Universidade de Aveiro
2021

VITÓRIA LOUREIRO DOS LOUROS

BIO- E FOTODEGRADAÇÃO COMO ESTRATÉGIAS PARA A REMOÇÃO DE ESTROGÉNIOS E ANTIBIÓTICOS DAS ÁGUAS RESIDUAIS

BIO- AND PHOTODEGRADATION AS STRATEGIES FOR THE REMOVAL OF ESTROGENS AND ANTIBIOTICS FROM WASTEWATERS

Universidade de Aveiro
2021

VITÓRIA LOUREIRO DOS LOUROS

BIO- E FOTODEGRADAÇÃO COMO ESTRATÉGIAS PARA A REMOÇÃO DE ESTROGÉNIOS E ANTIBIÓTICOS DAS ÁGUAS RESIDUAIS

BIO- AND PHOTODEGRADATION AS STRATEGIES FOR THE REMOVAL OF ESTROGENS AND ANTIBIOTICS FROM WASTEWATERS

Tese apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Doutor em Ciências e Engenharia do Ambiente, realizada sob a orientação científica da Professora Doutora Maria Helena Gomes de Almeida Gonçalves Nadais, Professora Auxiliar do Departamento de Ambiente e Ordenamento da Universidade de Aveiro, do Professor Doutor Valdemar Inocêncio Esteves, Professor Auxiliar do Departamento de Química da Universidade de Aveiro, e do Professor Doutor Jorge Humberto Gomes Leitão, Professor Associado do Departamento de Bioengenharia do Instituto Superior Técnico de Lisboa.

FCT Fundação para a Ciência e a Tecnologia
MINISTÉRIO DA CIÊNCIA, TECNOLOGIA E ENSINO SUPERIOR

UNIÃO EUROPEIA
Fundo Social Europeu

POCH

REPÚBLICA PORTUGUESA

Apoio financeiro da FCT e do FSE no âmbito do III Quadro Comunitário de Apoio.
Apoio via Bolsa de Doutoramento (SFRH/BD/112907/2015).

Cofinanciado por:

FCT Fundação para a Ciência e a Tecnologia
CENTRO 2020
PORTUGAL 2020
UNIÃO EUROPEIA
Fundo Europeu de Desenvolvimento Regional

Apoio financeiro do FEDER, através do CENTRO2020, e de fundos nacionais, através da FCT, no âmbito do projeto de investigação REM-AQUA (PTDC/ASP-PES/29021/2017).

Aos meus pais e irmãs.

o júri

presidente

Doutora Ana Isabel de Oliveira Andrade
Professora Catedrática, Universidade de Aveiro

Doutora Maria Arminda Costa Alves
Professora Catedrática, Faculdade de Engenharia da Universidade do Porto

Doutora Angelina Lopes Simões Pena
Professora Associada com Agregação, Faculdade de Farmácia da Universidade de Coimbra

Doutora Helena Maria Rodrigues Vasconcelos Pinheiro
Professora Associada, Instituto Superior Técnico da Universidade de Lisboa

Doutora Maria Isabel Aparício Paulo Fernandes Capela
Professora Associada, Universidade de Aveiro

Doutora Maria Cristina Guiomar Antunes
Professora Auxiliar, Universidade de Trás-Os-Montes e Alto Douro

Doutor Flávio Gonzaga Castro Santos Silva
Investigador Doutorado, Universidade de Aveiro

Doutora Maria Helena Gomes de Almeida Gonçalves Nadais (Orientadora)
Professora Auxiliar, Universidade de Aveiro

agradecimentos

A concretização desta etapa foi possível graças ao apoio de muitas pessoas. A todos aqueles que me acompanharam gostaria de deixar o meu profundo agradecimento.

À Professora Doutora Helena Nadais, que me incentivou a percorrer esta longa caminhada, por ter acreditado nas minhas capacidades, por todos os conhecimentos transmitidos e por todo o apoio nesta tese. Ao Professor Doutor Valdemar Esteves, pelos preciosos conhecimentos transmitidos e por estar sempre presente em todas as etapas deste trabalho. Por me incutir que desistir não é opção e que a persistência é o segredo para se conseguir obter resultados. Ao Professor Doutor Jorge Leitão pela inteira disponibilidade para ajudar, por todo o apoio e por todas as sugestões.

À Fundação para a Ciência e Tecnologia pelo apoio financeiro, sob a forma de uma Bolsa de Doutoramento (SFRH/BD/112907/2015) e ao projeto REM-AQUA, financiados pelo FEDER, através do PT2020 Partnership Agreement e centro2020, e por fundos nacionais (OE), através da FCT/MCTES. Ao CESAM pelo apoio financeiro (UIDP/50017/2020+UIDB/50017/2020), por parte da FCT/MEC através de fundos nacionais, e pelo cofinanciamento pelo FEDER, no âmbito do Acordo de Parceria PT2020, e COMPETE 2020.

Ao engenheiro Milton Fontes, pela possibilidade da recolha de amostras de lamas e efluente na Estação de Tratamento de Águas Residuais.

À Diana, a quem estarei eternamente grata, por estar sempre presente, pelo apoio incansável, pelos conhecimentos que me transmitiu, por todo o incentivo e por todas as sugestões. E acima de tudo pela excelente pessoa que foi e é para mim. Sem dúvida a pessoa que mais me acompanhou nos bons e maus momentos ao longo deste percurso. A sua ajuda foi fulcral para o desenvolvimento desta tese.

Aos meninos do laboratório 29.2.16, Guilaine, Valentina, Patrícia, Érika, Andreia, Liliana, Marta, Vânia, Luciana, Cindy e Diogo por todos os bons momentos passados, pelo apoio, pelas gargalhadas e também pela troca de conhecimentos. Ao Matheus, pela ajuda no laboratório, pelo apoio e incentivo e principalmente pela sua alegria contagiante que tornou os meus dias muito mais felizes no CESAM.

Às minhas amigas do coração, Raquel, Jukinha, Dalel e Andreia por estarem sempre presentes e pelo apoio incondicional.

À minha família, pais, irmãs, cunhados e sobrinhos, que são os pilares da minha vida. Por me alegrarem mesmo quando estou triste e por estarem sempre presentes. Ao meu namorado, pela força, paciência, companheirismo, e pelos momentos de incentivo.

À minha madrinha, que é e será sempre a minha maior motivação. Por me fazer acreditar que por muito que o percurso seja difícil, terei de lutar e ser forte para atingir todos os objetivos a que me proponho.

palavras-chave

Sulfadiazina, ácido oxolínico, estrona, 17α-etinilestradiol, biodegradação, adsorção, fotodegradação, fotossensibilizadores, modo de operação intermitente do reator UASB.

resumo

Nas últimas décadas, a ocorrência de contaminantes emergentes (CE) suscitou preocupações devido à sua presença no ambiente e ao seu potencial para causar efeitos ecológicos indesejáveis. A sua principal fonte são as descargas de estações de tratamento de águas residuais (ETARs), que não são eficazes na remoção destes poluentes.

A fim de avaliar o destino de CE, bem como de avaliar possíveis estratégias para atenuar a sua presença, o estudo dos processos naturais é de maior importância. Adsorção, biodegradação e fotodegradação aparecem como mecanismos significativos de remoção de CE em ambientes aquáticos.

Entre os CE, dois antibióticos, sulfadiazina (SDZ) e ácido oxolínico (OXA), e dois estrogénios, estrona (E1) e 17α-etinilestradiol (EE2), têm recebido especial atenção devido ao seu elevado consumo e persistência no ambiente.

Uma vez que os antibióticos SDZ e OXA resistem à biodegradação e tendem a permanecer na fase aquosa, e os estrogénios E1 e EE2 têm grande afinidade com a fase sólida, no presente trabalho foram investigadas a fotodegradação dos antibióticos e a adsorção e biodegradação dos estrogénios.

No que diz respeito à fotodegradação de SDZ e OXA, foi avaliado o efeito de fatores ambientalmente relevantes como o pH, a presença de frações de substâncias húmicas estuarinas e a salinidade. Os resultados obtidos indicaram que a pH mais elevado, e na presença de fotossensibilizadores, a fotodegradação da SDZ foi muito mais rápida do que em água ultrapura. Para o OXA foram obtidos resultados diferentes, tendo-se observado uma diminuição significativa da sua fotodegradação na presença de fotossensibilizadores. Estas observações podem explicar os resultados obtidos em matrizes ambientais, nomeadamente, o efluente final de uma ETAR, água doce e água salobra, nas quais a fotodegradação da SDZ ($t_{½}$ entre 2,32 h e 3,48 h) foi muito mais rápida do que em água ultrapura ($t_{½}$ = 6,76 h), enquanto que para o OXA foi muito mais lenta ($t_{½}$ entre 1,65 h e 4,03 h) do que em água ultrapura ($t_{½}$ = 0,99 h).

Nas ETARs os estrogénios são principalmente adsorvidos às lamas. Assim, foi desenvolvido um método simples, fiável e barato para a quantificação de E1 e EE2 em amostras de lamas frescas. Foram obtidos valores de recuperação de 103 % e 97 % para E1 e EE2, respetivamente. Relativamente à remoção de estrogénios em condições anaeróbias, foi investigada a influência de diferentes fatores. Obtiveram-se remoções mais elevadas de E1 da fase sólida das lamas a temperaturas mais elevadas (25 °C e 34 °C) e menor teor de lamas (2 g L^{-1}). No caso do EE2, foram observadas remoções mais elevadas com menor teor de lamas (2 g L^{-1} e 3 g L^{-1}). No entanto, não foi observado qualquer efeito da temperatura na remoção do EE2. A presença de nitrato não parece influenciar a remoção, tanto do E1 como do EE2 das lamas.

