characterized by a set of experimental techniques such as ATR-FTIR spectroscopy, ssNMR spectroscopy, differential scanning calorimetry (DSC) and thermogravimetry (TGA), all providing evidence of successful drug loading. The thermal decomposition of composites monitored by TGA occur mostly at a higher temperature relative to native ibuprofen, indicating an increase of thermal resistance of the incorporated drug in both matrices; moreover, the decomposition occurs in a two-step profile, more evident in MCM-41$_{\text{sil}}$, pointing to two different populations, one more bulk-like and another one in a closer interaction with the pore walls.

The detection of a glass transition by DSC and the absence of the melting peak of the native drug ($T_{m_{\text{Ibu}}} = 77 \degree$ C) confirmed the successful amorphization of Ibu in the composites. The calorimetric analysis corroborate TGA findings evidencing a bimodal heat flux discontinuity and a shift of the glass transition, that in native Ibu is around -40 °C as taken in the midpoint, towards higher temperatures: respectively -34 °C in MCM-41 and -24 °C in MCM-41$_{\text{sil}}$; the accentuation of this effect for ibuprofen loaded in MCM-41$_{\text{sil}}$ is interpreted as a consequence of greater interactions of the guest with the mesoporous host surface. Finally, the drug release studies were carried out in a phosphate buffer with a pH of 6.8, to simulate the intestinal fluid, at a constant temperature of 37 °C and 100 rpm and analyzed at 222 nm with UV-VIS spectroscopy. The time-release of ibuprofen was simulated according different kinetic models being interpreted in terms of guest-host interactions.

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References
Crosslinked starch/chitosan microparticles as reinforcement of thermoplastic corn starch

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Thermoplastic corn starch (TPS) materials are known to have limitations regarding their application as biodegradable material for short lifetime products. This study proposes the incorporation of starch/chitosan microparticles crosslinked with glutaraldehyde (SCM) in a TPS matrix plasticized with glycerol. The use of microparticles made of starch increase the compatibility with the TPS matrix. Morphological, thermal and water solubility and boiling water solubility were conducted on the SCM particles to assess their stability in different conditions. X-ray diffraction performed to the SCM indicated that crosslinked starch avoids retrogradation. Tensile strength, Young’s modulus and toughness were improved with the incorporation of 30 % (w/w) of SCM in the TPS. The elongation of these materials was also improved. A slight decrease in the water uptake was detected when SCM was incorporated in the matrix.

Introduction

Environmental concerns increase over the years related to the intensive use and waste disposable of common petroleum-based plastics, specially the plastics where the end use application is short lifetime, like disposable eating utensils, food packaging, films or trash bags, that originates a huge amount of waste, mostly non-biodegradable. Thermoplastic starch (TPS) is 100 % renewable and completely biodegradable and can be a potential solution to reduce the waste produced by those applications. However, it has some disadvantages that need improvement.

Starch is highly hydrophilic and has great limitations regarding its processability [1].

Native starch is composed of amylose, a linear polymer of D-glucose units linked by (1→4) bonds, and amyllopectin, a highly branched polymer of D-glucose units linked by (1→4) bonds, branched with (1→6) linkages at intervals of 20 units, approximately. The production of thermoplastic starch (TPS) requires the disruption of starch granules, in a process called gelatinization, where, when in contact with a solvent able to form hydrogen bonds and above a certain temperature, the amylose molecules are dissolved, the crystallites present in the amyllopectin molecules start melting and the hydrogen bonding among the molecules is lost, forming a paste [2]. Plasticizers, like glycerol, are used to decrease intermolecular hydrogen bonding in amylose and amyllopectin. This allows starch to be processed by extrusion. Despite particulate fillers, like graphene, or clays, lignocellulosic fibers, like cotton cellulose nanofibers or green coconut fibers, are the most common fillers in TPS, since they are readily available, are renewable and have been reported for improving mechanical properties of TPS, such as tensile strength [3].

Objectives

The incorporation of starch/chitosan microparticles crosslinked with glutaraldehyde in thermoplastic corn starch is a new approach for reinforcing TPS matrix. The similarities in the nature of the filler particles are expected to ensure a good compatibility and, therefore, to improve the mechanical properties of these blends.

Methods

Starch/chitosan microparticles (SCM) were prepared by solvent exchange technique, adapting procedures previously described in the literature for microparticles composed solely of starch [4]. The thermoplastic materials were prepared by melt mixing, using 30 % glycerol as plasticizer, as described by Carvalho et al. [5]. The SCM content was varied from 0 to 50 %. The mixing chamber temperature was 140 °C. The twin screws were operated at 200 rpm and the mixture time after loading was 5 min. The resulting mixture was then injected into a mold to produce dog-bone shaped specimens with 2 mm thickness and 80 mm length. The injection pressure was 10 bar, the temperature in the injection nozzle was set to 150 °C and the mold temperature to 60 °C. The characterization of SCM particles consisted on the evaluation of their stability in different conditions. Water and boiling water solubility were evaluated in terms of total soluble matter (TSM) to assess the efficiency of the crosslinking reaction. XRD measurements provided information about the crystallinity of these particles and their behavior with time. SCM were also morphological characterized by SEM and their thermal stability was evaluated by thermogravimetric analysis. Thermoplastic corn starch and its blends were characterized by tensile tests and water absorption.

Results

The SCM particles presented a roughly spherical shape with diameters between 10 and 20 μm. The quantification of total soluble matter is a simple technique to evaluate the effectiveness of the crosslinking reaction. The results showed that using 7.5 % of glutaraldehyde per 100 g of polysaccharide achieved the lower values of TSM either in water or boiling water, at both pH values tested (4 and 7). Thermogravimetric curves showed the onset of thermal degradation for corn starch is 311 °C and the onset of degradation for SCM occurs at the same temperature as for chitosan (280 °C). The residual mass fraction of SCM is 27 %. Considering the starch/chitosan ratio present in the microparticles, a residual mass of 19 % should be expected. The higher value obtained is a consequence of crosslinking with glutaraldehyde improving thermal stability of the material. X-
ray diffractions measurements (Figure 1) showed that native corn starch presents a crystalline structure with A-type polymorphism, that is common in cereal starches. At day 1 and day 30 after production, the SCM show peaks at $\theta = 13.2^\circ$ and $20.2^\circ$, resembling the Va-type microstructure [6], indicating the absence of retrogradation.

Figure 1. X-ray diffraction patterns for native corn starch and SCM, at day 1 and day 30 after production.

The mechanical performance of thermoplastic starch was evaluated by stress-strain curves. The shape of the stress-strain curves indicates that linear elastic behavior is present only at low strains, and is followed by extensive ductile deformation, without a defined stress yield point (Figure 2). This behavior is typical of thermoplastic starches. Strain hardening occurs during the plastic deformation regime, as a consequence of reorientation of molecular chains and/or crystalline regions in the direction of the applied stress. This contributes to increase the toughness of the material under tensile strain [7].

Figure 2. Representative stress-strain curve obtained for thermoplastic starch containing 30 % SCM and without SCM.

The ultimate tensile strength (UTS) reaches a value 58 % higher than neat TPS for 30 % SCM content and decreases afterwards. This reinforcement is a consequence of efficient stress transfer through a strong interfacial bond between the thermoplastic matrix and the microparticles. The observed 84 % increase in the elongation at break, for 30 % SCM may be due to the microparticles being able to undergo elastic deformation while maintaining strong physical bonding with the thermoplastic matrix, which is not a common behavior. The Young’s modulus and toughness also exhibit a maximum for 30 % SCM content. The combined increase in rigidity, strength and elongation at break for SCM contents up to 30 % leads to significant improvement in toughness, computed as the area under the stress-strain curves (Figure 3).

Figure 3. Toughness as a function of SCM content in thermoplastic starch.

Toughness is about 360 % higher for 30 % SCM content than for neat thermoplastic starch, which translates into a much higher capacity to absorb energy without fracture. Above this filler content, all properties decrease, due to inefficient dispersion of the microparticles, as mentioned before. Water absorption was measured along time under 100 % relative humidity for all materials. In all cases maximum absorption is attained after three days. The microparticles, despite being insoluble, are able to absorb water and therefore do not seem to affect the hydrophilicity of material, since results showed only a slight decrease in water absorption, when compared with neat TPS.

Conclusions

Particles made of corn starch and chitosan were successfully cross-linked with different amounts of glutaraldehyde. The particles synthesized with 7.5 g per 100 g of polysaccharide present the highest water resistance and a thermal resistance similar to the native corn starch. XRD results showed that, after cross-linking, these particles lose the ability to retrograde, which is an important feature to their application in thermoplastic starch matrices. The thermoplastic composite reinforced with 30 % of cross-linked particles presented the highest ultimate strength, the highest Young’s modulus, the highest elongation at break and the highest values for toughness, proving that cross-linked particles made of corn starch can effectively reinforce a thermoplastic corn starch composite. These composites also present lower water uptake than the thermoplastic starch without particles.

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References

Novel O/W green emulsions stabilized with nano-hydroxyapatite solid particles – Pickering emulsions

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Surfactant-free emulsions have been gaining increasing interest along the last years. In this context, Pickering emulsions, characterized by using solid particles as stabilizers provide an innovative, promising and green alternative to produce stable emulsions. In this work, Pickering emulsions were developed using sunflower oil dispersed in water and stabilized with nano-hydroxyapatite (n-HAp). The impact of nanoparticle’s concentration, and oil-water ratio were studied on the emulsion stability and type. Stability, droplet’s size and morphology were monitored during storage time by optical microscopy (OM), and cryo-scanning electron microscopy (Cryo-SEM). The presence of n-HAp at the oil-water interface was confirmed by both techniques. A model was developed to predict the stability behaviour of Pickering emulsions based on the distance between the n-HAp particles that remain free in solution.

Introduction

Emulsions consist on mixtures of two immiscible liquids, usually oil and water. Traditionally, their stabilization is achieved by one or various chemical emulsifiers. However, recently, some of these emulsifiers were related with allergic reactions and carcinogenicity leading to serious health problems [1].

Pickering emulsions appear as an alternative to traditional emulsifiers, since they use solid particles as emulsion stabilizers [2]. Briefly, the solid particles tend to be positioned in the interface between the two immiscible liquids, stabilizing droplets against coalescence [3]. The first Pickering emulsions date from 1900’s [4]; since then, this technology received significant attention, both at industrial and academic level, especially due to their attributes, such as the increased functionality, and the non-toxicity of the particles. Thus, Pickering emulsions become attractive to be used in applications such as pharmaceutical, agrochemical and food, since this process allows the formation of a protective shell around the oil or aqueous core, serving as encapsulation systems for drug and bioactive compounds-controlled release [2, 5].

Inorganic and organic particles have been used to stabilize Pickering emulsions. Among them, modified silica, proteins and fat crystals are some examples of suitable solid particles to stabilize these emulsions [6-8].

More recently, hydroxyapatite (HAp) has been described as a suitable Pickering stabilizer, since due of its hydrophilicity, HAp tends to stabilize O/W emulsions [9]. Several authors have worked in order to develop Pickering emulsions stabilized with HAp particles, however, in most cases, the produced emulsions use other polymers (dissolved in the opposite phase of HAp) to improve the interaction between oil and water phases [9, 10].

Objectives

The aim of this work was to developed stable O/W Pickering emulsions using n-HAp particles as stabilizers. The impact of process parameters, such as solids concentration and oil-water ratio, on emulsion stability and phase separation occurrence by creaming and sedimentation, were studied. Additionally, a model was developed to predict the type of emulsion formed (stable, unstable and gel form).

Materials and methods

A nano-hydroxyapatite aqueous paste (nanoXIM-HApCare, supplied by Fluidnova S.A.) and sunflower oil were used as aqueous and oil phase, respectively. For the production of the Pickering emulsions, a mixing system comprised by a high-speed dispersing and homogenizing device Miccra D-9, a thermostatic bath and a peristaltic pump, was used. Briefly, the oil phase was injected into the aqueous phase by using the peristaltic pump at 240 rpm (~43 mL/min), and the homogenizing device set at 11 000 rpm to ensure a prompt dispersion. The temperature was kept constant (20 ºC) using the thermostatic bath.

Results

The Pickering emulsions were developed considering parameters that can affect the stability. Thus, the effect of the solid (n-HAp) concentration and the oil-water ratio, on the emulsion stability was evaluated during two months of storage. Pickering emulsions stability was inferred from OM analysis (emulsion morphology and droplet’s interaction).

With respect to solids content, n-HAp aqueous paste concentrations from 0.5 up to 15 %wt were considered. After the emulsification process, all Pickering emulsions presented a morphology characterized by spherical and well distributed Pickering droplets. In the graphical abstract, it is possible to observe a typical OM image taken from a Pickering emulsion, as well as an image of cryo-SEM. At initial time (0 days), identical shape and size of the Pickering droplets was observed and achieved by OM for all emulsions tested with different solids concentrations. At low n-HAp concentration (1.5 %wt.), the produced Pickering emulsions become unstable after some hours; by OM analysis irregular shaped droplets were observed after two days, which indicated emulsion coalescence. Furthermore, gel-like type Pickering emulsions were produced when using n-HAp paste at 15 %wt.

The effect of oil-water ratio in the emulsion stability was also evaluated. In this case, Pickering emulsions were produced with oil-water ratios of 10-90, 20-80, 40-60 and 60-40. Pickering droplets with well-defined shape were obtained, for the various studied oil-water ratios, right after the emulsification process. Increased oil concentration lead to a higher concentration of Pickering droplets, i.e., more droplets were observed as a consequence of the higher oil content; highlighting that droplets became more tightly packed as the oil fraction increase.
In an attempt to predict the behaviour of the Pickering emulsion, as a function of the used oil-water ratio, total solids concentration, and droplet size, the distance between HAp-free particles was estimated as:

\[ l_{ss} = \left( \frac{m_{ss}^1}{c_{ss}^{final}} \right)^{1/3} \]  

where, \( m_{ss}^1 \) is the mass of n-HAp particle and \( c_{ss}^{final} \) is the concentration of n-HAp that will remain free in solution. Considering two layers of n-HAp at the interface, it is possible to estimate the Pickering emulsion stabilization through:

\[
\begin{aligned}
& l_{ss} > 1.7d_{HAp} \quad \text{unstable} \\
& 1.7d_{HAp} \geq l_{ss} > d_{HAp} \quad \text{stable} \\
& l_{ss} < d_{HAp} \quad \text{gel formation}
\end{aligned}
\]  

where \( d_{HAp} \) is the length of a HAp particle. Under these assumptions, it is possible to build a ternary diagram, shown in Figure 1 that describes the stability of Pickering emulsions with the defined regions: stable, unstable and gel. Non-stable Pickering emulsions are obtained when \( l_{ss} \) is 1.7 times the length of the n-HAp particle. When \( l_{ss} \) is lower than the length of HAp nanoparticles a Pickering emulsion with high viscosity is formed, i.e., a cream-like emulsion. If \( l_{ss} \) is between 1 and 1.7 times the length of n-HAp, a stable Pickering emulsion is obtained.

Figure 1 shows the emulsion behaviour predicted by the model, and different experimental points: the green symbols (*) represent stable Pickering emulsions, and the red symbols (○) represent unstable emulsions. Thus, it was possible to observe that, after two storage days, the assumptions predicted by the model described by Eq. 2 are in good agreement with the obtained experimental data.

**Figure 1.** Ternary diagram of O/W Pickering emulsions with HAp at 2 days of storage time. Assumption: \( l_{ss} > 85 \) nm – unstable, \( l_{ss} < 50 \) nm and oil fraction > 0.6 – gel formation and \( 85 \geq l_{ss} > 50 \) nm – stable.

**Conclusion**

In this study, stable Pickering emulsions were produced using HAp nanoparticles as stabilizers. The storage time revealed that some of the produced Pickering emulsions, in particular those produced at concentrations above 5 %wt., were stable over two months. The evaluation by different microscopy techniques confirmed the formation of spherical Pickering droplets and the HAp adsorbed at the oil-water interface revealed that the Pickering droplets have an oil core – HAp shell morphology. The distance between the HAp-free particles remaining in the solution allows the predication of the emulsion stability as a function of the used oil-water ratio and solids concentration, assuming the existence of two layers of n-HAp around the oil droplets.

**References**

In European Union (EU), fluorinated gases emissions have increased up to 60% since 1990, in contrast to all other greenhouse gases, whose emissions have been reduced. Hence, in order to accomplish the EU commitments related to Kyoto’s Protocol, their use has been controlled since 2006, with different legislations aiming to combat their effect into the atmosphere. A new EU regulation entered into force on 1 January 2015 (No 517/2014) targeting to cut the emissions of these gases by 2/3 by 2030, compared to levels in 2014. Due to their utility, economic value and industrial application, the worldwide production of fluorinated gases is expected to increase for the foreseeable future. For instance, hydrofluorocarbon emissions are projected to grow by nearly 140% between 2005 and 2020, mainly in air conditioning and refrigeration systems. Although there are technologies under development to avoid the growth of these emissions, they may not be successful in finding the right compound with the required properties for some specific applications. Therefore, actions to reduce these emissions are needed according to it, including the capture of these gases and the development of alternative, environmentally benign solvents to recovery and recycle these greenhouse gases. The focus of research that addresses the basis of future environmentally friendly processes is a crucial objective that must be pursued. Therefore, a key objective for the European chemical industry, in order to improve its competitiveness, is to manufacture high value products more efficiently and with a significantly reduced environmental impact.

Ionic liquids (ILs) have become recognized as greener media due to their properties, such as almost null volatility, tuneability and recyclability. After the discovery that fluorinated ionic liquids (FILs) can present 3 nanosegregated domains (polar, nonpolar and fluorous) [1], it is clear that FILs can be used as “3 in 1” solvents. Playing with the interactions and the size of different domains will allow the development of solvents designed for separation of fluorinated greenhouse gases.

The objective of this work is to develop climate-friendly processes based on fluorinated ionic liquids to recover and recycle fluorinated greenhouse gases. The most appropriate solvent will be custom designed and evaluated for each specific application [2]. The fluorinated gases that are usually used in air conditioning and commercial refrigeration equipment, such as R-134a (1,1,1,2-tetrafluoroethane), R-32 (difluoromethane), R-125 (pentafluoroethane) and R-410a (binary mixture of R-125 and R-32), will be studied. In order to evaluate the performance of fluorinated ionic liquids in this application, a sorption equilibrium isotherm will be measured using an ISOSORP 2000 high-pressure magnetic-suspension microbalance (Rubotherm GmbH, Bochum, Germany). The supported porous materials increase the mass transfer between the gas and the ionic fluids that are studied. This work will deliver both fundamental understanding of the behaviour of these new alternative solvents and the skills to manipulate that behaviour to process development and scale-up.

References
New eco-sorbents for hydrogen sulphide removal from biogas

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The H2S removal capacity from a real biogas stream by activated carbons (AC) produced from Maize Cob Waste (MCW) was tested. The AC samples were produced by both activation with CO2 and functionalisation with a by-product of anaerobic digestion - Liquid Digestate. The physically activated carbon (MCW-CO2.2) provided the highest H2S removal capacity with a value of 15.1 mg H2S/g due to its better textural properties and probable presence of oxygen active sites that favoured the catalytic oxidation of H2S on the AC surface; this AC can be considered an interesting adsorbent for H2S removal from a real biogas stream at the conditions currently applied in industrial plants.

Introduction
Biogas is the gaseous product obtained by Anaerobic Digestion (AD) of organic matter, being composed mainly by CH4 and CO2 and by several other components, such as H2O, H2S, siloxanes, NH3, O2, CO and N2, that are present in low concentrations. Before the biogas upgrading to biomethane, by using the common conditioning processes, H2S must be removed due to its poisonous. Among the different techniques that can be applied for H2S removal from biogas stream, the adsorption on Activated Carbons (ACs) is considered, in most cases, a safe, reliable and environment-friendly technique [1].

Maize Cob Waste (MCW) is a potential precursor to produce AC, since it is a lignocellulosic biomass characterised by high carbon content (40-45% w/w) and a low percentage of ashes (about 1-2% w/w), able to develop ACs with high surface areas and micro e meso porosity [2]. The main aim of this study is to assess the H2S removal capacity of AC produced from MCW by physical activation with CO2 and chemical activation with a by-product of anaerobic digestion - Liquid Digestate (LD). LD was the agent used due to its potential to add minerals and nitrogen-containing groups, that can enhance the H2S removal.

Material and Methods
MCW was collected from a Portuguese farmer located in Coruche. LD was collected from the digester of a Portuguese AD plant located in Lisbon region. MCW was air-dried up to a moisture content of 14% w/w. Afterwards, it was carbonized during 2 h at 450 °C (CAR-MCW). Also, MCW was submitted both to physical and chemical activation, while CAR-MCW was submitted only to chemical activation (CAR-MCW.LD) (Fig. 1). The ACs were characterized for elemental analysis (EA), proximate analysis (PA), pH at the point of zero charge (pHpzc), thermogravimetric analysis (TGA), textural properties (N2 at 77 K), and scanning electron microscopy with energy dispersive spectroscopy (SEM-EDS) analysis.

The H2S adsorption assays were carried out using real biogas produced in a completely stirred lab-scale digestor (2.1 L), under thermophilic conditions (50°C). The digester was operated with a previously hydrolysed Organic Fraction of Municipal Solid Waste.

![Figure 1. Methodology used in the production of ACs.](image)

The H2S adsorption capacity (qm25) of the produced ACs was assessed through breakthrough adsorption runs performed in a packed-bed column with a biogas flow of 400 mL/min. The H2S concentration was continuously monitored at the exit of the column by a biogas multichannel analyser (Gas Data GFM410) under STP conditions. The qm25 was calculated for the breakthrough of 50 ppmv by using a standard equation reported in ASTM D6646. The results were compared with those obtained with a commercial AC (CAC) specifically designed for biogas purification.

Results and Discussion
The chemically ACs MCW-LD and CAR-MCW.LD showed an ash-content similar to the commercial AC (28.6% w/w) with percentages of 34.2% and 24.7% w/w, respectively; the mineral impregnation performed with LD seem to be efficient as the amount of ashes in the ACs not impregnated with LD was lower (2.5% -5.0%). The physical activated carbons, MCW-CO2.1 and MCW-CO2.2, showed the highest conversion of the initial volatile matter providing a final fixed carbon content of 85.4% and 83.6% w/w, respectively. EA highlighted higher nitrogen and oxygen percentages in all the produced ACs when compared to CAC, suggesting the presence of potential surface functional groups. The higher nitrogen and oxygen contents observed in MCW-CO2.2 (1.6% and 20.5% w/w, respectively) than in MCW-CO2.1 (0.7% and 11.7% w/w, respectively) indicated that the nitrogen functional groups present in that AC have high thermal stability, and that longer activation time lead to a high level of oxygen incorporation in the AC structure. MCW-LD and CAR-MCW.LD showed a strong alkaline behaviour, with a pHpzc value (11.8) very close to that measured on CAC (11.9). Physically activated carbons...
provided a weaker alkaline behaviour than the chemical activated ones with values of pHpzc, ranging from 9.6 for MCW-CO2.2 to 9.7 for MCW-CO2.1.

MCW-CO2.1 and MCW-CO2.2 developed the highest surface area ($S_{BET}$ of 630 m$^2$/g and 896 m$^2$/g, respectively); the total porous volume for these AC were of 0.25 cm$^3$/g and 0.35 cm$^3$/g, respectively. According to IUPAC, they are characterised by a type I(a) N$_2$ (77 K) isotherm. Chemical ACs presented very poor $S_{BET}$ values (38 m$^2$/g for MCW-LD and 8 m$^2$/g for CAR-MCW-LD), with very low associated porosity (Table 1). These last AC showed textural properties closer to carbonized material than to AC [3].

The SEM-EDS analysis of MCW-CO2.1 and MCW-CO2.2 showed a well-developed porous structure, confirming the results obtained with the isotherm of N$_2$ at 77 K. The structure of both these ACs is more homogeneous than for CAC; on the other hand, the SEM-EDS spectra of chemically activated ACs showed a heterogeneous and poorly porous structure like CAC. Moreover, these ACs are characterised by the presence of inorganic surface aggregates, composed by Cl, K, Na, and Ca.

Figures 1 and 2 show the H$_2$S breakthrough adsorption curves for all the ACs. After an initial sharp increase of the slope that strongly influenced the breakthrough time, a plateau is generically achieved. None of the curves obtained reached the saturation ($C_{ad}/C_{in}=1$) during the assays, whose total time was controlled by the availability of biogas. The physical activated carbon MCW-CO2.2 provided the highest H$_2$S adsorption capacity at the breakthrough (15.11 mg H$_2$S/g); the other ACs showed adsorption capacities lower than 1 mg H$_2$S/g. The adsorption capacity calculated for MCW-CO2.2 is almost the double of the average value reported in literature (9 mg H$_2$S/g) [4]. Despite of the low adsorption capacity at the breakthrough calculate for MCW-CO2.1, it must be noticed that this AC is able to remove 78% of the initial H$_2$S concentration during 1600 s, which is similar to the performance obtained with MCW-CO2.2 (92%). After an initial adsorption phase that seems to be mainly controlled by physisorption, in which MCW-CO2.2 presented the best adsorption performance related to its more favourable porous properties, H$_2$S oxidation occurred, probably favoured by the presence of oxygenated functional groups on AC surface that acted as active sites for H$_2$S oxidation [5]. Saturation plateau was not reached probably due to a high number of active/pore sites yet available during the adsorption assays, and that were not occupied by the oxidation products. Although the chemically impregnated ACs presented well dispersed inorganic clusters able to act as H$_2$S catalytic oxidations sites, their weak porous system, affected significantly their H$_2$S removal capacity.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>MCW-CO2.1</th>
<th>MCW-CO2.2</th>
<th>MCW-LD</th>
<th>CAR-MCW-LD</th>
<th>CAC</th>
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<tr>
<td>$S_{BET}$</td>
<td>630</td>
<td>896</td>
<td>38</td>
<td>8</td>
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<tr>
<td>$V_{micro}$</td>
<td>0.21</td>
<td>0.33</td>
<td>0.01</td>
<td>N/C</td>
<td>0.1</td>
</tr>
<tr>
<td>$V_{total}$</td>
<td>0.25</td>
<td>0.38</td>
<td>0.02</td>
<td>0.01</td>
<td>0.4</td>
</tr>
<tr>
<td>$V_{micro}$</td>
<td>0.015</td>
<td>0.05</td>
<td>0.01</td>
<td>0.01</td>
<td>0.3</td>
</tr>
</tbody>
</table>

**Acknowledgments**

Financial support from ERANet LAC initiative and Portuguese Foundation for Science and Technology (FCT/MCTES) through the project ELAC2014/BEE0367, IF/01016/2014, and fellowship SFRH/BPD/93407/2013. Partial funding from REQUIMTE, financed by FCT/MCTES (UID/QUI/50066/2013) and ERDF under the PT2020 Partnership Agreement (POCI-01-0145-FEDER-007265).

**References**

Influence of the treatment of mesoporous silica surfaces in the release profile of a loaded drug

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The present work aims to obtain a drug delivery system using mesoporous silica as carrier to stabilize ibuprofen (IBP) drug in the amorphous state. Two mesoporous silica matrices (SBA-15): unmodified and methyl-functionalized were used with pore diameters of 6.8 and 6.7 nm, respectively. The amount of drug loaded was quantified by thermogravimetric analysis being 90% (v/v). Differential scanning calorimetry (DSC) and ATR-FTIR spectroscopy gave evidence of successful IBP incorporation in the matrices. The glass transition of IBP was detected by DSC showing that the drug is in the amorphous state; however a small fraction remains crystalline outside pores. The mobility studied by Dielectric Relaxation Spectroscopy of IBP inside pores was found much slower than that of bulk IBP. Drug delivery was monitored by UV-VIS spectroscopy showing: a) all the incorporated drug is released and b) it is faster from the functionalized silica due to suppression of guest-host hydrogen bonding.

This work was motivated by the need of the pharmaceutical industry for more water-soluble drugs, since low drug’s solubility limits bioavailability. An approach is to modify the physical state of the drug, obtaining it in the amorphous state. The latter is characterized by a high intrinsic disorder which promotes higher solubility, however lacking of the long-time stability of the crystal.

Therefore, the incorporation in SBA-15 mesoporous matrices was adopted as a strategy to achieve amorphization and to stabilize the amorphous state. The target drug was ibuprofen (IBP), a worldwide used non-steroidal and anti-inflammatory pharmaceutical drug. The work was preceded by the investigation of neat IBP phase transformations by differential scanning calorimetry (DSC) allowing identifying melting centered at 78 °C and a glass transition, which midpoint temperature (Tg,mid) is -41.4 °C. It was observed that IBP avoids crystallization entering in a glassy state, however easily converting to the crystalline form under manipulation.

As drug carrier, a mesoporous (100% Si) silica matrix was synthesized. Additionally, a methyl-functionalized silica matrix was used. Both non-functionalized (SBA-15) and functionalized (SBA-15_f) were characterized by scanning electron microscopy and transmission electron microscopy; from the textural characterization, pore sizes of, respectively, 6.8 and 6.7 nm were estimated. In the SBA-15 composite (IBP_SBA-15), the loaded drug was quantified by thermogravimetric analysis being 105.9% (w/w) and 90% (v/v). DSC and attenuated total reflectance – Fourier transform infrared spectroscopy gave evidence of successful IBP incorporation in both matrices. By DSC, a glass transition was detected in both IBP_SBA-15 (Tg,mid = -43.9 °C) and IBP_SBA-15_f (Tg,mid = -46.4 °C), showing that the drug is in the amorphous state; however a small fraction of IBP remains crystalline disappearing after the first melting. The mobility of IBP inside pores was studied by Dielectric Relaxation Spectroscopy (DRS), which allowed estimate the reorientational molecular time as much slower than the ones of bulk IBP. The comparison with a previous study by some of us [1] allowed to identify the type of molecular reorientation mechanism, as monitored by DRS, as originated by IBP molecules interacting with the inner pore surface. Drug delivery from the two silica matrices in pH 6.8, to simulate intestinal fluid, was monitored by UV-VIS spectroscopy indicating that all the drug is able to be released from both silicas. Moreover, it was observed a faster release from the functionalized silica, comparable to neat IBP dissolution as shown in the Graphical Abstract; this was interpreted as a consequence of the suppression of hydrogen bonding drug interactions due to the replacement of hydroxyl groups by methyl groups. IBP_SBA-15 composite showed a more controlled released, however, depending on the desired usage, both composites are promissory to build a drug delivery system.

Acknowledgements
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References
Silica aerogel reinforcement with different types of cellulose fibres

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* apt@eq.uc.pt

Four different type of cellulose fibres were used, namely raw pasta of eucalyptus, raw pulp of sodra (pine + spruce), white eucalyptus pulp and white sodra pulp. The influence of the amount and quality of fibres in the gelation time, density and monolithic nature of the aerogels has been assessed. In this study, we have obtained different results when we used TEOS or TMOS precursor for the density and shrinkage.

**Introduction**

The silica-based aerogels exhibit outstanding properties, with wide-ranging applications. However, these materials possess a number of issues which limit their large scale production, namely poor mechanical resistance and particle shedding [1]. Native silica aerogels are nanostructured materials with extremely low density (0.03–0.15 g/cm³), high specific surface area (500-1000 m²/g), low thermal conductivity (0.005–0.021 W/m.K). These properties are particularly suitable for applications in thermal insulation, aerospace applications, catalytic supports, among others [1]. But the low strength and brittleness of aerogels restrict their large-scale application. Many researches have used fibres as supporting skeletons to improve the mechanical properties of aerogels. Inorganic and organic fibres, including mineral [2], ceramic [3], aramid [4] and glass fibres [5] have been used.

**Objectives**

The objective of this work is to prepare hydrophobic silica aerogel/cellulose composites with low thermal conductivity and good mechanical properties.

**Methods**

In this work, we synthesized silica aerogel/cellulose composites from tetraethyl orthosilicate (TEOS) by a two-step acid-base catalysed sol-gel method. Tetramethyl orthosilicate (TMOS) has also been used as precursor in a one-step procedure. Four different type of cellulose fibres were used, namely raw pulp of eucalyptus, raw pulp of sodra (pine + spruce), bleached white eucalyptus pulp and bleached white sodra pulp. The influence of the amount and quality of fibres in the gelation time, density and monolithic nature of the aerogels has been assessed.

MTMS and HDMSO (5%) was also used as co-precursor for the aerogels surface modification.

**Results**

In table 1 the results obtained with TEOS are shown. The raw pulp of sodra with and without surface modification is the pulp that displays smaller density and smaller percentage of shrinkage. The use of the co-precursor, MTMS, lead to better results of shrinkage due to surface modification of the obtained monoliths, but the co-precursor HDMSO lead the better results of density. By increasing the amount of fibers introduced into the aerogel it was found that both the density and the shrinkage decrease considerably. Samples with raw sodra pulp whose surface was modified with MTMS or HDMSO are more flexible than the eucalyptus pulp counterparts.

Table 2 shows the results obtained by using TMOS as precursor. When we use MTMS as a surface modifier, we obtained an increase of density and shrinkage percentage of the aerogels / cellulose.

As the amount of fibers increases, there is a slight decrease in density and percentage shrinkage.

**Conclusion**

From the preliminary results obtained for the aerosols / cellulose synthesized with TEOS and TMOS we found that, those synthesized with TEOS present lower values of density and lower shrinkage, indicating that they have better properties for the intended purposes.

---

**Table 1. Results the aerogels/cellulose using TEOS with precursor.**

<table>
<thead>
<tr>
<th>Samples</th>
<th>Weight pulp (%)</th>
<th>Density dry hybrid gel (kg/m³)</th>
<th>Linear shrinkage gel (%)</th>
<th>Surface modification</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Raw pulp eucalyptus</td>
<td>2.5</td>
<td>614.5</td>
<td>48.3</td>
<td>MTMS</td>
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<tr>
<td></td>
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<td>638.5</td>
<td>40.8</td>
<td>HDMSO</td>
</tr>
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<td></td>
<td></td>
<td>463.4</td>
<td>47.2</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>427.8</td>
<td>36.6</td>
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<td></td>
<td>473.7</td>
<td>31.8</td>
<td>MTMS</td>
</tr>
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<td>24.2</td>
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<td>236.7</td>
<td>26.1</td>
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<td>Density dry hybrid gel (kg/m³)</td>
<td>Linear shrinkage gel (%)</td>
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<td>--------------------------</td>
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<tr>
<td>Raw pulp sodra</td>
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<td>49.0</td>
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<td>503.3</td>
<td>45.1</td>
<td>HDMSO</td>
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<tr>
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<td>274.8</td>
<td>27.4</td>
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<tr>
<td></td>
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<td>210.0</td>
<td>10.5</td>
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<tr>
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<td>167.3</td>
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<td>128.4</td>
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<td>588.2</td>
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<td>655.9</td>
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<td>605.1</td>
<td>49.8</td>
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<tr>
<td>White eucalyptus pulp</td>
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<td>577.0</td>
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<td>301.8</td>
<td>25.8</td>
<td>HDMSO</td>
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Table 2. Results the aerogels/cellulose using TMOS with precursor.