Finalmente, foi avaliado o desempenho do modo de operação contínuo (OC) e intermitente (OI) dos reatores de leito de lamas e fluxo ascendente (UASB) na remoção de E1 e EE2. Valores mais elevados de biodegradação (69,4 % *vs.* 43,3 % para E1 e 21,8 % *vs.* 8,0 % para EE2) e de adsorção (26,5 % *vs.* 5,7 % para E1 e 72,7 % *vs.* 31,0 % para EE2) foram obtidos com a OI em comparação com a OC. Assim, a OI dos reatores UASB pode ser uma alternativa promissora, sustentável e robusta para a remoção de E1 e EE2 das águas residuais.

keywords

Sulfadiazine, oxolinic acid, estrone, 17α-ethinylestradiol, biodegradation, adsorption, photodegradation, photosensitizers, intermittent UASB reactor.

abstract

Over the last few decades, the occurrence of emerging contaminants (EC) has raised concerns due to their ubiquitous presence in the environment and potential to cause undesirable ecological effects. Their main source are the discharges of wastewater treatment plants (WWTPs), which are not effective barriers to these pollutants.

In order to assess the fate of EC, as well as to evaluate possible strategies to attenuate their presence, the study of natural processes is of the greatest importance. Adsorption, biodegradation and photodegradation appear as significant mechanisms of removal of EC in aquatic environments.

Among the EC, two antibiotics, sulfadiazine (SDZ) and oxolinic acid (OXA), and two estrogens, estrone (E1) and 17α-ethinylestradiol (EE2), have received considerable attention because of their high consumption and persistence in the environment.

Since SDZ and OXA resist to biodegradation and tend to remain in aqueous phase, while E1 and EE2 have high affinity to solid phase, in the present work, the photodegradation of antibiotics and the adsorption and biodegradation of estrogens were investigated.

In what concerns SDZ and OXA photodegradation, the effect of environmentally relevant factors such as the pH, the presence of fractions of estuarine humic substances and salinity were evaluated. The results obtained indicated that at higher pH, and in the presence of photosensitizers, the SDZ photodegradation was much faster than in ultrapure water. Different results were obtained for OXA, with a significant decrease in its photodegradation in presence of photosensitizers. These observations may explain the results obtained in environmental matrices, namely the final effluent of a WWTP, fresh water and brackish water, in which the photodegradation of SDZ ($t_{½}$ between 2.32 h and 3.48 h) was found to be much faster than in ultrapure water ($t_{½}$ = 6.76 h). In contrast, OXA photodegradation was much slower in the final effluent of a WWTP, fresh water and brackish water ($t_{½}$ between 1.65 h and 4.03 h) than in ultrapure water ($t_{½}$ = 0.99 h).

In WWTPs estrogens are mainly adsorbed onto sludge. Thus, a simple, reliable and inexpensive method for the quantification of E1 and EE2 in fresh sludge samples was developed. Recovery values of 103 % and 97 % were obtained for E1 and EE2, respectively. Regarding the estrogens' removal under anaerobic conditions, the influence of different factors was investigated. A higher E1 removal from solid phase of sludge was obtained for higher temperatures (25 °C and 34 °C) and lower sludge content (2 g L^{-1}). In the case of EE2, higher removal was obtained for lower sludge content (2 g L^{-1} and 3 g L^{-1}). However, temperature had no effect on the removal of EE2. The presence of nitrate does not appear to influence the removal of both E1 and EE2 from sludge.

Finally, the performance of continuous (CO) and intermittent operation (IO) of Upflow Anaerobic Sludge Blanket (UASB) reactors on the removal of E1 and EE2 was assessed. Higher biodegradation values (69.4 % *vs.* 43.3 % for E1 and 21.8 % *vs.* 8.0 % for EE2) and adsorption values (26.5 % *vs.* 5.7 % for E1 and 72.7 % *vs.* 31.0% for EE2) were found with the IO, compared to CO. Thus, the IO of UASB reactors can be a promising, sustainable, and robust alternative for E1 and EE2 removal from wastewaters.

CONTENTS

PART II – Evaluation of the removal of estrogens in aqueous and sludge samples

CHAPTER 3 – Determination of estrone and 17α-ethinylestradiol in digested sludge by ultrasonic liquid extraction and high-performance liquid chromatography with fluorescence detection

CHAPTER 4 – Removal of estrone and 17α-ethinylestradiol by digested sludge using batch experiments

Final remarks

LIST OF FIGURES

CHAPTER 1 – Introduction

PART I – Evaluation of the removal of antibiotics in the aquatic environment

CHAPTER 2 - Photosensitized degradation of sulfadiazine and oxolinic in water

LIST OF TABLES

CHAPTER 1 – Introduction

PART I – Evaluation of the removal of antibiotics in the aquatic environment

CHAPTER 2 - Photosensitized degradation of sulfadiazine and oxolinic in water

PART II – Evaluation of the removal of estrogens in aqueous and sludge samples

CHAPTER 3 – Determination of estrone and 17α-ethinylestradiol in digested sludge by ultrasonic liquid extraction and high-performance liquid chromatography with fluorescence detection

CHAPTER 4 – Removal of estrone and 17α-ethinylestradiol by digested sludge using batch experiments

CHAPTER 5 – Removal of estrone and 17α-ethinylestradiol in upflow anaerobic sludge blanket reactors using different operation modes

GLOSSARY

ABBREVIATIONS AND SYMBOLS

ABBREVIATIONS

AAE	Aqueous Alkali Extraction
ACE	Acetone
ALK	Alkalinity
A/AO/AE	Anaerobic/Anoxic/Aerobic process
A/AO/O	Anaerobic/Anoxic/Oxic process
AO/A/AO	Anoxic/Anaerobic/Anoxic process
AOB	Ammonia-Oxidizing Bacteria
AO/O	Anoxic/Oxic process
AOP	Advanced Oxidation Process
ARB	Antibiotic Resistance Bacteria
ARG	Antibiotic Resistance Genes
AS	Activated Sludge
ASE	Accelerated Solvent Extraction
BF	Biological Filter
BOD_5	Biochemical Oxygen Demand
CAS	Chemical Abstract Services
CIP	Ciprofloxacin
CD	Carbon Dots
CO	Continuous Operation
COD	Chemical Oxygen Demand
DAD	Diode Array Detector
DAN	Danofloxacin
DIF	Difloxacin
DO	Dissolved Oxygen
DOC	Dissolved Organic Carbon
DOM	Dissolved Organic Matter
E1	Estrone
E2	17β-Estradiol
EC	Emerging Contaminant
EDC	Endocrine-Disrupting Chemical

EE2	17α-Ethinylestradiol
ENR	Enrofloxacin
ET	Enhanced Treatment
FA	Fulvic Acids
FB	Fixed Bed Bioreactors
FLD	Fluorescence Detector
FLU	Flumequine
F/M ratio	Food-Microorganism ratio
FS	Flat Sheet
GC	Gas Chromatography
GCB	Graphitized Carbon Black
GPC	Gel Permeation Chromatography
HA	Humic Acids
HF	Hollow Fibre
HLB	Hydrophilic–Lipophilic Balance
HPLC	High-Performance Liquid Chromatography
HRT	Hydraulic Retention Time
HS	Humic Substances
Hydr. Acid. CASS	Hydrolytic - Acidification Cyclic Activated Sludge System
IC	Inorganic Carbon
Inv. A/AO/O	Inversed Anaerobic/Anoxic/Oxic process
IO	Intermittent Operation
LC	Liquid Chromatography
LEV	Levofloxacin
LG	Lagoon
LIN	Linearity
LLE	Liquid-Liquid Extraction
LOD	Limit of Detection
LOQ	Limit of Quantification
LP	Liquid Phase
MAR	Marbofloxacin
MBR	Membrane Bioreactor
MLSS	Mixed Liquor Suspended Solid

MLVSS	Mixed Liquor Volatile Suspended Solid
Mol. Wt.	Molecular Weight
MOX	Moxifloxacin
MS	Mass Spectrometry
MS/MS	Tandem Mass Spectrometry
NOM	Natural Organic Matter
OC	Organic Carbon
OD	Oxidation Ditch
OLR	Organic Loading Rate
OXA	Oxolinic Acid
PPCP	Pharmaceuticals and Personal Care Products
QA	Quinolone Antibiotic
RHS	Reactive Halogen Species
ROS	Reactive Oxygen Species
RSD	Relative Standard Deviation
SA	Sulfonamide
SARA	Sarafloxacin
SDZ	Sulfadiazine
SMX	Sulfamethoxazole
SP	Solid Phase
SPE	Solid Phase Extraction
SPME	Solid Phase Microextraction
SRT	Solids Retention Time
SS	Suspended Solids
TDS	Total Dissolved Solids
TSS	Total Suspended Solids
TT	Tertiary Treatment
UASB	Upflow Anaerobic Sludge Blanket
UHPLC	Ultra-High-Performance Liquid Chromatography
ULE	Ultrasonic Liquid Extraction
UV	Ultraviolet
VSS	Volatile Suspended Solids
WWTP	Wastewater Treatment Plant

SYMBOLS

C	Concentration value at the inflection point or Concentration of compound at a given irradiation time
C_0	Initial concentration of the compound within the sample or Initial concentration of compound
C_{eff}	Final compound concentration in the liquid phase of treated effluent
$^3DOM^*$	Dissolved Organic Matter in the triplet state
F_{bio}	Compound mass biodegraded
F_{eff}	Compound mass in effluent
F_{inf}	Compound mass in influent
F_{sor}	Compound mass adsorbed onto sludge
k	Rate constant
k_{bio}	Biodegradation rate constant
k_d	Solid-water distribution coefficient
k_{ow}	Octanol/water partition coefficient
$^1O_2$	Singlet oxygen
$^{\bullet}OH$	Hydroxyl radicals
pK_a	Dissociation constant
r	Correlation coefficient
$ROO^{\bullet}$	Peroxyl radicals
SD	Standard deviation
S_w	Water solubility
$s_{y/x}$	Statistical parameter that estimates the random errors in the *y* axis
t	Time
$t_{½}$	Half-life time
V	Reactor volume
v_{up}	Upflow Velocity
X_{VSS}	Volatile suspended solids concentration inside the reactor
σ_y	Standard deviation of the response for replicate measurements

THESIS' MAIN OBJECTIVES AND LAYOUT

MOTIVATION AND RELEVANCE

The population growth has resulted in increased consumption of pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) worldwide (Kaur et al., 2019). These compounds are frequently used in human and veterinary medicine to treat various diseases, to control birth, and even as growth promoters (Ašperger et al., 2014; Cui et al., 2020; Ren et al., 2017; Romero et al., 2012). As PPCPs and EDCs are not completely ingested or metabolised, the environment is continuously loaded with these contaminants (Massé et al., 2014; Tran et al., 2018). Wastewater treatment plants (WWTPs) are not designed to efficiently remove PPCPs or EDCs, constituting a significant source of environmental contamination. Even at low concentrations, several risks have been reported regarding the presence of these contaminants in the environment; among them, the endocrine disruption of wildlife and the induction of bacterial resistance to antibiotics are the greatest concerns (Adeel et al., 2017; Brandt et al., 2013; Sturini et al., 2010).