Acknowledgements
This work was funded by the European Union – European Regional Development Fund, through Centro 2020 – Programa Operacional do Centro, under the Project - MATIS - Materiais e Tecnologias Industriais Sustentáveis. Consumables for the syntheses and characterizations performed at CIEPQPF research unit were funded by national funds through the FCT/MEC and when appropriate co-funded by FEDER under the PT2020 Partnership Agreement under the project POCI-01-0145-FEDER-006910 (FCT Ref. UID/EQU/00102/2013).

References
Selective recovery of platinum-group elements using Fe₃O₄@EG nanocomposites and their precursors

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The Platinum-group elements (PGE) consists in six precious metallic elements (Ru, Rh, Pd, Os, Ir and Pt), that due to their unique characteristics are widely used in many applications. More, due to their economic importance it is crucial to recycle these elements, and their recovery from solutions is an important step on that process. In this work, two magnetic composites had been synthesized using exfoliated graphite and magnetite nanoparticles as precursors. Afterwards, all materials had been investigated as sorbents for PGE removal, by carrying out batch experiments. Characterization results proved that composites were successfully synthesized and have different amounts of magnetite. Removal efficiency results have revealed a high dependency from pH and sorbent material for Pt, Ir and Rh. Pd and Ru had similar results, with the greatest removal efficiencies (~90%). The application of these composites for PGE removal constitutes a promising step in the recycling of some PGE.

Introduction
Platinum-group elements – ruthenium (Ru), osmium (Os), rhodium (Rh), iridium (Ir), palladium (Pd) and platinum (Pt) are considered precious metals having a high trading value [1]. These metals have similar physical and chemical properties, usually occur together in nature and are widely used in new technology applications, in auto and industrial catalysts and in jewelry and medical applications [2]. Due to the declining resources of precious metals, increasing worldwide demand and growing environmental concern, the recovery of these elements from leaching and waste solutions has become important [3]. Carbon-based nanocomposites have shown numerous potential applications owing to their excellent physico-chemical properties [4]. On the other hand, magnetite (Fe₃O₄) nanoparticles (NPs) are very well known for their applications in water treatment, and due to their facility to be collected and removed from the solution by applying an external magnetic field. So, the preparation of hybrid structures that combine the properties of graphene derivates and magnetite NPs will allow the implementation of magnetic separation technologies in water treatment units using sorption processes for the recovery of precious elements such as PGE.

Objectives
The main objectives of this work were:
- Synthesis and characterization of magnetic graphene-based nanocomposites;
- Evaluation of the removal efficiency of the synthetized composites and their precursors toward Pt, Ru, Rh, Pd and Ir, for different pH values in a natural matrix (spring water).

Methods
Exfoliated graphite (EG) was prepared by ultrasonic treatment of graphite powder. Fe₃O₄ NPs were obtained by oxidative hydrolysis of Fe(II)SO₄ in alkaline media. The two Fe₃O₄@EG nanocomposites were synthetized by different techniques: in the first method, the magnetic composite (Fe₃O₄@EG_01) was prepared by electrostatic assembly of EG and Fe₃O₄ colloidal NPs, using ultra-sonic treatment, while for the second method the magnetic composite (Fe₃O₄@EG_02) was prepared by in situ oxidative hydrolysis of Fe(II)SO₄. After synthesis, all materials were carefully washed and dried. For characterization, powder x-ray diffraction (XRD), transmission electron microscopy (TEM), Raman spectroscopy, infrared spectroscopy and magnetic measurements were carried out. Batch sorption experiments were carried out at 20 °C for 24h by contacting both nanocomposites and their precursors (50 mg/L) with an aqueous solution (spring water) of Ru, Rh, Ir, Pd and Pt (~1 μmol L⁻¹) adjusted to specified pH (5, 7 and 8). Magnetic nanocomposites and Fe₃O₄ NPs were separated from solution by applying an external magnetic field, while EG was removed by centrifugation. The initial and final PGE concentration in solution was determined by ICP-OES.

Results
The XRD patterns of both composites exhibit a narrow peak (2θ = 26.56°) characteristic of graphite, and the remained peaks observed in the diffractogram (not shown) are assigned to the inverse spinel structure of magnetite as indicated by the respective Miller indices. TEM images (Figure 1) suggested that graphite was successfully exfoliated, Fe₃O₄ NPs had a spherical shape and most of the Fe₃O₄ NPs were deposited on EG sheets (in composites). The images also confirmed higher density of magnetite in Fe₃O₄@EG_02 composite. The Raman spectra of both composites (Figure 2) exhibit the spectral signature of graphitic carbon-sp² materials (G and 2D bands), together with the D band associated with the structural defects in the carbon lattice, and a Raman band at 678 cm⁻¹ ascribed to A₁g mode for magnetite, that is more intense in the Fe₃O₄@EG_02 spectrum. The G band appears at 1580 cm⁻¹ and is assigned to the first-order scattering of the E₂g mode of C-sp² atoms in the 2D hexagonal lattice. This band is also observed respectively in the Raman spectra of graphite and EG (not shown) since is due to C-C bond stretching in graphitic materials, as such is common to all sp² carbon systems. The D band (sp³ carbon) at ca. 1350 cm⁻¹ observed in the Raman spectra of the Fe₃O₄@EG composites and EG, but not observed in the graphite Raman spectrum, is related to the presence of structural defects. The FTIR spectra of both composites also corroborate the presence of the magnetic phase in the materials, by the presence of a peak at 591 cm⁻¹ corresponding to the stretching vibration of Fe-O in the lattice. The magnetic properties analysis of composites were slightly different. The nanocomposite prepared by in situ oxidative hydrolysis (Fe₃O₄@EG_02) shows a
magnetization of 80 emu/g, while the one prepared by electrostatic assembly (Fe₃O₄@EG_01) shows a magnetization of ca. 45 emu/g. This difference is related with the percentage of Fe₃O₄ NPs in the sample.

Figure 1 - TEM images of graphite (a), EG (b and c), Fe₃O₄ NPs (d), Fe₃O₄@EG_01 (e) and Fe₃O₄@EG_02 (f) composites.

The efficiency results from the sorption essays show that for the different pH values and for all materials (composites and precursors), there are different tendencies for 3 groups of metals: Pt and Ir had low removal efficiency, with best results obtained at pH 7 and for Fe₃O₄@EG_02 composite; Rh had intermediate removal efficiency and the best results were obtained for Fe₃O₄@EG_02 and pH 8; while Pd and Ru had the highest removal efficiencies for all pH values and materials. The efficiency results can be seen in Table 1 and Figure 3 for pH 7. The Pd and Ru concentration were in some cases lower than the ICP-OES quantification limit.

Table 1. Removal efficiency (%) for pH 7.

<table>
<thead>
<tr>
<th>Material</th>
<th>Ir</th>
<th>Pd</th>
<th>Pt</th>
<th>Rh</th>
<th>Ru</th>
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<tr>
<td>EG</td>
<td>6.7</td>
<td>90.5</td>
<td>6.78</td>
<td>4.6</td>
<td>92.3</td>
</tr>
<tr>
<td>Fe₃O₄ NPs</td>
<td>10.2</td>
<td>85.2</td>
<td>12.1</td>
<td>53.1</td>
<td>87.3</td>
</tr>
<tr>
<td>Fe₃O₄@EG_01</td>
<td>9.7</td>
<td>90.5</td>
<td>8.9</td>
<td>38.4</td>
<td>95.0</td>
</tr>
<tr>
<td>Fe₃O₄@EG_02</td>
<td>15.1</td>
<td>88.6</td>
<td>12.6</td>
<td>50.6</td>
<td>92.0</td>
</tr>
</tbody>
</table>

Figure 3 – Removal efficiency for each element and each sorbent material.

Analyzing the results above, it is possible to conclude that the best removal considering all elements in spiked spring water is obtained for the nanocomposite with high magnetite grade. The results also allow us to conclude that Rh has more affinity to Fe₃O₄ NPs than for the carbon substrate, and Ir, Pt and Rh have very low affinity for EG. This behavior will allow to carry out selective removal of Pd and Ru, using the material with the highest carbon content.

Conclusions

Two magnetic composites were successfully synthesized using exfoliated graphite and Fe₃O₄ NPs as precursors, and were used for PGE removal application. In spring water with low PGE concentration, and considering the removal of all elements, the composite with highest Fe₃O₄ NPs (Fe₃O₄@EG_02) presented the best performance. However, due to the low affinity of Ir, Pt and Rh to the carbon substrate (EG) it is possible to carry out selective removal of Pd and Ru using EG as sorbent material. The highest removal efficiencies were always obtained for Pd and Ru, independently of the material used. This study highlights that is possible to effectively recover some PGE, even from low-concentration solutions, which is usually a challenge task.

Acknowledgements

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References

A new molybdenum trioxide hybrid decorated by 3-(1,2,4-Triazol-4-yl)adamantane-1-carboxylic acid: A promising reaction-induced self-separating catalyst for epoxidation

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Some catalysts exhibit reaction induced self-separating (RI-SS) behaviour, which is a promising concept in the field of catalysis. The RI-SS concept combines advantages of homogeneous and heterogeneous catalysis. The original insoluble catalyst is in situ converted to soluble active species, in the presence of H2O2; after the consumption of H2O2, the active species are structurally reverted into the original form of the catalyst, which precipitates and may be easily separated (e.g. via decantation/filtration). Another eco-friendly feature of RI-SS/H2O2 catalytic systems is that water is the co-product of the oxidant consumption. Very few molybdenum oxide compounds acted as RI-SS epoxidation systems, due to the need for water as co-product. The original solid material converts into soluble active species, and the latter revert to the original material after the consumption of the oxidant, precipitating and allowing ease of catalyst separation/reuse (in a similar fashion to heterogeneous catalysts). This compound was explored for a chemical reactions scope covering sulfoxidation, alcohol oxidative dehydrogenation, aldehyde oxidation, and olefin epoxidation.

In this work, a new one dimensional Mo-trioxide hybrid possessing double triazole bridges, [MoO3(tradeH)+H2O (1)] was synthesized via the reaction of the ligand tradeH with MoO3 under hydrothermal conditions. Hybrid 1 is thermally stable up to 270 ºC. It was active for Cy epoxidation using H2O2 at 70 ºC (Table 1), leading to 76 %/100 % conversion at 6 h/24 h (100 % CyO selectivity). The system 1/H2O2/Cy presented RI-SS behaviour; initially the reaction mixture was biphasic (white)-liquid(colourless), then it turned into a yellow-coloured solution, and finally it was again a biphasic solid-liquid mixture, as verified initially. Characterisation studies indicated that the chemical structure of the precipitated solid was similar to that of 1. On the other hand, the conditions of the catalytic reaction were different from those used to synthesise 1, and depending on the solvent media the solid may crystallise differently. The soluble active species (yellow in colour) seemed to a oxo(bisperoxo)molybdenum compound. Hybrid 1 was an effective catalyst for different catalytic reactions such as sulfoxidation, alcohol oxidative dehydrogenation, and aldehyde oxidation (Table 1).

Table 1. Chemical reaction scope for the catalytic system 1/H2O2.

<table>
<thead>
<tr>
<th>Chemical reaction</th>
<th>Reaction conditions</th>
<th>Reaction Products</th>
<th>Conversion (%)</th>
<th>Selectivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olefin epoxidation (cis-Cyclooctene)</td>
<td>70 ºC, 24 h</td>
<td>Cyclooctene oxide</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Oxidative dehydrogenation (Benzyl alcohol)</td>
<td>70 ºC, 24 h</td>
<td>Benzaldehyde / Benzoic acid</td>
<td>63</td>
<td>68/32</td>
</tr>
<tr>
<td>Aldehyde oxidation (Benzaldehyde)</td>
<td>70 ºC, 24 h</td>
<td>Benzoic acid</td>
<td>61</td>
<td>100</td>
</tr>
<tr>
<td>Sulfoxidation (Methylphenylsulfide)</td>
<td>35 ºC, 3 h</td>
<td>Methylphenylsulfone / Methylphenylsulfone</td>
<td>100</td>
<td>46/54</td>
</tr>
</tbody>
</table>

Acknowledgements

The Portuguese (PT) group acknowledges the Fundação para a Ciência e a Tecnologia (FCT) and the European Union for post-doctoral grant to P.N. (SFRH/BPD/110530/2015) co-funded by MCTES and the European Social Fund through the program POPH of QREN; and project POCI-01-0145-FEDER-030075 co-funded by POCI (Programa Operacional Competitividade e Internacionalização), FEDER, FCT. This work was developed in the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 [FCT ref. UID/CTM/50011/2013], financed by national funds through the FCT/MEC and when applicable co-financed by FEDER (Fundo Europeu de Desenvolvimento Regional) under the PT2020 Partnership Agreement.

References

Ultralight microcellular polyurethanes for the production of technical footwear components

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Today’s society demands lightweight and comfortable footwear products, which represents a market opportunity for the footwear industry, motivating the development of low-density (ultralight) materials. In this context, the application of low density microcellular polyurethane (PU) materials is an area of interest due to low weight, cost and reduction of raw materials consumption. The materials to develop must guarantee the maintenance and/or improvement of the final footwear performance. One of the possible strategies to be used for microcellular PU density reduction involves the modification of its cellular structure, i.e., by increasing the number of cells and improve the homogeneity of their distribution within the PU physical structure. This can be accomplished through the incorporation of hollow and/or expandable microparticles into thePU formulation. However, the additive incorporation should maintain and/or improve the physico-mechanical properties of the obtained PU, which requires a systematic study in what concerns the evaluation of the used amounts and relationship with properties.

Materials and Methods
The PUs were synthesized from a polyester-based polyol and methylene diphenyl diisocyanate (MDI). It corresponds to a base chemical system used for midsoles production at industrial level. The formulation was established based on an A/B (A: polyol, chain extensor and catalyst mixture; B: isocyanate) weigh ratio of 100/76. The selected additive was a commercial grade product, based on expandable thermoplastic microspheres with an average size of 10-16 µm, named MIC-A. Two types of formulations were assayed: the base formulation (PUB, base PU without MIC-A) and the modified formulations comprising the addition of 1, 2 and 3% (w/w, PU-basis) of MIC-A. The samples were identified as follows: PUXB or PUXAY, where X is the sample number, B correspond the base formulation, A identifies the samples containing MIC-A and Y the MIC-A content used.

The PU samples were produced in closed mold in order to control the density. Base PUs with target density of 45-50 (typical value), and modified PUs with target densities of 35 and 30 g/cm³, were produced by reducing the mass of the PU mixture transferred to the mold in 30 and 40% (relative to the base PU). The adopted synthesis procedure consisted on the mixture of the polyol, chain extensor, catalyst and MIC-A (when used) during 10 minutes at 500 rpm, under heating at 45°C. Next, the amount of the required MDI was added and the stirring was increased for 1500 rpm until the gel time was achieved (typically 5 s). After, a controlled amount of the reactive mixture was rapidly transferred to the mold, and the lid was set and fixed. Then, the PU was left to cure inside the mold during 240 s. After this time period, the PU was demolded and stored at ambient temperature in the absence of the light, for further studies. The PUs cellular structure was characterized by optical microscopy (OM), using a Nikkon Eclipse Ni-U optical microscope, equipped with a digital camera and the software NIS Elements Br. The cells size was measured based on the objectives calibration. Fourier Transform Infrared Spectroscopy (FTIR) was used to evaluate the effect of the additives incorporation on PU chemical structure. PUs spectra were obtained in ATR mode using a FTIR (ABB Model MB3000) equipped with a diamond crystal. The spectra were acquired in the range between 550 and 4000 cm⁻¹ using a resolution of 16 cm⁻¹, and by co-adding 32 scans per minute. The PUs density was evaluated according to the ISO 2420:2002 standard, and the impact absorption properties evaluated according to the Footwear Technological Center (CTCP) internal method using a flat base punch. The return energy and the maximum deceleration are the most relevant parameters in the assessment of the material’s impact absorption properties. The reference values are: maximum deceleration ≤280 m.s⁻² and energy return between 30% and 50%, being these values established for complete footwear.

Results
The assessment of the cellular structure by OM allowed to observe differences between the base PU and the modified ones (MIC-A incorporation). Figure 1 shows the images of OM obtained for all the PU samples. By comparing the obtained images, a reduction of cell size was noted as the MIC-A content increases; namely the size varied from 16-286 µm for the PUB sample to 5-157 µm for the PUB5A. Regarding cells homogeneity, the appearing of a rise amount of cells will small
size was evidenced, as the MIC-A content increased. Also, the presence of the MIC-A on the cell wall was identified, allowing the reduction of the cell wall density and, consequently, the final PU density. The density results are presented on Table 1. Analyzing these data, it can be noticed that the PU density varied from 0.50 for sample PUB1 to 0.30 g/cm³ for PU5A3. These values are in good agreement with the experimental strategy implemented for the PU samples production, once the PU mass transferred to the mold was reduced in 30% and 40%, respectively for the target densities of 0.35 and 0.30 g·cm⁻³.

![Figure 1. OM images of microcellular PUs (magnification 100X): (a) base formulation (PUB) and formulations modified with different MIC-A contents: (b) (PUA1) 1%; (c) (PUA2) 2%; and (d) (PUA3) 3%.](image1)

Regarding PUs FTIR analysis, namely the spectra shown in Figure 2, the vibration assigned at 3330 cm⁻¹ correspond to the NH stretching from the urethane, while the one at 1720 cm⁻¹ is identified as the carbonyl groups from the urethane and ester (polyol) stretching, being maintained in all samples. Moreover, when comparing the PUB spectrum with those of the modified samples, no additional vibrations are identified, pointing for the absence of chemical reactions between the additive and the PU matrix. Thus, it can be concluded that the MIC-A incorporation has no influence on the chemical structure of the resultant PU. Relatively to the impact absorption properties, these are related with the comfort characteristics. The dynamic analysis of the material ability to absorb the impact on the heel, when subjected to a standard impact weight, is correlated with the degree of impact absorption of the material. The maximum deceleration and the percentage of energy are the most important properties once they represent, respectively the damping and resilience. Regarding to the maximum deceleration, lower values represent a better capacity to absorb the impact. On the other hand, higher values of energy return show an improved resilience. Regarding the results of the impact absorptions of the PUs, presented on Table 1, a general analysis shows that all the produced PUs present values of maximum deceleration and energy return that fulfill specifications values: maximum deceleration ≤280 m·s⁻² and energy return between 30% and 50%, despite these values being established for complete footwear (composed by midsole and sole), which is expected to have higher performance. It can be also noted that: (1) The maximum deceleration values varied between 183-266 m·s⁻² while the return energy varied between 36-46%; (2) The base formulation sample, PUB2 (density 0.45 g·cm⁻³) has the lowest value of maximum deceleration (183 m·s⁻²) and the highest value of energy return (46%); (3) The samples PUIA2 (density 0.36 g·cm⁻³) and PU2A3 (density 0.34 g·cm⁻³) show a maximum deceleration values of 201 m·s⁻² and 186 m·s⁻², respectively, and an energy return value of about 37%. Moreover, the density reduction results, in a general way, in PUs (PU3A1, PU4A2 and PU5A3) with higher maximum deceleration values, increasing from 210.29 to 232.93 m·s⁻² with the rise of MIC-A content from 1 to 3%. However, the energy return of this samples does not present a similar behavior, since it varies from 36.34% to 40.73% when the MIC-A content rises from 1 to 2% (PU3A1, PU4A2), while for a MC-A content of 3% the energy return decreased to about 35.94% (PU5A3). Based on these results, it can be concluded that the low density PUs, present adequate impact absorption properties, being suitable for the production of midsoles for ultralight safety shoes.

![Figure 2. FTIR spectra for PU base formulation and PUs modified with 1, 2 and 3% of MIC-A.](image2)

### Conclusion
This work presented a simple strategy that allows the production of low density microcellular PUs. The tested additive proved to be suitable to reduce PUs density without impair material’s physico-mechanical properties. The impact absorption properties evidenced the suitability of the developed PUs for the production of midsoles for ultralight safety shoes.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Density (g·cm⁻³)</th>
<th>Maximum Deceleration (m·s⁻²)</th>
<th>Return Energy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PU1B</td>
<td>0.50</td>
<td>266.64</td>
<td>42.88</td>
</tr>
<tr>
<td>PU2B</td>
<td>0.45</td>
<td>183.03</td>
<td>46.04</td>
</tr>
<tr>
<td>PU1A2</td>
<td>0.36</td>
<td>201.09</td>
<td>36.76</td>
</tr>
<tr>
<td>PU2A3</td>
<td>0.34</td>
<td>186.18</td>
<td>37.22</td>
</tr>
<tr>
<td>PU3A1</td>
<td>0.31</td>
<td>210.29</td>
<td>36.34</td>
</tr>
<tr>
<td>PU4A2</td>
<td>0.31</td>
<td>225.22</td>
<td>40.73</td>
</tr>
<tr>
<td>PU5A3</td>
<td>0.30</td>
<td>232.93</td>
<td>35.94</td>
</tr>
</tbody>
</table>

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POCI-01-0145-FEDER-006984 (LA LSRE-LCM) and UID/AGR/00690/2013 (CIMO), financed by FEDER, through POCI-COMPETE2020 and FCT. Project n.º 017570 - ExtraLightSafeShoe – Development of polymeric solutions for technical footwear, co-financed by COMPETE2020 through PT2020 and FEDER.
Stabilizing simvastatin in the amorphous state into nanostructured matrices

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Among the different strategies developed by the pharmaceutical industry to turn more soluble the existing drugs, the modification of its physical state is a promising route, namely their amorphization, since the amorphous state is characterized by a high intrinsic disorder that promotes higher solubility. In the present work, a drug delivery system has been prepared and characterized aiming to improve the water solubility of a drug taking advantage of the amorphous state. The studied drug is simvastatin (SIM) that belongs to statins family, frequently used to reduce the levels of cholesterol in blood but also with high efficiency in the bone regeneration. In order to achieve SIM’s amorphization, the drug was incorporated in SBA-15 mesoporous matrices. Therefore, unmodified (SBA-15) and functionalized by methylation (SBA-15_F) mesoporous silica matrices were synthesized and used as drug carriers. The characterization of the silica surface was performed by scanning electron microscopy and transmission electron microscopy; their pore sizes were estimated by textural analysis as 6.8 nm for SBA-15 and 6.7 nm for SBA-15_F. Attenuated total reflectance, NMR and thermogravimetric analysis evidence efficient inclusion in matrices. By TGA a loading percentage of 49% (w/w) in the composite was determined corresponding to a pore filling of 98% (v/v). Bulk SIM’s phase transformations were studied by differential scanning calorimetry (DSC), allowing identifying melting as an exothermic peak centred at 140ºC. Upon cooling from the melt, crystallization is avoided, and SIM solidifies in a glassy state. The glass transition, detected in subsequent heating, is clearly seen with a midpoint temperature (Tg,mid) of 33ºC. In the composite SBA-15_SIM, the glass transition is also detected at 59ºC showing that the incorporated drug is in the amorphous state, however with an increased Tg (Tg, CIM = 30 ºC). The mobility hindrance inside pores is confirmed by Dielectric Relaxation Spectroscopy. Cytotoxicity essays show as at higher concentrations SIM is less cytotoxic when in composite. Release essays followed by UV-VIS spectroscopy suggest a faster release from the unmodified silica.

Acknowledgements

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Introduction
Most of the commercial resins used in the production of biobased composites are obtain from petroleum, and therefore there are several end-of-life disposal problems associated, because they do not biodegrade for decades or even centuries, under current waste processing treatments [1]. With that in mind, a solution that allow the substitution of petroleum-based polymers has to be found and so there is a great demand for new biodegradable products. From the various biobased materials available, which are potential alternatives to petroleum-based polymers/resins, starch has received, in the last years, a special attention. This is because starch is a cheap plant-based polysaccharide, with an annual growth cycle, produced in large quantities and so can be used as feedstock on the production of resins for biobased composites [1]. In comparison to petroleum-based polymers, the use of starch as a resin has been limited to perishable goods and applications due to its low water resistance and unsuitable mechanical properties [2]. The use of cassava starch in lightweight particleboards has been studied by Monteiro et al. with the add chitosan and wood fibers [3]. The starch acted not only as adhesive of wood particles, but also to produce a foam system, permitting to obtain particleboards with low density and good internal bond strength. In this adhesive system for particleboard production, chitosan and wood fibers were used as additives. Chitosan solutions (5 % w/w) are commonly used as an additive (crosslinker agent) [4] to increase the water resistance of starch resins, but the use of these solutions increases the amount of water in the bond, which induces a long post-pressing drying stage. An alternative adhesive system using potato starch with local origin is currently under study.

Objectives
Evaluate both the impact of the use of chitosan solutions (5 % w/w) as an additive in potato starch based adhesive.

Methods
ABES (Automatic Bonding Evaluation System) is a powerful and versatile technique for the evaluation of adhesive “mechanical cure”, allowing the determination of the shear strength as a function of resin type, catalytic system, resin load, cure temperature, time and substrate [5] (Figure 1).

In the last years, due to the increasing environmental awareness, sustainable solutions are being sought for the use of composites produced from renewable materials, such as wood and other lignocellulosic materials, as well as natural adhesives. A cheap, eco-friendly polysaccharide found all over the world is starch, which can be used as adhesive in wood composites. A new biobased foamable sour cassava starch resin was developed to be used in particleboard production. This formulation is used chitosan solution as additive, a common crosslinker used in starch resins. The impact of the use of chitosan solution on adhesive performance was studied using ABES (Automated Bonding Evaluation System). This equipment was used to evaluate the cure of the mix starch + chitosan solution, as well the mix of starch + water. The performed tests were all carried out at the same pressing temperature and using the same pressing times.

It allows the determination of the isothermal strength development rate of the bond, at temperatures ranging between ambient and 260 °C and may be used to explore many types of bonding systems. The evolution of the bond strength (σ) can be estimated using the model established by Costa et al. (see Equation 1) [6]:

\[
\sigma = \frac{\sigma_{\text{max}}}{1 + \lambda} \left(A + \tanh \left(\frac{t - t_0}{\lambda}\right)\right)
\]

\[A = \tanh \left(\frac{t_0}{\lambda}\right)\]

where \(t_0\) is the resin gel time (s), \(\lambda\) is the dynamic curing constant (s) and \(\sigma_{\text{max}}\) is the maximum shear strength (MPa).

A pair of relatively thin adhering strips (beech wood) were used to place the solutions to be tested. Each of the obtained solutions were applied at the end of the strip and the two strips were overlapped to form miniature bonds typically measuring 20 x 5 mm (Figure 2).

A solution of chitosan was previously prepared with water, propionic acid and chitosan. In order to understand the influence of chitosan solution on the cure of starch-based resins several adhesive samples (four formulations) were prepared, adding different amounts of chitosan solution to a fixed amount of potato starch (5 g) supplied by Diverembal. Due to the expected impact of water in the performance of starch based resins another four adhesive samples were prepared with corresponding solid content.
Each adhesive sample was then evaluated at a temperature of 120 ºC, for several pressing time.

Results
The major results from this study are presented in the following figures (figure 3 and 4). In Figure 3, it can be observed the direct results of the application of both the ABES methodology and the mathematical models for one of the samples (adhesive mixture), specifically the mixture consisting on potato starch (5 g) + water (5 g). Although, the model has been developed to represent the shear strength evolution during the bonding of beech strips with urea formaldehyde resins, resin commonly used in particleboard, a very good fit of the model to the experimental data can be observed, which validated the use of model to evaluate our resins (mixture of potato starch with either water or chitosan).

In Figure 4, the values of $\sigma_{\text{max}}$ (model parameter) for the different mixtures starch + additive used to bond beech strips, both using water (blue) and chitosan (orange), are presented. The impact of the amount of different additives, water and chitosan, have different behavior, on one hand the amount of water seems not to have any impact on $\sigma_{\text{max}}$, on the other hand the use of chitosan not only gives higher $\sigma_{\text{max}}$ but also $\sigma_{\text{max}}$ evolution is different. There is an initial increase, but for values higher than those for 7.5 g of chitosan + 5 g of potato starch (150 %) $\sigma_{\text{max}}$ seems not to change.

Conclusions
The use of chitosan solution significantly increases the maximum shear strength, due to the well-known cross linking effect, but for amounts of chitosan higher than 7.5g of chitosan on 5g of potato starch, the effects stables. Contrary to the expected, the increase of the amount of water added does not appear to influence the performance of the adhesive. So, the ABES equipment does not detect the influence of water on $\sigma_{\text{max}}$ - maximum shear strength and further studies have to be carried out.

Acknowledgements: This work was financially supported by: project POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013), funded by European Regional Development Fund (ERDF) through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI), and by national funds through FCT – Fundação para a Ciência e a Tecnologia; project “LEPABE-2-ECO-INNOVATION” – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

References
BioFPro a web based software for correlation and prediction of biofuels properties
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Introduction
There have been some rare attempts to generate free software in the scope of calculating biofuels properties. An important attempt has been provided by Talebi et al. [1], who presented a user-friendly public domain software package (the BiodieselAnalyser) to estimate properties of biodiesel. The new software presented in this work, BioFPro, contains an extensive property database constructed and continuously updated for the prediction of biofuels thermophysical properties. BioFPro has a web interface largely based on JavaScript, a programming language that provides great flexibility for web visualization and calculation resources. Because of its popularity, JavaScript has plenty plugins available that reduce the amount of coding necessary during software development. BioFPro is designed for the calculation of biofuels thermodynamic properties using an integrated approach which includes the trilogy prediction, interactivity and development. Given the variety of properties and methods available, BioFPro is useful for day-to-day practical, teaching, and research purposes. Furthermore, BioFPro is easily integrable with spreadsheet software, such as MS-Excel®, to expand the data manipulation and analysis capabilities.

Structure and utilities of BioFPro
The user interface of BioFPro is implemented with HTML, CSS, and JavaScript. JavaScript programs interact with the HTML interface structure to receive and process the user’s requests, while CSS scripts set the formatting rules for the web pages that are generated dynamically. The dynamic portions of the interface, including real-time property calculations and plotting, are implemented in JavaScript using the jQuery framework. The software is accessed via internet and the homepage automatically loads a guide containing buttons for calculating biofuel properties (e.g., for biodiesel, bioalcohols, natural gas, also diesel). By activating each of the buttons the user will have a new page with two main buttons: “General properties and standards and property selection”. The former allows the calculation of general properties usually related with biofuels quality and standards (ASTM or CEN); and the latter directs the user for detailed property calculations. Take biodiesel as an example. By clicking on the “property selection button”, a selection menu will appear as shown in Figure 1. Here, a selection can be made for any of the listed properties or the user can access the general property database for various biodiesels.

Figure 1. Example of property selection interface for biodiesel.

On clicking in a circular button (property), the interface for calculations with models for the selected property will appear. For density, the following is displayed:

Figure 2. An interface to estimate the biodiesel density from several models within a given range of pressure and temperature.

All the property calculation interfaces have the same visual form as in Figure 2. Taking for instance the density of biodiesel represented in Figure 2: 1- orange buttons provide: info, data processing tools, specialized database for selected property, general database, save and open calculated results, and plotting tools; 2- upper left box (method selection); 3- upper right box (composition of fuel, with possible selection of biofuels from a previously established database); 3- lower left box (T and p...
ranges selection); 5- middle lower box (calculation of property in user selected T and p ranges using the best available equations for each fuel from the database); 6- pvT properties of diesel and biodiesel blends. After filling all the cells, the user clicks the “calculate” button and a results page is displayed as in Figure 3.

Figure 3. Results for the biodiesel density query using the GCVOL equation of state (EoS).

Subsequently, this page can be saved as a text or as a CSV file and opened in MS-Excel® for further processing. The orange section contains the data processing button, which in turn, links to “Fitter” that is dedicated to data fitting (see Figure 4).

All the property interfaces have detailed thermodynamic information on models used for calculations (accessed in “Methods / info” fields in upper left box). This is exemplified in Figure 5 for Tait EoS.

Figure 4. Fitter tool. Fitting biodiesel pvT data to an EoS.

Figure 5. Information for Tait EoS (clicking in info field of Figure 2).

References

Link to MS-Excel®
The property values calculated with a selected model can be exported/saved in an Excel book as mentioned in Figure 3, and processed later (compared with experimental data, plotting, etc) as exemplified in Figure 6.

Figure 6. Processing BioFPro data of Figure 2 in MS-Excel.
Modified carbon nanotubes as alternative platforms for the adsorption of immunoglobulin G

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Introduction

Nowadays, one of the main interests of biopharmaceutical industries is the production of highly purified antibodies for applications in research, immunodiagnostics and immunotherapy [1]. For these applications, antibodies require high levels of purification and to be of high quality, i.e. to keep their biological function. However, the production cost of these therapeutic molecules is still very high due to the complexity of the downstream process, making the use of antibodies highly limited [2].

Currently, the most widespread used technique to capture antibodies for large-scale production is the protein A affinity chromatography [3]. However, this technique has significant problems such as high cost, low reusability, ligand leakage and harsh elution conditions [3]. Consequently, it is urgent to find new techniques for the purification of antibodies to decrease their production costs. One of the promising alternatives for IgG purification processes consist in the use of carbon materials, namely carbon nanotubes (CNT), since they have been described as efficient materials for the adsorption of biomolecules, such as proteins, DNA oligomers and aptamers [4]. In the past few years, the functionalization of CNT was shown to be an interesting and challenging methodology to increase their biocompatibility and processability, which are essential features for their application in the purification of biomolecules. CNT’s high surface area, capacity to establish π-π interactions, and good chemical, mechanical and thermal stabilities, make of them a very interesting material for separation and detection of both nonpolar and polar compounds [7].

From a chemical reactivity point of view, CNT can be separated into two different zones: the tips and the sidewalls. The tips are similar to the structure of a fullerene hemisphere and are relatively reactive. The sidewalls can be approximately considered as curved graphite and whatever the diameter of the CNT, the reactivity of the sidewalls is considerably lower than that of the tips. Therefore, most reactions are expected to occur at the tips first and then, at the sidewalls, principally in the areas where defects are present [8].

The main objective of this work was to selectively absorb IgG from rabbit serum using modified CNT.

Antibodies (Ab’s) are molecules with high specificity for binding and inactivation of antigens, such as bacteria and virus. Ab’s, such as immunoglobulin G (IgG), have been the subject of research for the treatment of infectious diseases and as new therapies for cancer. However, Ab’s are among the most expensive therapeutic options due to the lack of a cost-effective purification method. Thus, there is an urgent need for new purification methods for the IgG production.

The main objective of this work was to determine the best operating conditions for purifying IgG from rabbit serum using pristine and modified carbon nanotubes (CNT). Experimental conditions, such as pH, serum and IgG concentrations were optimized. At the best conditions, IgG with a purity of 51.3% and a recovery yield of 99.9% was obtained. The used CNT were chemically, structurally and morphologically characterized by Brunauer–Emmett–Teller(BET) specific surface areas ($S_{BET}$) and transmission electronic microscopy (TEM).