Among PPCPs, the antibiotics sulfadiazine (SDZ) belonging to the group of sulfonamides (SAs) and oxolinic acid (OXA), a quinolone antibiotic (QA), and among EDCs, the natural estrogen estrone (E1), and the synthetic estrogen 17α-ethinylestradiol (EE2) have raised remarkable concerns due to their ubiquitous presence in the environment and their potential to cause undesirable effects on ecosystems and human health (Atkinson et al., 2012; Lulijwa et al., 2020; Tran et al., 2018). To better understand the fate and persistence of antibiotics and estrogens, as well as to draw possible strategies to mitigate their presence, naturally occurring processes must be considered. Adsorption, biodegradation and photodegradation appear as significant removal mechanisms for these pollutants in aquatic environments (Silva et al., 2016a).

Some studies report that in conventional systems currently implemented in WWTPs, E1 and EE2 can not be effectively removed from the treated effluent (Baronti et al., 2000; Braga et al., 2005a, 2005b; Cargouët et al., 2004; Servos et al., 2005; Ternes et al., 1999). Moreover, due to the properties of these pollutants and their high affinity to solid phase, they are mostly retained in the sludge, being adsorption onto sludge the dominant removal mechanism (Clara et al., 2004; Ternes et al., 2004; Tran et al., 2018). Generally, sludge is applied as fertilizer on agriculture fields, and thereby contribute to the indirect application of estrogens to soils and a concomitant increase in their occurrence in the environment (Ikehata et al., 2007; Martín et al., 2010). Therefore, and owing to the affinity of estrogens to solid phase, it is crucial to assess the influence of different factors on the removal of these pollutants, as well as to find efficient alternative approaches for their removal

from contaminated wastewater and sludge. The analytical difficulties in the identification and quantification of estrogens adsorbed onto the sludge solid phase (due to the extraction procedure needed), contributed to the limited information available in the literature. Consequently, the development of a simple and effective method for determining E1 and EE2 in the solid phase is of great importance, as it will be addressed in Chapter 3.

Recently, important developments were achieved in anaerobic treatment systems mainly due to the advantages that they present comparatively to conventional/aerobic systems. The success of these systems is based on the improved capacity to develop microbial consortia well adapted to the degradation of a large variety of substrates. The intermittent operation (IO) of Upflow Anaerobic Sludge Blanket (UASB) reactors is a strategy for the degradation of substrates that are difficult to metabolize. IO favours the thriving of key microbial groups that perform the degradation of those substrates (Couras et al., 2014) and consists of a periodical interruption of the reactor feed being thus formed by a sequence of alternate feed and feedless periods (Nadais et al., 2011). In anaerobic systems, some substrates are easily metabolized whilst others are not immediately metabolized and may be adsorbed onto the microbial aggregates as is the case of estrogens (Xu et al., 2014; Zeng et al., 2009). This results in the adaptation of the microbial consortium towards the metabolism of recalcitrant substrates. Therefore, the IO of anaerobic reactors is a promising option for the removal of estrogens from wastewaters, due to both the high tendency of estrogens for adsorption onto the sludge aggregates and the high capacity of these systems to develop microbial consortia well adapted for the metabolism of recalcitrant substrates as estrogens. In this context, the study of the performance of IO and continuous operation (CO) of UASB reactors for the removal of E1 and EE2 from wastewaters is of high relevance. For this purpose, the adsorption and biodegradation mechanisms must be considered, as will be provided in PART II – Evaluation of the removal of estrogens in aqueous and sludge samples.

Regarding photodegradation, estrogen elimination by light action has been well-documented in the literature (Atkinson et al., 2011; Bertoldi et al., 2019; Chowdhury et al., 2010; Ren et al., 2017; Silva et al., 2016a, 2016b; Zhang and Li, 2014). Photodegradation can occur in two ways, namely the direct and indirect way (Oliveira et al., 2019; Periša et al., 2013). In the direct way, the contaminant itself absorbs the light, which induces its chemical transformation (Oliveira et al., 2019, 2016). In the indirect way, other substances also present in the water absorb the light, generating reactive oxygen species (ROS) (Oliveira et al., 2019, 2016). These substances, named photosensitizers, can have a dual role, acting as photosensitizers accelerating the

photodegradation of the compound, or having a filter effect retarding the photodegradation by screening sunlight or scavenging reactive species (Oliveira et al., 2019).

In what concerns the antibiotics, some authors remarked that SDZ and OXA resist to biodegradation (Biošić et al., 2017; Lai and Lin, 2009; Li et al., 2020). In addition, it has been stated that SDZ and OXA have low affinity to solids (Lin et al., 2018; Yan et al., 2014). On the other hand, in the case of the antibiotics SDZ and OXA, there is still a lack of information regarding their direct and indirect photodegradation. Therefore, photodegradation may be the feasible process to mitigate the persistence of SDZ and OXA in the environment, as will be addressed in PART I – Evaluation of the removal of antibiotics in the aquatic environment, chapter 2.

OBJECTIVES AND OUTLINE

The main objective of this thesis is to contribute with strategies to lower the presence of E1, EE2, SDZ and OXA in the environment. This goal will be attained by improving the biological anaerobic degradation of E1 and EE2 and the photodegradation of SDZ and OXA. The removal was assessed in two types of matrices (solid and liquid in the case of biodegradation, and liquid in the case of photodegradation). This thesis is divided in two main parts: Part I – Evaluation of the removal of antibiotics in the aquatic environment; and Part II – Evaluation of the removal of estrogens in aqueous and sludge samples; including a total of five chapters organized as follows:

Chapter 1 – Introduction

The introductory chapter comprises a detailed literature review in order to support the established objectives. For this purpose, an overview of the state-of-the-art is described regarding the effects, sources, possible pathways and fate in the environment of PPCPs and EDCs. In addition, the occurrence of PPCPs and EDCs under study, namely SDZ, OXA, E1 and EE2, and their removal in WWTPs worldwide, is discussed. The influence of photodegradation, adsorption and biodegradation processes on the removal of pollutants under study are also herein addressed.

Part I – Evaluation of the removal of antibiotics in the aquatic environment

Chapter 2 - Photosensitized degradation of sulfadiazine and oxolinic acid in water

The second chapter describes a study of the direct and indirect photodegradation of SDZ and OXA. The influence of different factors such as the presence of several fractions of estuarine

humic substances (HS) (humic acids (HA), fulvic acids (FA), and XAD-4 fraction), salinity and the presence of $^1O_2$ and $^{\bullet}OH$ scavengers on the photodegradation of SDZ and OXA are assessed.

To accomplish these main purposes, the following points are highlighted:

i. Understanding of the fate and persistence of the antibiotics SDZ and OXA under photodegradation when present in real matrices, namely fresh, brackish and wastewater;
ii. Evaluation of the influence of water characteristics, such as the presence of organic matter, pH or salinity, and the role they play on SDZ and OXA photodegradation rate.

The work detailed in Chapter 2 is published in the following articles:

Louros, V. L., Silva, C. P., Nadais, H., Otero, M., Esteves, V. I., & Lima, D. L. D. (2020a). Photodegradation of sulfadiazine in different aquatic environments – Evaluation of influencing factors. *Environmental Research*, 188, 109730. https://doi.org/10.1016/J.ENVRES.2020.109730

Louros, V. L., Silva, C. P., Nadais, H., Otero, M., Esteves, V. I., & Lima, D. L. D. (2020b). Oxolinic acid in aquaculture waters: Can natural attenuation through photodegradation decrease its concentration? *Science of The Total Environment*, 749, 141661. https://doi.org/10.1016/J.SCITOTENV.2020.141661

Part II – Evaluation of the removal of estrogens in aqueous and sludge samples

Chapter 3 – Determination of estrone and 17α-ethinylestradiol in digested sludge by ultrasonic liquid extraction and high-performance liquid chromatography with fluorescence detection

Chapter 3 presents a method for the determination of E1 and EE2 in fresh sludge samples. The methodology developed is carried out without any clean-up procedure, simplifying the procedure and reducing analysis time and costs. Concentration levels of E1 and EE2 in real sludge, collected from an operating WWTP located in Aveiro, Portugal, using the developed method are presented.

The main objectives of this chapter are the following:

i. Development of a fast, simple and efficient method to determine E1 and EE2 in fresh sludge samples;
ii. Application of the developed method to determine the concentration levels of E1 and EE2 in real sludge, collected from an operating WWTP.