Methods

The influence of external diameter CNT ranges, namely <10, 10-20, 20-40 and 60-100 nm, pH and serum concentration (1:5, 1:15, 1:20 and 1:30) was tested. CNT with a diameter range of 10–20 nm were functionalized by heating them at reflux with HNO₃ (7M) at 130°C (CNTox). The CNTox material was heat treated under an inert atmosphere (N) at 400°C (CNTox-400) and at 900°C (CNTox-900), to selectively remove surface groups [9]. The obtained samples were characterized by N₂ adsorption–desorption isotherms. Brunauer–Emmett–Teller (BET) specific surface areas ($S_{BET}$) were calculated from nitrogen adsorption assays. The Barrett–Joyner–Halenda (BJH) method was applied to the desorption branch of the N₂ adsorption isotherms to obtain the pore size distribution curves and cumulative volume of pores [9]. Surface chemical groups were characterized and quantified by temperature-programmed desorption (TPD) [9]. The CNT morphology was evaluated by transmission electron microscopy (TEM). The pH of the point of zero charge ($pH_{ZPC}$) of the CNT samples was determined by a drift method [9].

The selective adsorption of IgG was carried out using functionalized CNTs. Experimental conditions, such as pH, rabbit serum dilution and material/serum ratio were optimized.

Results

A preliminary study on the influence of CNT diameter on the IgG recovery was performed using CNT with external diameter ranges: 10–20, 20–40, and 60–100 nm. The characterization studies are presented in Table 1.

The results presented in Table 1 show that the surface area increases with the decrease in CNT external diameter. The surface areas of modified 10-20 nm CNT, determined by the BET method, revealed that the oxidation treatment led to an increase in the surface area of the resulting material [9]. Figure 1 shows TEM analysis where the sidewalls of CNT could be observed.

To evaluate the influence of the external diameter on IgG adsorption, it was used CNTs with the different external diameters at pH 5. The CNT 60-100 nm presented the poor results. A low percentage of IgG purification (30.7 %) and yield (45.9 %) were obtained, probably due to the large diameter of the CNTs, which reduces the surface area and not allowing...
proteins adsorption. On the other hand, CNT 10-20 nm were more effective, presenting a complete IgG adsorption. Thus, this CNT was selected for its surface modification and further IgG adsorption studies.

Table 1. Characterization of CNT with different external diameters [9].

<table>
<thead>
<tr>
<th>CNT range (nm)</th>
<th>SBET (m² g⁻¹)</th>
<th>dp (nm)</th>
<th>Vp (cm³ g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10-20</td>
<td>73</td>
<td>3.6</td>
<td>0.242</td>
</tr>
<tr>
<td>20-40</td>
<td>67</td>
<td>3.8</td>
<td>0.269</td>
</tr>
<tr>
<td>60-100</td>
<td>35</td>
<td>4.0</td>
<td>0.081</td>
</tr>
</tbody>
</table>

Figure 1. TEM images of pristine CNT 10-20 nm.

The characterization of modified CNT is presented in Table 2. For example, the surface area of CNTox is around 1.3 times higher than that of pristine CNT. This occurs because oxidative treatment with HNO₃ creates sidewall defects and can open up the end caps of the CNT, therefore increasing the porosity [9]. Effect of CNT surface chemistry on direct physical adsorption is a commonly used noncovalent method. The main driving forces involved in this process are hydrophobic, electrostatic, and π-π stacking interactions between CNT sidewalls and proteins, and hydrogen bonding [9].

Table 2. Characterization of modified CNT [9].

<table>
<thead>
<tr>
<th>Material</th>
<th>S_BET [a]</th>
<th>pH_ZPC</th>
<th>CO [b]</th>
<th>CO₂ [b]</th>
<th>CO⁺/CO₂ [b]</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNT</td>
<td>96</td>
<td>3.0</td>
<td>800</td>
<td>412</td>
<td>1212</td>
</tr>
<tr>
<td>CNTox</td>
<td>100</td>
<td>4.0</td>
<td>623</td>
<td>223</td>
<td>846</td>
</tr>
<tr>
<td>CNTox-900</td>
<td>111</td>
<td>6.9</td>
<td>176</td>
<td>37</td>
<td>213</td>
</tr>
</tbody>
</table>

[a] in m² g⁻¹ [b] in μmol g⁻¹

The IgG adsorption conditions were optimized by using the different CNT, serum concentration and pH. At the best conditions, it was found that the most promising material was the CNTox-900, revealing an IgG recovery yield of 99.9% and a purity of 51.3% (initial serum IgG purity: 20-25%). The back extraction of the IgG from this material was also evaluated with buffer solutions at various pH. From the results obtained, no IgG desorption was observed, indicating a strong interaction between IgG and the material, allowing these IgG-CNT materials to be used as biosensors.

Conclusions

IgG was efficiently adsorbed on multiwalled carbon nanotubes by a simple contact method. The diameter of the CNTs as well as their surface functionalization with oxygen-containing groups were found to be determinant for the efficient formation of IgG-CNT hybrids. Thus, CNT can be promising materials as biosensors, since they have IgG as a biological component useful for the detection of specific diseases.

Acknowledgements

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References

Neon adsorption on metal-organic frameworks over wide temperature and pressure ranges

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Neon @ Co\(_3\)(ndc)\(_3\)(dabco)

Several metal-organic frameworks (MOFs) were studied with the objective of evaluating their performance for eventual application in adsorption-based coolers. From the huge amount of available MOF structures, a preliminary selection of suitable MOFs was performed through molecular simulation techniques. Three MOFs were selected: HKUST-1, UiO-66, and Co\((ndc)(dabco)\) – and experimental adsorption equilibria measurements were performed to evaluate the MOFs’ properties for Neon adsorption. The measurements were performed spanning temperature and pressure ranges of 77 – 500 K and 0 – 100 bar, respectively. The measurement of adsorption equilibria in such broad thermodynamic conditions is not common and the obtained results showed that Neon can be stored in the three MOFs evaluated. The Co\((ndc)(dabco)\) MOF presents higher adsorption capacity, followed by HKUST-1, and, finally, UiO-66.

Introduction

Cryogenic temperatures are commonly used in space applications, and the employed coolers generally use mechanical compressors that promote vibrations potentially harmful for diverse equipment. Thermal compressors based in sorption cooling can achieve low temperatures and avoid undesirable vibrations. These devices employ a thermal swing for sorption and subsequent gas release. Continuous cooling power is ensured by using several adsorbent beds working cyclically. The efficient operation of the coolers is highly dependent on the properties of the chosen sorbent. In this work, several metal-organic frameworks (MOFs) were studied to evaluate their potential of application in adsorption-based coolers. Molecular simulation techniques were used to select the more promising MOFs among the huge amount of available MOF structures. Three MOFs were selected, namely HKUST-1 [1], UiO-66 [2], and Co\((ndc)(dabco)\) [3], and experimental adsorption equilibria measurements were performed to evaluate the MOFs’ properties towards Neon adsorption spanning temperature and pressure ranges of 77 – 500 K and 0 – 100 bar, respectively.

Methods and Materials

HKUST-1 (also known as CuBTC) was synthesized by BASF under the trademark Basolite® C300 and purchased from Sigma-Aldrich. UiO-66 and Co\((ndc)(dabco)\) were synthesized and purchased from the Materials Center at Technical University Dresden (Germany). Adsorption equilibria of Neon were determined experimentally by standard gravimetric and volumetric techniques. Single-component adsorption isotherms of Neon in the 273 K to 353 K range were measured using a high-pressure magnetic-suspension balance ISOSORP 2000 (Rubotherm GmbH, Germany). Neon adsorption equilibria within 77 – 500 K and up to 100 bar were determined using a volumetric apparatus built-in-house [4].

Results

Adsorption equilibria of Neon on HKUST-1 (77 – 500 K; 0 – 100 bar), UiO-66 (77 – 400 K; 0 – 70 bar), and Co\((ndc)(dabco)\) (77 – 320 K; 0 – 100 bar) were experimentally measured. The good operation of the volumetric unit was validated against the results obtained using the high-precision Rubotherm balance. Co\((ndc)(dabco)\) presents the higher Neon uptake, followed by HKUST-1, and UiO-66. At 150 K and 16 bar, Co\((ndc)(dabco)\) adsorbs 3.0 mol/kg, while the HKUST-1 adsorbs 2.6 mol/kg, and UiO-66 only 1.6 mol/kg.

Conclusions

Adsorption equilibria measurements of Neon on three MOFs were determined spanning wide temperature (77 – 500 K) and pressure (0 – 100 bar) ranges. Reporting adsorption equilibria in such broad thermodynamic conditions is not usual, and, to the best of our knowledge, it is the first time neon adsorption on MOFs is reported within this pressure and temperature ranges. The obtained results showed that Neon can be stored in the three MOFs evaluated. The Co\((ndc)(dabco)\) MOF presents higher adsorption capacity, followed by HKUST-1, and, finally, UiO-66.

Acknowledgements

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References


Titanium oxide-based materials for photo-catalysis of dyes and benzene

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Titanium dioxide (TiO₂), based materials were tested regarding its ability to degrade the dye Rhodamine B (RhB) using UV and visible light, as well as the ability to degrade the volatile organic compound – benzene, using solar light. The pure TiO₂ compounds show a blue shift in the UV-vis analysis of the photodegradation of RhB indicating that photosensitization occurs. This phenomenon was not perceived in samples with added graphene oxide (GO) and carbon nanotubes (CNT). The sample with the highest amount of photosensitization depicted lower photodegradation of benzene than a sample without a blue shift.

Introduction
Photocatalysis can solve contamination issues in low level organic polluted air and wastewaters. Several inorganic oxides have been used as photocatalysts. Titanium oxide (TiO₂) is the most often chosen oxide, due to its strong oxidation ability and consequently high efficiency, good thermal, photo and chemical stabilities allied with its cost effectiveness compared with other oxides. TiO₂ has three main crystallographic polymorphs: anatase, brookite and rutile. Anatase is the most used phase as photocatalyst because of its high activity. Nonetheless, the mixture of different phases such as anatase with brookite [1] or rutile [2] has proved to possess high photocatalytic degradation ability, surpassing that of the single phases. Additionally, materials such as graphene oxide (GO) [3] and carbon nanotubes (CNT) [4] can increase TiO₂ photocatalytic activity. The desirable TiO₂ based material also should have high specific surface areas to allow for the organic pollutants to contact with the photocatalyst’s surface.

Objectives
The aim was to produce TiO₂ based materials capable of degrading the dye Rhodamine B (RhB) using different light sources (UV- and visible light). Photocatalytic tests were also conducted using solar light, to understand if the liquid degradation tests with RhB could help predict which samples are best to degrade a volatile organic compound (VOC), such as benzene.

Methods
We proposed an environmental friendly alternative to produce TiO₂-based photocatalysts relatively to the procedures proposed in the literature [5]. Macro- and meso-porous TiO₂ materials were produced without the use of surfactant templates, just by dropping the tetra-n-butyl-orthotitanate precursor into water, followed by thermal treatments at different temperatures. In some of the samples, GO and CNT were also used. Hydrothermal treatments were performed in some of the samples to achieve the crystallization of the intended crystallographic phase. The photocatalytic materials were characterized regarding their structural and morphological features via scanning electron microscopy (SEM), X-ray, Raman spectroscopy, Fourier-transform infrared spectroscopy (FTIR) and -196 °C N₂ adsorption-desorption isotherms analyses. The photocatalytic ability was also studied monitoring the degradation of Rhodamine B (RhB) as well as that of benzene, a VOC, in the gas-solid phase.

Results
Obtained TiO₂ and TiO₂-based materials present meso and macro pores in their structure (figure 1), thus increasing their surface area, which enhances the contact area between dye or benzene improving photo-degrading rates. Each TiO₂ sample, except one, showed a noticeable blue shift in the UV-Vis spectra of RhB degradation as a function of irradiation time, thus showing photosensitization phenomena. On the contrary, the TiO₂/carbon-based composites, prepared with GO and CNT, were efficient in the photodegradation of RhB, without displaying said photosensitization effect. The sample with the greater blue shift presents less photocatalytic ability in the degradation of benzene, compared to the sample that showed no blue shift.

Conclusion
The addition of GO and CNT helped to decrease the photosensitization observed in liquid phase photocatalytic tests. All the samples with GO, along with some with CNT, performed better using visible light than UV light, in the degradation of RhB. On the other hand, the sample displaying higher photosensitization effects in the liquid-solid phase had worse photocatalytic ability to degrade benzene under solar light, than the sample without photosensitization.
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References
Enantioselective separation of racemic mandelic acid using aqueous biphasic systems with chiral ionic liquids

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This work proposes the development of a versatile enantioseparation platform for the chiral resolution of racemic mandelic acid (MA). ABS using chiral ionic liquids (CILs) simultaneously as chiral selectors and phase forming agents is proposed. Five ABS were studied to determine the CIL structure and salt role on the ABS aptitude to separate MA enantiomers. Optimization studies were further pursued, as representative CIL-based ABS were employed in optimization studies, where the MA content, temperature, tie-line length, salt and phases weight ratio were studied. The influence of these parameters is shown to be highly dependent on the CIL-based ABS, nevertheless the results attained suggest that the key driving the enantioseparation in these ABS is a combination of the enantiorecognition ability of a given CIL with the solubility of MA in the corresponding CIL-rich phase. By closely manipulating the two conditions of the purification, good enantioselective separations can be achieved.

Introduction

The optical isomers of a molecule can be discriminated by the human body, notwithstanding their very similar physical and chemical properties (apart from their optical rotation).[1] This is of utmost importance in drug development, as the pharmacological activity usually results from only one of the enantiomers (the eutomer), while the other (the distomer) may be inert, less potent or even toxic. To this day, the production of enantiopure drugs is a key challenge for the pharma industry.[2] Considering the increased difficulty in the production of pure enantiomers by direct synthesis, the synthesis of racemates followed by their chiral resolution is a simpler, more flexible and cheaper alternative. This approach is commonly achieved by chromatography and crystallization, yet other methods, such as enantioselective liquid-liquid extraction can provide a better balance between costs, operational versatility and scale-up opportunities. Aqueous biphasic systems (ABS) are good candidates to turn enantioseparations not only into more biocompatible but also more versatile approaches.[3] Ionic liquids are alternative solvents with an enormous degree of structural diversity, allowing the design of task-specific solvents and, by their introduction in ABS, of highly performant extraction/separation approaches. Being made up of ions, if one can select/develop chiral structures to function as cations, anions or both, the opportunity of creating chiral ionic liquids (CILs) emerges.[4],[5]

Objectives

Given the limited application of CILs in the development of ABS for chiral resolution purposes, it is here intended to contribute towards the enlargement of CIL-based ABS database and to provide further insight on their enantioseparation aptitude. In an initial stage of this work, the phase diagrams of ABS composed of five CILs based on quinine, L-proline and L-valine and three salts (viz. K₂PO₄, K₃HPO₄ and K₂CO₃) were determined and characterized. The low toxicity, significant water solubility and proved chiral recognition aptitude of this set of CILs recently synthesized by some of us showcase the interest of their implementation in chiral ABS. Their enantioseparation aptitude was further evaluated and optimized using mandelic acid, a key precursor in chiral pharmaceuticals manufacturing, as model chiral compound.

Methods

Biphasic mixture points were selected to conduct studies on racemic mandelic acid enantioseparation. The systems were gravimetrically prepared by adding the correct amounts of CIL, salt and water along with equal amounts of two aqueous solutions of R-mandelic acid and S-mandelic acid both prepared at the same concentrations to yield the desired final content in the ABS. Throughout this work, the evaluation of distinct conditions was carried out: CIL’s structure, enantiomers content, temperature, tie-line lengths (TLLs), salt and mixture points along the same tie-line (TL). The CILs were placed in contact with the mandelic acid enantiomers in aqueous solution under constant stirring to promote specific interactions between the CIL and the target enantiomers. The salt was added after such a period to induce liquid-liquid demixing. To this, a period of equilibration followed, to guarantee complete separation of the phases and partition of the enantiomers among phases. The phases were then separated and weighted. CIL-rich phases were submitted to HPLC-DAD analysis for mandelic acid enantiomers’ quantification. In order to estimate the average extraction/enantioseparation parameters and the corresponding standard deviations, triplicates were performed.

The percentage extraction efficiencies of R and S-mandelic acid (EE₉,MA and EE₅,MA, %) were separately determined in accordance with the equation 1:

\[ EE_{R,S-MA} \% = \frac{m_{R,S-MA}^{\text{en}}} {m_{R,S-MA}^{\text{in}}} \times 100 \]  

where \( m_{R,S-MA}^{\text{en}} \) is the mass of R or S-mandelic acid present in the CIL-rich phase and \( m_{R,S-MA}^{\text{in}} \) is the mass of R or S-mandelic acid originally added to the ABS. The enantiomeric excess (e.e., %) present in the CIL-rich phase was calculated in accordance with equation 2:

\[ \text{e.e., %} = \frac{m_{R-MA}^{\text{en}} - m_{S-MA}^{\text{en}}}{m_{R-MA}^{\text{en}} + m_{S-MA}^{\text{en}}} \times 100 \]

in which \( m_{R-MA}^{\text{en}} \) and \( m_{S-MA}^{\text{en}} \) are the mass of S and R-mandelic acid present in the CIL-rich phase, respectively.

Results and Discussion

After an initial screening where all five CILs were ranked according to their relative ability to separate mandelic acid...
enantiomers, a maximum e.e. of 17.37 ± 1.92 % was achieved with [C2C2C2Pro]Br. The most and least promising CILs were object of detailed optimization, comprising the parameters: mandelic acid content, temperature, TLL, salt and phases’ weight ratio. With the CIL structure playing a central role, all remaining conditions were shown to influence (less) the enantioseparation. Such impacts are highly dependent on the ABS nature: while temperature was the main factor improving the enantioseparation ability of [C1Qui][C1SO4]-based ABS, [C2C2C2Pro]Br-based ABS was mainly influenced by the salt used and the phases weight ratio. Based on the optimization results it seems that the saturation of the CIL-rich phase rules the enantioseparation: S-mandelic acid (the enantiomer with higher affinity for this set of CILs) remains in the CIL-rich phase, while R-mandelic acid partitions to the K3PO4-rich phase.

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References
Hydrogen peroxide oxidation of potato Starch

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Although starch biodegradable and non-toxicity properties make it an alternative to products based on fossil fuel, its structure and functional properties limit its industrial applications. To overcome these disadvantages chemical, physical or enzymatic modifications can be applied. The present study describes the effect of oxidation reaction with different concentration of hydrogen peroxide (1 and 10 g/kg starch) in potato starch. A gradual increase in carboxyl content with increasing oxidant concentration was observed due to the oxidation of the hydroxyl groups to carboxyl groups. Although SEM analysis showed that the oxidation reaction does not induce changes in the granules morphology, the second derivative FTIR and NMR analyses confirmed the occurrence of oxidation reaction.

Starch is the most common carbohydrate present in plants and can be found in leaves, fruits, seeds, pollen grains, roots and stalks. The major sources of starch are cereals (40 to 90%), tubers (65 to 85%), roots (30 to 70%) and vegetables (25 to 50%) [1]. The main commercial species are maize, cassava, potato, wheat and rice. Starch properties are influenced by genetic and environmental factors.

Starch is composed by two macromolecules: amylose, a mainly linear polymer of D-glucose units linked by α-1,4 bonds, and amyllopectin, a highly branched polymer of D-glucose units also linked by α-1,4 bonds, with branching points resulting from α-1,6 linkages [2]. Starch structure and its functional properties have some limitations, relatively to its solubility, texture, adhesion and tolerance to the heating temperatures used in industrial processes, which often hinder its practical application. However, this can be overcome by chemical, physical or enzymatic modifications [1].

Starch oxidation has been used to improve its properties and hydrogen peroxide, sodium hypochlorite, permanganate and UV radiation are some examples of treatments that can be applied [3]. During oxidation reaction the oxidant agent reacts with the free hydroxyl groups leading to the formation of carbonyl and carboxyl groups [4]. On the other hand, starch is depolymerized, due to the hydrolysis of glucosidic linkages [5]. The oxidant concentration, reaction time, pH and the presence of metal ion catalysts determine the degree of oxidation in the modified starches, and hydrogen peroxide is often use to produce both starches with high and low degree of oxidation [6]. The aim of this work was to study the impact of the oxidation reaction with different concentration of hydrogen peroxide in potato starch.

Methods

Starch oxidation. Native potato starch was oxidized at 30 °C with hydrogen peroxide according to the procedure described above: starch (100 g) and distilled water (250 ml) were charged into a 500 ml jacketed flask reactor equipped with mechanical stirrer and a thermometer. The reaction temperature was controlled by circulating water from a temperature controlled water bath in the reactor jacket. After adjusting pH to 4 with acetic acid (850 g/l), hydrogen peroxide (1 and 10 g/kg starch) was added dropwise and the reaction was continued for 8 h with constant agitation. The reaction product was washed with distilled water until pH 6 was reached. This mixture was filtered under vacuum to recover the starch and dried at 45 °C during 48 h in an oven.

Carboxyl content, of the oxidized samples was determined by volumetric titration according to Demiate (2000). 500 mg of sample were suspended on 300 ml of distilled water and boiled for 10 min with constant agitation. Phenolphthalein as indicator was added to the cooled solutions and these samples were titrated with NaOH 0.025 M until the appearance of the first permanent pink color. The results reported are an average for at least three determinations.

\[
\text{COOH} \% = \frac{V_{\text{sample}} (\text{ml}) \times M_{NaOH} \times 0.045 \times 100}{W (g)}
\]

where \(V_{\text{sample}}\) is the volume of NaOH required for the sample (ml), \(M_{NaOH}\) is the molarity of NaOH and \(W\) is the sample weight (d.b.).

Infrared (IR): spectra were recorded with a Vertex 70 spectrometer (Bruker), equipped with an ATR cell with a A225/Q PLATINUM ATR Diamond crystal with single reflection accessory. Each spectrum was an average of 64 scans taken with 4 cm\(^{-1}\) resolution in the 4000-500 cm\(^{-1}\) range. Savitzky–Golay method was used to obtain second derivatives by means of 17 points smoothing filter and a second order polynomial.

Nuclear Magnetic Resonance (NMR): spectra were recorded on a Bruker Avance III—400 spectrometer operating at a frequency of 400 MHz, using a mixture of deuterated dimethyl sulfoxide and deuterium oxide (90:10) as solvent. Chemical shifts were reported in part per million (ppm) relative to the resonance of TMSP (0.00 ppm) as an internal standard.

Scanning electron microscopy (SEM) was obtained using a FEI Quanta 400FEG ESEM/EDAX Genesis X4M equipment. The samples were sputtered with gold/platinum before being analyzed.

Results and Discussion

Carboxyl content of native starch and starches oxidized with different concentration of hydrogen peroxide are summarized in Table 1. The results obtained showed a gradual increase in carboxyl content (from 0.164 % to 0.271 %) with increasing hydrogen peroxide concentration. The presence of carboxyl groups in the oxidized starches results from the oxidation reaction of the free hydroxyl groups in the glucose molecule.
Table 1. Carboxyl content of native potato starch and starch oxidized with different concentrations of hydrogen peroxide.

<table>
<thead>
<tr>
<th>Starch</th>
<th>Carboxyl content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Native Potato</td>
<td>0.164 ± 0.007</td>
</tr>
<tr>
<td>Oxidized with 0.1 % H₂O₂</td>
<td>0.173 ± 0.008</td>
</tr>
<tr>
<td>Oxidized with 1 % H₂O₂</td>
<td>0.271 ± 0.012</td>
</tr>
</tbody>
</table>

Infrared spectra of native potato starch and starch oxidized with 1 % H₂O₂ are presented in Figure 1. Spectra analysis allowed to identify the characteristic bands of potato starch, namely, at 3291 cm⁻¹ O-H stretching, at 2933 cm⁻¹ C-H stretching, at 1636 cm⁻¹ the typical band relative to H₂O bending vibration, at 1460 and 1370 cm⁻¹ C-H bending, at 1244 cm⁻¹ CH₂ out-of-plane bending and at 1149 cm⁻¹ C-O stretching [7]. Finally, the three bands between 900 and 1100 cm⁻¹, specifically, 1077, 1047 and 930 cm⁻¹ were assigned to the C-O-C glucose unit vibrations [8].

Although the spectra allowed to identify the characteristic bands of starch, the changes caused by the oxidation reaction were not detected due to signals overlapping and bands suppression. Thus, the second derivative was applied. The results demonstrated a slight decrease in bands intensity, namely at 2930 and 2887 cm⁻¹ (C-H stretching), 1244 cm⁻¹ (CH₂ out-of-plane bending) and 1460 cm⁻¹ (C-H bending), due to the conversion of the CH₂OH-6 into carboxyl groups (COOH-6).

The ¹H NMR spectrum analysis (Figure 2) shows the typical hydrogens resonances namely, at δ 5.12 and 4.80 ppm the H-1 and H-1’ assigned to the α-1,4 and α-1,6 bonds, at δ 5.43 ppm the hydroxyl groups OH-2 and OH-3, at δ 4.55 ppm the hydroxyl OH-6, at δ 1.3 ppm the signal of the aliphatic chain (-CH₂)₉ of lipids and at 0.9 ppm the -CH₃ groups of aliphatic protons (from proteins and lipids). Finally, between δ 3.32 - 3.80 ppm the signals assigned to the protons H-2, H-3, H-4, H-5 and H-6.

The SEM images (Figure 3) show that native and oxidized potato starch granules both present a very similar morphology with an oval or spherical shapes, with large dispersion of sizes (10 to 30 µm) and smooth surfaces, confirming that these oxidation conditions do not induces changes in granules morphology.

**Conclusion**

Native potato starch was oxidized with different hydrogen peroxide concentrations and the results demonstrate that carboxyl content increases with hydrogen peroxide concentration increases. NMR and FTIR analyses with application of mathematical spectra treatment (second derivative) confirmed the occurrence of oxidation. On the other hand, SEM analysis indicate that the oxidation reaction did not affect granules morphology.

Acknowledgements

This work was financially supported by: project POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013), funded by European Regional Development Fund (ERDF) through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI), and by national funds through FCT – Fundação para a Ciência e a Tecnologia; project “LEPABE-2-ECO-INNOVATION” – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

References

Development of acrylic/alkyd hybrid polymers

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In the present work acrylic/alkyd hybrids were developed to obtain a resin exhibiting properties from both materials, promoting chemical reaction between them and eliminating the presence of organic solvents. Conventional emulsion polymerization and mini-emulsion were used, adding a pre-made alkyd resin (AR) and unsaturated monomers (UM). FTIR and NMR spectrophotometry analyses were used to confirmed chemical reaction between the alkyd resin and the unsaturated monomers. Stable hybrids with 10 % of alkyd resin were produced by mini-emulsion process, when anionic surfactants were used, while colloidal destabilization was observed with conventional emulsion. NMR analyses confirmed the decrease of 67 % of double bounds from the fatty acids of the alkyd resin, suggesting free radical polymerization with the unsaturated monomers.

Introduction
The reduction of VOC emissions is an important concern in the coating industry since there are an increasing awareness about environmental and health issues, leading to legislation that obligate the producers to create eco-friendly products. Therefore, the replacement of solvent-based binders by their aqueous-based replacements has been an important research topic during the last years. Alkyd resins exhibit great properties and are commonly used as binders, but they are usually dissolved in organic solvents. Acrylic aqueous emulsions have been gaining popularity as an alternative to alkyd resins. Unfortunately, they lack some properties as gloss and applicability. Synthesis of hybrid acrylic/alkyd polymers has been studied, particularly as a colloidal dispersion in which the two distinct polymers exist within the same particles. This is a way of guarantying the production of a binder that has the properties of both materials (the ability of oxidative cure in alkyd and the low tendency to yellow in acrylic resin) and at the same time overcome the environmental concerns. The main problem in producing hybrid acrylic/alkyd emulsions is the fact that alkyd is a hydrophobic polymer due to the presence of fatty acids in it composition. Hence the diffusion from the monomers droplets to the micelles during polymerization process is very unlikely [1]. Mini-emulsion process is an alternative to this method, since it does not require the diffusion through the aqueous phase. In this process, small monomer droplets are formed, promoting polymerization reaction within the droplets. [2] In the present work, acrylic/alkyd hybrids were produced by conventional and mini-emulsion polymerization, using a pre-made alkyd resin and acrylic monomers (and styrene). NMR spectrophotometry analyses were made to confirmed polymerization reaction.

Methods
Pre-emulsions were prepared by dispersing unsaturated monomers (UM, namely styrene, butylacrylate, acrylic acid and hydromethylmethacrylate) or UM with one alkyd resin (AR) into a surfactant solution by magnetic stirring. Anionic (A1 and A2) and non-ionic (NI) surfactants were used. Mini-emulsion was produced by shearing the pre-emulsion with an Ultra Turrax during 20 min at 10 000 rpm. Polymerization process was carried out in a 500 mL jacketed flask reactor equipped with mechanical stirrer and a condenser. Water and surfactant were added to the reactor and heated to 82 °C. Seeded emulsion was first charged into the reactor and mixed for 10 minutes. Then the remaining pre-emulsion and the initiator solution were added during 2 hours. The reaction was maintained at 85 °C for 1 hour to ensure complete polymerization. Finally, the produced emulsion was filtered and neutralized using an ammonia solution until pH 7-8 was obtained.

Particle size distribution was performed on a Beckman Coulter LS230 with Polariodization Intensity Differential Scattering (PIDS) assembly nd an obscuration of 45 %. The dispersion was diluted to avoid agglomeration. Infrared (IR) spectra were recorded on a Bruker Avance III—600 spectrometer operating at a frequency of 600 MHz, using CDCl3 as solvent. Chemical shifts were reported in parts per million (ppm), relative to the resonance of TMS (0.00 ppm) as an internal standard.

Results and Discussion
Conventional emulsion polymerization process was first used to produce hybrids with different proportions of alkyd resin: unsaturated monomers (AR:UM) from 1:1 to 1:9 using A1. The experimental solid content (Scexp) was determined weighting samples before and after drying at 125 °C. The deviation between this value and the theoretical solid content (Stheo) was also calculated (Table 1).

Table 1. Results obtained for different alkyd resin: unsaturated monomers ratio.

<table>
<thead>
<tr>
<th>AL:UM ratio</th>
<th>HC01</th>
<th>HC02</th>
<th>HC03</th>
<th>HC04</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:1</td>
<td>SC (%)</td>
<td>58</td>
<td>51</td>
<td>46</td>
</tr>
<tr>
<td>1:2</td>
<td>SC (%)</td>
<td>37</td>
<td>39</td>
<td>44</td>
</tr>
<tr>
<td>1:5</td>
<td>SC (%)</td>
<td>36</td>
<td>24</td>
<td>6</td>
</tr>
<tr>
<td>1:9</td>
<td>RAL segregation</td>
<td>30min</td>
<td>60 days</td>
<td>60 days</td>
</tr>
</tbody>
</table>

When the AR:UM ratio was decreased from 1:1 to 1:5 a reduction in the deviation value from 36 to 3 was observed. The boiling point of AR is higher than 125 °C so it was expected that the difference between experimental and theoretical values occurs due to the evaporation of free-monomers. The results showed a decrease in polymerization rate with alkyd content.
increases. This may be associated with the chain transfer phenomena which produces relatively inactive radicals [3]. Segregation of alkyd resin was observed in HC01, HC02 and HC03, and this process was accelerated with the addition of higher amounts of alkyd resin (30 minutes). The particle size determination (Figure 1) showed two distributions sizes (0.1 and 1 µm) which can explain the segregation phenomenon. In conventional emulsion polymerization process there is migration from monomer droplets to the micelles. During monomers transfer to the growing polymer particles the concentration of the alkyd resin drastically increases leading to the particles coalescence and the alkyd resin segregation forming a rigid yellow layer [4].

![Particle size distribution for different alkyd resin: unsaturated monomers ratio.](image)

Although segregation was not observed using the proportion of 1:9 (AR:UM), the formation of creaming after 10 days was detected, due to particles aggregation which forms a small layer on emulsion surface. These results indicate the need of promoting the polymerization reaction within the droplets formed, without the requirement of diffusion to the micelles. If the droplet size is small enough, the interfacial area of the monomer is large enough to capture free radicals and start the polymerization process [5]. Therefore, mini-emulsion process was studied for a AR:UM proportion of 1:9. Three hybrids were produced adding different surfactants: HMN01 and HMN03 using anionic surfactants (A1 and A2, respectively) and HMN02 using a non-ionic surfactant (N1). Emulsions produced with anionic surfactants were stable, however non-ionic surfactants induce alkyd resin segregation. Emulsions particle size confirmed that the Ultra Turrax stirring destroyed the larger particles (1 µm), leading to a much less polydisperse system.

![Figure 2. FTIR spectra of: a) AR b) SAR and c) HMN03.](image)

To confirm chemical reaction between the alkyd resin and the vinylic monomers in HMN03, NMR spectrophotometry was used (Figure 3). The signal at δ 5.32 ppm was assigned to vynilic protons (CH=CH) and the two signals between δ 6.5-7.2 ppm were assigned to aromatic protons from styrene. Comparing the integral area of these signals a reduction of 67 % was verified, suggesting the occurrence of the reaction between the acrylic monomers and alkyd resin. The remaining double bounds were still available for oxidative cure process [6].

![Figure 3. NMR spectra of: a) AR b) SAR and c) HMN03.](image)

**Conclusion**

An acrylic/alkyd stable emulsion was produced with 1:9 alkyd:acrylic ratio using a mini-emulsion process. In hybrids synthesized by conventional emulsion process, segregation of the alkyd resin or creaming were observed. The introduction of the alkyd seems to affect the acrylic polymerization when added in higher amounts. NMR spectrophotometry confirmed that 67 % of the double bounds of the alkyd resin reacted, suggesting the occurrence of the polymerization reaction.
Paints are complex mixtures composed mainly by four constituents: resins, fillers, solvents and additives. Each of these components gives certain characteristics to paints and therefore it is of real importance to identify and quantify them. In this work, FTIR is combined with Partial Least Squares (PLS) regression models. Upon collecting spectral data, models for the quantification of polymer, calcium carbonate, and water content in styrene-acrylic, vinyl and acrylic type paints were developed and evaluated according to some performance indexes for achieving models with good prediction features. Moreover, it was possible to formulate and produce a new paint based on quantified components content of an unknown paint given by the corresponding PLS model. Furthermore, both paints were subjected to quality control tests and the assessed properties were consistent. It can be stated that these PLS models can be used to quality control purposes of paints and for predicting paint composition.