This study resulted in the following publication:

Louros, V. L., Lima, D. L. D., Leitão, J. H., Esteves, V. I., & Nadais, H. G. (2019). Determination of estrone and 17α-ethinylestradiol in digested sludge by ultrasonic liquid extraction and high-performance liquid chromatography with fluorescence detection. *Journal of Separation Science*, 42(8), 1585–1592. https://doi.org/10.1002/jssc.201801114

Chapter 4 – Removal of estrone and 17α-ethinylestradiol by digested sludge using batch experiments

In the fourth chapter, the influence of different factors on the removal of E1 and EE2 from synthetic wastewater using active sludge (non-sterilised sludge) under anaerobic conditions is investigated.

The main goal of this chapter is:

i. Evaluation of the influence of temperature, mixed liquor suspended solids (MLSS) content, and nitrate supplementation on the removal of E1 and EE2.

This study resulted in the following submitted article:

Louros, V. L., Sousa, A. F., Lima, D. L. D., Santos, P., Leitão, J. H., Esteves, V. I., & Nadais, H. G. Estrogens in wastewaters: can different operating conditions improve their removal by digested sludge?

Chapter 5 – Removal of estrone and 17α-ethinylestradiol in upflow anaerobic sludge blanket reactors using different operation modes

Chapter 5 reports the performance of the CO and IO of UASB reactors for the removal of E1 and EE2 from wastewaters and the results for the removal efficiency of estrogens for both systems are presented, taking into account the main removal mechanisms. In order to accomplish this, the contributions of adsorption and biodegradation on the removal of estrogens under CO and IO are determined. In this context, the following objectives are outlined for this chapter:

i. Comparison of the performance of the CO and IO of UASB reactors for the removal of E1 and EE2 from wastewaters;
ii. Evaluation of the main mechanisms (biodegradation and adsorption) responsible for estrogen removal.

This work resulted in the following article:

Louros, V.L., Lima, D.L.D., Leitão, J.H., Esteves, V.I. & Nadais, H.G. (2021) Impact of UASB reactors operation mode on the removal of estrone and 17α-ethinylestradiol from wastewaters. *Science of The Total Environment*, 764, 144291. https://doi.org/10.1016/j.scitotenv.2020.144291

Final Remarks

A Final Remarks section is dedicated to point out the main conclusions, identify knowledge gaps and propose research guidelines for future studies.

REFERENCES

Adeel, M., Song, X., Wang, Y., Francis, D., Yang, Y., 2017. Environmental impact of estrogens on human, animal and plant life: A critical review. Environ. Int. 99, 107–119. doi:10.1016/j.envint.2016.12.010

Ašperger, D., Tišler, V., Zrnčić, M., Mutavdžić Pavlović, D., Babić, S., Horvat, A.J.M., Kaštelan-Macan, M., 2014. HPLC–DAD–FLD Determination of veterinary pharmaceuticals in pharmaceutical industry wastewater with precolumn derivatization using fluorescamine. Chromatographia 77, 1059–1066. doi:10.1007/s10337-014-2685-x

Atkinson, S.K., Marlatt, V.L., Kimpe, L.E., Lean, D.R.S., Trudeau, V.L., Blais, J.M., 2012. The occurrence of steroidal estrogens in south-eastern Ontario wastewater treatment plants. Sci. Total Environ. 430, 119–125. doi:10.1016/j.scitotenv.2012.04.069

Atkinson, S.K., Marlatt, V.L., Kimpe, L.E., Lean, D.R.S., Trudeau, V.L., Blais, J.M., 2011. Environmental factors affecting ultraviolet photodegradation rates and estrogenicity of estrone and ethinylestradiol in natural waters. Arch. Environ. Contam. Toxicol. 60, 1–7. doi:10.1007/s00244-010-9515-4

Baronti, C., Curini, R., D'Ascenzo, G., Di Corcia, A., Gentili, A., Samperi, R., 2000. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. Environ. Sci. Technol. 34, 5059–5066. doi:10.1021/es001359q

Bertoldi, C., Rodrigues, A.G., Fernandes, A.N., 2019. Removal of endocrine disrupters in water under artificial light: the effect of organic matter. J. Water Process Eng. 27, 126–133. doi:10.1016/J.JWPE.2018.11.016

Biošić, M., Mitrevski, M., Babić, S., 2017. Environmental behavior of sulfadiazine, sulfamethazine, and their metabolites. Environ. Sci. Pollut. Res. 24, 9802–9812. doi:10.1007/s11356-017-8639-8

Braga, O., Smythe, G., Schafer, A., Feitz, A., 2005a. Steroid estrogens in primary and tertiary wastewater treatment plants. Water Sci. Technol. 52, 273–278.

Braga, O., Smythe, G., Schäfer, A., Feitz, A., 2005b. Fate of steroid estrogens in Australian inland and coastal wastewater treatment plants. Environ. Sci. Technol. 39, 3351–3358. doi:10.1021/es0501767

Brandt, E.M.F., de Queiroz, F.B., Afonso, R.J.C.F., Aquino, S.F., Chernicharo, C.A.L., 2013. Behaviour of pharmaceuticals and endocrine disrupting chemicals in simplified sewage treatment systems. J. Environ. Manage. 128, 718–726. doi:10.1016/j.jenvman.2013.06.003

Cargouët, M., Perdiz, D., Mouatassim-Souali, A., Tamisier-Karolak, S., Levi, Y., 2004. Assessment of river contamination by estrogenic compounds in Paris area (France). Sci. Total Environ. 324, 55–66. doi:10.1016/j.scitotenv.2003.10.035

Chowdhury, R.R., Charpentier, P., Ray, M.B., 2010. Photodegradation of estrone in solar irradiation. Ind. Eng. Chem. Res. 49, 6923–6930. doi:10.1021/ie901796x

Clara, M., Strenn, B., Saracevic, E., Kreuzinger, N., 2004. Adsorption of bisphenol-A, 17β-estradiole and 17α- ethinylestradiole to sewage sludge. Chemosphere 56, 843–851. doi:10.1016/j.chemosphere.2004.04.048

Couras, C.S., Louros, V.L., Grilo, A.M., Leitão, J.H., Capela, M.I., Arroja, L.M., Nadais, M.H., 2014. Effects of operational shocks on key microbial populations for biogas production in UASB (Upflow Anaerobic Sludge Blanket) reactors. Energy 73, 866–874. doi:10.1016/j.energy.2014.06.098

Cui, J., Fu, L., Tang, B., Bin, L., Li, P., Huang, S., Fu, F., 2020. Occurrence, ecotoxicological risks of sulfonamides and their acetylated metabolites in the typical wastewater treatment plants and receiving rivers at the Pearl River Delta. Sci. Total Environ. 709, 136192. doi:10.1016/J.SCITOTENV.2019.136192

Ikehata, K., Jodeiri Naghashkar, N., Gamal El-Din, M., 2007. Degradation of aqueous pharmaceuticals by ozonation and advanced oxidation processes: a review. Ozone Sci. Eng. 28, 353–414. doi:10.1080/01919510600985937

Kaur, H., Hippargi, G., Pophali, G.R., Bansiwal, A.K., 2019. Treatment methods for removal of pharmaceuticals and personal care products from domestic wastewater. Pharm. Pers. Care Prod. Waste Manag. Treat. Technol. 129–150. doi:10.1016/B978-0-12-816189-0.00006-8

Lai, H.-T., Lin, J.-J., 2009. Degradation of oxolinic acid and flumequine in aquaculture pond waters and sediments. Chemosphere 75, 462–468. doi:10.1016/J.CHEMOSPHERE.2008.12.060

Li, H., Duan, L., Wang, H., Chen, Y., Wang, F., Zhang, S., 2020. Photolysis of sulfadiazine under UV radiation: Effects of the initial sulfadiazine concentration, pH, NO3− and Cd2+. Chem. Phys. Lett. 739, 136949. doi:10.1016/J.CPLETT.2019.136949

Lin, H., Li, H., Chen, L., Li, L., Yin, L., Lee, H., Yang, Z., 2018. Mass loading and emission of thirty-seven pharmaceuticals in a typical municipal wastewater treatment plant in Hunan Province, Southern China. Ecotoxicol. Environ. Saf. 147, 530–536. doi:10.1016/j.ecoenv.2017.08.052

Lulijwa, R., Rupia, E.J., Alfaro, A.C., 2020. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. Rev. Aquac. 12, 640–663. doi:10.1111/raq.12344

Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2010. Multi-residue method for the analysis of pharmaceutical compounds in sewage sludge, compost and sediments by sonication-assisted extraction and LC determination. J. Sep. Sci. 33, 1760–1766. doi:10.1002/jssc.200900873

Massé, D.I., Saady, N.M.C., Gilbert, Y., 2014. Potential of biological processes to eliminate antibiotics in livestock manure: an overview. Anim. an open access J. from MDPI 4, 146–63. doi:10.3390/ani4020146

Nadais, H., Barbosa, M., Capela, I., Arroja, L., Ramos, C.G., Grilo, A., Sousa, S.A., Leitão, J.H., 2011. Enhancing wastewater degradation and biogas production by intermittent operation of UASB reactors. Energy 36, 2164–2168. doi:10.1016/j.energy.2010.03.007

Oliveira, C., Lima, D.L.D., Silva, C.P., Calisto, V., Otero, M., Esteves, V.I., 2019. Photodegradation of sulfamethoxazole in environmental samples: The role of pH, organic matter and salinity. Sci. Total Environ. 648, 1403–1410. doi:10.1016/j.scitotenv.2018.08.235

Oliveira, C., Lima, D.L.D., Silva, C.P., Otero, M., Esteves, V.I., 2016. Photodegradation behaviour of estriol: An insight on natural aquatic organic matter influence. Chemosphere 159, 545–551. doi:10.1016/j.chemosphere.2016.06.046