Introduction

The paints are a complex mixtures consisting essentially of four main compounds: i) the resin (containing the polymer); ii) fillers; ii) solvents (water in aqueous-based paints) and; iv) additives. Each one of these components provides the paint with certain characteristics, and it is of real importance to identify and quantify them. Several methods have been used to identify and quantify the paint components, among them near-IR, NIR [1]. Although NIR (12820-4000 cm\(^{-1}\)) has already been applied to the quality control in the paint industry, only few studies are available that use middle IR (MIR) spectroscopy (4000-400 cm\(^{-1}\)) to control paint quality and more specifically in predicting the components of paints [2]. The main advantage of the middle IR spectroscopy is its ability in the identification of functional groups of a sample. Since each paint component has a typical mid-IR spectrum, the respective paint spectrum will be the sum of the spectra of all paint components present, which is affected by its ratio. In this work, PLS models were applied to derive calibration models that relate the mid-IR spectra of paints with their different components.

Methods

Sample formulation, three different paints containing different polymers were made: acrylic, styrene-acrylic and vinyl type paint. After obtaining these base paints, 8 dilutions were performed with water. Each of these paints contained polymer, water and some solvents, commonly used in paint formulations. None of these paints contained fillers. Also industrial paints made by Barbot Tintas S.A, were developed using the MatLab®2016 software. Infrared (IR) spectra, were recorded with a Spectrum Two spectrometer (PerkinElmer), equipped with an ATR cell with diamond crystal with single reflection accessory. Each spectrum was an average of 8 scans, taken with 2 cm\(^{-1}\) resolution in the 4000-400 cm\(^{-1}\) range.

Models were developed using the MatLab®2016 software. The data obtained by mid-IR spectroscopy were the input variables (X), corresponding to 1800 of intensity values of each spectrum, while the properties to be predicted (the contents of certain components of the paints) were the output variables (Y). For the development of the PLS models, external validation was implemented to compute the number of latent variables (LV). The number of latent variables was defined by minimising the root mean squared error of prediction (RMSEP) of the validation set, which is given by:

$$\text{RMSEP} = \sqrt{\frac{\sum(y_i - \hat{y}_i)^2}{n_{\text{real}}}}$$

where \(\hat{y}_i\) refers to the estimated values of the validation data set, \(n_{\text{real}}\) is the total number of samples in that set [3]. Therefore, the data was divided into different data sets: training, validation and test, in the proportion of 50%, 25% and 25%, respectively. The training dataset (the largest) and validation dataset were used to configure the models, to determine the model parameters, and to evaluate the number of latent variables, while the test datasets were used to assess the performances of the models when applied to the new datasets. Taking into account, the defined percentage distribution, the original data was allocated in the three datasets in order to the three datasets have observations spread over the whole range of the output variables.

Performance indexes (PI). Three statistical parameters were used for evaluating the performance indexes of the model: Nash-Sutcliffe Efficiency (NSE), Percent Bias (PIBIAS) and Root Mean Squared Error (RSR, observations standard deviation ratio). The developed model can be evaluated and classified as: very good (VG), good (G), satisfactory (S) and unsatisfactory (US) according to the ranges of values specified in Table 1. The model classification (MC) is defined by the worst value of the RSR, NSE and PBIAS performance indexes in each case.

<table>
<thead>
<tr>
<th>Rating</th>
<th>RSR</th>
<th>NSE</th>
<th>PBIAS</th>
</tr>
</thead>
<tbody>
<tr>
<td>V</td>
<td>0.00</td>
<td>0.90</td>
<td>IPIBIAS&lt;5</td>
</tr>
<tr>
<td>G</td>
<td>0.50</td>
<td>0.80</td>
<td>IPIBIAS&lt;10</td>
</tr>
<tr>
<td>S</td>
<td>0.60</td>
<td>0.60</td>
<td>IPIBIAS&lt;15</td>
</tr>
<tr>
<td>US</td>
<td>0.70</td>
<td>0.60</td>
<td>IPIBIAS&gt;20</td>
</tr>
</tbody>
</table>

Results and Discussion

One hundred and seventy samples were analysed. Of these, 71 were styrene-acrylic paints, 45 were vinilic and 54 acrylic. The total number of samples analyzed for each model can be found in Table 2, as well as the range in which they are found. In Figure 1, one spectrum of each paint type analysed is shown where T3, T2, and T1 are spectra of acrylic, vinyl, and styrene-acrylic paints, respectively.
After obtaining the FTIR spectra of all paint samples, it was then possible to elaborate the models for the quantification of three principal constituents of paints: polymer, calcium carbonate and water, and classify them according to the PI (Table 3). This table does not present the PI values of the models for training data set because the MC was very good for all cases analysed.

Table 2. Samples and range of components in paints.

<table>
<thead>
<tr>
<th>Paints Component</th>
<th>Samples analyzed</th>
<th>Range of components (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Styrene-acrylic</td>
<td>71</td>
<td>2.24 - 40.8</td>
</tr>
<tr>
<td>Polymer</td>
<td>45</td>
<td>15.74 - 57.41</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>45</td>
<td>0.1 - 88.88</td>
</tr>
<tr>
<td>Water</td>
<td>63</td>
<td>0.1 - 88.88</td>
</tr>
<tr>
<td>Vinylic</td>
<td>45</td>
<td>5.68 - 49.58</td>
</tr>
<tr>
<td>Polymer</td>
<td></td>
<td>0.1 - 88.31</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>48</td>
<td>0.1 - 88.62</td>
</tr>
<tr>
<td>Water</td>
<td>63</td>
<td>0.1 - 88.88</td>
</tr>
<tr>
<td>Acrylic</td>
<td>54</td>
<td>5.17 - 45.16</td>
</tr>
<tr>
<td>Polymer</td>
<td>21</td>
<td>15.5 - 46.75</td>
</tr>
<tr>
<td>CaCO₃</td>
<td>48</td>
<td>0.1 - 88.62</td>
</tr>
</tbody>
</table>

The lowest RMSEP was obtained with 8 latent variables for all models. For quantification of the polymer content, all performance indexes were very good, and the PBIAS values were positive, which means that polymer quantification in any of the paints is overestimated. The model for calcium carbonate was not evaluated for vinylic paints, due to the reduced number of samples analysed. While, the model for styrene-acrylic paints is underestimated (e.g., the amount of carbonate obtained through the model is less than the actual amount present in the paint) for acrylic paints is overestimated.

In relation to the quantification of water content, satisfactory performance indexes for styrene-acrylic paints were obtained, being the model underestimated. Concerning the other two paints (vinylic and acrylic) the models were overestimated. In the elaboration of the models, only three components of paints had been considered although they are complex mixtures, which affect the intensity of IR spectral bands.

Figure 2A and Figure 2B present the estimated versus observed polymer and water content, for vinylic paints, respectively. The figures also show the good agreements between experimental values and the model predictions.

Table 3. PI values of PLS models for paint components.

<table>
<thead>
<tr>
<th>Paints Component</th>
<th>Polymer (%)</th>
<th>CaCO₃ (%)</th>
<th>Water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Val</td>
<td>8</td>
<td>Te</td>
</tr>
<tr>
<td>Styrene-acrylic</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NSE</td>
<td>1.00</td>
<td>0.98</td>
<td>-</td>
</tr>
<tr>
<td>PBIAS</td>
<td>-0.16</td>
<td>2.90</td>
<td>-</td>
</tr>
<tr>
<td>MC</td>
<td>VG</td>
<td>VG</td>
<td>-</td>
</tr>
<tr>
<td>NSE</td>
<td>0.93</td>
<td>0.90</td>
<td>0.86</td>
</tr>
<tr>
<td>PBIAS</td>
<td>1.90</td>
<td>0.35</td>
<td>1.55</td>
</tr>
<tr>
<td>RSR</td>
<td>0.27</td>
<td>0.32</td>
<td>0.37</td>
</tr>
<tr>
<td>MC</td>
<td>VG</td>
<td>VG</td>
<td>G</td>
</tr>
<tr>
<td>NSE</td>
<td>0.94</td>
<td>0.90</td>
<td>0.97</td>
</tr>
<tr>
<td>PBIAS</td>
<td>0.61</td>
<td>4.25</td>
<td>1.78</td>
</tr>
<tr>
<td>RSR</td>
<td>0.24</td>
<td>0.31</td>
<td>0.17</td>
</tr>
<tr>
<td>MC</td>
<td>VG</td>
<td>VG</td>
<td>VG</td>
</tr>
</tbody>
</table>

In sum up

Predicting paint components from FTIR can be used to quality control purposes of paints and for predicting paint composition.

Acknowledgements

This work was financially supported by: project POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013), funded by European Regional Development Fund (ERDF) through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI), and by national funds through FCT – Fundação para a Ciência e a Tecnologia; project “LEPABE-2-ECO-INNOVATION” – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

References

Covalent organic frameworks as support for Ni nanocatalysts for CO₂ methanation

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CO₂ methanation, also called Sabatier reaction (1) is the reaction in which carbon dioxide (CO₂) reacts with hydrogen (H₂) to form methane (CH₄) [1]. This reaction is of great interest since it is the most energetically advantageous reaction to valorise CO₂ as carbon source, with respect to thermodynamics, decreasing the CO₂ emissions to atmosphere. However, in order to achieve CO₂-neutral emissions to the atmosphere, the hydrogen used in the process should be produced from sustainable sources.

\[
\text{CO}_2 + 4\text{H}_2 \leftrightarrow \text{CH}_4 + 2\text{H}_2\text{O} \quad \Delta H_{298K}^{\text{rxn}} = -165 \text{ kJ mol}^{-1} \quad (1)
\]

Despite being a thermodynamically favourable reaction, Sabatier reaction has significant kinetic barriers since the reduction of the fully oxidized carbon to methane is an eight-electron process, requiring a catalyst to reduce reaction barriers and to achieve considerable activity and selectivity [2].

Many group VIII transition metals have been found to be active, and from these, supported nickel-based catalysts are the most commonly used, due to its good combination of activity, selectivity and price [3–5]. Along with nickel, ruthenium is another of the most effective metals for CO₂ methanation reaction, and it has also been studied on different supports; however, it has the drawback of being a noble metal [6,7].

Different supports have been tested, mainly metal oxides, such as Al₂O₃, SiO₂ and TiO₂ [4], but also new types of supports, such as zeolites [8], carbon nanofibre-reinforced foams [9], and recently, metal-organic frameworks [5].

In this work, we propose the use of covalent organic frameworks (COFs) as supports for Ni nanoparticles, to be used as catalysts for CO₂ methanation reaction, with the objective to develop new catalysts, more efficient, and working at lower temperature.

COFs are crystalline porous materials where the designed building blocks are connected via covalent bonds. These materials enable the integration of the organic building blocks into an ordered structure with atomic precision, allowing the creation of predesigned skeletons and nanopores. Their in-built covalent bond architecture provides them with unique properties, such as low mass densities, high thermal stabilities, and permanent porosity [10]. These materials can have great potential as catalyst support systems since their high surface area allows a better distribution of the active sites.

TpBD-Me: COF was synthesised using as building blocks triformalylphloroglucinol (Tp) and o-tolidine and 1,4-Dioxane anhydrous as solvent. The mixture is sonicated in order to obtain a homogeneous dispersion and after that acetic acid 6 M is added to the mixture. After degassed with N₂, the pressure tube is closed and placed at 100 °C for 3 days.

After washing and purification of the COF support, incipient wetness impregnation was performed to incorporate the nickel precursor (Ni(acac)₂) on the support, using dichloromethane as solvent and different reduction procedures were performed to reduce the Ni nanoparticles. The tested reduction procedures were the most commonly used thermal treatment with hydrogen at high temperatures (sample Ni@COF_TT), and solid-state reduction with sodium borohydride (NaBH₄) as reducing agent (sample Ni@COF_SS).

As comparison, an activated carbon supported Ni catalyst was also prepared by incipient wetness impregnation with Ni(acac)₂ as precursor and a thermal treatment with hydrogen for the reduction of the nanoparticles (sample Ni@AC).

Finally, a commercial Ni on alumina catalyst was characterized and tested for comparison (sample Ni@Al₂O₃).

Both the COF and activated carbon supports were characterized by TEM, N₂ physisorption and in the case of COF, by SAXS to evaluate the crystallinity and TGA to evaluate the thermal stability. The prepared catalysts were also characterized with the same techniques, after reduction of the metal.

The CO₂ methanation catalytic tests were performed in a Microactivity reactor from PID Eng & Tech, where 100 mg of catalyst were diluted in silicon carbide (SiC) and placed in a stainless-steel reactor. The conversion and selectivity of the catalysts were evaluated by temperature-programmed experiments at atmospheric pressure, using a gas feed of 5 % CO₂, 20 % H₂, balanced with N₂. The outflow was analysed by a GC equipped with an FID detector for methane and a CO₂ and CO analyser (Rapidox 3100 from Cambridge Sensors) equipped with an IR detector.

The sample Ni@COF_TT presented nanoparticles with a mean width of 13±8 nm, as determined by TEM (Figure 1). However, this sample presented a complete loss of crystallinity of the COF support and a massive decrease in the surface area. The Ni@COF_SS sample presented nanoparticles with a narrow size distribution with a mean width of 3 ± 1 nm (Figure 1) and with no apparent loss in crystallinity of the COF, as assessed by SAXS.

Regarding the prepared Ni@AC sample, the nanoparticles present a mean width of 10±4 nm.

The three prepared samples and the commercial Ni@Al₂O₃ sample were tested for CO₂ methanation and the observed trends are discussed in terms of conversion and selectivity of carbon dioxide to methane.

We successfully prepared, characterized and tested Ni nanocatalysts over different supports for CO₂ methanation, in order to assess the possibility of using COFs as support for more efficient metal catalysts.
Figure 1. TEM images of (a) Ni@COF_TT (b) Ni@COF_SS and (c) Ni@AC.

Acknowledgements
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References
Development of new lead-free medical X-ray protective clothing

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Throughout life, humans are daily exposed to the effects of radiation. These radiations can have natural or artificial origins and efforts should be concentrated to reduce their harmful effects. Radiation protection is of fundamental importance is the medical sector, mainly in clinical radiology. As such, all efforts should be directed towards controlling and reducing radiation exposure, and in this context protective clothing plays a key role. The main objective of this work is the development of a lead-free protective clothing with enhanced protection. X-ray attenuation properties were assessed on a set of tissues coated with Bi2O3, Bi, W and SnO. A high attenuation of 98% was obtained for the tissues coated with 20% of Bi2O3.

Figure 1. wt% of the metals in the formulation.

In the last decades, the number of radiological examinations and interventions has been increasing, which has given rise to constant concern with the protection of medical personnel and patients from the harmful effects of exposure to X-rays [1].

During many years, the adopted protective medical clothing consisted of metallic powder lead incorporated in polymer matrices [2]. However, the high weight of these garments encouraged the search for alternative solutions to increase comfort for the user while minimizing fatigue and orthopedic problems resulting from prolonged use [3,4]. On the other hand, the high toxicity and problems associated with incineration of metallic lead are serious problems for health and environment [4,5]. Thus, new composite materials with lower lead or lead-free content have been developed, containing one or more chemical elements (e.g. tungsten, bismuth, tin) to produce lighter and flexible garments, which may confer a degree of protection equal to or greater than that of traditional protective clothing [6]. Alternatively, metals/metal oxides with high atomic number and high density, such as Bi, W, Sn, Sb or their combinations, can impart higher shielding protection with the advantage of lower toxicity when compared to lead [7].

Yaffe et al. [8] developed aprons containing barium, tungsten and lead and found that the combination of lead with tungsten and barium imparted practically the same degree of attenuation as a pure lead apron with a material mass reduction of 25-30%. McCaffrey et al. [9] measured the attenuation properties of metal oxides containing materials of Bi2O3, Gd2O3 and BaSO4, either individually (monolayer) or bilayer. Materials containing Bi2O3 (in mono- and bilayer) showed radiation attenuation per unit mass equal to or greater than that of other commercial lead-based materials.

There are several commercial and patented lead-free solutions on the market for X-ray protection, namely containing bismuth, tin, barium and antimony. However, there is a great deal of discussion about the efficiency of many of these products, since the degrees of attenuation indicated by manufacturers are generally only equivalent to that of lead for a given X-ray energy [10].

This work aims to develop lead-free protective clothing for X-rays that is lighter, more flexible, lower cost and with a higher degree of protection for X-rays compared to the clothes that currently exist, incorporating metals / compounds / metal oxide with nano- or micrometric size.

The application was made using technologies of additivation of the materials by coating. The steps involved were as follows:

- Preparation of the polyurethane formulation according to the manufacturer's specifications;
- Dispersion of the materials in the formulation;
- Application of the formulation to the tissue;
- Drying and thermosetting the coating in the tissue.

Cotton fabrics coated with different percentages (wt%) of metals were prepared focused on the use of high atomic number elements with a lower degree of toxicity than lead. In this context, metals (Bi and W), metal oxides (Bi2O3 and SnO) and metal compounds (BaSO4) were dispersed in polyurethane-based polymer matrices.

All formulations prepared are summarized in Table 1.

<table>
<thead>
<tr>
<th>Table 1. Composition of polyurethane formulations prepared with various metals, metal compounds, metal oxides and their respective weight percentages.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Matrix</strong></td>
</tr>
<tr>
<td>-----------</td>
</tr>
<tr>
<td>Polyurethane</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
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<td></td>
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<tr>
<td></td>
</tr>
</tbody>
</table>

Initially, formulations were made with 20 wt% of the materials to determine which had the best attenuation to X-ray. The results shown in the Graphical Abstract revealed that these materials have a good attenuation to the X-rays, except SnO that has a clearly lower attenuation when compared with the other materials. Among them Bi2O3 is the one with the best performance. After this result, formulations with different...
amounts of Bi$_2$O$_3$ were prepared as shown in Figure 2, to assess the attenuation to X-rays.

The X-ray attenuation values obtained for these tissues at 45 kV increase with the increase of the content of the metal oxide in the formulation. For the 10 wt% Bi$_2$O$_3$ polyurethane formulation the attenuation is 51 % and for 70 wt% it is 100 %.

From the results of attenuation to X-rays at 45 kV, it can be concluded that Bi$_2$O$_3$ shows better results than the other materials. The results showed a radiation attenuation higher than 99 % for fabrics coated with ≥50 wt% of Bi$_2$O$_3$.

Regarding the use of metal vs. metal oxide; in general, formulations produced with the metal oxide appear to have a slight improvement in the attenuation. For example, for the formulation with 20 wt% Bi vs. 20 wt% of Bi$_2$O$_3$ an attenuation of 89 % vs. 98 %, respectively, was observed.

Acknowledgements

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References

Nanomaterials application in water disinfection for human consumption

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Introduction

There are innumerable factors associated with the scarcity of drinking water in the world. The achievement of innovative technologies for water treatment becomes crucial in order to guarantee the supply of drinking water and reduce global pollution. Nanomaterials have proven to have great antibacterial action, which make them quite efficient in water disinfection. In this work, eight different magnetic nanoparticles (MNP’s) were synthesized, having in their base iron oxides, and were characterized using different techniques. Two different bacteria were used to evaluate the nanoparticles bacterial removal efficiency, Escherichia coli (gram-negative) and Staphylococcus aureus (gram-positive), respectively. Several tests with different experimental conditions were carried out in a batch reactor. The results showed that the MNP’s were efficient in the removal of the two studied bacteria.

Methods

Eight different nanoparticles were synthesized by diverse methods. Iron oxide nanoparticles (FeO), copper nanoparticles (CuFeO) and iron and copper nanoparticles with a physical mixture of carbon nanotubes (FeO/CNT and CuFeO/CNT) were synthesized by the co-precipitation method. The incipient impregnation method was used to synthesize silver nanoparticles (5% Ag/FeO) and silver nanoparticles with carbon nanotubes (5% Ag/CNT). Iron oxide nanoparticles coated with carbon (C-FeO@CVD750) and iron oxide nanoparticles coated with nitrogen-doped carbon (N-C-FeO@CVD750) were synthesized by the chemical vapour deposition method (CVD).

All the nanoparticles were characterized using different techniques as thermogravimetric analysis, nitrogen adsorption at -196 °C, temperature programmed reduction, transmission electron microscopy and X-ray dispersion. Finally, particles were applied in batch tests in order to evaluate the bacterial removal efficiency using an E. coli and S. aureus suspensions with a bacterial density of 10⁶ CFU/ml.

Results

Table 1 shows the results of thermogravimetric analysis and nitrogen adsorption at -196 °C for all nanoparticles synthesized.

<table>
<thead>
<tr>
<th>MNP’s</th>
<th>BET (m²/g)</th>
<th>V_p (cm³/g)</th>
<th>% Carbon</th>
<th>% Metal</th>
</tr>
</thead>
<tbody>
<tr>
<td>FeO</td>
<td>161</td>
<td>0.324</td>
<td>-</td>
<td>97</td>
</tr>
<tr>
<td>CuFeO</td>
<td>256</td>
<td>0.280</td>
<td>-</td>
<td>96</td>
</tr>
<tr>
<td>5% Ag/FeO</td>
<td>134</td>
<td>0.290</td>
<td>-</td>
<td>98</td>
</tr>
<tr>
<td>FeO/CNT</td>
<td>178</td>
<td>0.425</td>
<td>25</td>
<td>75</td>
</tr>
<tr>
<td>CuFeO/CNT</td>
<td>226</td>
<td>0.351</td>
<td>11</td>
<td>89</td>
</tr>
<tr>
<td>5% Ag/CNT</td>
<td>157</td>
<td>0.385</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>C-FeO@CVD750</td>
<td>&lt;5</td>
<td>0.014</td>
<td>1</td>
<td>99</td>
</tr>
<tr>
<td>N-C-FeO@CVD750</td>
<td>197</td>
<td>0.488</td>
<td>87</td>
<td>13</td>
</tr>
</tbody>
</table>

MNP’s synthesized by CVD method present a lower surface area than metal-based nanoparticles due to the high temperatures used during their synthesis. MNP’s with carbon nanotubes in their structure present intermediate specific surface area values between those obtained for the metal-based nanoparticles, composed only by the oxides, and for the CNTs.

The results shown in the graphical abstract revealed that the three most promising nanoparticles in bacterial removal were C-FeO@CVD750, CuFeO/CNT and 5% Ag/FeO. Table 2 shows the percentages of bacterial removal for MNP’s when in contact with the two bacterial suspensions studied (E. coli and S. aureus).

As can be seen in Figures 1 and 2, these three MNP’s achieved maximum bacterial removal efficiency for longer contact times and higher MNP’s concentrations. The conditions considered...
most promising for bacterial removal were contact time of 1 min and MNP’s concentration of 50 mg/L. However, for these conditions, the maximum log reduction value obtained was 3. Comparing the removal efficiencies obtained for the three MNP’s to different bacterial suspensions, there was a small loss of efficiency when in contact with S. aureus suspension.

<table>
<thead>
<tr>
<th>MNP’s</th>
<th>E. coli removal (%)</th>
<th>S. aureus removal (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-FeO@CVD750</td>
<td>99.4</td>
<td>92.0</td>
</tr>
<tr>
<td>CuFeO/CNT</td>
<td>100</td>
<td>93.0</td>
</tr>
<tr>
<td>5% Ag/FeO</td>
<td>96.7</td>
<td>95.9</td>
</tr>
</tbody>
</table>

Figure 1. Kinetic tests for the E. coli removal

Table 2. S. aureus and E. coli removal (1 min; 50 mg/L)

Conclusions
The most efficient MNP’s were C-FeO@CVD, CuFeO/CNT and 5% Ag/FeO, presenting high bacterial removal efficiencies for short contact times with the suspension. The most appropriate conditions for bacterial removal were established as a contact time of 1 min and a MNP’s concentration of 50 mg/L. These conditions were used in S. aureus tests and the results showed that there was a small loss of efficiency for the three MNP’s comparing with those obtained with E. coli in the same conditions. However, this loss is almost negligible, so it is possible to conclude that the three MNP’s were efficient in the removal of the two studied bacteria (E. coli and S. aureus, gram-negative and gram-positive, respectively).

Acknowledgements
This work is a result of projects: “AIProcMat@N2020 - Advanced Industrial Processes and Materials for a Sustainable Northern Region of Portugal 2020”, with the reference NORTE-01-0145-FEDER-000006, supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF) and of Project POCI-01-0145-FEDER-006984 – Associate Laboratory LSRE-LCM funded by ERDF through COMPETE2020 - Programa Operacional Competitividade e Internacionalização (POCI) – and by national funds through FCT - Fundação para a Ciência e a Tecnologia and project POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013), funded by European Regional Development Fund (ERDF) through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI), and by national funds through FCT – Fundação para a Ciência e a Tecnologia; project “LEPABE-2-ECO-INNOVATION” – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF).

References
Valorization of wastes from pulp and paper industry: effect of distinct formulations and stabilization conditions in the properties of granules to be used as soil improvers

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Valorization of wastes from pulp and paper industry is necessary to reduce environmental impacts of thermochemical conversion of biomass for heat and power production and to boost the re-use of wastes which otherwise would require landfilling.

The granulation of different waste materials (fly ash, composted biological sludge and biological sludge effluent) proved to be suitable to produce new products that can be used as soil improvers. Distinct formulations allow us to obtain a wide range of different new products with variable properties (bulk density, organic matter and elemental composition). Stabilization conditions are another key factor in the control of the physical-chemical properties of the materials produced (pH, EC, chloride content and soluble elements).

Table 1. Distinct formulation tested with waste materials (% of waste materials per kg of new product, in dry basis).

<table>
<thead>
<tr>
<th>Sample name</th>
<th>Fly ash</th>
<th>Composted biological sludge</th>
<th>Biological sludge effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>G FA_I 1</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G FA_I 2</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G FA</td>
<td>100</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>G FA_BSE</td>
<td>99.5</td>
<td>0.5</td>
<td>-</td>
</tr>
<tr>
<td>G FA_CBS 1</td>
<td>88</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>G FA_CBS 2</td>
<td>79</td>
<td>21</td>
<td>-</td>
</tr>
</tbody>
</table>

After drying, the granulated materials were characterized. Physical-chemical properties (moisture, loss-on-ignition, bulk density, particle size distribution), and elemental composition (major and minor elements) were determined. The granulated materials were leached according to EN 12457, and the extracts were characterized to determine pH, EC, chloride content and soluble elements (macro- and micronutrients and potentially toxic elements by ICP-MS).

Results showed that the distinct granules formulations presented different bulk density: granules with CBS presented lower bulk densities, (range: 976 – 1068 kg m⁻³) compared to the remaining formulations (1300 kg m⁻³ on average). This is due to the higher bulk density of FA (900 kg m⁻³) relatively to the CBS (500 kg m⁻³). LOI analysis showed that G FA_CBS 1 and G FA_CBS 2 presented higher contents of organic matter than the other granules. Materials with higher % of CBS on their formulation resulted in granules with upper LOI value, due to the high LOI content observed in the CBS residue (84%), contrary to the low value observed for FA (0.9%). Elemental composition (as determined by FRX) showed that granules with FA only (G FA, G FA_I 1 and 2) and G FA_BSE presented higher concentrations of Al, Ca and Si than granules G FA_CBS 1 and 2. On the other hand, G FA_CBS 1 and 2 showed higher concentrations of S than the remaining granules, due to the higher sulphur content in biological sludge. Contents of Ba, Sr and Zn are higher in granules G FA, G FA_I 1 and 2, and G FA_BSE, since FA is enriched on these elements comparatively to biological sludge.

Granules G FA_CBS 2 presented lower contents of potentially...
toxic elements, including As, Co, Cu and Zn than the remaining granules. Contents of Cu and Zn were clearly lower for G FA CBS 2 (88.1 and 430 mg kg⁻¹, respectively) compared to the other granules (G FA: 210 and 620 mg kg⁻¹, respectively; G FA_BSE: 200 and 700 mg kg⁻¹, respectively due to the dilution effect promoted by the incorporation of biological sludge in granulated materials, since this waste material presented lower contents of these PTEs than biomass FA.

Extracts obtained in leaching tests showed that granules G FA, G FA_I 1 and 2, and G FA_BSE presented values of pH higher than G FA_CBS 1 and 2, due to the higher % of the highly alkaline FA in the formulations (FA presented pH values around 13, while the composted biological sludge had a neutral pH).

Results also clearly showed lower values in all parameters in materials stabilized outdoors (pH: 9.89; EC: 0.47 mS cm⁻¹; [Cl]: 102 mg L⁻¹) compared to granules subject to indoor stabilization (pH: 11.4; EC: 3.4 mS cm⁻¹; [Cl]: 782 mg L⁻¹). Lower values observed for these properties in materials stabilized outdoors are due to the leaching that materials were subjected through meteorological conditions during stabilization process (air moisture and raining).

Distinct formulations allow us to obtain products with variable bulk density, organic matter and elemental composition. Stabilization conditions of the granules determine the physical-chemical properties of the materials produced (pH, EC, chloride content and soluble elements). Granules with longer stabilization time and/or stabilized outdoors presented lower pH, EC, chloride content and soluble elements than the materials stabilized indoors. This will influence the reactivity of the granule products when applied to soil and it will affect the impact that the granules will have on soil properties.

The impact of formulation and stabilization conditions of waste-based granules on the properties of the materials produced will be discussed in detail in this presentation. Implications for further use of the different materials as soil improvers will also be presented and discussed thoroughly. All formulations (G FA, G FA_BSE and G FA_CBS) appear be suitable for the improvement of soils properties, particularly for acidic soils and/or poor in plant nutrients. Granules that contain higher % of CBS in their formulation can be preferentially used in soils poor in organic matter. Materials stabilized outdoors can be preferentially used in agricultural/forest/mining soils with potential salinity problems. The low EC and chloride content of these materials can prevent soil salinization problems and potential plant stress problems due to the toxicity induced by chlorides. It is however necessary to further assess the fate of salts, soluble ions, and chlorides during the stabilization of granules outdoors as it may be necessary to collect and treat leachates whenever these are formed during this process.

Acknowledgements
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References
Microencapsulation by spray coagulation method: Study of the alginate crosslinking by different calcium salts

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Introduction
The use of biopolymers such as alginate has been growing in the last decades due to properties such as non-toxicity and biodegradability. In this work, the microencapsulation of curcumin and safranin, used as model compounds for hydrophobic and hydrophilic active principles, respectively, was studied with alginate matrices by the spray coagulation technique. The alginate ionic crosslinking with three calcium sources (calcium chloride (CaCl2), calcium gluconate and calcium lactate) was considered. The obtained microcapsules were characterized in terms of load, encapsulation efficiency (EE), rehydration capacity, and particle size. The release profiles of both active principles were also studied. Through FTIR analysis the contribution of the calcium source, and the presence of the active principles inside the microcapsules, was identified, while the TGA evidenced an increasing of the thermal stability due to the alginate crosslinking effect, particularly when CaCl2 was used. The overall results showed that CaCl2 is the most effective calcium source for alginate crosslinking, while calcium lactate lead to a more gradual release of the active principles.

Materials and methods
Microencapsulation. The first step for the microencapsulation process corresponded to the preparation of the active principles aqueous suspension (for curcumin), and solution (for safranin), both with a concentration of 4% (w/w). For the curcumin suspension, 20 ml of distilled water were mixed with 100µl of Tween80® and 100 mg of curcumin. The mixture was homogenized during 20 minutes by using a homogenizer (CAT Unidrive X, Germany) at 11000 rpm. Afterwards, 800 mg of alginate were added to the suspension, which was then kept under stirring until the alginate dissolution was complete. For the safranin aqueous solution, 20 ml of distilled water and 100 mg of safranin were stirred until the complete dissolution of safranin was reached. After, 800 mg of alginate were added and the solution was kept under stirring until its full dissolution. 250 ml of the coagulation solution (using one of the three calcium sources), with concentration of 4% (w/v), were prepared. Then, the curcumin suspension or safranin solution, was introduced into a syringe and placed on the syringe pump of the atomization system (Nisco VarJ30 system (Zurich, Switzerland)). The flow rate and the pressure of the atomizing gas (nitrogen) were established at 0.3 ml/min and 0.1 bar, respectively. At the end of the atomization, the coagulation stage was maintained for further 4 hours at room temperature, in order to promote the microspheres (Mic) crosslinking. Following, the obtained Mic were recovered by filtration, washed twice with distilled water and freeze-dried for further tests. Empty Mic (EMic, Mic without active principle) were also produced.

Mic characterization. The Mic samples were characterized by optical microscopy (OM), using the optical microscope Ni-U (Nikon Eclipse) equipped with a digital camera and a NIS-Elements Documentation software, in order to access their morphology. The encapsulation efficiency (EE), load, dry residue, rehydration capacity of the freeze-dried Mic were evaluated. The release profile of curcumin was evaluated on ethanol, while for safranin water was used. The Mic particle size was evaluated by laser diffraction, using the equipment Malvern Mastersizer 3000, equipped with a dispersion unit Hydro 2000 (Malver, United Kingdom). The FTIR spectra were obtained on a ABB Inc. FTIR, model MB3000 (Quebec, Canada) in transmittance mode by using KBr pellets with a sample concentration of 1% (w/w). Spectra were recorded between 550
and 4000 cm⁻¹ at a resolution of 16 cm⁻¹ and by co-adding 32 scans. Thermogravimetric analysis (TGA) was done by using a NETZSCH model TG 209F3 Tarsus (Selb, Germany) equipment. The used temperature range was from 32 to 600°C, with a heating rate of 10°C.min⁻¹. The experiments were made under nitrogen flow (50 mL/min).

Results

**Optical Microscopy.** The OM analysis allowed to check the spherical conformation of the EMic, being also noticed a predominance of a pear shape, mainly for the Mic crosslinked with CaCl₂ and Ca-glu. The curcumin Mic had a spherical conformation, and it was possible to observe the presence of small curcumin crystals on their surface. Regarding the safranin Mic, a predominant spherical conformation was observed. However, the occurrence of Mic deformation was also noticed, especially for the Mic crosslinked with CaCl₂.

**Encapsulation Efficiency.** The EE was estimated based on the quantification of the active principle loss to the crosslinking solution during the coagulation stage. The results, shown in Table 1, revealed that all the samples presented high EE values. When CaCl₂ is used, higher EE was obtained, followed by Ca-glu and Ca-lact. For curcumin Mic, due to its hydrophobicity it wouldn’t be expected a significant loss for the aqueous solution, during the coagulation stage. Regarding safranin Mic, the high values were not expected, once safranin is a hydrophilic compound with a good chemical compatibility with the coagulation aqueous solution, being thus expected a higher loss during the coagulation stage.

**Dry Residue and Rehydration Capacity.** The dry residue (%) allows the quantification of the Mic solid content, based on the weight measured before and after the freeze drying. The results presented in Table 2 showed that a higher dry residue was obtained for the Mic crosslinked with Ca-glu, followed by the Mic crosslinked with CaCl₂ and finally the Mic crosslinked with Ca-lact. Regarding the rehydration capacity, this parameter is related with the Mic ability to absorb water and recover the initial shape. The results (Table 3) evidenced a higher capacity for the Ca-glu crosslinked Mic, in all the cases, followed by the Ca-lact Mic and finally, the CaCl₂ ones.