Periša, M., Babić, S., Škorić, I., Frömel, T., Knepper, T.P., 2013. Photodegradation of sulfonamides and their N 4-acetylated metabolites in water by simulated sunlight irradiation: kinetics and identification of photoproducts. Environ. Sci. Pollut. Res. 20, 8934–8946. doi:10.1007/s11356-013-1836-1

Ren, D., Huang, B., Xiong, D., He, H., Meng, X., Pan, X., 2017. Photodegradation of 17α-ethynylestradiol in dissolved humic substances solution: Kinetics, mechanism and estrogenicity variation. J. Environ. Sci. (China) 54, 196–205. doi:10.1016/j.jes.2016.03.002

Romero, J., Feijoó, C.G., Navarrete, P., 2012. Antibiotics in aquaculture – use, abuse and alternatives, in: Health and Environment in Aquaculture. InTech, pp. 159–198.

doi:10.5772/28157

Servos, M.R., Bennie, D.T., Burnison, B.K., Jurkovic, A., McInnis, R., Neheli, T., Schnell, A., Seto, P., Smyth, S.A., Ternes, T.A., 2005. Distribution of estrogens, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants. Sci. Total Environ. 336, 155–170. doi:10.1016/j.scitotenv.2004.05.025

Silva, C.P., Lima, D.L.D., Groth, M.B., Otero, M., Esteves, V.I., 2016a. Effect of natural aquatic humic substances on the photodegradation of estrone. Chemosphere 145, 249–255. doi:10.1016/J.CHEMOSPHERE.2015.11.068

Silva, C.P., Lima, D.L.D., Otero, M., Esteves, V.I., 2016b. Photosensitized Degradation of 17β-estradiol and 17α-ethinylestradiol: Role of Humic Substances Fractions. J. Environ. Qual. 45, 693–700. doi:10.2134/jeq2015.07.0396

Sturini, M., Speltini, A., Maraschi, F., Profumo, A., Pretali, L., Fasani, E., Albini, A., 2010. Photochemical degradation of marbofloxacin and enrofloxacin in natural waters. Environ. Sci. Technol. 44, 4564–4569. doi:10.1021/es100278n

Ternes, T., Kreckel, P., Mueller, J., 1999. Behaviour and occurrence of estrogens in municipal sewage treatment plants — II. Aerobic batch experiments with activated sludge. Sci. Total Environ. 225, 91–99. doi:10.1016/S0048-9697(98)00335-0

Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A., 2004. A rapid method to measure the solid-water distribution coefficient (Kd) for pharmaceuticals and musk fragrances in sewage sludge. Water Res. 38, 4075–84. doi:10.1016/j.watres.2004.07.015

Tran, N.H., Reinhard, M., Gin, K.Y.-H., 2018. Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. Water Res. 133, 182–207. doi:10.1016/J.WATRES.2017.12.029

Xu, Y., Xu, N., Llewellyn, N.R., Tao, H., 2014. Occurrence and removal of free and conjugated estrogens in wastewater and sludge in five sewage treatment plants. Environ. Sci. Process. Impacts 16, 262–270. doi:10.1039/C3EM00199G

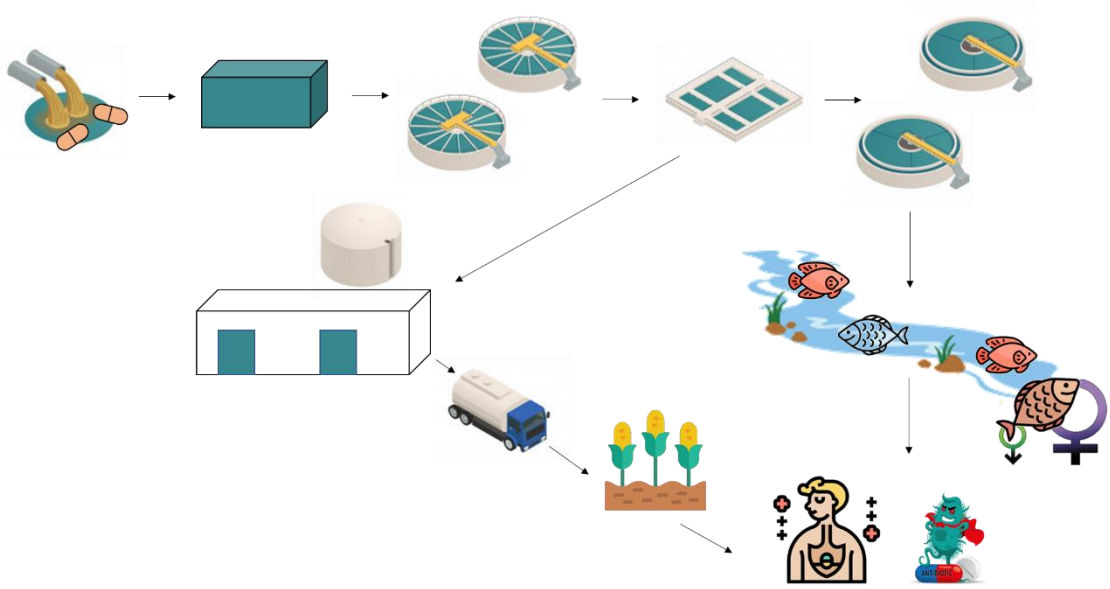
Yan, Q., Gao, X., Huang, L., Gan, X.M., Zhang, Y.X., Chen, Y.P., Peng, X.Y., Guo, J.S., 2014. Occurrence and fate of pharmaceutically active compounds in the largest municipal wastewater treatment plant in Southwest China: Mass balance analysis and consumption back-calculated model. Chemosphere 99, 160–170. doi:10.1016/j.chemosphere.2013.10.062

Zeng, Q., Li, Y., Gu, G., 2009. Nitrate-dependent degradation of 17α-ethinylestradiol by acclimated activated sludge under anaerobic conditions. J. Chem. Technol. Biotechnol. 84, 1841–1847. doi:10.1002/jctb.2255

Zhang, A., Li, Y., 2014. Removal of phenolic endocrine disrupting compounds from waste activated sludge using UV, H2O2, and UV/H2O2 oxidation processes: Effects of reaction conditions and sludge matrix. Sci. Total Environ. 493, 307–323. doi:10.1016/J.SCITOTENV.2014.05.149

CHAPTER 1

INTRODUCTION



SUMMARY

The emergent consumption of pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs) worldwide, both in human and veterinary medicine, has triggered a remarkable increase in the occurrence of these pollutants in the environment. The main concern is that these drugs are very persistent and harmful to human populations and aquatic wildlife, and that the wastewater treatment plants (WWTPs) are not effective barriers to their removal. Consequently, these compounds are continuously introduced into the environment in various ways. The discharge of wastewater effluents is one of the main routes, however, the application of sludge in agriculture is also an important route of contamination. Even at relatively low concentrations, the presence of PPCPs and EDCs in the environment is a significant concern due to their potential impacts on human health and aquatic living organisms, which is considered a major threat to global health.

The antibiotics sulfadiazine (SDZ) and oxolinic acid (OXA), and the estrogens, estrone (E1) and 17α-ethinylestradiol (EE2), are particularly concerning contaminants, due to the induction of bacterial resistance to antibiotics, and endocrine disruption, respectively. In the last decades, important developments were achieved in the knowledge of the occurrence, fate and removal processes of PPCPs and EDCs in the environment. However, much still needs to be investigated in order to understand the removal of these compounds not only from wastewater effluents but also from sludge.

The *Introduction* chapter is intended to provide a summary of the effects, sources, fate, occurrence and removal processes of PPCPs and EDCs in the environment.

CONTENTS

1.1. PPCPS AND EDCS IN THE ENVIRONMENT

The growing rate of globalization and industrialization has markedly depleted natural resources as a result of the economic and social changes (Aroh, 2018). Consequently, pollution is the main global challenge that has increased considerably worldwide, undermining the economic growth and the physical health of billions of people (FAO, 2020). The rapid urbanization and the consequent overuse of pharmaceuticals have caused their occurrence in the environment worldwide (Kaur et al., 2019). Moreover, human consumption of pharmaceuticals is expected to increase in the future years, as a result of the continued ageing population and the improvement of life quality worldwide. In addition, the consumption of pharmaceuticals in veterinary medicine is also increasing, for the treatment of diseases, for growth promotion and to improve feed efficiency (Andreu et al., 2007; Jones et al., 2004; Van der Aa et al., 2011). Since the 1970s and 1980s, environmental monitoring of the so-called emerging contaminants (EC) has gained interest within the scientific community due to their potential to enter the environment and cause adverse ecological and/or human health effects (Rosenfeld et al., 2011), even at concentrations of the order of micrograms per litre or lower (Kim and Aga, 2007). However, these compounds are not commonly monitored in the environment (Rosenfeld et al., 2011).

EC are defined as synthetic or naturally occurring chemicals that include several classes of pollutants as pesticides, surfactants, pharmaceuticals and personal care products (PPCPs) and endocrine-disrupting chemicals (EDCs). These pollutants are regularly detected in groundwater, surface water, drinking water, municipal wastewater, and food sources (Ikehata et al., 2007; Rosenfeld et al., 2011). The monitoring of these pollutants in the environment is often hampered by the low concentrations in the environment, as well as by the complexity of the matrices of the environmental samples (Barreiros et al., 2016; Caliman and Gavrilescu, 2009).

The 2030 Agenda for Sustainable Development recognizes water pollution as a priority problem (FAO, 2020). This Agenda aims to influence future policies and strategies to ensure the control of water quality with the main emphasis on minimizing the release of hazardous chemicals and materials to the environment (FAO, 2020). To mitigate the effects of the presence of contaminants in the environment, the European Water Framework Directive established and supervised every 4 years a list of priority water pollutants (European Union, 2000). Despite efforts by political structures to implement the monitoring of a wide range of known pollutants in waters, a large number of other contaminants were not considered in the list of substances to be regulated.

The growing interest in preserving aquatic ecosystems and the potential risk of contamination of public water supplies has encouraged several intervention studies. The identification and quantification of PPCPs and EDCs in the environment has been one of the focuses of previous studies, but one of the main concerns of environmental scientists and engineers is the development of efficient processes to remove them.

1.1.1. EFFECTS AND SOURCES

During the years 2000-2010, the consumption of pharmaceuticals has increased by 36 % worldwide (Yadav et al., 2019). As aforementioned, these compounds are widely prescribed for both human and veterinary medicine for the treatment of diseases, to promote health, to improve feed efficiency and to acquire lean muscle mass in farm animals (Adeel et al., 2017; Yadav et al., 2019). The main issue is that the widespread and increasing use of these compounds leads to their continuous introduction into the environment outgrowing their removal rate (Caliman and Gavrilescu, 2009; Kaur et al., 2019).

The effects of the presence of PPCPs and EDCs in the environment are not entirely known. Notwithstanding that pharmaceuticals are not considered to constitute a significant threat to human health through the consumption of drinking water and fish, some studies have shown that the effluents from the WWTP contaminated with PPCP and EDC have strongly contributed to the toxicity of water, rather than pollutants considered as a priority (Caliman and Gavrilescu, 2009). Thus, new concerns arise related to the persistence of these pollutants, their biological activity and their degradation by-products (Caliman and Gavrilescu, 2009). Published studies sustain the premise that the occurrence of PPCPs and EDCs contributes to the environmental damage and suspected detrimental effects on ecosystems and human health (Barreiros et al., 2016; Ikehata et al., 2007; Marti and Batista, 2014; Ying et al., 2002). Indeed, different undesirable effects due to the exposure to PPCPs' and EDCs', such as the aquatic toxicity, the resistant pathogenic bacteria, the endocrine disruption in living organisms as mimicking or antagonizing actions of steroid hormones (Jobling et al., 1998), among others (Adeel et al., 2017; Kim and Aga, 2007) have been reported. It is noteworthy that the lifetimes of PPCPs and EDCs in aquatic systems determine the magnitude of the effects and the potential threats to drinking water supplies (Caliman and Gavrilescu, 2009).

Some studies pointed out that PPCPs can cause adverse effects on human health such as gastrointestinal disturbances, allergies, dizziness, headache, and skin rashes (Rasul and Majumdar, 2017; Wagman and Wentland, 2007). However, other more harmful effects as the aquatic toxicity and the development of antibiotic-resistant bacteria have also been reported (Rasul and Majumdar, 2017; Zeghioud et al., 2019). Bacterial resistance phenomenon are currently recognized as one of the most serious threats to public health, since both animals and animal waste are potential reservoirs of multi-resistant genes that can be transmitted directly or indirectly to humans through contact and food consumption (Caruso, 2016; WHO, 2020). This phenomenon may occur due to the evolution of antibiotic resistance genes (ARGs) and bacteria (ARB), which reduces the therapeutic potential of antibiotics against human and animal pathogens (Tran et al., 2018).

In the case of EDCs, their presence in water bodies can cause endocrine disruption and negatively affect the sexual and reproductive systems of wildlife, fish and humans (Barreiros et al., 2016; Gabet-Giraud et al., 2010; Hamid and Eskicioglu, 2012; Ikehata et al., 2007; Ying et al., 2002). Regarding human health, different effects have been reported, such as increased breast cancer incidents in women (Moore et al., 2016), prostate cancer in men (Nelles et al., 2011), decreased fertility and feminisation of men (Sumpter and Jobling, 2013) or undescended testicles (Chen and Hu, 2010). In wildlife, the health effects include reproductive abnormalities (Rose et al., 2013), induction of vitellogenin production (Kidd et al., 2007), feminisation of males (Rodgers-Gray et al., 2001) and masculinization of females (Matthiessen and Gibbs, 1998).

When considering the sources of discharges of a specific pollutant into the environment, many factors must be taken into account. These include the behaviour of the compound in different matrices (aqueous or solid), the structural changes that the compound may undergo after its administration, the anthropogenic or natural origin of the source, or even if it comes from an accidental discharge, point or diffuse source. After consumption, these contaminants are mostly absorbed, distributed, partially metabolized and often excreted in unmetabolized form or as active metabolites by the urine and/or faeces (Ikehata et al., 2007). Some studies have shown that approximately 50 % to 90 % of pharmaceuticals administered are excreted via urine and faeces as the original unmodified compounds or as metabolites (Massé et al., 2014; Tran et al., 2018).

Figure 1.1 illustrates the different routes of PPCPs and EDCs entering the environment. The main route of entry of PPCPs and EDCs into the environment is human and animal excretion (Yadav et al., 2019) which leads to a possible discharge through the domestic wastewaters, ending up in the wastewater treatment plants (WWTPs). However, despite the treatment systems applied to the effluent, WWTPs are not designed to remove these contaminants, allowing them to enter the

aquatic environment through a few pathways (Al Aukidy et al., 2012; Brandt et al., 2013; Verlicchi et al., 2012; Zhang et al., 2016). Consequently, discharge into water bodies causes the contamination of surface water or even groundwater (Ikehata et al., 2007).

Besides that, another very important issue arising from the treatment processes of WWTPs is contaminated sludge. Several drugs are hydrophobic compounds and during the biological treatment (aerobic and/or anaerobic) are transferred to the sludge, being able to remain intact (Tran et al., 2018; Y. Zhou et al., 2012b). The resulting sludge is conditioned, dehydrated and then deposited in a landfill, incinerated or applied to land as fertiliser (Ikehata et al., 2007). During the digestion process, the pharmaceutical compounds can be degraded or remain intact. Therefore, these pollutants can be infiltrated into groundwater aquifers or be flushed by surface water runoff (Ikehata et al., 2007).

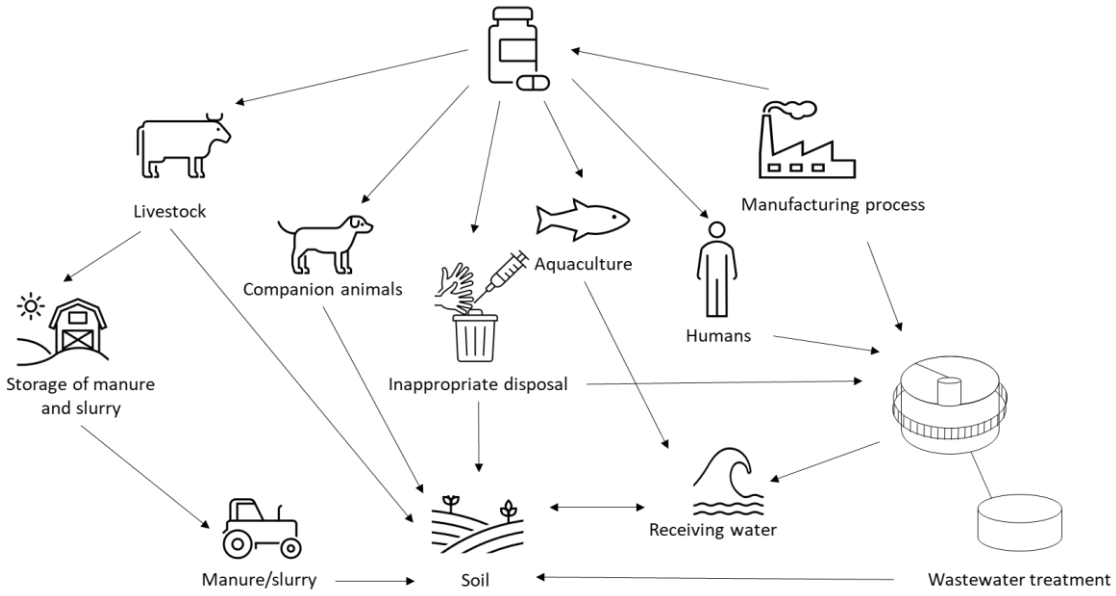


Figure 1.1 Routes of PPCPs and EDCs entering the environment (adapted from Boxall (2004) and Ikehata et al (2007)).

Other ways PPCPs and EDCs enter the environment are the effluents from hospitals (Duong et al., 2008; Verlicchi et al., 2010), pharmaceutical industries (Queiroz et al., 2012), sewer leakage/sewer overflow (Launay et al., 2016; Wolf et al., 2012), landfill leachates (Queiroz et al., 2012; Yi et al., 2017), and surface runoff from urban or agricultural areas where treated wastewater/sludge or manure waste are applied for irrigation activities (Al Aukidy et al., 2012; Chee-Sanford et al., 2009; Queiroz et al., 2012; Sidhu et al., 2013). Furthermore, the inadequate

disposal of unused, surplus or expired drugs also contributes to the presence of these compounds in the environment (Boxall, 2004; Brooks et al., 2008; Ikehata et al., 2007; Melo et al., 2009).

In the veterinary field, these compounds are used in aquaculture for the treatment of fish or shrimp and in intensive livestock treatments (Ikehata et al., 2007). Aquaculture industry can take different regimes, the classification is based on levels of production control and whether or not artificial feeding is required. In extensive breeding the animals are fed exclusively naturally, while in semi-intensive breeding the species, although they also consume natural feed, are additionally fed with artificial supplements. In the intensive diet, there is human control of breeding conditions in order to make the most of production and maximise it. In this case, the fish are fed exclusively on artificial feed, where antibiotics are excessively used to prevent fish diseases or treat infections due to bacterial diseases (Romero et al., 2012; Zeghioud et al., 2019). Different aquaculture production regimes can take place in different environments - marine/ brackish waters or freshwaters. Aquaculture industry has been facing a huge development in the last few years, more than all other sectors of animal food-production, now accounting for 50 % of the world's fish used for food (FAO, 2020; Romero et al., 2012). The residues from aquaculture are directly released into surface waters and the residues from livestock can enter the environment indirectly through the application of slurry and manure as fertilizers (Boxall, 2004; Melo et al., 2009). As a result, PPCPs and EDCs can be transmitted to humans through the food chain. Consequently, the contamination of the environment by PPCPs and EDCs is recognized as an emerging issue of concern by the general public and the government agencies (Yao et al., 2011). The most suitable approach will always be to reduce the consumption of potentially hazardous compounds, however and if this is not possible, these substances should be treated and removed as close as possible to the primary sources (Ikehata et al., 2007).