**Release Profile.** The release profile of both, curcumin and safranin compounds, was lower when CaCl₂ was used (5.70% of curcumin in ethanol, and 4.15% of safranin in distilled water), followed by the Ca-glu Mic (8.30% for curcumin and 4.42% for safranin). Ca-lact crosslinked Mic give rise to systems with higher release (12.10% for curcumin and 4.76% for safranin). The curcumin’s release was restricted by the formation of curcumin crystals inside the Mic. For safrin Mic, gradual release was achieved during the evaluation period, without reaching a plateau indicating that the analysis should be extended for longer periods.

The overall results revealed that the alginate crosslinking can be influenced the calcium salts dissociation and the calcium diffusion to the Mic inner. The conditions used on this study were chosen in order to guarantee the calcium salts dissociation (once the solutions pH was above the pKₐ values of the calcium salts). Regarding the calcium diffusion to the Mic inner, for the system alginate/CaCl₂, the molar ratio between the calcium ions (Ca²⁺) and the alginate G residues (Ca²⁺/G) was 45, which means that the CaCl₂ solution at 4% (w/v) has a Ca²⁺ concentration 45-fold higher than the required for total alginate crosslinking, favouring this reaction. In the same manner, for the Ca-lact, the Ca²⁺/G ratio was 25, but for this calcium salt the complete dissociation of Ca²⁺ was not achieved, due to interaction between the negatively charged alcohol groups on the lactate complex and the Ca²⁺ [2], which decreased the dissociation capacity, resulting on a less effective alginate crosslinking. Regarding the Ca-glu, the Ca²⁺/G ratio was 11, and the dissociation was complete, being more efficient than the Ca-lact.

**Table 1. Encapsulation Efficiency (w/w) %).**

<table>
<thead>
<tr>
<th>Crosslinking agent</th>
<th>Mic CaCl₂ Ca-glu Ca-lact</th>
</tr>
</thead>
<tbody>
<tr>
<td>Curcumin</td>
<td>99.3% 98.5% 97.9%</td>
</tr>
<tr>
<td>Safranin</td>
<td>98.5% 97.6% 97.4%</td>
</tr>
</tbody>
</table>

**Table 2. Dry Residue (w/w) %.**

<table>
<thead>
<tr>
<th>Crosslinking agent</th>
<th>Mic CaCl₂ Ca-glu Ca-lact</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMic</td>
<td>5.9% 6.6% 5.7%</td>
</tr>
<tr>
<td>Curcumin</td>
<td>8.7% 10.2% 9.9%</td>
</tr>
<tr>
<td>Safranin</td>
<td>9.5% 11.1% 10.2%</td>
</tr>
</tbody>
</table>

**Table 3. Rehydration Capacity (w/w) %.**

<table>
<thead>
<tr>
<th>Crosslinking agent</th>
<th>Mic CaCl₂ Ca-glu Ca-lact</th>
</tr>
</thead>
<tbody>
<tr>
<td>EMic</td>
<td>12.1% 39.6% 15.5%</td>
</tr>
<tr>
<td>Curcumin</td>
<td>11.5% 23.7% 15.2%</td>
</tr>
<tr>
<td>Safranin</td>
<td>18.2% 42.9% 32.6%</td>
</tr>
</tbody>
</table>

**Particle Size Analysis.** The particle size analysis showed that the larger particle size, based on the volume distribution, was obtained for the Ca-glu based system (mean sizes of 131.46, 78.85 and 91.46 μm for curcumin, safranin and empty Mic), while the CaCl₂ based system gave rise to the smallest ones (mean sizes of 91.65, 60.10 and 80.31 μm, respectively for curcumin, safranin and empty Mic). FTIR and TGA analysis. The FTIR analysis was used to access the effectiveness of the encapsulation of curcumin and safranin on the alginate matrix, and also evaluate the contribution of the crosslinkers in the Mic’s chemical structure. The spectra analysis evidenced the curcumin and safranin presence, proving their encapsulation, and the contribution of the calcium crosslinkers was also identified. The TGA was used to evaluate the thermal stability of the Mic. Pure alginate, curcumin and safranin were also analyzed. The degradation profile obtained showed an increase of the thermal stability due to alginate crosslinking, once the active principles degradation shifted to higher temperatures due to the crosslinked polymeric matrix protection, particularly when calcium chloride was used.

**Conclusions**

Analyzing the overall results obtained for the EE, it can be concluded that the CaCl₂ is the most effective calcium source for alginate crosslinking, being followed by calcium gluconate and finally calcium lactate. From the point of view of the curcumin and safranin release profile, calcium lactate lead to a more gradual release than the other two calcium sources.

Acknowledgements

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References

Emulsions preparation based on ternary phase diagrams: comparative study using two oils (miglyol and sweet almond) with two distinct surfactants (Twee 80 and Saponin)

Introduction
Emulsions are systems formed by mixing components presenting three distinct characteristics: hydrophilic, lipophilic, and amphiphilic. In other words, water, oil, and surfactant (or a combination of surfactants), forming, for certain levels of compositions, a macroscopically homogeneous system that presents important technological features. In fact, because of its versatile character, the technological application of emulsions is vast, including food, pharmaceutical and cosmetics industries. Among the different types of emulsions, micro and nanoemulsions present the most appealing properties due to their higher stability and possibility to serve as potential carriers for functionalities delivery.

Nowadays, an important topic in the field of emulsions is the introduction of natural products to act as surfactant. This is partly motivated by consumer demands for more sustainable, natural and environmentally friendly formulations, which is also connected to the current environment concerns and legislation. Thus, industry and researchers are searching for products with friendly-natural label, more specifically to surfactants, which have a high economic importance and face problems due to the large utilization of synthetic forms [1]. This is particularly valid in certain areas, e.g. the food industry, which accounts for around 67% of the global market volume [2].

Recent studies are being focused on the use of a highly surface-active group named Saponins [3], whose properties (biological and physicochemical) lead to a number of industrial applications [4]. The abundance of Saponins in nature result in a wide range of natural matrices for their commercial production, being Quillaja Bark one of them [5]. In this context, the objective of this work is to perform a preliminary study concerning the preparation of emulsions, building the ternary phase diagrams either using a traditional or natural surfactant, to help in the decision of the best formulations.

Methods
The studied surfactants were Tween 80 and Saponin from Quillaja Bark. Two oils with different chemical composition and viscosity (Sweet almond oil (SAO) and Miglyol 812) were selected as the lipophilic constituent. To build the entire diagram, different samples covering the appropriate range of compositions were prepared using a Vortex system. The prepared samples were analyzed macroscopically to identify the type of emulsion formed. Thereafter, the more suitable compositions were prepared by using a high-pressure homogenizer (HPH) and the droplet size determined by laser diffraction (LD) technique.

Results
Figure 1 shows the ternary diagrams in terms of mass fraction for the systems: a) Water + Tween 80 + Miglyol 812, b) Water + Tween 80 + Sweet Almond Oil and c) Water + Saponin + Sweet Almond Oil. The different samples were classified in: 1 Phase, 2 Phases, 3 Phases, Gel or microemulsion (ME), giving a first general analysis of the mixture state according to composition. Potentially, the 1 phase and the gels are the most interesting forms to explore from a technological point of view. Tween 80, a commercial surfactant formed emulsions in the entire range of compositions. The ME zone for these systems comprise the use of high amounts of surfactant, which is not commercially desirable as the compositions with low level of surfactant are the most attractive ones. For all the three studied systems, an area corresponding to gel formation was observed for water compositions between 0.15 and 0.5 and surfactant mass fraction higher than 0.5.

The Saponin-based system, using SAO also gives rise to the formation of emulsions, but only when composition comprised more water than oil (probable o/w emulsions). However, some compositions, namely the ones with high oil content (probable w/o emulsions), were difficult to prepare (in some cases impossible) and no emulsion was formed.

Comparing both surfactants, the Tween 80 has a strong ability to form emulsions in the entire range of composition, while the Saponin not. However, considering the top of the ternary diagram (high amount of water), the Saponin was able to form emulsions with only one phase. The droplet size of the sample 18, for the three tested systems, was determined by LD technique. The average particle size in number are shown in Table 1.
Table 1. Average particle size, in number, for the three tested systems.

<table>
<thead>
<tr>
<th>System</th>
<th>Average Particle Size in number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween 80/Miglyol 812</td>
<td>0.0230 μm</td>
</tr>
<tr>
<td>Tween 80/SAO</td>
<td>1.0085 μm</td>
</tr>
<tr>
<td>Saponin/SAO</td>
<td>0.5682 μm</td>
</tr>
</tbody>
</table>

For the nanoemulsion production, using the HPH system, the composition of sample 18 was chosen, and the effect of the number of cycles (3, 6, 9, 12 and 15 cycles) on particle size was analyzed. Table 2 shows the results achieved for the system using Saponin as emulsifier.

Table 2. Droplet size by HPH for Saponin/SAO system.

<table>
<thead>
<tr>
<th>Number of cycles</th>
<th>Droplet Size (μm)</th>
<th>Number of cycles</th>
<th>Droplet Size (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.5682</td>
<td>9</td>
<td>0.0254</td>
</tr>
<tr>
<td>3</td>
<td>0.0551</td>
<td>12</td>
<td>0.0250</td>
</tr>
<tr>
<td>6</td>
<td>0.0321</td>
<td>15</td>
<td>0.0224</td>
</tr>
</tbody>
</table>

As expected, the average droplet size decreased as the number of cycles increased turning the emulsion to the nanoscale, which might increase the stability as reported in some works [6]. The influence of the number of cycles was higher at the beginning (application of the first cycles), comparatively with the latter. After six cycles the size tends to stabilize, reaching a plateau.

Conclusion
The ternary diagrams using the commercial surfactant (Tween 80), and two oils (Miglyol 812 and Sweet Almond Oil), pointed out for the existence of different typology of samples (ME, gel 1, 2 and 3 phases). The alternative natural surfactant, Saponin, was not effective for some compositions, but in the range of o/w emulsions gives rise to emulsions with lower particle size, comparatively with the ones prepared with Tween 80. The HPH technique showed to be effective, reducing both droplet size and sample heterogeneity. The results showed that as the number of cycles increase the droplet size decrease until reaches a point where a stable final droplet size is achieved.

Acknowledgements
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References
The effect of treating the carbon supports on the copper electrocatalytic activity towards the electroreduction of CO2

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The carbon support effect on the electrocatalytic reduction of CO2 was studied using nanostructured carbon materials, which were treated using different oxidizing routes or functionalized with heteroatoms. The effects of the pretreatment on the carbon support materials was evaluated by N2 adsorption and X-ray photoelectron spectroscopy (XPS). It was found that the chemical surface properties and surface area were significantly modified after treating the carbon supports. The electrocatalytic activity was measured using linear sweep voltammetry, which showed that the introduction of oxygen or the functionalization with heteroatoms have a major impact on the electroreduction rate of CO2.

Introduction

The increasing emission of CO2 to the atmosphere due to the global consumption of fossil fuels for energy production is considered as the main cause for the climate change and the serious environmental problems that our society is already dealing with. To overcome these environmental challenges and mitigate the anthropogenic climate change, the electrochemical reduction of CO2 became a very attractive strategy since it combines renewable electricity and captured CO2 in useful fuels/chemicals. CO2 is a highly stable molecule and its electroreduction is kinetically hindered due to the multiple electron steps and high energy barriers involved. Therefore, proper catalysts are required to facilitate the reaction. However, to the date this technology suffers from the low efficiency and poor selectivity of the electrocatalysts [1].

The selection of a suitable support for the catalyst became a crucial step for the development of new catalysts. Carbon is considered one of the best supports in the field of heterogeneous catalysis in particular for the electroreduction of CO2. However, carbon supports require proper treatments to improve textural and surface chemical properties [2].

To address these challenges, the effect of different pretreatments of carbon black supports to the electroreduction of CO2 were investigated. Also, the effect of modified carbon black supports on the activity of Cu/C specimens was evaluated.

Results and discussion

Figure 1 shows an example of FTIR spectra of the pristine and treated Vulcan carbon black samples. From these results, it can be concluded that the untreated carbon samples have traces of oxygen groups. This oxygen was assigned to the chemisorption oxygen after the synthesis [3]. In addition, there is a small amount of CH bonds (2800-2980 cm⁻¹) [4]. After acidic oxidation, it was expected the presence of bands at about 3400, 1630 and 1060 cm⁻¹ attributed to the presence of hydroxyl groups (-OH), carboxyl (-COOH), and carbonyl (-C=O) groups. The carbon samples treated either with an acid or with a base showed and an increment in these functional groups. The acid treated samples showed a C=O stretching at about 1700 cm⁻¹, a -OH bending at 1390 cm⁻¹ and a C-O stretching at about 1230 cm⁻¹, all assigned to the carboxylic group.

The textual modifications of the treated carbon samples were evaluated by N2 physisorption. All samples analyzed can be described by a type IV isotherm with a H3 hysteresis, which is typical of mesoporous solids with slit-shaped pores [5]; Table 1 summarizes the results obtained from the N2 physisorption analysis of the Vulcan carbon samples analyzed. Pristine Vulcan sample presents a S BET of about 279 m² g⁻¹ and a mesopore volume of about 0.48 cm³ g⁻¹. Treating the carbon black samples led to a decrease in the surface area assigned to a partial destruction of the initial textural structure. This decrease is more pronounced for the chemically treated samples. In addition, a slight increase of the mesopore volume was observed after chemical oxidation, which may be associated to the collapse of the pore walls due to the introduction of the oxygen groups.

Figure 1. FTIR spectra of the Vulcan carbon substrates after treated.

To study the electrochemical behavior of the carbons supports for the electrochemical reduction of CO2 it was used a three-electrode cell composed by a carbon rod as counter electrode, a Ag/AgCl electrode as a reference and the carbon samples as the working electrode. The support electrolyte used was a 0.1 M of KHCO3 aqueous solution – see graphical abstract.

As example, Figure 2 shows the linear sweep voltammetry of pristine and annealed Vulcan supports with a nominal Cu content of 20 wt. %.

In case of the electrolyte saturated in nitrogen, the electrical current measured was associated to the hydrogen evolution reaction (HER), whereas for the electrolyte saturated with CO2 it was associated to both hydrogen formation and electroreduction of CO2. Even though the current density obtained for the solution saturated with CO2 is similar for both
specimens, the contribution of the hydrogen evolution reaction is greatly diminished when annealed carbon supports were used. In addition, it can be seen a decrease in the current density at -1.8 V in the presence of CO₂. This can be assigned to the adsorption of CO₂ reduction products, which inhibits the HER and thus decreasing the total cathodic current [6]. Further results will be presented comparing the electrochemical behavior of the different carbon samples after chemical treatments.

Figure 2. Linear sweep voltammograms of the untreated and annealed Vulcan carbon samples with a nominal Cu content of 20 wt.% in 0.1 M of KHCO₃ (solid line refers to N₂ saturated solution and dash line refers to CO₂ saturated solution) at 20 mV s⁻¹.

Table 1. Textural parameters of untreated and treated Vulcan carbon samples.

<table>
<thead>
<tr>
<th>sample</th>
<th>( S_{\text{BET}} ) (m² g⁻¹)</th>
<th>( V_{\text{meso}} ) (cm³ g⁻¹)</th>
<th>( D_p ) (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>279.1</td>
<td>0.48</td>
<td>2.2</td>
</tr>
<tr>
<td>Annealed</td>
<td>257.5</td>
<td>0.46</td>
<td>3.1</td>
</tr>
<tr>
<td>Acid-treatment</td>
<td>211.6</td>
<td>0.55</td>
<td>3.1</td>
</tr>
<tr>
<td>Basic-treatment</td>
<td>214.7</td>
<td>0.52</td>
<td>3.9</td>
</tr>
</tbody>
</table>

Acknowledgements
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References
A novel approach based on Encapsulated Ionic Liquids (ENILs) is proposed for overcoming the mass transfer constraints in CO₂ physical absorption by ILs. Experimental gravimetric analyses with the 4 ILs selected (EmimTCM, BnimTCM, BnimDCN, and BnimOcSO₄) were carried out. Following, ENIL materials were prepared by encapsulation of ILs in hollow carbon sub-microcapsules with a porous shell. The experimental gravimetric analysis evidenced that the ILs maintain their CO₂ absorption capacity after encapsulation, whereas the absorption rate is ca. 50 times higher for ENILs than neat ILs. ENIL tests in fixed bed operation at different operating conditions yielded bed utilization values dependent on the CO₂ solubility in the ENIL, while equivalent mass transfer zone lengths were obtained for all the materials. The results demonstrate the fast CO₂ mass transfer rates in ENILs -related to the high contact area provided- allows overcoming the mass transfer limitations controlling the CO₂ rate of physical absorption by ILs.

Introduction
The development of more energetically efficient and clean technologies for CO₂ capture is receiving increasing attention from academy and companies [1]. In this context, ionic liquids (ILs) have been widely considered promising CO₂ absorbents due to their low volatility, tunable solvent capacity and high chemical and thermal stability [2]. However, CO₂ physical absorption by ILs in commercial packing column may be strongly kinetically controlled. To this respect, the evaluation of IL solvent performance at industrial scale by modeling with professional process simulators has allowed considering a more consistent set of criteria (thermodynamic, kinetic, technical, energetic, and economic) for selecting the ILs with optimized properties for CO₂ capture. The Encapsulated Ionic Liquid (ENIL) concept has been proposed as a promising alternative to overcome the mass transfer rate limitations [3]. ENILs consist of hollow carbonaceous submicrocapsules (C-cap) filled with ILs [4]. Due to ENIL morphology, the specific contact area is drastically increased with respect to the neat IL, enhancing the rate of mass transfer phenomena but maintaining the properties of the ILs as solvents [3].

Objectives
The objective of this work is to evaluate the potential application of ENIL materials in the capture of CO₂ by physical absorption with ILs. Firstly, process simulations using COSMO-based/Aspen Plus methodology are carried out to evaluate the behavior of a large set of ILs in the physical absorption of CO₂ in commercial packed columns. From this theoretical analysis, a selection of 4 ILs with similar absorption and markedly different kinetic is made. The ILs selected were characterized using a high-pressure microbalance to obtain CO₂ absorption capacities and CO₂ diffusion values. The next step is the synthesis and characterization of ENIL materials using the previously selected ILs. The ENIL were tested in gravimetric essays to analyze their CO₂ sorption capacity and rate. Finally, fixed-bed sorption experiments were carried out in order to evaluate if the application of the proposed ENIL systems allows overcoming the mass transfer rate limitations of the CO₂ physical absorption by the free ILs.

Methodology
COSMO-based/Aspen Plus methodology was applied to model the CO₂ physical absorption by IL in a column with a commercial packing using Equilibrium- and Rate-based models [5]. ILUAM database [6] was used to incorporate 44 ILs as pseudo-components in Aspen Plus v9.0 (they are not available as conventional compounds in the extensive Aspen Properties database). RADFRAC column model implemented as default in Aspen Plus v9.0 was used. The recovery of CO₂ was calculated considering equilibrium and rate-based models.

The measurements of CO₂ solubility (mg CO₂/g IL) in ILs and ENILs were performed in a gravimetric high-pressure sorption analyzer (ISOSORP GAS LP-flow, Rubotherm) equipped with a magnetic suspension balance (MSB). A full description of the thermogravimetric experimental methodology is available in previous works [7]. The CO₂ absorption isotherms for the four selected ILs and their corresponding ENILs were obtained along with CO₂ diffusion coefficients.

The fixed-bed experiments were carried out in a Microactivity unit (PID Eng&Tech, Spain) provided with a stainless-steel tube of 9.5 mm of internal diameter and 15 cm of length. The outlet gas flow was analyzed by an Agilent 7820A gas chromatograph and a TCD detector. For each ENIL, sorption capacity, Yoon and Nelson [8] kinetic constants and lengths of the mass transfer zone was calculated from the breakthrough curves.

Results
If we compare the CO₂ recoveries achieved for 44 commercial ILs in a packed column at fixed operating conditions using RADFRAC Equilibrium mode (only thermodynamics is considered) and Rate-based mode (mass transfer kinetic is also taken into account) simulations, there is a wide range of recoveries for the studied ILs. When the absorption kinetics is taken into account in the calculations by using the Rate-based mode, drastic reductions in the CO₂ recovery are obtained at identical operating conditions. From these results, we have selected four ILs (EmimTCM, BnimTCM, BnimDCN and BnimOcSO₄) that show similar absorption efficiency attending to the thermodynamics, but show different kinetic behavior. From this IL, ENIL materials were prepared following the
methodology reported by our group in previous works [4] (Figure 1). Figure 2 compares the kinetic curves of CO2 uptake by ILs and ENIL materials. As can be seen, the uptake of CO2 by neat ILs is much slower than for ENIL materials.

![Figure 1: (A) SEM and TEM images (B, C) of C_cap used as support for ENIL preparation.](image)

![Figure 2: Kinetic curves of CO2 uptake in ENIL and neat IL at 301.5 K and pCO2 = 1 bar for A) EmimTCM, B) BmimTCM, C) BmimDCN and D) BmimOcSO4.](image)

Noticeably, the saturation time is greatly reduced and nearly the same (~ 10 min) for all prepared ENILs, regardless of the IL used. These results indicate that the CO2 uptake rate is governed by the high contact area conferred to the IL by the morphology of the material.

Conclusions
Encapsulated ionic liquids (ENIL materials) are proposed for overcoming the mass transfer limitations that neat ILs present in the physical absorption of CO2. From RADFRAC absorption columns on Equilibrium and Rate-based modes of Aspen Plus EmimTCM, BmimTCM, BmimDCN, and BmimOcSO4 were selected for the experimental determination of CO2 solubilities and diffusivities by gravimetric analysis. The results are in agreement with those from process simulation, yielding almost the same CO2 solubility values (3 mg·g\(^{-1}\) at 301 K) for the four ILs tested and markedly different CO2 diffusivity values. In order to solve these mass transfer limitations, ENILs were prepared with the selected ILs and tested in an MSB equipment, showing that the solubility of the IL loaded is maintained while the mass transfer rate is enhanced, and the saturation time is reduced by two orders of magnitude. Finally, the ENILs were tested in CO2 capture using fixed beds, showing no significant differences in absorption rate and a bed utilization determined mainly by the solubility of CO2 in the ILs. Therefore, these results demonstrate the fast CO2 mass transfer rates in ENILs - related to the high contact area provided - allows overcoming the mass transfer limitations that typically control the rate of physical absorption of CO2 in neat ILs.

Acknowledgements
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References
New promising microporous niobium and vanadium silicates for mercury removal from aqueous solutions

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* elainefabre@ua.pt

Mercury is a hazardous toxic metal and its serious impacts to human health and environment make necessary its removal even at very low concentrations. In this work, two new microporous materials, AM-11 (niobium silicate) and AM-14 (vanadium silicate) have been tested for Hg(II) removal from aqueous solutions. Small doses of these solids (3.5-14.0 mg L\(^{-1}\)) have removed most of the metal in solution (81-99%). Well known isotherms were applied for equilibrium study and the best fits have been obtained with Langmuir and Temkin equations, for AM-11 and AM-14, respectively. The ion exchange capacities obtained from Langmuir isotherm for AM-11 (161 mg g\(^{-1}\)) and AM-14 (304 mg g\(^{-1}\)) highlight the promising potential of these new sorbents for water treatment applications.

Introduction
The contamination of the aquatic systems by the discharge of effluents containing toxic metals is a global concern. Among the major pollutants, mercury is one of the most dangerous due to its serious impacts on biota and human health. It is present as a priority hazardous substance in 2013/39/EU directive and occupies the third position in the list of the most dangerous substances of the Agency for Toxic Substances & Disease Registry (ATSDR) [1]. The main industrial activities responsible for the release of mercury include chloralkali, pulp and paper, oil refining, electrical, rubber processing and fertilizer producers [2].

The sorption processes, such as adsorption and ion exchange, have emerged as good alternatives for waters treatment. Their increasing use is attributed to the ease of operation, efficiency, selectivity, and large range of application [3]. Many synthetic materials like zeolites evidence high surface area, affinity to various target metals, and excellent ion exchange capacity, which make them especially attractive for sorption applications. Microporous solids like the titanium silicates ETS-4 and ETS-10 have been reported as good cation exchangers for mercury and cadmium removal [4,5]. As an extension of these works, in this study two novel solids, a niobium silicate (AM-11) and a vanadium silicate (AM-14), have been applied for mercury elimination from aqueous solutions.

AM-11 has a microporous structure composed by octahedral units of niobium with tetrahedral silicon and medium pore size of 4 Å. The counter ion present in this solid is NH\(_4^+\) and its theoretical ion exchange capacity is 2.12 meq g\(^{-1}\). Concerning the vanadium silicate AM-14, the medium pore size is 6.8 Å, counter ion is sodium, and theoretical ion exchange capacity is 5.2 meq g\(^{-1}\).

Batch experiments were performed with the objective to investigate the ability of these two innovative sorbents. The main goals here involve providing potential sorbents and to contribute knowledge to the understanding of sorption processes in wastewater treatment.

Materials and Methods
The materials applied were synthesized according to Rocha et al. for AM-11 [6] and Brandão et al. for AM-14 [7]. The sorption experiments were carried out in batch systems, at 22 °C, and the contaminated solutions were prepared diluting the standard solution in high purity water (18 M\(\Omega\) cm) to the desired Hg(II) concentration of 1 mg L\(^{-1}\). The pH of the solutions were adjusted to 6 with 0.1 M NaOH. Known masses of the solids were added into the solutions and samples were taken at increasing times, filtered through a 0.45 µm Millipore filter, adjusted to pH < 2 with HNO\(_3\), stored at 4 °C and then analyzed. A blank solution (without solid) was always run as control. The mercury quantification was performed by cold vapour atomic fluorescence spectroscopy (CV-AFS). The morphology of the AM-11 and AM-14 powders was assessed by scanning electron microscopy (SEM).

Results and discussion
The morphology of the AM-11 and AM-14 is shown in Figure 1. These solids have presented very distinct features, AM-11 has needles shape with ca. 10 µm in length while AM-14 crystals are thin plates with size of ca. 1-2 µm.

Figure 1. SEM images of AM-11 (a) and AM-14 (b).

Regarding the Hg(II) removal, in Figure 2 are presented the normalized mercury concentrations versus time for two different masses of solids in solution, 6.5 and 14.0 mg L\(^{-1}\) of AM-11, and 3.5 and 6.5 mg L\(^{-1}\) of AM-14. The trends found indicate that both materials can reduce satisfactorily the content of mercury ions in solution. Despite the notable differences in the structure, the two solids exhibit similar removal satisfactorily, for example, with the dosage of 6.5 mg L\(^{-1}\) it is possible to observe a Hg(II) decrease of 92 % for both materials. In the case of AM-11, with only 14 mg L\(^{-1}\) all the metal in solution has been removed. Even applying very small amounts of AM-14 (3.5 mg L\(^{-1}\)), this solid shows high uptake performance, removing 81 % of the mercury. Concerning the equilibrium time, it is approximately (96 h) for both, although concentration ratios of AM-11 decrease smoothly until 192 h.
To ion exchange equilibrium was also analyzed. Well known isotherms embodying two and three parameters have been studied, namely: Langmuir, Freundlich, Temkin, Dubinin-Radushkevich, Sips, Redlich-Peterson and Toth. Taking into account the calculated values of the adjusted coefficient of determination ($R^2_{adj}$) and the average absolute relative deviation ($\text{AARD}$), Langmuir isotherm was the best model for AM-11 data ($\text{AARD}=3.58\%$ and $R^2_{adj}=0.980$). According to this model, all the active sites are identical with equal energy of sorption, the Hg(II) is sorbed in monolayer, and the maximum uptake is 161 mg g$^{-1}$ [8,9]. The experimental data attained a plateau, which indicates that the ion exchange capacity of AM-11 has been achieved under the operating conditions. Temkin equation provided the best fit of AM-14 data ($\text{AARD}=3.92\%$ and $R^2_{adj}=0.985$). As stated by this model, the sorption energy has a uniform distribution of the binding energies, up to some maximum energy [9]. The ion exchange capacity given by the Langmuir model is 304 mg g$^{-1}$ while the maximum experimental value was 280 mg g$^{-1}$, suggesting that the capacity of the solid is above the observed in this study. In line with the latter statements, the isotherm does not achieve its plateau stage.

Conclusions

The ability of two microporous materials has been investigated in this work. Both solids have presented high affinity to capture mercury from aqueous solutions. Even very small dosages are able to remove considerably the metal from solution, since 14 mg L$^{-1}$ of AM-11 are able to eliminate all Hg(II) from solution. The isotherms of Langmuir and Temkin have provided the best fits to AM-11 and AM-14 data, respectively. The ion exchange capacities of the solids according to Langmuir model are 161 mg g$^{-1}$ for AM-11 and 304 mg g$^{-1}$ for AM-14, which highlights the excellent performance of both sorbents in water treatments.

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References

The Navigator Company
Sistema de Identidade da Marca
Poster Session

MODELING, SYNTHESIS AND INTEGRATION OF CHEMICAL PROCESSES
**1H NMR spectroscopy combined with partial least squares regression for predicting gasoline composition**

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Proton nuclear magnetic resonance spectroscopy allows the expeditious and accurate quantitative evaluation of gasoline composition. Partial least squares regression models were developed using training and validation sets of a database containing 212 gasoline samples spectra and the respective composition values, obtained by the reference method. The multivariate data analysis was performed for five different compounds/groups: aromatic, olefin, benzene, methyl tert-butyl ether and oxygen content. The results show that the models can predict gasoline composition with high accuracy, presenting determination coefficients above 0.90 for the test data set. The statistical parameters obtained in terms of standard prediction errors are, in most cases, lower than the reproducibility values of the reference method. These facts indicate that this methodology is a powerful alternative for predicting gasoline composition.

### Introduction

Due to the advances in technology and to the new energy sources growth, petroleum industry is facing one of the most transformative periods in its history. Making products expeditiously available is crucial and that can be achieved by reducing the quality control process duration.

Proton nuclear magnetic resonance (1H NMR) is a fast and simple technique that allows the quantification of hydrogen atoms in aromatic and aliphatic groups [1]. The 1H NMR spectrum is a result of energy level transitions which are related to the different orientation of nuclear spin when the nuclei is under a magnetic field [2]. One of the main advantages of this technique compared with other spectroscopic techniques is that the peak areas arising from the 1H nuclei in different molecular positions are proportional to their concentration [1]. This technology, combined with chemometrics, provides a powerful tool for products characterization. Moreover, this methodology can easily be implemented on-line, which is of particular interest for production control.

This study focuses on the prediction of the gasoline composition. Hydrocarbons and oxygenated compounds present in gasoline must obey to specification requirements. Therefore, the aim is to develop statistical models based on 1H NMR spectroscopy to estimate aromatic, olefin, benzene, methyl tert-butyl ether (MTBE) and oxygen contents.

### Methods

A total of 212 gasoline samples, 95 of which were formulated in laboratory exclusively for the models’ development purposes, were used in this study. The remaining samples are of two different kinds of gasoline produced in Matosinhas’ refinery. The database range of values for each property is presented in Table 1.

The 1H NMR spectra was recorded with a picoSpin NMR spectrometer operating at 80 MHz. The conditions for spectra recording are the following: spectral width 4000 Hz, with a 58 μs pulse length, 4096 data points and 128 scans. The samples were diluted in deuteriochloroform with 1% of tetramethylsilane (TMS), which is used as reference. The spectral range selected for subsequent data analysis is from 0.5 to 7.5 ppm.

### Results

The multivariate calibration method implemented in this work is partial least squares (PLS) regression, which is one of the most commonly used regression modelling technique for quantitative analysis. PLS finds the directions of greatest variability by comparing both spectral and target property with the new axes, named PLS components. They represent the best correlation with the target property values, which were previously measured by gas chromatography [3]. Each spectrum was pre-processed prior to model calibration by normalization followed by multiplicative scatter correction (MSC). The data was divided into training, validation and test data sets. The pre-processing, data division and PLS regression tools were developed in Excel using Visual Basic for Applications (VBA). The following two statistical parameters were calculated for evaluating the models’ performance: coefficient of determination ($R^2$) and standard error of prediction (SEP).

<table>
<thead>
<tr>
<th>Property</th>
<th>Minimum</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Olefin (% v/v)</td>
<td>0.1</td>
<td>12.8</td>
</tr>
<tr>
<td>Aromatic (% v/v)</td>
<td>0.2</td>
<td>12.8</td>
</tr>
<tr>
<td>MTBE (% v/v)</td>
<td>0.0</td>
<td>0.2</td>
</tr>
<tr>
<td>Oxygen (% m/m)</td>
<td>1.2</td>
<td>23.2</td>
</tr>
<tr>
<td>Benzene (% v/v)</td>
<td>0.1</td>
<td>4.1</td>
</tr>
</tbody>
</table>

Table 1. Range of values for gasoline samples composition.

The models were implemented in Excel using VBA. The reference method performed for five different compounds/groups: aromatic, olefin, benzene, methyl tert-butyl ether and oxygen content. The results show that the models can predict gasoline composition with high accuracy, presenting determination coefficients above 0.90 for the test data set. The statistical parameters obtained in terms of standard prediction errors are, in most cases, lower than the reproducibility values of the reference method. These facts indicate that this methodology is a powerful alternative for predicting gasoline composition.
method [3]. Total oxygen content prediction model is the one that presents better results, with negligible errors of prediction.

Table 2. Performance indexes of the developed models for test dataset.

<table>
<thead>
<tr>
<th>Property</th>
<th>$R^2$</th>
<th>SEP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic</td>
<td>0.948</td>
<td>1.144</td>
</tr>
<tr>
<td>Olefin</td>
<td>0.931</td>
<td>0.530</td>
</tr>
<tr>
<td>MTBE</td>
<td>0.977</td>
<td>0.624</td>
</tr>
<tr>
<td>Oxygen</td>
<td>0.986</td>
<td>0.083</td>
</tr>
<tr>
<td>Benzene</td>
<td>0.952</td>
<td>0.052</td>
</tr>
</tbody>
</table>

Figure 1. Aromatic content: Model predicted versus reference method.

Figure 2. Olefin content: Model predicted versus reference method.

Figure 3. Benzene content: Model predicted versus reference.

Figure 4. MTBE content: Model predicted versus reference.

Figure 5. Oxygen content: Model predicted versus reference.

Conclusions

The chemometric analysis, through PLS regression modeling, associated with $^1$H NMR spectroscopy proved to be a powerful tool and a valuable alternative to the reference method for gasoline composition determination. Furthermore, $^1$H NMR spectroscopy can potentially be used to determine other gasoline properties, such as octane numbers and distillation curve, which would give further value to this methodology.