As mentioned above, there are no regulated discharge limits for these pollutants either from municipal or industrial WWTPs, although some directives have been published in the European framework. The first milestone in the European water policy was the Directive 2000/60/EC, which targeted prioritizing substances presenting a high risk. The more recent Decision 2015/495/EU widened the group of compounds to be monitored, including three antibiotics and recommended attention to treatment options. In Portugal, the Valormed organization has a collection point not only for unused or expired chemicals but also for materials used in product packaging and wrapping. However, its collection depends on the participation of the population. In 2007, the REACH (Registration, Evaluation, Authorisation of Chemicals) regulation was implemented in Portugal, aiming to control the use of toxic chemicals in industry and providing

information on the possible hazards of about 30,000 chemicals and how to deal with the risks they present.

1.1.2. PATHWAYS AND FATE

The occurrence and fate of PPCPs and EDCs in the environment is an emerging concern and is a subject of study for several researchers. As mentioned above, most of the hazardous compounds reach the environment and consecutively may undergo different degradation and transport routes (Figure 1.2). The distribution of PPCPs and EDCs into the environment can be influenced by several factors such as their physicochemical properties and the region-specific environmental conditions, in particular, the pattern of consumption of these pollutants by the population, the water consumption per person per day, the water catchment characteristics (e.g. land use, population size, and population density), the treatment processes applied in WWTP as well as the environmental conditions (i.e. temperature, sunny days and its intensity) (Caliman and Gavrilescu, 2009; Melo et al., 2009; Pérez-Lucas et al., 2019; Tran et al., 2018). The most prominent physicochemical properties are the molecular weight, the octanol/water partition coefficient (K_{ow}) and the water solubility (S_W), which is heavily influenced by the temperature, pH, ionic strength, and natural organic matter concentration (Caliman and Gavrilescu, 2009; Kaur et al., 2019).

Pollutants transformation and transport includes differentiated processes, such as chemical degradation, biodegradation, photodegradation, leaching, volatilization, surface runoff and adsorption or desorption (Lima et al., 2012). Degradation is recognized as the key process for the reduction of pollutants in the environment. Biodegradation is defined by the process in which the structure of the pollutant is transformed or altered by the action of microorganisms (through metabolic or enzymatic action) into a simpler compound (Michael et al., 2013). In contrast, degradation can also be a non-biotic process, in which hydrolysis, oxidation and photodegradation are included (Michael et al., 2013; Periša et al., 2013). Chemical degradation includes some processes such as oxidation, reduction and polymerisation. Oxidation and reduction reactions modify the parent compound into products with progressively increased water polarity and solubility (Patel et al., 2019). Afterwards, conjugation reactions modify either the original contaminant or its products previously formed. Conjugation reactions generate hydrophilic derivatives that are easily excreted or more effectively degraded enzymatically (Patel et al., 2019). Certain conjugation reactions take place in the original contaminant without any previous oxidation

(Patel et al., 2019). In polymerisation process, smaller molecules, called monomers or building blocks, are chemically combined to create larger molecules or a macromolecule (Shrivastava, 2018).

Leaching is the vertical downward transfer of contaminants, as an the effect of rain or irrigation water, through the soil layer and the unsaturated zone, finally reaching groundwater (Pérez-Lucas et al., 2019). The highest leaching takes place for compounds that tend not to be adsorbed and/or that are persistent, or when soils have low organic matter content and sandy texture or even under weather conditions favourable to this transport pathway such as higher precipitation and lower temperatures (Pérez-Lucas et al., 2019). In the volatilization process, the pollutants are transferred from the dissolved to the gaseous phase (Tran et al., 2018). The volatilization process strongly depends on the physicochemical properties of the contaminant and the operational conditions of the treatment processes used in WWTP (i.e. temperature, atmospheric pressure, among others) (Tran et al., 2018). Surface runoff is another important route for pollutants' transport due to the direct application of a range of compounds in agricultural fields and irrigation water (Pedersen et al., 2003).

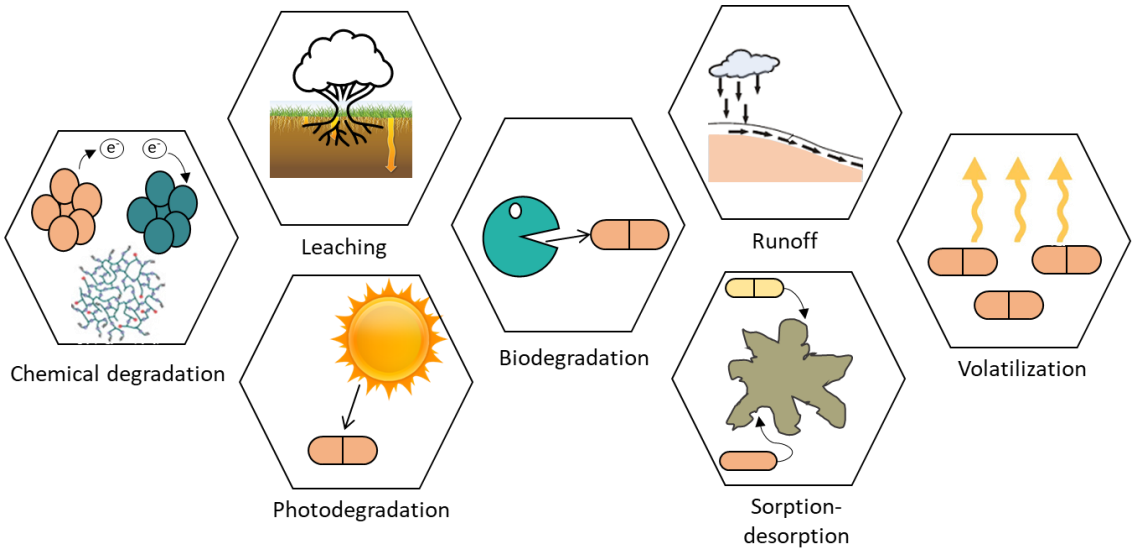


Figure 1.2 Pathways of EDCs and PPCPs degradation and transport.

Adsorption or desorption are important pathways of immobilization/mobilization of pollutants, particularly during sewage treatment processes (Ikehata et al., 2007). Adsorption is a physicochemical process in which molecules of a fluid, liquid or gas, bind to the outer and inner surfaces of a particulate matter (adsorbent) (Crini et al., 2019). Different binding mechanisms can

occur in the adsorption process, such as physical binding influenced by van der Waals forces, hydrogen bonds or electrostatic interactions and chemical binding induced by ionic and covalent interactions (Crini et al., 2019).

To better understand the fate of PPCPs and EDCs in the environment as well as to devise possible strategies to mitigate their presence, different processes must be considered. Photochemical degradation, adsorption and biodegradation appear as the most meaningful removal mechanisms for these pollutants in the environment. Photodegradation, adsorption and biodegradation will be analysed in detail in Chapter 2, Chapter 4 and Chapter 5, respectively.

1.2. PPCPS AND EDCS UNDER STUDY

Among PPCPs and EDCs, antibiotics and estrogens, respectively, have received considerable attention by the scientific community due to their presence in the environment and their harmful effects on ecosystems and human health (Barreiros et al., 2016; Cui et al., 2020; Marti and Batista, 2014; Ying et al., 2002; Zeghioud et al., 2019). Antibiotics are organic compounds capable of selectively weaken or affect the functions of live organisms (Bian and Zhang, 2016). Estrogens are considered as the most important female sex hormones for the maintenance of health status in both reproductive and non-reproductive systems (Cui et al., 2013).

Sulfonamides (SAs) and quinolones (QAs) embrace two of the most important families of antibiotic agents that are used worldwide for the treatment of a broad range of infections in humans and animals (Cui et al., 2020; Liu et al., 2020; Toledo-Neira et al., 2016). According to data reported in the literature, SAs have been identified as the 3rd most used group of veterinary antibiotics in Europe (Conde-Cid et al., 2018). In a survey conducted by Lulijwa et al. (2020), 14 antibiotics were detected in aquaculture facilities exceeding the maximum residue limits for the respective country. The SAs are the main contributors for exceeding this values, representing approximately 42.9 % of antibiotics analysed, followed by QAs with 35.7 % (Lulijwa et al., 2020). Among these compounds, one SA, namely sulfadiazine (SDZ), and one QA, particularly oxolinic acid (OXA), contribute to exceeding the regulated value (Lulijwa et al., 2020). The antibiotics SDZ and OXA are widely used in veterinary medicine and food-animal production to prevent diseases, or as chemotherapeutic agents to control diseases (Cui et al., 2020; Lai and Lin, 2009; Liu et al., 2020). For instance, the annual human and veterinary consumption of SAs was around 7890 tonnes in China and 369 tonnes in the United States in 2013 (Liu et al., 2020). In the case of QAs, more than 1820 tonnes are consumed in China and 277 tonnes in the United States in 2011 (Janecko et al., 2016; Liu et al., 2020; WHO, 2020).

The essential SAs structural characteristics are the benzene ring with two substitutes for each other, an amino group in the fourth position, and the singly substituted 1-sulfonamido group (Sica, 2008). QAs belong to the class of organic compounds of the aromatic heterocyclic series characterized by a double-ring structure formed by a benzene ring and a pyridine ring attached to two carbon atoms.