Acknowledgements

This work was financially supported by the project POCI-01-0145-FEDER-006939 (LEPABE – UID/EQU/00511/2013) and by QOPNA research project (FCT UID/QUI/00062/2013), both funded by European Regional Development Fund (ERDF) through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds, through FCT – Fundação para a Ciência e a Tecnologia.

Research grant of A. L. Leal, financially supported from FCT Doctoral Program PD00158/2012, Ref: PD/BDE/128624/2017 and Galp Energia, is also acknowledged.

References


Modeling clathrate hydrate formation/inhibition using a modified CPA EoS

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A modified Cubic Plus Association (CPA) EoS is applied here in combination with the van der Waals-Platteeuw (vdWP) model, enabling the simultaneous description of liquid, gas and hydrate phases. Single and mixed guest hydrate formers are analyzed in the presence of the most common hydrate inhibitors. CPA is the most successful model for hydrate phase formation and inhibition in the Oil and Gas industry. However, without using a large number of binary interaction parameters between water and the inhibitor it is usually not possible to describe simultaneously their SLE and VLE, while accurately describing the hydrate phase. The present model was applied using a maximum of two binary interaction parameters between water and the hydrate inhibitors. This in turn increased the predictive capacity of the approach, while being able, in most cases, to describe freezing point depressions, VLE and hydrate dissociation curves.

Introduction
The possible formation of gas hydrates is one of the main concerns for flow assurance. Even in the presence of small quantities of light gases, water can form stable solid cages, which can lead to plugging of pipelines and damages to equipment. Thus, it is crucial to have models capable of predicting this type of phases and their equilibria with liquid and gas phases.

The cubic plus association (CPA) equation of state (EoS) is a simple, yet accurate, model for the description of mixtures containing associating compounds like water. It is also one of the most applied models for the description of hydrate formation and inhibition. Knowing this it is important to analyze the capacity of the modified version of this model. [1-3]. Thus, an approach is tested here where the binary interaction parameters $k_{ij}$ and $\beta_{ij}$ are fitted to SLE and VLE data and applied to the hydrate calculations. The hydrate phase is described using the van der Waals-Platteeuw (vdWP) model with proprietary Kihara parameters taken from Multiflash [4].

Results
From the initial studies with the model it was noted that, considering only a constant $k_{ij}$ it was possible to describe simultaneously the water freezing point depression between water and inhibitor and the hydrate formation/inhibition. For some of the compounds, like diethylene glycol and glycerol, this also enabled a good description of VLE for a good range of conditions. However, for most of the compounds it was necessary to introduce a $\beta_{ij}$ (binary interaction parameter for the volume of association). The accuracy of such method, while inferior to what would be obtained using two sets is still able to describe the experimental data.

Figures 1 and 2 present results for dissociation curves of hydrates formed from natural gas, when inhibited by methanol and ethylene glycol.

![Figure 1. Hydrate dissociation curve for water + natural gas with different wt % of methanol in the liquid phase. Lines are results with the equation of state. Data from Lee and Kang [5].](image1)

To analyze the description of VLE and SLE, when considering the use of both $k_{ij}$ and $\beta_{ij}$, the mixture water + ethylene glycol is studied in Figure 3, for the freezing point depression and in Figure 4 for VLE at two different pressures. Table 1 introduces the binary interaction parameters between water and the hydrate inhibitors.

Conclusions
Using the proposed approach accurate results for hydrate formation/inhibition are obtained up to high concentration of hydrate inhibitor. Despite the degradation of both VLE and SLE results, due to their simultaneous fitting, the VLE results are still accurate for most mixtures, while the SLE results tend to overestimate the melting temperatures at water compositions above 0.2 mole fraction.
Figure 2. Hydrate dissociation curve for water + natural gas with different wt % of ethylene glycol in the liquid phase. Lines are results with the equation of state. Data from Lee and Kang [5].

Figure 3. Water rich phase of the water + ethylene glycol SLE. Line – Results with the equation of state, without considering the solid complex. Data from Ott et al. [6].

Acknowledgements
This work was funded by KBC Advanced Technologies Limited (A Yokogawa Company) under project "Extension of the CPA model for Polyfunctional Associating Mixtures". This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement.

References

Table 1. Binary interaction parameters applied for water + hydrate inhibitor.

<table>
<thead>
<tr>
<th>Hydrate inhibitor</th>
<th>$k_{ij}$</th>
<th>$\beta_{ij}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>methanol</td>
<td>-0.115</td>
<td>-0.0008</td>
</tr>
<tr>
<td>ethylene glycol</td>
<td>-0.085</td>
<td>-0.0004</td>
</tr>
<tr>
<td>triethylene glycol</td>
<td>-0.190</td>
<td>-0.0005</td>
</tr>
</tbody>
</table>

Figure 4. VLE description of water + ethylene glycol at two temperatures. Lines are results with the equation of state. Data from Chiavone-Filho [7].
Heat exchanger network retrofit of an aromatics plant process unit using a hybrid methodology

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Introduction

Methods for heat exchanger network (HEN) synthesis are well recognised by major energy-intensive consumers, such as refinery and petrochemical industries, to design and improve their process units through a more efficient and economic use of energy [1]. Such methods can provide optimal designs of new plants with minimum energy consumptions, and also improve heat recovery of existing plants through retrofit designs. In the last decades, different retrofit approaches have been proposed by either increasing heat transfer area, reassigning existing exchangers and/or using heat transfer enhancements able to increase heat transfer coefficients [2]. Three known approaches are pinch analysis, mathematical programming methods and hybrid methods. Pinch analysis is a thermodynamic-based method with tools such as composite curves and grand composite curves for energy targets estimation, and also grid diagrams for network retrofit design. Mathematical programming methods are based on optimization formulations, which are developed from a HEN superstructure. This represents a set of alternative retrofit designs in which the optimization algorithm searches for the optimal network satisfying energy/cost requirements. Hybrid methods involve the combination of both methods mentioned above [3,4].

In this work, a hybrid approach is considered to retrofit the Pre-Distillation Unit of Matosinhos Refinery’s Aromatics Plant aiming at maximum heat recovery. Tools provided by pinch analysis are used to quantify minimum energy targets and identify zones of inefficient heat integration in the present HEN. These are then redesigned using mathematical programming methods following the sequential approach [4]. In this work, phase change of process streams is explicitly incorporated in the definition of the HEN superstructure.

Hybrid Methodology

The retrofit problem is decomposed in two phases: 1) application of pinch analysis tools to determine minimum energy targets and identify inefficient zones of the current network; and 2) optimal redesign of these zones to attain minimum energy targets with minimum investment, and using the sequential approach [4].

Aromatics plants of petroleum refineries are energy-intensive consumers. The Pre-Distillation Unit of Matosinhos Refinery’s Aromatics Plant is here subjected to a heat integration and optimization study, in order to maximize heat transfer efficiency and thus reduce operating costs. For this purpose, a hybrid methodology, combining pinch analysis and mathematical programming tools, is developed for the retrofit of heat exchanger networks with minimum investment cost, and respecting minimum energy requirements. These are estimated through the construction of composite curves provided by pinch analysis. A grid diagram is used to represent the current network and to locate inefficient zones of heat integration. The inefficient zones are then removed using mathematical programming tools through the implementation of the sequential methodology. First results are very encouraging, with an estimated payback period of about 1 year and 2 months.

Hybrid Methodology

<table>
<thead>
<tr>
<th>HYBRID METHODOLOGY</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Stage: Pinch Analysis</td>
</tr>
<tr>
<td>- Composite Curves for an HRAT;</td>
</tr>
<tr>
<td>- Grid Diagram of actual HEN to locate inefficient zones of heat integration;</td>
</tr>
<tr>
<td>- Input Data</td>
</tr>
<tr>
<td>2nd Stage: Retrofit to attain maximum heat recovery with minimum investment</td>
</tr>
<tr>
<td>- Minimum number of units (MILP model);</td>
</tr>
<tr>
<td>- Optimal HEN configuration and streams branches parameters (NLP/MINLP models).</td>
</tr>
</tbody>
</table>

Pinch Analysis: Pinch analysis is performed using Aspen Energy Analyzer. For a certain value of HRAT (heat recovery approach temperature), composite curves are constructed giving the location of pinch point(s) and the minimum utilities consumption. A grid diagram of the current network is then drawn, based on which inefficient zones are identified, namely those where heat is transferred across the pinch point(s). These will be redesigned in the second phase using optimization tools, while well-allocated units are left out of the optimization study.

Optimal Retrofit: Here, the sequential methodology is used [4]: each subnetwork delimited by pinch point(s) is treated independently and for each of them one first determines stream matches and in a second stage the optimal HEN. The heat recovery of the global network is fixed at the energy targets determined in the pinch analysis. Using process streams data of inefficient zones previously identified, a MILP model is first formulated to determine the minimum number of matches \(N_{(m)}\) and their respective heat loads distribution. The partitioning of the temperature range into intervals is done considering strict-pinch case and bubble and dew-points of streams with phase change. Next, based on the MILP model solution, a superstructure of each subnetwork is generated and a NLP model is formulated to find the optimal configuration (streams interconnections, flows rates and temperatures) that minimize the investment in new heat exchanger units. The heat transfer areas of new and modified units were determined considering constant and by default values of heat transfer coefficients. If streams with phase change exist, their enthalsy are modelled as piecewise linear functions of temperature with different slopes below the bubble-points, between bubble and dew-points and above the dew-points. Binary variables are needed to describe these enthalsy functions and thus the NLP model becomes a MINLP model. All models are solved using GAMS coupled with adequate solvers (such as CONOPT and BARON).

Results and Discussion

The Pre-Distillation Unit has currently 14 heat exchangers installed with 23 MW of utilities consumption (medium pressure
steam, air cooling and cooling water), corresponding to 8.35 MEUR/year in operating costs (OPEX). The network is represented in Figure 1. Based on existing heat exchangers design data, HRAT is set at 10 °C. With this value, pinch analysis resulted in a single pinch point located between 144.6 and 154.6 °C and dividing the overall network into two subnetworks. Heat exchangers transferring heat across the pinch point (HEATX-5, HEATX-6 and HEATX-7) are considered to define an inefficient zone of the network to be retrofitted. Exchangers HEATX-1, HEATX-2 and HEATX-8 are considered well-allocated and thus excluded from the optimal retrofit problem. The remaining streams were considered to make best use of heat transfer between process-to-process streams. Also, in the retrofit study, it was considered only cooling water as the cold utility (HEATX-11, HEATX-12 and HEATX-13). Minimum hot and cold utilities consumptions are 1.5 MW and 13.6 MW, respectively, achieving an energy savings of 7.9 MW, approximately. These results correspond to an OPEX of 3.01 MEUR/year, i.e., a saving of 5.35 MEUR/year, using medium pressure steam and cooling water as utilities system.

Figure 1: Actual HEN of Pre-Distillation Unit.

The optimal retrofit obtained using the sequential methodology is shown in Figure 2. The MILP model resulted in 6 and 7 new and modified units (NU), above and below pinch point respectively, together with their corresponding heat loads. Below the pinch point, the NLP model resulted in an optimal retrofitted network with a heat transfer area of 2659 m². Above the pinch point, the MINLP model (handling phase-change streams) indicated that 1833 m² of heat transfer area is needed.

Figure 2: Proposed retrofit of the HEN.

Conclusions
This work presents a hybrid methodology, combining pinch analysis and mathematical programming tools, to retrofit the HEN of the Pre-Distillation Unit of Matosinhos Refinery’s Aromatics Plant. In the retrofit design project, well-allocated heat exchangers were excluded from the optimization study. Inefficient zones of energy consumption were located and removed to achieve maximum energy recovery. The proposed solution requires 13 new heat exchangers (including modifying ones), and an estimated investment cost of 3.41 MEUR/year with an operating costs of 3.01 MEUR/year, considering cooling water as the cold utility. With the retrofit design solution, it is achieved a 34 % of energy savings, which corresponds to an annual energy saving of 5.35 MEUR/year. A payback period of 1.13 years were expected for this retrofitting project. Further work will be developed using more realistic heat transfer coefficients and implementing superstructures with different utility systems.

Acknowledgements
This work was financially supported by: project POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy – UID/EQU/00511/2013), funded by European Regional Development Fund (ERDF) through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI), and by national funds through FCT – Fundação para a Ciência e a Tecnologia; project “LEPABE-2-EKO-INNOVATION” – NORTE-01-0145-FEDER-000005, funded by Norte Portugal Regional Operational Programme (NORTE 2020), under PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF), and also FCT/COMPETE project UID/EQU/00102/2013 (Chemical Process Engineering and Forest Products Research Centre, CIEPQPF). Research Grant of Vanessa Araújo financially supported from FCT Doctoral Program PD00158/2012, Ref: PD/BDE/113545/2015. Support from Galp energia is also acknowledged.

References
Advanced multi-granularity analytics for industry 4.0

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Data collected from industry present a variety of data structures, reflecting the evolution of industrial processes, measurement systems and IT infrastructures. Data analytics platforms must adapt to this new reality and keep the pace of its evolution in order to continue providing effective solutions to practitioners for dealing with the large data resources now available. In this context, one prevalent feature of industrial data has been largely overlooked: the existence of data with multiple granularities. The multi-granularity nature of data is directly connected to their aggregation in the time domain, an aspect that induces inner dependencies that current frameworks cannot address in a consistent and rigorous way. Furthermore, multi-granularity data have been often mistakenly treated as multivariate data, where in fact the meaning of the observations is completely different. This result in suboptimal methodologies and outcomes not reaching the desired goals. In this paper, we highlight such differences and discuss current multi-granularity frameworks for effectively handling industrial data sets.

Introduction

With the evolution in sensing technology, delocalized acquisition systems, communication infrastructures (including the Internet of Things) and storage/retrieving facilities for huge amounts of data (using cloud technology), the nature of data presented to plant engineers and data scientists has been changing significantly. Modern data sets are high-dimensional, contain structured data of different types (sensor data, spectra, images, etc.) as well as unstructured data (fault and alarm tags, operators notes, etc.), noise, outliers, missing segments, dynamic features and multi-granularity characteristics that need to be properly accounted for, in order to extract meaningful and useful information for the purposes of process monitoring, diagnosis, control and optimization. This is the new reality found in Industry 4.0 scenarios, to which Chemical Engineers must adapt, and take advantage of [1, 2].

Among these data characteristics, multi-granularity data structures are arguably the ones receiving less attention from the scientific community. In multi-granularity data, the recorded values contain information about the process with different levels of aggregation (different resolutions). Granularity arises from the implementation of aggregation rules that merge multiple (high resolution) samples into a single (low resolution) observation. Another source of granularity is found in situations where variables represent measurements made on composite samples collected during a certain time period (e.g., production lot, working shift, etc.), and therefore the recorded values regard specific windows of time. For more details on data multi-granularity (or multiresolution; these two terms will be used interchangeably), please refer to the following publication [3].

Objectives

The current lack of multi-granularity tools is especially strange as data aggregation is increasingly taken as a viable solution to handle the flood of data arising from high throughput industrial data streams. Therefore, in this presentation two complementary situations are addressed:
(i) How to handle multi-granularity data sets when they arise;
(ii) How to optimize the granularity of data for enhancing the methods performances.

A general framework is proposed to handle both types of problems, as depicted in the Graphical Abstract. For space constraints, we devote the remaining of this short abstract to the second challenge (optimizing data granularity), but in the presentation both complementary dimensions will be addressed.

Methods – Optimal Selection of Data Granularity

Even when the data is available at a single granularity (or resolution) it is not guaranteed that their native resolution is the best resolution for achieving the analysis goals. This is particularly relevant in predictive analytics tasks. For instance, the variables may be collected at a high resolution in order to capture the local variability from the standpoint of process control or monitoring, while for process modelling it might be preferable to adopt low resolution representations in order to obtain more parsimonious and stable models. Therefore, during model building it is not only desirable to select the set of predictors most suitable for estimating the response (as in the case of classical stepwise selection methodologies), but also to consider at which resolution they should be included in the model. In this way, a framework was proposed for building multiresolution empirical models for continuous processes (MR-EMC) [4] that contemplate the definition of the multi-granularity structure as an additional degree of freedom for model building.

In brief terms, MR-EMC simultaneously selects the best variables to be included in the model and searches for the best resolution for each variable. The search space for the variables’ resolution is constrained between the native resolution of the variables and a given maximum resolution defined by the user.

One way to implement MR-EMC is by extending the predictors space with variable duplicates at all resolutions. This extended matrix is then the base for simultaneous variable and resolution selection, following a procedure similar to the standard stepwise forward algorithm [5]. However, it should be noted that while the search space includes all duplicates at different resolutions, only one of them is included in the model (i.e., the same variable cannot included in the model at different resolutions).

Results

To illustrate the advantage of MR-EMC, a simulated Continuous Stirred Tank Reactor (CSTR) was used to generate data and then build Partial Least Squares (PLS), Dynamic Partial Least Squares (DPLS) and MR-EMC models for
estimating the output concentration. This simulator, returns readings for seven variables: (i) feed stream concentration of compound A, (ii) feed stream temperature, (iii) heating fluid inlet temperature, (iv) fluid level in the reactor, (v) outlet concentration of compound A, (vi) outlet stream temperature and (vii) heating fluid outlet temperature. The full specification of the simulated process can be found in [6].

In this study, all variables are recorded at high resolution and subject to measurement noise. As mentioned before, for modelling purposes, the output concentration was selected as the response variable and the other six variables form the predictors set. To assess the consistency of the results, 100 replicates were made and for each replicate 1000 observations were generated for training the models and another 1000 observations for testing their performance. The performance was evaluated through the Mean Squared Error (MSE) based on the noise free values for the concentration. The median MSE over the 100 replicates is represented in Figure 1. From this figure, it is clear that PLS presents the worst performance as it only uses the native resolution of the variables and has no information for modelling the process dynamics. When time-shifted replicated are added to the DPLS model, it is observed a significant improvement over PLS. However, the MSE of DPLS is still larger than that obtained with MR-EMC.

As MR-EMC is not accounting for process dynamics, these results demonstrate that choosing the appropriate resolution for each variable is more important that including information about the process dynamics. In subsequent studies, it was also observed that a dynamic version of MR-EMC produces slightly better estimates, being again verified that resolution selection in the main driver for an accurate estimation of the response.

Conclusions

Even though a large variety of industrial processes generate data with multi-granularity structures, the current modelling and analysis methodologies are not able to accommodate for this pervasive aspect, nor to take advantage of its presence. Furthermore, multi-granularity structures are often erroneously taken as multirate data, leading to the adoption of inadequate analysis procedures. To address this situation, a series of multiresolution frameworks for data analysis have been proposed by the authors and are currently available.

Figure 1. Median MSE over 100 replicates of the CSTR case study. For reference, note that the variance of the noise is $4 \times 10^{-6}$.

These approaches are applied to two distinct scenarios. In the first case, multiresolution is already present it the data and thus the focus is to explore the best way to incorporate it during model building. In the second scenario, data is originally single-resolution and the goal is to introduce multiresolution in order to improve the predictive performance. Both scenarios will be addressed in the communication, where further results for the comparison of multiresolution approaches against their single-resolution counterparts are presented that further corroborate they consistently overcome their single-resolution counterparts.

Acknowledgements

The authors acknowledge financial support through project 016658 (references PTDC/QEQ-EPS/1323/2014, POCI-01-0145-FEDER-016658) co-financed by the Portuguese FCT and European Union’s FEDER through the program “COMPETE 2020”.

References

Structured multiblock approaches for high-dimensional predictive modeling

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*marco@eq.uc.pt.

Current predictive approaches are strongly focused on optimizing accuracy metrics, leaving little room to incorporate a priori knowledge about the processes under analysis and relegating to a secondary concern the interpretation of results. However, there is often information available regarding the structure of the processes, which could be used in benefit of the analysis and to enhance the interpretation of results. The importance of this issue is not new and has motivated the development of multiblock approaches for improving interpretation while maintaining the quality of predictions. In this communication, two classes of multiblock frameworks are addressed. One class is based on the existence of a priori knowledge for building the blocks of variables, while methods from the second class are able to infer the system structure in a data-driven way. The introduction of such block structures constrain the predictive spaces for the sake of enabling interpretable elements in the final model. We show that such constraints do not usually compromise the methods’ performances when compared to their unconstrained counterparts, and sometimes even led to improvements in prediction ability, due to the use of more parsimonious and robust models.

Introduction

With the increasing availability of data in the Big Data era, there is a strong emphasis for adopting data-driven modeling approaches. In the domain of predictive modeling, the mainstream methods tend to treat all variables in the same way, and the different methodologies available provide different solutions to the way variables are selected and/or combined in order to compose the final model. We call these methods “single-block”, as they treat all predictors equally.

Taking a closer look at the recurrent structures found in industrial data and to the way systems generating them actually work, one can notice that, most often, not all variables are actively contributing to the phenomenon under study (as assumed in multivariate methods) nor are they bringing independent and isolated pieces of information to the model (for which variable selection schemes would be adequate). Rather, the prevailing structure seems to present the form of clusters of variables—modules—composed by sets of variables, where each cluster is relative to a given functional mechanism. Variables falling in the same cluster exhibit some degree of mutual correlation, and may be aggregated in a supper level in a hierarchical way at a higher level of abstraction.

In this context, instead of multivariate or variable selection schemes for handling high-dimensional systems, methods should enable the definition and selection of modules of variables that better reflect the system structure, which can then be selected for integrating the model according to their predictive power [1, 2]. This setting calls for methods that are able to handle simultaneously several heterogeneous blocks of variables in their formulation, called hereafter as multiblock methods.

Two classes of multiblock methods can be identified upon a close analysis of the technical literature. On one hand, there are methods where the blocks of variables are defined based on a priori knowledge about the system’s structure, e.g., when variables regard different process units, arise from different analytical measurement sources or are related to distinct and identifiable functional modules of the system. On the other hand, one can find methods where such knowledge is not explicitly known, but a modular structure is believed to exist, that must be inferred and extracted from the available existing data.

Objectives

The purpose of this communication is to briefly provide an overview of both classes of multiblock methods and to illustrate their application with resort to a real world case study.

Multiblock Methods for Predictive Data Analysis

In this section, a short presentation of the methods addressed in this work is provided. For more details on the implementation and use of these methods, we refer the interested readers to the references cited.

Class 1. Composition of the blocks is known a priori. Belong to this class all multiblock methods that assume the composition of the different blocks to be known a priori, i.e., the following mapping can be established using background knowledge: variable $\rightarrow$ block. This attribution is often possible to be made when there is sufficient knowledge about the system, and the way variables are naturally organized regarding how they contribute to the final outcome. Several multi- and megavariate methods fall in this category, and we will address the mainstream ones, namely: Concatenated PLS (CPLS) [3], Hierarchical PLS (HPLS), Multiblock PLS (MPLS) [4], as well as recent advances in this field, such as Sequential Orthogonalised PLS (SO-PLS) [5].

Class 2. Composition of the blocks is unknown. When the composition of the blocks is unknown, it must me induced from data. Network Induced Supervised Learning Regression method (NI-SL) is a method proposed by Reis [1, 2], aiming at bringing interpretation features to the forefront of the analysis goals. The method was divided in two stages. Stage 1 (Network-Induced Clustering) aims at finding functionally related groups of variables (clusters), which will form meaningful X blocks with predictive power for Y. The second stage consists in developing a predictive model, based on variates computed for the blocks induced in the first stage. For such, classical PLS models are developed separately between
each X block and the Y response, and a predefined number of latent variables are retrieved from each block (in the present study five latent variables were retrieved from each block). These latent variables are gathered into a super block and a forward stepwise regression procedure is used to select the subgroup of latent variables that lead to the best fit.

NI-SL can also belong to Class 1 if the cluster compositions are known beforehand, in which case the NI-clustering stage is bypassed and the method start immediately in the second stage of modelling. This is the case for the example addressed in this work, which will be described in the next section.

Results

We illustrate the application of multi-block methodologies with resort to an example from the wine production industry. More specifically, this example is focused on the prediction of ageing time in Madeira wine, based on different analytical measurement sources: volatile profile (1st block), the polyphenols and two furanic compounds (2nd block), the organic acids quantification (3rd block) and the ultraviolet-visible spectra (4th block). The volatile profile was analysed by gas chromatography coupled to mass spectrometry (GC-MS), preceded by solid phase extraction; the second block of data was obtained by High-Performance Liquid Chromatography combined with Photodiode Array Detection (HPLC-DAD; direct injection); organic acids (the 3rd block of variables) were also quantified by Liquid Chromatography combined with Photodiode Array Detection; UV-Vis absorbance spectra (4th block of variables) was done in a Perkin-Elmer Lambda 2 spectrophotometer (Waltham, MA, USA). More information about this case study and results is available elsewhere [6].

A total of 26 samples were analysed, covering a range of 20 years (2-3 wine samples were taken per ageing year, with 2 year intervals). All samples correspond to wines produced from the same grape variety (Malvasia) and were supplied from the same Madeira wine producer.

Due to space constraints, the analysis will be focused on the predictive capabilities of the methods, assessed according to the following procedure. For each multiblock algorithm, 50 models were estimated using the datasets described above and Monte Carlo assignment of samples. In each Monte Carlo assignment, the dataset is randomly divided into a test set (20%) and a training set (80%). The training set is used to calibrate the model and to determine the respective hyperparameters based on 10-fold cross validation method. This procedure is repeated 50 times originating 50 models for each multiblock method. The test sets are used for prediction based on which one computes the root mean square error of prediction (RMSEP), for each Monte Carlo run and each method.

Table 1 presents the mean RMSEP obtained for the several multiblock methods studies in this article. The methods leading to better performance are CPLS (with a new scaling methodology developed by the authors; see [6]) and SO-PLS, followed by NI-SL. The first two methods present superior predictive performances than the best linear multivariate methodologies applied to each block separately – the best single-block method was PLS applied to the 2nd block of variables, that led to a mean RMSEP of 1.17 years (see all results in [7]).

Table 1. Average root mean square error of prediction and interquartile range (IQR) for each multiblock method obtained by the Monte Carlo Cross-Validation procedure.

<table>
<thead>
<tr>
<th>Method</th>
<th>RMSEP</th>
<th>IQR (75%-25%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPLS</td>
<td>0.93</td>
<td>0.61</td>
</tr>
<tr>
<td>HPLS</td>
<td>1.48</td>
<td>0.44</td>
</tr>
<tr>
<td>MBPLS</td>
<td>1.34</td>
<td>0.54</td>
</tr>
<tr>
<td>NI-SL</td>
<td>1.17</td>
<td>0.85</td>
</tr>
<tr>
<td>SO-PLS</td>
<td>0.97</td>
<td>0.49</td>
</tr>
</tbody>
</table>

Conclusions

The results presented clearly indicate that it is indeed possible to synergistically combine different sources of information for improving the predictive performance of the methods, even though the single-block methods based on the Polyphenol Content already lead to interesting predictive results.

Moreover, the multiblock methodologies bring other interpretational dimensions to the analysis, namely regarding the importance of the different blocks for predicting the response and their redundancy or overlap, which are not addressed in this short abstract.

Acknowledgements

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References

Polymeric catalytic membranes for biodiesel production – Effect of the addition of k-carrageenan in PVA membranes

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Composite catalytic membranes were prepared with poly(vinyl alcohol) (PVA) and k-carrageenan (KCA) blends and cross-linked with hexamethylene diisocyanate (HDI). The phase inversion process is performed after crosslinking. CaO derived from oyster shells was synthesised, treated with hydration cycles and employed as catalyst dispersed in the polymeric matrix. The catalytic membranes were used in the biodiesel synthesis by transesterification of soybean oil. Swelling degree, SEM and elemental analyses were used to characterise the membranes. The catalyst leaching was also determined. The characterisation results indicate that the phase inversion process partially removes the KCA, affecting the membrane structure with pore formation. The results show that the swelling is higher the higher the membrane porosity, and the presence of KCA minimises the catalyst leaching.

Introduction

Biodiesel, considered a promising alternative to the fossil diesel, is conventionally produced by transesterification of vegetable oils using homogeneous catalysts [1]. The substitution by the heterogeneous route allows the reduction of the washing water consumption and the necessity to treat it. In addition, heterogeneous catalysts can be recovered and reused in the process [2]. The literature has indicated the good catalytic performance of CaO, generally produced by thermal decomposition of CaCO3 [3]. Mollusk shells and eggshell are examples of rich sources of CaCO3, besides they are low value-added residues. The main disadvantage of CaO, due to the lower basicity and lower contact area of the material, is the long reaction time required to reach satisfactory conversions [4].

Other challenges associated with conventional biodiesel production such as the costly purification step, thermodynamic equilibrium and limited mass transfer can be minimised through the process intensification technologies. The membrane reactor offers advantages by combining chemical reaction and product separation in a single operating unit. In addition, the continuous removal of the products from the reaction medium makes it possible to overcome the limitation caused by the chemical equilibrium of the transesterification reaction, but it is still a little-explored technology [5,6].

This work aims to contribute to the technical feasibility of the use of membrane reactor in the biodiesel production. Composite catalytic membranes prepared with PVA/KCA blends, in different compositions, were tested in the transesterification of soybean oil.

Methods

Catalyst preparation. CaO was obtained by calcination of CaCO3 of oyster shells at 900 °C for 4 h, under N2 flow. Two hydration-dehydration-calcination cycles were performed with calcined powder to develop porosity. In the hydration step, the material remains in suspension in water for 4 h. In the dehydration step, the product is dried at 120 °C and calcined, closing the cycle.

Membrane preparation. The PVA was dissolved at 115 °C in a solution of N,N-dimethylformamide (DMF) and dimethyl sulfoxide (DMSO). Before the addition of the KCA, the temperature was reduced to 90 °C. The catalyst (20 wt% of the total polymer mass) was added to the mixture after complete KCA dissolution. The mixture is poured onto a Petri dish in the presence of liquid N2 and kept in the refrigerator for 2 h. The membrane is then suspended and held in a cold solution of DMF/DMSO and HDI overnight. The amount of HDI is used in order to obtain a crosslinking degree of approximately 14%. The crosslinked membrane is subjected to the phase inversion process with dry methanol and kept in a closed box until its use.

Membranes with different mass ratios of PVA/KCA (100/0, 95/05, 90/10 and 80/20) were prepared and coded (% PVA% KCA).

Membrane characterisation. The membranes were characterised by swelling degree, Scanning Electron Microscopy (SEM) and elemental analysis. Swelling experiments were initiated by immersing the membrane samples in appropriate solvents (water, methanol, glycerol or soybean oil) for 24 h. The samples were then removed, weighed (Wwet) and dried in a vacuum oven for 24 h at 100 °C. They were again weighed (Wd). Equation 1 was used to calculate the swelling degree.

\[
\text{Swelling} \,(\%) = \frac{W_{\text{wet}} - W_d}{W_d} \times 100
\] (1)

The catalyst leaching was determined after the phase inversion step and at the end of the catalytic test by burning the membrane. Kinetic study. The experiments were carried out in a batch reactor with soybean oil and a large excess of methanol to avoid mass transfer resistance. The membranes were cut into small pieces and added to the reaction medium, which was kept at 60 °C. The samples were analyzed by gas chromatography and the membrane activity was evaluated as the maximum reaction rate.

Results and discussion

Membrane preparation. A visible mass of polymer of membranes with PVA and KCA was lost in the phase inversion.

Membrane characterisation. The elemental analysis reveals that the membranes contain sulfur varying in the order PVA80/KCA20 (0.15%) < PVA90/KCA10 (1.43%) < PVA95/KCA05 (1.77%). Since sulfur is present in the KCA molecule, it was expected to find a concentration varying in reverse order. This result indicates that the KCA was the
polymer lost during the membrane preparation and that the PVA concentration affects the retention of KCA in the matrix. The SEM images of PVA100KCA0 and PVA80KCA20 are shown in Figure 1. It is possible to observe a dense structure in the sample prepared only with PVA, and the presence of pores in the polymer structure prepared with PVA and KCA.

![SEM images of the surface and side view of PVA and KCA membranes.](image)

Figure 2. SEM images of the surface (x 3,000) and the side view (x 70) of 100PVA0KCA (a) and 80PVA20KCA (b) membranes.

Figure 2 shows the graph with the results of swelling analysis. An increase in swelling degree can be observed with increasing concentration of KCA in the membrane preparation. The increase can be explained by the presence of pores throughout the thickness of the membranes, which allow the solvent molecules to access the polymer structure easily.

![Swelling analysis graph.](image)

Figure 2. Graph of the swelling analysis.

The values of leached CaO are shown in Table 1. The retention of the catalyst is higher the higher the KCA concentration in the membrane preparation.

![Graph with experimental data and kinetic curves.](image)

Figure 3. Graph with experimental data and kinetic curves.

**Conclusions**

Composite catalytic membranes with PVA/KCA blends were prepared and tested in biodiesel production. CaO derived from oyster shells was treated and used as a catalyst. The presence of KCA during the polymer structure formation and its subsequent elimination in the phase inversion step had a pronounced effect on the morphological properties, swelling degree and catalyst retention. An increase in KCA concentration increased the presence of pores throughout the thickness of the membranes. The evaluation of the membrane catalytic activity was compromised due to the observed leaching of CaO.

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Phase inversion</th>
<th>Reaction</th>
<th>Reaction time</th>
</tr>
</thead>
<tbody>
<tr>
<td>95PVA05KCA</td>
<td>6.6</td>
<td>5.2</td>
<td>3 h</td>
</tr>
<tr>
<td>90PVA10KCA</td>
<td>4.3</td>
<td>4.8</td>
<td>3 h</td>
</tr>
<tr>
<td>80PVA20KCA</td>
<td>3.4</td>
<td>4.7</td>
<td>5 h</td>
</tr>
</tbody>
</table>

**Kinetic study.** A kinetic model based on the Eley-Rideal mechanism was used to adjust the experimental data (Figure 3). Since the model was developed for heterogeneous catalysis, without considering membrane diffusional resistances, and presented a good fit for the reaction results, there is a strong indication that the activity of the leached catalyst ensured the total conversion of the oil.

The maximum reaction rates, calculated from the slope of the curves, follow the order 0.55 < 0.61 < 0.94 mol min kg⁻¹ for the sequence 80PVA20KCA, 90PVA10KCA, 95PVA05KCA, respectively. According to data from Table 1, the velocity is higher the higher the mass of the leached catalyst.  

Acknowledgements

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References

(Nitro)phenols reactivity in mixed acid benzene nitration

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Phenol-benzene mixtures were nitrated in mixed acid systems, employing smooth conditions for enabling both phenol detection in the reaction medium and the construction of a simple kinetic model for this highly reactive species nitration while supporting the mechanism of phenol as the nitrophenols precursor. Benzene (phenol-free) nitration was also carried out, in reaction conditions similar to the ones employed in the industrial production of mononitrobenzene, aiming the detection of all reaction by-products formed by phenol nitration, while also evidencing its high reactivity. The effect of some operating conditions, namely the reaction temperature, sulfuric acid concentration and mixed acid composition, were evaluated, which allowed verifying that upon phenol formation, nitrophenols are formed by consecutive reactions, i.e., mononitrophenols are nitrated into dinitrophenols that are the trinitrophenol precursors.

Introduction

Mononitrobenzene (MNB) production results from an exothermic reaction between benzene (Bz) and nitric acid, catalysed by a strong acid [1], typically sulfuric acid, originating thus the so-called mixed acid, a mixture of sulfuric and nitric acid. Industrially, Bz nitration has been improved over time [2,3]; however, despite the achieved process improvements and the reaction high selectivity towards MNB formation, Bz nitration is always accompanied by side reactions. In the MNB production process the main reaction by-products are dinitrobenzenes and nitrophenols (NPs) [4,5]. Efforts have already been made to understand and reduce the NPs formation [4–7]. Nonetheless, despite the achieved conclusions, which allowed to relate the reaction parameters with NPs formation, their formation mechanism is still uncertain. It is postulated that NPs formation starts via Bz oxidation to phenol (Ph) in the organic phase by action of undissociated nitric acid [8]. Then, it diffuses to the aqueous phase and by sequential nitration, Ph can originate mono-, di- or tri-nitrophenols – see Graphical Abstract. Ph presence in Bz nitration medium was confirmed in a work of ours, as well as its nitration into MNPs, supporting thus Burns and Ramshaw [8] theory.

Despite the studies done concerning Bz nitration, there is information lacking in the literature regarding the formation of nitrophenolic by-products, namely the NPs interconversion, i.e., mononitrophenols (MNsPs) being converted into dinitrophenols (DNPs) and DNPs originating trinitrophenol (TNP) – Graphical Abstract – data typically not presented in Bz nitration studies [4,5]. At industrial level, it is important to predict the NPs concentrations for certain operating conditions in order to choose the proper alkaline agent to be used for extracting NPs from the produced MNB, thus ensuring that it is complying with the final product specifications. Consequently, an objective of the present study was to display all the NPs concentration profiles, to show how the reaction conditions can affect the NPs formation.

Once Ph is expected to be formed during mixed acid Bz nitration, but to our knowledge this species was never detected in this nitration system, the present work also aimed to evidence Ph’s high reactivity towards nitration, even in smooth reaction conditions, and to support its role as the NPs precursor; the construction of a simple kinetic model was also aimed for the reasons described above. To achieve to enumerated objectives a set of laboratory batch tests were performed using a variety of nitrating conditions.

Experimental

The nitration of Bz (Ph-added and Ph-free) was carried out discontinuously, in a 0.75 L jacketed reactor equipped with a tantalum air-impelled stirrer and a Teflon coated thermocouple. During the reaction time samples were collected and prepared to be analysed in a HPLC-DAD apparatus.

Results and Discussion

Figure 1 discloses the obtained Ph, 2-MNP and 4-MNP concentration profiles (total of both reaction phases). Due to the employed soft reaction conditions (low temperature and low sulfuric acid concentration), the DNPs and TNPs formation was negligible. It is noteworthy that even for the employed smooth reaction conditions, Ph concentration became vestigial 10 minutes after the reaction start. This evidences this compound high reactivity, explaining thus the difficulty of detecting it in the usual nitrating reaction mediums in which, typically, the initial reaction temperature is higher than 80 °C.

The obtained results are in accordance with the ones previously obtained by us, namely in what concerns to Ph high reactivity and its role as the MNPs (and consequently as the NPs) precursor in mixed acid Bz nitration systems.

Using the gotten concentration profiles, assuming perfectly stirring conditions and the existence of a single (pseudo-homogenous) liquid phase (thus neglecting mass transfer limitations) during Ph-added Bz nitration and considering a pseudo-first order kinetics, a simple kinetic model was constructed – Figure 1.

Despite the small deviation between the fitted model and the MNPs experimental data, the proposed model expresses satisfactorily the Ph, 2-MNP and 4-MNP concentration profiles. Regarding Ph-free Bz nitration, the chosen reaction conditions were closer to the industrial ones, although softer, to enable the NPs interconversion observation. Figure 2 displays the achieved results for the reaction temperature (one of the analysed parameters) effect on the NPs formation.
Figure 1. Phenol (○), 2-mononitrophenol (□) and 4-mononitrophenol (Δ) total concentration profiles and fitting of the proposed model to experimental data. Reaction conditions: \( T_{\text{initial}} = 15^\circ \text{C} \), % wt. \( \text{H}_2\text{SO}_4 = 38.1 \); % wt. \( \text{HNO}_3 = 2.1 \). X_{\text{HNO}_3} < 1\% at the reaction end.

Acknowledgements
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References
Introduction
Statistical process control is a fundamental part of the industrial landscape since the 1930’s. In the former times, it was carried out using univariate control chart approaches, such as the celebrated Shewhart, CUSUM and EWMA charts. However, with the technological evolution, more measurements are performed across the industrial processes, collecting information about many different operation variables. This situation calls for more advanced monitoring methodologies that are able to cope with new demands for data handling and processing, namely the increasing volume and speed at which they are collected [1]. In this context, multivariate methodologies, such as Principal Component Analysis (PCA), have been applied, with very interesting results, to monitor chemical processes and detect special causes [2], in what is usually called Multivariate Statistical Process Control (MSPC), or more specifically, PCA-MSPC (to distinguish from other methodologies for conducting MSPC). PCA-MSPC is quite effective for detecting abrupt faults, especially in sensors and the process, showing less sensitivity to detect faults in actuators, such as valves. These are abnormal events that occur in narrow windows of time, with relatively fast dynamics. However, there are other types of events that have been neglected from the analysis so far, namely equipment degradation.

Complex reactions and phenomena occurring in chemical processes often have a negative effect, in the long term, on product quality, equipment conditions or energy consumption [3]. However, process monitoring usually does not look into these dynamics, leaving possible process improvement unattended. In fact, equipment degradation is often seen as a task of the Reliability & Maintenance department, and discarded from the concerns of process engineers and unit managers. This silo based mindset is hindering further improvement to take place in industry, and is a paradigm that needs to be shifted as soon as possible, even because Industry 4.0 opens many new opportunities for integrating maintenance and process related activities.

Objectives
In this context, the objective of this work is to develop an industrial simulator that could act as a test bed for developing algorithms for monitoring simultaneously process monitoring and equipment degradation methods. More specifically, we have developed an industrial simulator, programmed several faults with a variety of magnitudes, implemented fault detection and diagnosis methods, and tested new approaches for detecting equipment degradation.

Methods
The methodologies used to conduct process monitoring (i.e., fault detection and diagnosis) are based on PCA and MSPC control charts, whereas contribution plots were employed for diagnosis [4-6]. Regarding process & equipment degradation, PCA in conjunction with Wavelet based Multiresolution Analysis (MRA) were synergistically combined to detect the slower or long term dynamics that affect chemical processes, but whose effects are often masked by the normal variability of processes and the effective action of controllers (See Figure 1) [7].

To develop and test these methodologies, an industrial system was simulated to generate the necessary reference data as well as to have access to the process’s ground truth which is not available in reality. The system consists of a non-isothermal Continuous Stirred-Tank Reactor (CSTR) under feedback control with the needed flexibility to simulate a wide spectrum of faulty conditions with different magnitudes and durations.

The phenomena were simulated in such a way that accurately reproduces common process operation, including the normal variability, valve operation and faults, fouling phenomena, etc. The code contemplates two integration time grids, to emulate the operation of a continuous processes under control.
Results
The outcomes of the PCA-MSPC methodology revealed that process variability in Normal Operating Conditions (NOC) is greatly explained by the actions of feedback control. The detection of abrupt faults is fairly robust in sensor and process fault types. However, faulty valves are more difficult to be detected. A remark to be retained is that fault diagnosis through contribution plots performed rather poorly in some cases, especially regarding faults in valves; yet, in other failure classes, contribution plots do provide indication to where a problem might be.

Conclusions
Concerning the detection of abrupt faults, the PCA-MSPC methodology demonstrated to be an effective and robust solution. However, fault diagnosis had mixed results with the contribution plots failing for some classes of faults.

Finally, PCA-MRA showed promising results as it was able to detect the simulated process & degradation dynamics, paving the way to the development of a model that predicts the state of a process degradation based on process measurements.

References
Image-based process monitoring using multiscale and multivariate image analysis: A pilot scale implementation study and results

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With this work, we establish the validity and usefulness of the Multiscale and Multivariate Image Analysis (MSMIA) approach for image-based process monitoring, proposed by M. Reis [1], by applying it online in a pilot scale hardware system. The MSMIA methodology combines wavelet texture analysis and multivariate image analysis, for detecting both abnormal changes in texture and color. The approach was implemented in a pilot scale system and applied to several controlled testing scenarios. The scenarios include normal operation conditions (NOC) and faults of different types and magnitudes. Therefore, it was possible to assess the monitoring potential of the proposed methodology to detect abnormal process conditions under various types of faulty conditions related to the size, shape and color of the image attributes. Results confirm the sensitivity of MSMIA to perform image-based fault detection when applied on real images.

Introduction
Multiscale and Multivariate Image Analysis (MSMIA), is a state-of-the-art approach for performing image-based process monitoring. It consists of the synergistically combination of Principal Component Analysis (PCA) and Wavelet Analysis of the unfolded multi-channel images; after which monitoring statistics are computed that capture the main features of the color and texture of the collected images. As any process monitoring approach, MSMIA comprises two phases [2]. In the first phase, process stability is analyzed and the normal operation condition models (NOC) estimated. In this stage, the PCA and MSPC parameters are computed from NOC data. The second phase is dedicated to monitor new samples, if the process was found to be stable in Phase 1. During this phase, images are processed by MSMIA and classified as normal or abnormal.

Objectives
The objective of this work is to test, in real world conditions, the process monitoring methodology based on online image analysis, known as MSMIA. An experimental apparatus was assembled, the software code adapted for online use and several testing scenarios were contemplated for assessing the methodology sensitivity and robustness.

Methodology
MSMIA contemplates several stages of image processing (Figure 2). In these stages, the several color channels are split and processed with Two-Dimensional Wavelet Transforms (WT); in this case, WT are applied with five decomposition levels along three directions (horizontal, vertical and diagonal). Thus one coarser matrix and 15 detail matrices are obtained for each channel. The energy of all detail matrices, in all color channels, is calculated and used for building the multivariate statistic for process monitoring. For RGB images, the dimension of the vector of images features (energies) is 45. These energies are strongly correlated and therefore are monitored using PCA-MSPC. In the first phase, Principal Component Analysis (PCA) is applied to the energy vectors in order to reduce the dimensionality of the analysis space and to model the correlation between the monitoring variables. PCA parameters are estimated and the number of components to retain, selected. In this case, three principal components were selected.

After the application of PCA, multivariate statistics (Hotelling’s T² and Q) were computed for each image [3]. The training set was composed by 50 observations of NOC process images and was used to derive the PCA model (see Figure 1 for an example). A validation set, also composed by 50 observations, was then used to obtain the control limits for these statistics, avoiding overfitting and reducing the rate of future false alarms.

In phase 2 the methodology is applied to new samples, for which the statistics Hotelling’s T² and Q are obtained. In this work, we have considered the monitoring of a process producing colored pellets. Three types of faults were considered: changes in the nominal size of the pellets; changes in shape and changes in color. The faults can be total (affecting the entire sample) or partial (the change occurs only in a part of the sample).

Results
The MSMIA approach was able to detect all kinds of simulated process faults, with one of its two monitoring statistics. In some cases, one statistic was less efficient for some type of faults, while in other cases the other multivariate statistic was less efficient, for other types of faults. By using both statistics simultaneously they complement each other. The performance of each statistic is resumed in Table 1.

In Figure 3 is possible to observe the monitoring statistics resulting from applying MSMIA to a testing scenario composed by several faults. Each fault corresponds to one of the 6 regions depicted: (a) – NOC (no fault – see Figure 1); (b) - medium size faults; (c) - large size faults; (d) - color fault #1; (e) - color fault #2 faults; (f) – simultaneous color and shape fault. It is possible to verify that the two statistics have different performances depending on the type of fault, but they end up complementing each other quite nicely.
Table 1. Summary of MSMIA statistics detection performance in different scenarios.

<table>
<thead>
<tr>
<th>Fail type</th>
<th>Hotelling’s T²</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medium size (total)</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Big size (total)</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Big size (partial)</td>
<td>+(f)</td>
<td>-</td>
</tr>
<tr>
<td>Shape (total)</td>
<td>+(f)</td>
<td>++</td>
</tr>
<tr>
<td>Shape (partial)</td>
<td>-(f)</td>
<td>+</td>
</tr>
<tr>
<td>Color (total)</td>
<td>+(f)</td>
<td>++</td>
</tr>
<tr>
<td>Color (partial)</td>
<td>+(f)</td>
<td>++</td>
</tr>
</tbody>
</table>

Legend of Table 1: + detected; ++ better detected; - not detected; (f) some false alarms; (ff) many of false alarms

These results illustrate the detection potential of MSMIA methodology as a process monitoring tool for texture matrices, based on image data. It effectively allows the simultaneous detection of abnormalities in size distributions, shape and color, in an efficient and affordable way.

Conclusions
With this work, we experimentally confirmed MSMIA potential for online image-based process monitoring. A variety of testing scenarios were employed, where pellets with different sizes, colors and shapes were used to set NOC conditions and faults. MSMIA robustly detected deviations from normal conditions, through one of the monitoring statistics. Future applications abound in Industry 4.0 environments, and will be explored in future work.

Figure 1. Example of an image used to derive the NOC data set.

Figure 2. MSMIA framework

Figure 3. Hotelling’s T² & Q control charts for one type of testing scenario.

References
A coarse-grain computer simulation approach of triblock copolymers

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The advent of the transferable MARTINI coarse-grain (CG) model boosted the applicability of computer simulations to study the self-assembly of complex systems. However, despite a great paperwork developed in the recent years, a general model for Triblock copolymers (TBC) is still lacking. In this study, the CG model was developed to reproduce, by computer simulations, the essential physico-chemical characteristics of TBC in aqueous solutions. For this purpose, an archetypical normal and reverse TBC with different amphiphilic character were chosen. Our CG approach was validated by comparing selected computer simulation results with experiments carried in our laboratory and literature data.

Introduction

TBCs are constituted by a combination of polyethylene glycol (PEG) and polypropylene glycol (PPG) units to form the non-ionic moiety. Thus, the amphiphilic character can be tuned by varying the PEG to PPG unit ratio. TBCs are biodegradable, relatively cheap, biocompatible, which makes them excellent co-solvents for biological applications.[1] TBCs exhibit remarkable characteristics such as a high thermal and chemical stability, thermo-responsiveness, self-assembly in diverse morphologies and low volatility, among others. Furthermore, TBCs are liquid at room temperature and have the ability to solvate a wide range of polar and non-polar compounds. TBCs are used as solvents in many extraction/separation processes, commonly by phase separation under the cloud point temperature conditions. The intricated TBCs micelle morphologies dramatically increases the complexity of the inter micelle interactions, making the control of their properties a tough question for researchers. Computer simulations can enhance our acquaintance and shed light in the interactions involved in TBC micelle solutions.

Objectives

The main aim of this work is to provide a CG molecular dynamics (MD) approach able to mimic the phase behavior of TBCs in aqueous solutions. Thus, a transferable model following the MARTINI [2] philosophy is proposed. Experiments developed in our laboratory were used to validate the computer simulation approach. In this work, one normal and one reverse TBC, with the same PPG and PEG content but differently distributed, were chosen.

Methods

The binodal curve of both normal and reverse TBC from 1 to 18 wt% were obtained by visual determination of the onset turbidity of a solution heated in a temperature-controlled water bath. These systems were replicated by computer simulations where the diffusion coefficient, the micelle size distribution and the aggregation number were compared to analyze the effect of the micelle surface shape (PEG distribution alongside the micelle surface).

The CG MD simulations were carried with the Gromacs 5.1.4 package.[3] The potential energy function comprised the bond stretching, angle bending, and dihedral torsion for bonded interactions. The Lennard-Jones (LJ) potential and the Coulomb term were considered for non-bonded interactions. The simulation samples were built with Packmol. In all of the simulations performed in this work, the same protocol was followed: an energy minimization step using the steepest descent algorithm to prevent short-range contacts between atoms prior to two short equilibrium steps in the NVT and NpT ensembles, respectively. Otherwise stated, the duration of the final NpT production run was 1 microsecond of simulation time. MD simulations of aqueous solutions at 1, 5 and 20 wt% of TBC concentrations were carried out under the experimental cloud point temperature conditions.

Results and conclusions

Figure 1.I) shows the experimental cloud points obtained for both TBC at different concentrations. Both systems have the same PEG and PPG content, thus the same amphiphilic character, however the cloud point temperature strongly differs as soon as the concentration is raised from 1% wt. This difference presumably comes from the micelle crown of PEG (orange) formed once the TBC moieties arrange in micelles as illustrated in the Graphical Abstract. The reverse TBC exhibits flower-shape micelles because the PEG segments form loops resembling flower petals. Conversely, the PEG peripheral units in the normal TBC point outwards from the micelle core, organizing in a star-like shape. The results in Figure 1.I) indicate that the micelle surface plays an important role. The inter micelle interactions of both TBC micelles obtained in the MD simulations were evaluated through the coefficient of diffusion (Dco) and the results are shown in Table 1. At 1 wt%, the normal and reverse TBC arranged in a dispersed micelle distribution with similar Dco value of 0.166 × 10⁻⁵ cm²·s⁻¹ and 0.158 × 10⁻⁵ cm²·s⁻¹, respectively (Figures 1.III.i and iv). However, raising the concentration to 5 wt% the system exhibited a markedly difference in Dco, with the reverse TBC system displaying a lower Dco, pointing out to a higher degree of linked micelles, also clearly visible in the simulation snapshot (Figure 1.IV). In this regard, at TBC concentrations above 1 wt%, the reverse TBC micelles interact more strongly with each other, promoting a higher degree of self-aggregation and slowing down the dynamics of the system. In fact, at 20 wt%, the Dco in reverse TBC was one order of magnitude (Figure 1.IIIi) lower than the normal TBC, indicating a reduction in the mobility of the reverse TBC micelles, even when both systems were densely packed. The micelles obtained in each reverse and normal TBC were
characterized in detail using an in-house code based on the Hoshen – Kopelman cluster counting algorithm. Thus, the micelle diameter and the aggregation number are presented in Table 1. The micelle growth above 1 wt% of concentration in normal TBC was smoother, denoting low micelle fusion events if one compares the \( \varnothing \) and \( N_a \) in both copolymers. Furthermore, the reverse TBC micelles were slightly bigger compared with the normal TBC. This accentuates the easiness of reverse TBC micelles to form aggregates of linked micelles as shown in Figure 1.II.v. These results suggest that the reverse TBC system requires less energy to coalesce and separate into two macroscopic phases, which is reflected in its lower cloud point temperature compared to the normal TBC, as presented in Figure 1.I).

Table 1. Diffusion coefficient (\( D_{cd} \)) in (\( \text{cm}^2 \cdot \text{s}^{-1} \)) units, aggregation number (\( N_a \)) and averaged micelle diameter (\( \varnothing \)) in nanometers were obtained after 1 microsecond of simulation time.

<table>
<thead>
<tr>
<th></th>
<th>Normal TBC</th>
<th>reverse TBC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>( D_{cd} \</td>
<td>( N_a \</td>
</tr>
<tr>
<td>1 \text{ wt%}</td>
<td>0.166</td>
<td>10</td>
</tr>
<tr>
<td>5 \text{ wt%}</td>
<td>0.071</td>
<td>15</td>
</tr>
<tr>
<td>20 \text{ wt%}</td>
<td>0.030</td>
<td>21</td>
</tr>
</tbody>
</table>

Acknowledgements
This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. The authors are also grateful for the national fund through the Portuguese Foundation for Science and Technology (FCT) for the doctoral grant SFRH/BD/101683/2014 of F.A. Vicente. S.P.M. Ventura acknowledges FCT for the contract IF/00402/2015 under the Investigador FCT 2015. G. Pérez-Sánchez acknowledges the research contract under the project CENTRO-01-0145-FEDER-000005: SusPhotoSolutions: Soluções Fotovoltaicas Sustentáveis. N. Schaeffer acknowledges financial support by BATRE-ARES project (ERA-MIN/0001/2015) funded by ADEME and FCT.

References
The grassroots and retrofit design of hydrogen distribution systems has been actively investigated since the 2000’s either using mathematical programming or simply relying on insight-based methods. This paper intends to present the optimisation model that has been developed so far for the retrofit design of Galp’s Sines refinery hydrogen network. The retrofit design model depicted here represents only one part of a complex optimisation framework, which includes data conditioning, reconciliation, and Pinch Analysis. The objective is to find the optimal flowsheet configuration that leads to the lowest operational expenses with minimal capital expenditure involved such as new connections, PSA units, and compressors and minimal configuration complexity. For the current flowsheet configuration, a total annual cost saving of approximately 3,400 u.year\(^{-1}\) can be easily obtained just by redirecting some of the off-gas streams that are being disposed of to the fuel gas system to a PSA unit. This solution requires, however, the revamping of the existing PSA unit since the former has already reached its full capacity. A payback period of 1.1 years is expected after the proposed solutions are implemented. This project was carried out as part of a joint venture between Galp, IST, and the U. Coimbra.

General Overview
The recent environmental regulations to cut down pollutant gas emissions, the strict sulphur and aromatics specifications, the increasing drive to process heavy-sour crude oils, and the refining market trends toward gasoline and middle distillate fuels led to profound changes in the oil and gas industry. All over the world, crude oil refineries are being subject to increased hydrogen demands, mostly in hydrotreating, hydrocracking, and more recently, hydrosulfurising operations. In the recent past, catalytic reforming processes have been the conventional source of hydrogen in oil refineries. However, the current trends toward low-aromatic gasoline are constraining the use of hydrogen from catalytic reformers. As a result, many oil refineries are often confronted with temporary hydrogen shortfalls to meet product quality specifications, as well as to boost the hydrogen partial pressures, which have a considerable impact on hydroprocessing reactors’ conversion, yields, and catalyst life. A comprehensive description of the mathematical programming and insight-based approaches available for the design of hydrogen networks can be found in the literature [1].

Deterministic mathematical-based model
The deterministic mathematical model discussed in this section has already been presented elsewhere [2]. We borrowed some of the ideas and nomenclature of Deng et al. [3–6], amongst other sources. The retrofit design model was developed in GAMS with Microsoft Excel\(^{®}\) serving as the interface to the end user. The retrofit design problem is classified as a mixed-integer nonlinear programming problem owing to the existence of bilinear terms, concave univariate functions, and discrete variables. The former is particularly difficult from a computational perspective due to the presence of nonconvex terms which led us to exploit several relaxation techniques such as the piecewise-linear McCormick’s envelopes and other convexification techniques. The objective of the retrofit design model is to minimise the total annual cost, which comprehends both operational expenses as well as capital expenditure. The industrial data supplied as input include flows, compositions, and pressures. The flow rate measurements must be corrected before the optimal flowsheet configuration is found to eliminate the occurrence of systematic and random errors. The same cannot be said for the composition measurements due to the lack of analysers. The MINLP problem can be summarised as follows:

- **Objective:** Minimise the total annual cost (TAC)
- **s.t.**
  - Material balance constraints
  - Pipeline connection constraints
  - Pressure restrictions
  - Additional equipment restrictions
  - Operating expenses and capital expenditure

The nonconvex mixed-integer nonlinear programming problem comprises 2,840 equations, 1,550 continuous variables, and 177 integer variables. The best flowsheet configuration was obtained using ANTIGONE on an Intel\(^{®}\) Core\(^{TM}\) i7-8550U @ 1.80 GHz 8 CPUs, 16 GB RAM, 512 GB SSD with CPLEX and CONOPT selected as MILP and NLP subsolvers. The computational effort required to find global optima is highly dependent on the domain partitioning scheme chosen, as well as the number of disjoint segments per variable. With respect to the domain partitioning scheme, we can opt for a uniform or a logarithmic partitioning scheme. In this paper, we will consider a logarithmic domain partitioning and ten disjoint segments per variable. It is not our aim to provide a detailed description of the mathematical aspects behind the generalised disjunctive programming such as the Big-M and Convex Hull relaxations. The interested reader is strongly recommended to search for more information in the literature.

Case study
To illustrate the benefits of modelling an oil refinery hydrogen network in an interactive development environment and not just lingering on some spreadsheet calculations, we will investigate two different scenarios and compare their results in terms of the total annual cost and the resultant configuration complexity. The hydrogen network comprises two steam-methane reformers, one platformer, one hydrotreater, five hydrotreaters, one diolefins hydrosomerisation unit, and one PSA unit. We will not present
the current flowsheet configuration nor the one that leads to the highest total annual cost savings due to space restrictions. Still, the interested reader is recommended to read Marques et al. [2] for a deeper understanding of the hydrogen network at hand. In Scenario 1 the off-gas streams from the SR light gas oil and vacuum gas oil hydrotreaters are routed to the existing PSA unit, whilst the off-gas stream from the SR heavy gas oil hydrotreater is sent to the SR light gas oil hydrotreater where it is admitted to one of the unit’s make-up gas compressors along with the hydrogen-rich stream from the SR heavy naphtha hydrotreater. The optimisation results for Scenario 1 are depicted in Table 1.

Final remarks
In this paper, we have discussed an alternative approach for the retrofit design of hydrogen networks which does not rely on the representation of the hydrogen composite curves but on exact optimisation algorithms. We pointed out some of the difficulties of solving nonconvex MINLP problems such as the occurrence of multiple local optima and several degenerate solutions. After investigating different scenarios, we concluded that even for the current flowsheet configuration and operating conditions it is possible to reduce the production of “on-purpose” hydrogen by investing in additional compressors and PSA units. A total annual cost savings of nearly 3,400 u.year⁻¹ can be achieved simply by putting into practice some of the proposed solutions. Even if we had not considered the revamping of the existing PSA unit it would have been possible to reduce the total annual costs by 2,150 u.year⁻¹ only by introducing some changes to the actual flowsheet configuration, e.g. new pipeline connections.

Table 1. Galp’s Sines Refinery hydrogen network optimisation results. The operating expenses and capital expenditure are indicated in arbitrary currency units per year, following Galp’s confidentiality policy rules.¹

<table>
<thead>
<tr>
<th></th>
<th>Base Case</th>
<th>Scenario 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>“On-purpose” hydrogen supply, Nm³.h⁻¹</td>
<td>105,401</td>
<td>91,394   -13.4%</td>
</tr>
<tr>
<td>“On-purpose” hydrogen production cost, u.year⁻¹</td>
<td>111,995</td>
<td>96,850   -13.4%</td>
</tr>
<tr>
<td>Power consumption, kW</td>
<td>19,750</td>
<td>20,164   2.1%</td>
</tr>
<tr>
<td>Electricity costs, u.year⁻¹</td>
<td>12,594</td>
<td>12,858   2.1%</td>
</tr>
<tr>
<td>Equivalent fuel gas heating value, u.year⁻¹</td>
<td>24,900</td>
<td>14,239   -42.8%</td>
</tr>
<tr>
<td>Operating expenses, u.year⁻¹</td>
<td>99,688</td>
<td>95,469   -4.2%</td>
</tr>
<tr>
<td>Capital expenditure, u.year⁻¹</td>
<td>798</td>
<td>778      2.6%</td>
</tr>
<tr>
<td>Total annual cost, u.year⁻¹</td>
<td>99,688</td>
<td>96,266   -3.4%</td>
</tr>
<tr>
<td>Total annual cost savings, u.year⁻¹</td>
<td>3,422</td>
<td>3,422    1.1%</td>
</tr>
</tbody>
</table>

Acknowledgements
A word of gratitude to Fundação para a Ciência e Tecnologia and Galp SPGS S.A. for their financial support through grant No. PDE/BD/52614/2014.

References

¹ Currency units can be provided upon request and with the permission of Galp’s Legal Department.
Introduction

Reactive polyurethane hot melt adhesives (PU-HMR) are prepolymerized from polyester- and/or polyether-ethers and diisocyanates. The urethane linkage (-NH-CO-) is a result of the reaction between the diisocyanate (-NCO) groups and polyols’ hydroxyl groups (-OH). They form block copolymers composed of alternating soft and hard blocks or segment. The soft segment provides elastomeric character to the polymer while the hard segment ensures dimensional stability. PU-HMR are moisture-curing polyurethane adhesives applied typically at a temperature between 85 °C and 140 °C in the form of a melt, then solidifying at room temperature. The final curing is attained at 24 to 48 hr. The initial bond strength of PU-HMR is activated by the solidification of the polymer and the final strength is achieved by the reaction of free NCO with both wood and moisture [2]. Reactive hot melts have two different stages as a moisture-curing adhesive. Firstly, initial bonding strength is developed after the cooling process, when the crystallization of the soft segments occur. This first stage is called physical crosslinking. Then, the isocyanate groups start to react with the moisture, being this second stage called chemical crosslinking. One of the important properties that distinguishes PUR hot melts from other PUR adhesives is the green strength. This property is the immediate adhesive strength prior to curing [3].

Objectives

HotPUR project is promoted by Lorcol, in cooperation with Faculdade de Engenharia da Universidade do Porto, Instituto Politécnico de Viseu and Associação Rede de Competência e Polímeros, as R&D partners, and Vicaima as the final user of the product to be developed. The project’s main objective is to provide Lorcol with a new line of products: reactive polyurethane hot-melt adhesives, PU-HMR. This will allow Lorcol to fill a gap in its product portfolio. The company will be able to offer a complete range of adhesives for the wood industry. In this work, different formulations of PU-HMR with different polyols and isocyanates were produced in order to evaluate the effect of the most relevant parameters on bonding performance and though their ability for edge banding in doors manufacture. Different polyols and isocyanates were tested. Adhesive characteristics such as the final viscosity, open time, wood bonding and thermal properties, were studied. The green strength, as well as the final cure were evaluated by ABES.

The synthesis of polyurethane materials was developed by Otto Bayer in 1937 and they became the most important organic polymers for technological applications. Polyurethane hot melt adhesives (PU-HMR) have attracted high attention and have been widely used, because of their features such as good green strength, excellent adhesion to multiple substrates and good resistance to heat [1]. These type of adhesives are prepared from polyester- and/or polyether-based polyols and diisocyanates. In this work, PU-HMR synthesis using different formulations was performed in order to evaluate the influence of the most important parameters on bonding performance and though their ability for edge banding in doors manufacture. Different polyols and isocyanates were tested. Adhesive characteristics such as the final viscosity, open time, wood bonding and thermal properties, were performed.

Methods

The synthesis was carried out in a 5-necked glass reactor equipped with mechanical stirrer, thermometer, and nitrogen gas inlet system. The reaction temperature was controlled with an oil bath. Firstly, the polyols and the filler were put inside the reactor, then the mixture homogenized, followed by the adding of the MDI under nitrogen atmosphere. Inert atmosphere was maintained throughout the reaction to avoid the ingress of atmospheric moisture. The reaction temperature was around 90-100°C. At the end of the reaction the hotmelt is collected for aluminum cartridges (for bonding tests) and for aluminum foil packaging (for physical and chemical characterization). The characterization of PU-HMR included the measurement of Brookfield viscosity, softening temperature and the thermal analysis using TGA (Thermogravimetry) and DSC (Differential Scanning Calorimetry). These are important parameters for adhesive quality control and provide important information about crystallinity. In wood industry applications, high initial bonding strength is advantageous and important since for certain applications, it is crucial a short setting time in order to assure high productivity. One of the techniques that can be used to evaluate this property is Automated Bonding Evaluations System (ABES), which has already proven to be an excellent tool for bonding performance evaluation of other type of adhesives (e.g. UF (urea-formaldehyde) adhesives) [4] [5]. Thus, the curing time for the different PU-HMR were evaluated using ABES [4,5]. This equipment is based on a single lap shear test, the joint is small (60-100 mm2) and the conditions such as temperature, pressing time can be adjusted.
Results

Polyurethane prepolymers were synthesized with molar excess of diphenyl methane diisocyanate (MDI) and with different polyols. Different formulations were performed in order to evaluate the influence of the most important parameters on bonding performance and though their ability for edge banding in doors. Amorphous and crystalline polyols and isocyanates with different molecular weights and reactivity, were tested. In table 1, the results of different formulations synthesized, using the same value of %NCO and NCO/OH, are presented. Also, for comparison purposes, a commercial adhesive was also characterized.

Table 1. Results of the characterization tests of PU-HMR samples.

<table>
<thead>
<tr>
<th>PU-HMR Samples</th>
<th>T_{Ball Softening} (ºC)</th>
<th>Viscosity (cP) at 150ºC</th>
<th>Open time (s)</th>
<th>Green strength</th>
</tr>
</thead>
<tbody>
<tr>
<td>Commercial adhesive</td>
<td>61 - 67</td>
<td>30 000</td>
<td>&lt; 60</td>
<td>Good</td>
</tr>
<tr>
<td>1L</td>
<td>70 - 71</td>
<td>49 000</td>
<td>5</td>
<td>Poor</td>
</tr>
<tr>
<td>2L_BD</td>
<td>57 - 58</td>
<td>30 000</td>
<td>45</td>
<td>Good</td>
</tr>
<tr>
<td>3L</td>
<td>56 - 58</td>
<td>17 000</td>
<td>&lt; 60</td>
<td>Very Good</td>
</tr>
</tbody>
</table>

From the achieved results, it is possible to conclude that the formulation 1L shows the highest value for melting temperature and viscosity, but a very small open time and a poor green strength. The 2L_BD and 3 L samples showed a behavior similar to the one of the commercial adhesive. On the other hand, the adhesive 3L has a very good green strength.

Figure 1 shows the thermograms of the studied PU-HMR adhesives. It can be observed that the melting temperature of commercial adhesive has a higher melting point than the formulations 1L, 2L_BD e 3L.

Performance evaluation of the polyurethanes produced in this work were assessed using ABES. Firstly, it was tried the application of the conventional method to our resins. However, it was not possible to control the adhesive load applied in the beech strip. In order to overcome this drawback, a hot melt dispenser gun was used to apply resins in beech strips. Different pressing times, using a constant pressure of 2.5 bar were used. Then a study concerning the behavior of the synthesized formulations over time was carried out in order to evaluate the final curing time. Figure 3 shows the ABES curve for different adhesive formulations (commercial adhesive and formulations 1L, 2L_BD e 3L) for a pressing time of 30 s for different pull-out times.

Figure 1. Thermogram of formulations: Commercial adhesive, 1L, 2L_BD, 3L (endo ↓).

Figure 2. Evolution of the specific shear strength over a week obtained using ABES equipment.

Conclusions

From this study, it was verified that different PU-HMR formulations have different properties, being possible to adjust them by changing the synthesis parameters, which ensure the obtainment of an excellent tailor-made product, with very distinguished results. It was also showed, that ABES is a very sensitive technique able to quantify the strength of the PU-HMR. One of main difficulty for ABES evaluation is the high PU-HMR’s viscosity making the hot melt application very challenging.

Acknowledgements

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References:

Company Overview

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Poster Session
MULTISCALE AND MULTIDISCIPLINARY ENGINEERING EDUCATION
A simple laboratory approach for the determination and characterization of ternary phase diagrams for Chemical Engineering undergraduate students

E.V. Capela, J.H.P.M. Santos, I. Boal-Palheiros, J.A.P. Coutinho, S.P.M. Ventura, M.G. Freire*

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maragfreire@ua.pt

In this work, a simple experimental protocol to determine liquid-liquid phase diagrams of aqueous two-phase systems (ATPS) on a Chemical Engineering course is described. To this end, the liquid-liquid phase diagrams composed of water, PEG (200, 400, 600 and 1000 g mol⁻¹) and sodium carbonate (Na₂CO₃) must be determined through the cloud-point titration method at the laboratory room temperature. Throughout this laboratory set of experiments, the liquid-liquid ternary phase diagrams, tie-lines, tie-line lengths and critical points of ATPS were determined. This novel educational approach could be potentially used to teach and help understanding 3-component liquid-liquid equilibrium as well as the formation of biphasic systems to undergraduate students, without requiring the use of volatile organic solvents.

In recent years, liquid-liquid extraction has gained great relevance in the chemical engineering, biochemistry and biotechnology fields. In this context, the design of more eco-friendly and cheaper protocols for liquid-liquid extraction/purification of (bio)molecules is imperative from an industrial point of view. Aqueous two phase systems (ATPS) fit within liquid-liquid extraction systems and are formed by two hydrophilic solutes dissolved in water above certain concentrations [1]. Due to their water-rich phases, simplicity, high resolution capacity and ability to be scaled-up, ATPS have emerged as prominent purification platforms. Beyond their industrial value, phase diagrams bear also a pedagogical interest since they are an essential subject to study and have a decisive role in the academic laboratories. Phase diagrams are an efficient tool to represent this type of information, enabling to grasp at a glance whether two or more substances are mutually miscible, or how pressure or temperature can be tuned to achieve a particular set of equilibrium conditions [2].

In this sense, it is proposed a two task laboratory experiment, consisting in the determination of binodal (solubility) curves of ternary systems constituted by water, PEG (200, 400, 600 and 1000 g mol⁻¹) and sodium carbonate (Na₂CO₃), and the determination of tie-lines, tie-line lengths and critical points for each polymer-salt ATPS. The first task was utterly experimental, and the second required the combination of experimental data with calculations. The experimental procedure used for defining the binodal curves was based on the cloud point titration method [3], in which the endpoint is the appearance (or disappearance) of turbidity when the system changes from one phase to two phases (or vice versa). The determination of the tie-lines by the combination of experimental and calculated data allows a considerable reduction in laboratory time, which is a most important issue not only for this lab experiment but in general, when scheming novel industrial separation processes. Also, the cloud point titrations may often present ill-defined endpoints, a disadvantage that may be overwhelmed by the correlation of the experimental data. This experiment may be part of Chemical Engineering course (e.g. 3rd year) and similarly oriented courses where separation processes are an important issue. It was designed to be taught in two practical laboratory classes and also to include more modern computational methods in the elaboration of the phase diagrams. Students were able to translate the theory on how to determine phase diagrams and characterize them to the practice, and also on the real application of calculus software to solve chemical engineering challenges. If lectured in laboratory, this experimental exercise will allow students to become acquainted with the principles behind the basic thermodynamics of ternary liquid-liquid systems and/or ATPS and with the necessary tools for the characterization of such systems. In addition, the influence of using different polymers as phase-forming components in the ability to form ATPS and in the effect on the parameters that characterize each ATPS it will be two important questions to be analyzed by the students.

This lab work was thought as a way to update the curriculum and to reflect environmental issues for Biotechnology, Chemical Engineering, Chemistry and Biochemistry students at an intermediate level. The ATPS presented herein will familiarize students with a viable and more eco-friendly alternative to conventional liquid-liquid extractions and separation processes in which the use of organic volatile solvents is avoided, and using techniques that from an administrative point of view offer low operational costs.

Acknowledgements

This work was developed within the scope of the project CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID/CTM/50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement. E.V. Capela, J.H.P.M. Santos and S.P.M. Ventura acknowledges FCT for the PhD grants SFRH/BD/126202/2016, SFRH/BD/102915/2014 and the IF contract IF/00402/2015, respectively. M.G. Freire acknowledges the European Research Council (ERC) for the Starting Grant ERC-2013-StG-337753.

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References
The purpose of this work is to promote an active learning based on the joint use of several methodologies (flipped classroom, peer instruction or gamification) in several subjects of the Chemical Engineering Degree at the Technical University of Madrid. Specifically, this approach has already been implemented in Process Control (4th course) and will also be applied in Process and product design (4th course) and Chemical Reactors (3rd course) during the next academic year. In order to implement the mentioned methods, the following material has been developed: screencasts, concept tests, trivia contest and simulations besides the traditional material (slides and text). The results obtained in Process control subject after the implementation of this methodology for the first time show increasing student motivation, higher participation in class and better results (marks) in the subject. The same results are expected for the other two subjects.

**Methodology**

Flipped classroom is applied using educational videos/screencasts and complemented with traditional material as slides or texts. The student learns by himself in advance the contents of the topic to be discussed in the following class; the peer instruction is implemented using concept test, and finally, gamification is based on the use of games (like trivia) to learn and understand in a funnier way. It is important to remark that the methodology applied is not based on a single one but on the application and integration of several ones. In order to implement the mentioned methods, the following material has been developed: screencasts, concept tests, trivia contest and simulations besides the traditional lecturing material (slides and text).

**Results and conclusions**

The results obtained in Process control subject after the implementation of this methodology for the first time show increasing student motivation, higher participation in class and better results (marks) in the subject. The same results are expected for the other two subjects.

**References**

Exploring the non-ideal behavior of coiled and straight tubular reactors

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Flow characterization in chemical reactors is a challenging and important topic in chemical engineering education. This work describes a simple and inexpensive experimental set-up for determining residence time distribution (RTD) curves in tubular reactors, using a conductivity cell to measure the outlet concentration of a pulse injection of an inert tracer. The experimental results are compared with different model alternatives (e.g. laminar flow, cascade of n-CSTR, axial dispersion) and used to evaluate the characteristic parameter(s). In a second stage, the saponification of ethyl acetate is performed in the tubular reactors to evaluate steady state conversion. The results are compared with predicted conversions obtained with the total segregation model and the suggested RTD model alternatives. The experimental procedure and calculations were performed by several students at University of Aveiro and proved to be very useful to grasp the underlying concepts.

Introduction

In undergraduate chemical reaction engineering courses the flow in tubular reactors is usually considered ideal, i.e. plug-flow reactor (PFR). However, non-ideal behavior is important to assess reactor performance and for scale-up studies. The Residence Time Distribution (RTD) technique is a powerful tool to evaluate the hydrodynamic behavior of chemical reactors. Experimental RTD curves can be obtained by simple procedures and compared to theoretical flow models [1].

The experimental set-up comprises two alternative jacketed reactors - a straight tube and a coiled tube (see Graphical Abstract), preceded by a static micro-mixer device. The tracer can be injected “before” or “after” the micro-mixer. At the reactor outlet, a conductivity cell connected to a data acquisition interface allows the online measurement of effluent concentration [2]. The system is equipped with two reservoirs for the reagents used in the test reaction, i.e. the saponification of ethyl acetate (“A”) with sodium hydroxide (“B”) in excess. The reagents are fed to the tubular reactor unit through a peristaltic pump. Further details of the experimental set-up can be found elsewhere [3].

Methodology

The experimental procedure is carried out in three main steps: (i) the conductivity cell is calibrated; (ii) a pulse input experiment is performed using a sodium hydroxide solution as tracer; (iii) the reactor is continuously fed with both reagents (“A” and “B”) and the outlet conductivity signal (AK) is registered when steady state is achieved.

The experimental RTD curve, $E(t)$, is obtained from Eq.(1)

$$E(t) = \frac{C_{exp}(t)}{I_{exp}}$$  \hspace{1cm} (1)

where $C_{exp}(t)$ represents the outlet concentration of the tracer at instant $t$. The residence time, $\bar{t}$, and the variance, $\sigma^2$, are calculated Eq. (2) and Eq.(3), respectively:

$$\bar{t} = \int_0^\infty t E(t)dt$$ \hspace{1cm} (2)

$$\sigma^2 = \int_0^\infty (t-\bar{t})^2 E(t)dt$$ \hspace{1cm} (3)

The experimental conversion of ethyl acetate, $x_{exp}$, is calculated by Eq.(4),

$$x_{exp} = \frac{\Lambda_B K - h_B K}{C_{Ac}(\Lambda_A O^- - \Lambda OH^-)}$$  \hspace{1cm} (4)

where, $\Lambda_B K$ is the conductivity of reagent “B” at inlet conditions, $C_{Ac}$ is the feed concentration of reagent “A”, and $(\Lambda_A O^- - \Lambda OH^-)$ is obtained from the calibration curves.

Theoretical conversion, $x_{model}$, was calculated by Eq.(5) (microfluidic hypothesis):

$$x_{model} = \int_0^\infty x(t) E(t)dt$$  \hspace{1cm} (5)

where $x(t)$ represents conversion in a segregated element of fluid considering an irreversible first-order reaction:

$$x(t) = 1 - \exp(-kt)$$  \hspace{1cm} (6)

Here, $k$ is the pseudo first-order kinetic constant at the operation temperature (ca. 24-25°C), obtained from literature.

Table 1 displays the exit age distribution curves, $E(t)$, for three models, namely: laminar flow – Eq.(7); “n” equal-sized CSTR in series – Eq.(8); and, Asymmetric Model – Eq.(9), which is a semi-empirical dispersion model used for describing chromatographic peaks [4]. Other alternatives have been considered by the students such as, for example, Levenspiel’s compartment models and dispersion models [1].

<table>
<thead>
<tr>
<th>Table 1. RTD models.</th>
<th>Model and $E(t)$ equation</th>
<th>Obs.</th>
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</thead>
<tbody>
<tr>
<td><strong>Laminar Model</strong></td>
<td>$E(t) = \left(\frac{t}{\tau}\right)^n H\left(t - \frac{\tau}{2}\right)$</td>
<td>$\tau = \frac{8\bar{t}}{\sigma^2}$, space time</td>
</tr>
<tr>
<td><strong>n-CSTR</strong></td>
<td>$E(t) = \left(\frac{t}{\tau}\right)^n e^{-t/\tau}\left[1 - \frac{t}{\tau}\right]_0^{\infty}$</td>
<td>$\tau_i = \frac{\tau}{n}$, space time in each tank</td>
</tr>
<tr>
<td><strong>Asymmetric Model</strong></td>
<td>$E(t) = h_{\text{max}} \exp\left[\frac{t}{\tau} - 1\right] \left[n(1 + b) - b\right] H\left(t - (\tau_i - \frac{n\tau_i - \sigma^2}{2\sigma^2})\right]$</td>
<td>$H$, unitary step function; $h_{\text{max}}$, peak maximum height; $\tau_{\text{max}}$, time at peak maximum; $\sigma$, standard deviation, $\sigma^2 = n \tau_i^2$; $a$, asymmetry factor; $b = \frac{2\sigma^2 - \tau_i^2}{4\sigma^2}$</td>
</tr>
</tbody>
</table>


Results

Typical results for the pulse input experiments with the coiled tubular reactor are presented in Fig. 1. The curves correspond to different tracer injection points, namely after (μ = 251 s, σ^2 = 1480 s^2) and before (μ = 254 s, σ^2 = 1780 s^2) the micro-mixer compelling the students to question its effect on the overall reactor performance.

Figure 1. Tracer test experimental results for the coated tubular reactor, with injection of the tracer after (○) and before (●) the micro-mixer – see Graphical Abstract.

In Fig. 2, the experimental RTD curve (injection before mixer) is compared with diverse theoretical curves. The most striking differences signaled by the students were the deviation from ideal behavior (PFR) and the lower experimental value of μ, in comparison to space time, τ. This is due to experimental errors in the evaluation of reactor volume (V_r) or flow rate (Q_r), compatible with a stagnant zone model.

Figure 2. RTD curves for the coated reactor: experimental results (○), “Fitted” – Eq.(9); “Laminar” – Eq. 7; n-CSTR model – Eq.(8) with n = 1, 60 and 120.

The slightly asymmetric peak was easily fitted with the dispersion model represented by Eq.(9). The n-CSTR model – Eq.(8) proved suitable provided τ is replaced by μ. Overall, the calculations allowed the students to develop their skills and understand the underlying concepts of reactor modeling. The experimental and theoretical RTD curves for the straight tubular reactor are shown in Fig. 3, along with the results for the coated reactor. In this case, the laminar flow model – Eq.(7) provides a better fitting. With these results the students understand the strong relation between reactor geometry and ideal/non-ideal behavior of real equipment and grasp the potential of the RTD technique to diagnose flow behavior.

Figure 3. Experimental RTD curves for the coated (○) and straight (●) tubular reactors; “Laminar model” – Eq. 7.

In terms of results for the saponification reaction in the coated reactor, the experimental conversion (x exp = 0.28 ± 0.03) the conversion calculated with the total segregation model - Eq.(5), using the experimental RTD curve (x model = 0.25), were very similar.

Conclusion

This work presents a simple and inexpensive experimental set-up and procedure to promote the development of fundamental concepts concerning the characterization of non-ideal behavior of chemical reactors, based on the stimulus/response RTD technique. The results were obtained using straight and coated tubular reactors, and were modeled using different alternatives: laminar flow, n-CSTR in series, dispersion-based models.

Acknowledgements

The authors acknowledge the collaboration of R. Santos and P. Carvalho during the installation of the data acquisition system, A. Morais for making the glass coated reactor, A.I. Caço for all the help provided before and during the laboratory classes, and A. Barros-Timmons for her enthusiasm and continuous improvement goals. A special word goes to the Chemical Engineering 4th year students J. Conceição, M. Sousa, S. Gonçalves, D. Rocha, J. Igléssias, and M. Teixeira for their contribution, opportune observations and analyses. Finally, to the Chemistry Department for providing the facilities.

References

Pushing the dynamics and the outputs of laboratory courses

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Experimental courses contribute to the development of practical skills as well as a series of soft skills. Furthermore these courses are also useful to demonstrate the relevance of the fundamental courses taught previously and help the students to go through a self-evaluation process of the command they have of theory. When large numbers are involved, i.e. 2 or 3 classes with circa 15 students, continuous supervision can be a challenging task, especially if the flow and dynamics of practical work are to be kept throughout the entire semester. In this work we present and discuss the set of activities that are being used in 4th year Chemical Engineering laboratory classes in order to push the dynamics and the outputs in these courses.

Background
The chemical engineering laboratories EQ1 and EQ2 are compulsory courses of the fourth year (1st and 2nd semesters, respectively) of the Integrated Master in Chemical Engineering at University of Aveiro (MIEQ). This set of laboratory courses covers concepts related to transport phenomena, applied thermodynamics, reactor engineering and separation, control and separation process which have been previously taught. In particular EQ1 focuses on equilibrium based and steady state processes, while EQ2 addresses non-equilibrium and non-steady state processes.

Objectives
Laboratory courses are demanding, time consuming, and actually expensive. However, sometimes, these courses are not always very efficient due to idle periods, significant percentage of copying or at least consultation of reports from previous years, and more often than not, boring oral presentations at the end of the semester. Therefore, there is a need to push the dynamics and the outputs of laboratory courses. Hence, in this work we report on a multi-tactic approach implemented in our laboratory courses which take place either sequentially or simultaneously in order to increase the dynamics. As regards the outcomes, different evaluation methods and levels of data treatment combined with distinct methods of presentation and discussion, help developing both practical and soft skills.

Summary of Laboratory Activities & Dynamics
Both EQ1 and EQ2 courses involve 8-10 different experiments per semester which operate simultaneously on a rota shift basis. Each group carries out 5 out of the whole set of experiments. All practicals consist of 2 week assignments (1 in the lab + 1 in the computer room). Additionally a field trip is also included in the courses. These activities include complimentary ones namely: individual quiz (MT); data treatment & results discussion and reporting. Reporting is varied during the semester and it includes short reports (RS), full reports (FR) and questionnaires (Q).

<table>
<thead>
<tr>
<th>Evaluation component</th>
<th>%</th>
<th>Evaluation component</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>FRA**</td>
<td>10</td>
<td>FRB</td>
<td>15</td>
</tr>
<tr>
<td>SR1</td>
<td>10</td>
<td>Quizzes</td>
<td>15</td>
</tr>
<tr>
<td>SR2</td>
<td>10</td>
<td>FT</td>
<td>5</td>
</tr>
<tr>
<td>SR3</td>
<td>10</td>
<td>PP</td>
<td>10</td>
</tr>
<tr>
<td>Q</td>
<td>10</td>
<td>CA</td>
<td>5</td>
</tr>
</tbody>
</table>

*Weights can vary slightly each semester; ** The topic of the FR and corresponding public presentation are known from day 1.

The practical laboratory classes begin with a 10 min quiz, to verify if students are prepared to carry out the experimental activity. The relevance of this quiz is twofold: 1) it forces students to prepare and plan carefully the laboratory work and 2) provides information whether students understand the fundamental aspects as well as safety issues. Once the experimental activities are all set up and running, the teacher evaluates the quizzes. This allows detecting faulty concepts, misconceptions and potential difficulties. At the end of each experimental work, students upload their experimental observations and data in our E-Learning system, simulating the laboratory notebook. This is important for freezing the
Experimental observations and data whilst preventing plagiarism. In the following week, students work in the computer room where they prepare the written reports. Besides SR and FR, Questionnaires have also been used and are discussed next. FR, are evaluated at 3 different moments: 1) at the beginning of the semester a first version is submitted following a fully detailed template which is corrected and commented by the teacher; 2) the end of the semester, students submit a revised version of this document which includes control charts and appropriate error analysis and 3) students make a public presentation (oral or poster). Additionally, a field trip to visit a nearby industry or a research facility, targeting a specific process, available at smaller scale in the laboratory is generally included in these courses.

Questionnaires
As mentioned above, an alternative to SR and FR is the use of questionnaires. Although there are known formal well-stablished procedures for designing questionnaires for data collection or detailed instruments for deep knowledge evaluations[1], the questionnaires implemented in this course result from a dynamic interaction between the students and the teacher about specific laboratory experiments and are performed during the computer room session. First, the students treat all the experimental results. This is followed by a discussion with the teacher upon which students are challenged to address 2 or 3 specific questions related to the fundamental aspects beyond the experimental results. At the end, a small report is delivered. This classroom activity allows assessing how the elements of a group interact between them, helps developing further skills such as problem solving and enhances their critical thinking in a reduced period.

Control Charts / Error Analysis
The last FR offers a unique opportunity for reviewing all experimental data and results obtained during the whole semester for a specific experiment. Students can collect the information from all and then analyze the overall performance of their main experimental topic by preparing control charts[2]. In turn, the error analysis can be performed taking into account more replicas. This complementary treatment of data helps to consolidate knowledge, and to demonstrate and assimilate the importance of the error analysis.[3]

Public Presentations
This activity takes place at the end of the course. PP are based on FR and involve a sequence of steps: flash presentations by each group, followed by a conventional oral presentation or poster session. The use of the English language is strongly encouraged. Besides the lecturers responsible for the “Chemical Engineering Laboratories”, students, researchers and lecturers of the Department are encouraged to participate in the evaluation of this activity following a systematic procedure based on a table which addresses different parameters. At coffee break, a pool takes place and as a result each group is appointed a second presentation /poster which at the very end, they will have to present, with minimum support (i.e. the karaoke).

Outputs & Student’s perspectives
The efficiency of such intense and diverse activities was assessed using students’ responses to quality assurance questionnaires collected over a 9 year period which reflects the evolution since 2012, when this strategy started to be gradually implemented. Figure 2 summarizes the results obtained.

![Figure 2. Radar plot of answers to quality assurance questionnaires collected over 9 years.](image)

Conclusions
A general diversified and dynamic strategy has been implemented in practical laboratory courses which consists in a multi-tactic approach and that can be adapted to any practical laboratory courses. Although it may seem a stunning amount of work, if it is well distributed along the semester, it has proven efficient whilst keeping the rhythm of work and pushing outputs. Students’ evaluation results over the years prove that the implementation of this strategy has been perceived by the as reduction of work load, yet not a reduction of the level of difficulty. Furthermore, the overall course performance has improved.

Acknowledgements
Our students! For responding positively and with enthusiasm. The Chemical Engineering group for accepting and supporting various ideas & active participation. Chemistry Department & CICECO-Aveiro Institute of Materials for providing the facilities. CICECO-Aveiro Institute of Materials, POCI-01-0145-FEDER-007679 (FCT Ref. UID /CTM /50011/2013), financed by national funds through the FCT/MEC and when appropriate co-financed by FEDER under the PT2020 Partnership Agreement

References
Contribution to Innovation in Chemical Engineering Education Using Online Resources

Introduction

Considering the evolution of technologies and platforms to develop and deliver resources online, all the actors involved can contribute with innovative approaches to improve teaching and learning activities, namely in Engineering education. In addition to the platforms that allow publishing and making available different types of materials in the Web, there are technologies that facilitate remote interaction with experiments and the collaborative development of resources supported in several programming languages.

In engineering courses, collaborative work is of great importance and the use of innovative technologies can offer the environment and the necessary tools to promote and simplify teamwork and interaction between teachers and students and to provide teaching and learning conditions that respond to current societal challenges [1].

This article presents examples of online resources that can be used in Chemical Engineering and in other educational contexts, which can contribute to increase teacher productivity and improve students’ learning process.

Methods

Several examples of various types of resources are available on the Web for different topics. The Portal of Virtual Laboratories of Chemical Processes – LabVirtual (http://labvirtual.eq.uc.pt/) is a significant example of a platform for sharing knowledge and a tool to support teaching and learning activities at different levels of Chemical Engineering [2]. As a result of another project, a set of multimedia materials was also developed as a tool to learn about the characterization of nanoparticles [3].

The Jupyter Notebook environment is an open-source project that can be used to support the development and sharing of interactive data and scientific computing using several programming languages, such as MATLAB.

In this context, teachers have a programming environment to develop and share educational materials through the Web, combining different types of resources, such as text, images and code, into a single document. This article aims to contribute to innovation in Chemical Engineering education by presenting some examples of online resources supported by online experimentation and the Jupyter Notebook environment, which can be used to increase teacher productivity and enhance the students learning process.

The improvement of the teaching and learning activities, namely in Engineering education, should be a permanent concern of all involved actors and the existence of multiple tools and platforms to develop and provide online resources should be considered as an additional motivation to develop and implement innovative processes and materials. By providing materials and experiments online, students have the ability to use and access them without severe scheduling restrictions. Using Jupyter Notebook, teachers have a programming environment to develop and share educational materials through the Web, combining different types of resources, such as text, images and code, into a single document. This article aims to contribute to innovation in Chemical Engineering education by presenting some examples of online resources supported by online experimentation and the Jupyter Notebook environment, which can be used to increase teacher productivity and enhance the students learning process.
Given the large flexibility to structure and organize the content of the notebooks, they can support different teaching approaches and learning strategies, and allow the interaction with remote and virtual laboratories.

As an example, Figure 2 shows the Web interface of a remote laboratory to control a nonlinear three-tank system. In this framework, the user can observe the behavior of the system through the camera image and the temporal evolution of references and outputs of the system, verifying the performance of the closed-loop system.

Several examples of notebooks and remote experiments can be developed with content tailored to specific topics, using, for example, data fetched from the remote system to present various approaches for data analysis and processing.

Acknowledgements
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References
Developing a framework for assessing the teaching effectiveness in Chemical Engineering education

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Evaluating the effectiveness of teaching and learning of core knowledge outcomes and professional skills is a highly challenging task that has not yet been satisfactorily addressed up to this date at higher education level. The iTech European project consortium developed a framework for assessing the effectiveness of various pedagogical methodologies in chemical engineering education, in particular those aimed at promoting important core competencies related to employability, in a range of geographical and educational contexts. The framework was tested on a range of subject areas from various branches of engineering, and other areas. The results of this broader testing encompassed a much more diverse student body with varying educational experiences and a wider range of different teaching methodologies. The outcomes of this testing are highlighted and the benefits of such an objective approach to evaluating teaching effectiveness is discussed.

Introduction

Addressing demographic changes is amongst the key focal points of the European Union (EU) and one of the EU headline targets is: “The share of early school leavers should be under 10% and at least 40% of the younger generation should have a tertiary degree” [1]. Two of the proposed ‘Europe 2020’ flagship initiatives are of special relevance to education and training:

• The “Youth on the Move” initiative; and
• The “Agenda for new skills and jobs” initiative, which highlights the need to upgrade skills and to boost employability. Progress has to be made to improve the identification of training needs, increase the labor market relevance of education and training, facilitate individuals’ access to lifelong learning opportunities and guidance, and ensure smooth transitions between the worlds of education, training and employment [2].

The reasoning of the iTech project was directly connected with the second flagship initiative of “Europe 2020”. The changing technology needs of a global knowledge economy are challenging the nature of engineering practice, demanding far broader skills than simply the mastery of scientific and technological disciplines [3]. The educators at the universities have a tendency to place emphasis predominantly on the fundamental knowledge and research methodologies in their courses rather than practical experience and engineering practice [4]. Thus, a more extensive involvement of industrial experts in the curriculum design and delivery is required to ensure that relevant competencies are developed. In addition, it is important that robust criteria of assessing the effectiveness of the core knowledge and competency delivery are implemented across the higher education sector to ensure the achievement of key aims of European education policies. After all, it is generally recognized that ‘What gets measured, gets improved’ [4]. Thus, evaluating the effectiveness of teaching and competency development will enable the identification of the most effective ways of promoting these competencies and ultimately promote the improvement of their delivery.

Objective

The iTech EU project [5] main objective was to develop a framework for assessing the effectiveness of various pedagogical methodologies in Chemical Engineering education, particularly those aimed at promoting important core competencies related to employability, in a range of geographical and educational contexts.

Methods

The iTech project methodology towards the development of the assessment framework was the following:

1 – Review the learning outcomes of chemical engineering education;
2 – Promote closer involvement of employer organizations in chemical engineering curriculum formation;
3 – Establish state-of-the art in assessing the effectiveness of teaching of core chemical engineering knowledge;
4 – Define various indicators of the effectiveness of teaching in chemical engineering higher education;
5 – Investigate in more depth methods of effectively acquiring employability competencies;
6 – Use decision making technology and multi-objective optimization to identify the most appropriate evaluation methods;
7 – Test the framework at partner institutions focusing on various pedagogic methodologies.

Results

The main outcome of iTech was the development of a tool allowing the framework exploitation by any higher education institution interested in the assessment, comparison and communication of their teaching effectiveness for a given course. The developed assessment framework considers 6 metrics, evaluated in a scale ranging from 1 to 5, as follows (see Graphical Abstract):

1 – Strategic nature of the course/discipline. Metric 1 deals with the importance of a teaching unit (course) for the global learning outcomes of the study program. Does this teaching unit (course) bring necessary knowledge and skills and is it adapted to what the graduates are expected to apply in professional situation?
2 – Relevance of the proposed formation. Metric 2 assesses the content of the teaching unit (course). Does it allow to reach a sufficient level, and does it cover all it should?

3 – Relevance of the proposed pedagogy. Metric 3 clearly relies on the pedagogy and on the chosen teaching method. Does it allow an efficient acquisition of the skills and knowledge to be taught?

4 – Perception of relevance of the pedagogical approach. Metric 4 assesses the perception of the teaching unit (course) by the students, from a qualitative and organization point of view.

5 – Evaluation of acquisitions. Metric 5 deals with knowledge acquisition by students as measured mostly by the marks at the end of the teaching unit (course).

6 – Evaluation of transfer. Metric 6 assesses not only the teaching efficiency of a single teaching unit (course) but gives also a measure of the whole formation. The evaluation of transfer must be performed in professional context, during internship if possible, or during the early career years.

The estimation of these 6 metrics requires dissemination of the online questionnaire available at the iTeach website [5] to the interested parties on the educational process (i.e. the stakeholders). The gathered opinions must be uploaded into the developed tool which automatically converts them into a single classification, varying 1 to 5, for each stakeholder and metric: A – Academics; E – Employers; G - Graduates (i.e. graduated in less than 5 years); S – Students.

Table 1 lists the equations and the weight that each stakeholder holds for metric’s estimation.

<table>
<thead>
<tr>
<th>Metric</th>
<th>Equation</th>
<th>Eq.</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>M1=(2A+G+2E)/5</td>
<td>(1)</td>
</tr>
<tr>
<td>2</td>
<td>M2=(2A+G+E+S)/5</td>
<td>(2)</td>
</tr>
<tr>
<td>3</td>
<td>M3=(2A+2G+S)/5</td>
<td>(3)</td>
</tr>
<tr>
<td>4</td>
<td>M4=S</td>
<td>(4)</td>
</tr>
<tr>
<td>6</td>
<td>M6=(A+2G+2E)/5</td>
<td>(5)</td>
</tr>
</tbody>
</table>

Equations listed in Table 1 result from discussions that took place in focus group sessions including stakeholders from Portugal, UK, Germany, France, Slovakia and Macedonia. The equation to estimate metric 5 (Eq. (6)) is not shown in Table 1 because it is based solely on student’s marks, and so it is not retrieved from the online questionnaire. To assess the evaluation of acquisitions by the students when employing different pedagogical methodologies, Eq. (6) considers the average mark (AM) and standard deviation (STD) in the course and cohort, regarding to the assessment year (y) and the previous 3 years.

\[
M5 = \left( \frac{AM^{course}}{\sum_{i=1}^{n} AM_{i}^{course}} / 3 \right) \times 3
\]

Hence, marks increase should be as high as possible, but above the cohort performance to have the highest impact on M5. Similarly, a decrease in the standard deviation is aimed, indicating a more uniform understanding of the cohort (or the absence of students who were lost in some parts of the course).

Moreover, with such arrangement, metric 5 is independent of the grading scale.

Pilot testing of the assessment framework was performed in the countries of the iTeach consortium members (Portugal, UK, France, Germany, Slovakia and Macedonia) for Chemical Reaction Engineering courses, engaging 89 Academics, 101 Graduates, 57 Employers and 217 students (total of 464 stakeholders). The results showed no major differences among partners testing different pedagogical approaches. The main ascribed reason for that is that Chemical Reaction Engineering course is a classical one in Chemical Engineering programmes, highlighting the robustness of the developed framework. Since the latter can be further extended to other topics aside Chemical Engineering, it was piloted for assessing other courses, mostly in engineering programs. Disperse Generation is a course at FEUP from the 5th year of the Integrated Master in Electrical and Computers Engineering (6 ECTS). The assessment resulting from the inputs of 74 stakeholders (15 A, 13 E, 23 G and 23 S) is shown in the Graphical Abstract. Disperse Generation was quite positively assessed by the stakeholders; metrics 1-4 and 6 are all ranked above 4, but metric 5 had a value of 3.0. The latter means that in the current year the pedagogical methodology neither improved nor declined the teaching effectiveness, which was expected since the pedagogical methodology was equal to that used in previous years (i.e. traditional lectures with problem-based activities).

Conclusions

A framework was developed to assess teaching effectiveness of a course, being particularly useful to check the influence of adopting different pedagogical approaches. Although it is expectable that some metrics would not change significantly with different pedagogical approaches, the framework metrics can also be used to compare different courses/institutions teaching the same or similar subjects.

Acknowledgements

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References


The knowledge of equilibrium and mass transfer phenomena is fundamental for the accurate modeling and design of rate-controlled separations. One may cite distillation, dehumidification, extraction and absorption carried out in packed columns, which are frequently covered by Chemical Engineering curricula. The present work focuses the efficiency of a packed column for the distillation of an ethanol-water mixture, where two approaches are compared: the Height Equivalent to a Theoretical Plate and the Height of a Transfer Unit. The column contains small glass spheres randomly dispersed, and two distinct internal flow rates are analyzed. The convective mass transfer coefficients are obtained from experimental data, and compared with estimations provided by various literature correlations, adequate for the type of packing and system under study. Bravo and Fair, and Billet and Schultes correlations were more suitable to calculate the mass transfer coefficients.

Figure 1. Packed distillation column setup.

The NRTL model was chosen as the equation of state (EoS) for the determination of the activity coefficients, since it was the model that presented less temperature deviations face to the experimental values obtained by Kojima et al. (1969). The physical properties of the liquid and the gas mixtures were calculated using literature models [4,5]. The diffusivities of the light component in the liquid and gas phases were calculated respectively by Hsu and Chen, and Wilke and Lee methods; the density of the liquid and gas phases were determined respectively by Jouyban-Acre model and the ideal gas law; the viscosity of the liquid and gas phases were computed respectively by Grunberg and Nissan and Wilke methods. The physical properties of the pure components were also determined using literature correlations or using Aspen Properties V8.4 software. The surface tension of the liquid phase
has a significant impact in the correlations presented above. Therefore, they were evaluated using three different methods to calculate this property [6]: by weighting the surface tensions of pure components; by Tamura et al. method; and by Jouyban-Acree model. The Tamura et al. method presented a better fit the experimental values (error = 3%) obtained by Vásquez et al. [7] (1995).

Table 1. HETP determined via definition and empirical correlations.

<table>
<thead>
<tr>
<th></th>
<th>HETP (m)</th>
</tr>
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<tbody>
<tr>
<td>Reboiler Power (J/s)</td>
<td>68</td>
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<tr>
<td>Internal flow rates</td>
<td>1.74</td>
</tr>
<tr>
<td>(mmol s⁻¹)</td>
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<tr>
<td>Ponchon-Savarit</td>
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<td>McCabe-Thiele</td>
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<tr>
<td>Grandville equation</td>
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</tr>
<tr>
<td>Rule of thumb</td>
<td>0.033</td>
</tr>
<tr>
<td>Kister equation</td>
<td>0.188</td>
</tr>
</tbody>
</table>

Table 2. HTU and overall mass transfer coefficients for both heating powers, using NRTL as EoS.

<table>
<thead>
<tr>
<th></th>
<th>68</th>
<th>96</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reboiler Power (J/s)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Internal flow rates</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(mmol s⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HTU (m)</td>
<td>0.165</td>
<td>0.164</td>
</tr>
<tr>
<td>Kxa (exp)</td>
<td>12.0</td>
<td>17.1</td>
</tr>
<tr>
<td>Kxa (exp)</td>
<td>6.1</td>
<td>8.6</td>
</tr>
</tbody>
</table>

The assessment of the packed bed design was made following three approaches: (i) design equation and HTU constant over the column; (ii) HTU varies point to point; and (iii) the sum of HETP from each theoretical stage of equilibrium. The column height obtained by the three approaches, using Bravo and Fair, and Billet and Schultes correlations, to determine overall mass transfer coefficient from the gas-side, gave similar results with an in the range of 15-30%.

Acknowledgements
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