Natural estrogen estrone (E1) and the synthetic estrogen 17α-ethinylestradiol (EE2) are reported as the EDCs with higher disrupting potency (Johnson and Sumpter, 2001) and have been identified as major sources of estrogenic activity (Atkinson et al., 2012; Tran et al., 2018). E1 and

EE2 can have a key role in both reproductive and non-reproductive tissues as liver, heart, muscle, bone and brain (Cui et al., 2013). These compounds are mainly excreted by humans, but also by livestock and wildlife (Adeel et al., 2017; Yu et al., 2004). The concentrations of estrogens present in human excretion may be highly variable depending on population structure (in terms of gender, pregnancy, menstruating women or menopausal women, etc.), age, individual metabolism, urine or faeces sampling duration, and/or mode of administration of contraceptive (oral or intravenous) (Xu et al., 2012). Human urine is often cited as a keyway of contamination by natural and synthetic estrogens in the aquatic environment (Jobling et al., 2006).

In this work, two antibiotics and two estrogens were studied: SDZ and OXA; E1 and EE2, respectively. Their structures and physicochemical properties are presented in Figure 1.3 and Table 1.1, respectively.

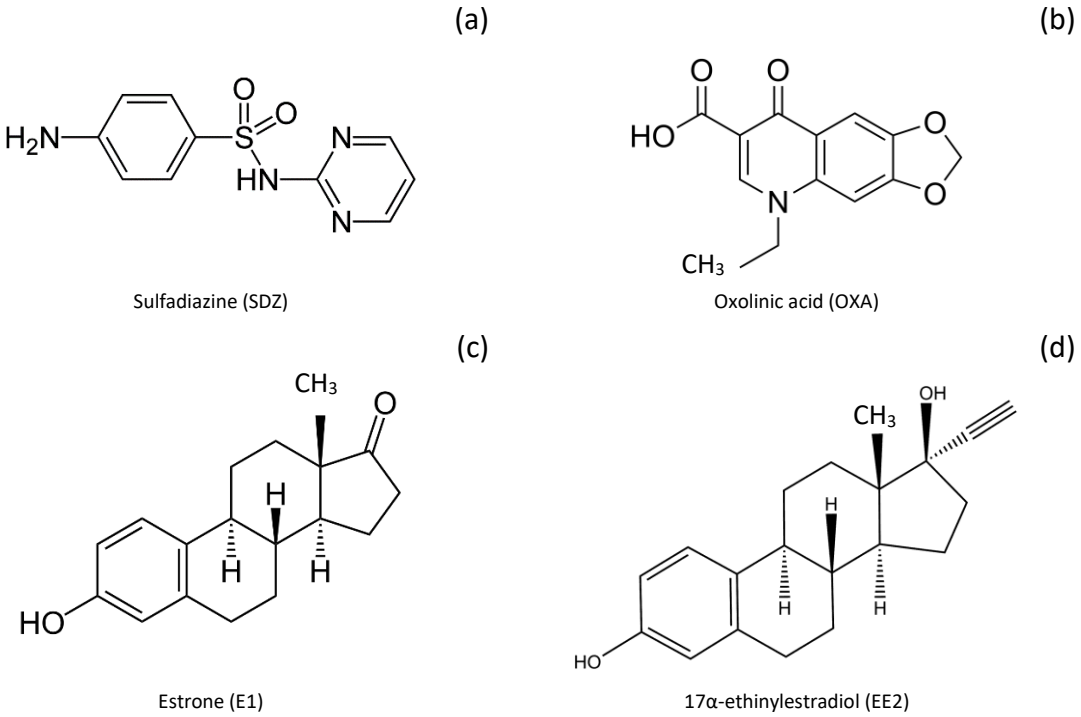


Figure 1.3 Molecular structures of (a) SDZ, (b) OXA, (c) E1 and (d) EE2 (Boreen et al., 2005; Rodríguez et al., 2011; Ying et al., 2002).

Table 1.1 Physicochemical properties of SDZ, OXA, E1 and EE2.

Compound	Group	Molecular formula	CAS [a]	Mol. wt. [b] (g mol^{-1})	S_W [c] (mg L^{-1})	pK_a [d]	Log K_{ow} [e]	Reference [f]
SDZ	Sulfonamide	$C_{10}H_{10}N_4O_2S$	68-35-9	250.3	77	2.01; 6.99	-0.09	[1, 2, 3, 4]
OXA	Quinolone	$C_{13}H_{11}NO_5$	14698-29-4	261.2	4.1 [g]	6.9	0.68	[5, 6]
E1	Natural estrogen	$C_{18}H_{22}O_2$	53-16-7	270.4	13	10.3 to 10.8	3.43	[7, 8, 9]
EE2	Synthetic estrogen	$C_{20}H_{24}O_2$	57-63-6	296.4	4.8	10.2 to 10.7	4.15	[7, 8, 9]

[a] CAS, chemical abstract services.

[b] Mol. wt., molecular weight.

[c] S_W, water solubility at 20 °C.

[d] pK_a, dissociation constant.

[e] Log K_{ow}, octanol/water partition coefficient.

[f] [1] Acosta-Rangel (2018); [2] Batista et al. (2014); [3] Boreen et al.(2005); [4] Lian et al. (2015); [5] Lützhøft et al. (2000); [6] Zeghioud et al. (2019); [7] Hamid and Eskicioglu (2012); [8] Sarmah et al.(2006); [9] Ying et al. (2002).

[g] At a pH of 7.

SDZ is an SA that comprises a pyrimidine with a 4-aminobenzenesulfonamido group at the 2-position and has the molecular formula $C_{10}H_{10}N_4O_2S$. SDZ is one of the most commonly used antibiotics in veterinary medicine (Li et al., 2020; Lulijwa et al., 2020). This compound has also been used in human medicine for the prevention and treatment of certain types of bacterial infections, such as urinary tract infections, chancroid, and other infections (Kokulnathan et al., 2021). According to a study carried out by Lulijwa et al.(2020), SDZ is used by more than 73 % of the 11 major fish production countries. The main reasons for its high consumption worldwide are its broad-spectrum, low cost and stability (Cui et al., 2020). SDZ presents a water solubility (S_w) of 77 mg L^{-1} and therefore is relatively water-soluble, being easily disseminated in the aquatic environment (Lian et al., 2015; Sukul and Spiteller, 2006).

OXA is a QA with a molecular formula of $C_{13}H_{11}NO_5$, commonly used in human and veterinary medicine for the treatment of bacterial infections (Fekete and Schmitt-Kopplin, 2007; Hagren et al., 2005). This synthetic antibiotic has as main functions of its anti-infective activity, an antibacterial drug, an enzyme inhibitor, an antimicrobial agent and an antifungal agent (Barry et al., 1984). The increasing use of OXA may be related to its broad-spectrum, high potency, good bioavailability and stable properties (Lin et al., 2010; Sturini et al., 2014). OXA consists of a quinolinemonocarboxylic acid with the carboxyl group at position 7 as well as oxo and ethyl groups at positions 4 and 1, respectively, and a dioxolo ring fused at the 5- and 6-positions. This contaminant is considered as a weak acid with pK_a at 6.9 and presents a S_w value of 4.1 mg L^{-1} (Lützhøft et al., 2000; Samuelsen et al., 1992; Zeghioud et al., 2019). Therefore, it is practically insoluble in acidic solutions but very soluble in alkaline solutions (Samuelsen et al., 1992).

The natural estrogen E1 with a molecular formula $C_{18}H_{22}O_2$ is considered as one of the major mammalians estrogens and is an aromatized C18 steroid with a 3-hydroxyl group and a 17-ketone. This compound can also be produced *in vivo*, using androstenedione or testosterone via estradiol. E1 could be metabolized to 16-alpha-hydroxyestrone, which may be reduced to estriol by estradiol dehydrogenase. E1 is produced mainly in ovaries, placenta, and peripheral tissues (especially adipose tissue) through the conversion of adrostenedione. Therefore, pregnant women are responsible for the largest production of E1, excreting up to 787 µg d^{-1} (Adeel et al., 2017; Johnson et al., 2000). This excretion is followed by menopausal women (4.0 µg d^{-1} to 31.5 µg d^{-1}), menstruating women (3.5 µg d^{-1} to 9.32 µg d^{-1}) and other women (7 µg d^{-1}) (Adeel et al., 2017; Johnson et al., 2000). Men excrete daily about 1.6 µg to 3.9 µg of E1 (Adeel et al., 2017; Johnson et al., 2000). The estrogen EE2 with a molecular formula $C_{20}H_{24}O_2$, is a synthetic hormone widely used as the estrogenic component of oral contraceptives (Silva et al., 2012). A study carried out by Johnson and Williams (2004) indicated that about 17 % of the total female population are ingesting daily 26 µg of EE2, of which about 40 % is excreted in faeces (6 µg d^{-1}) and urine (4.5 µg d^{-1}).

Both E1 and EE2 are hydrophobic compounds with an octanol/water partition coefficient (log K_{ow}) values of 3.43 and 4.15, respectively, and consequently low S_w (13 mg L^{-1} and 4.8 mg L^{-1}, respectively) (Ying et al., 2002). Thus, these compounds have a high affinity to solids (Michael et al., 2013).

1.2.1. Occurrence of SDZ and OXA in the environment

The presence of pharmaceuticals in the environment was first revealed in the 1970s (WHO, 2020). Since then several studies have been undertaken and different pollutants have been detected in different parts of the world. The pharmaceutical concentration detected in environmental samples depends not only on the matrix (surface water, wastewater or drinking water) but also on the sampling site and sampling date (Tran et al., 2018).

A recent study conducted by Cui et al. (2020) in China found that, comparatively with other SAs, SDZ presented a very high detection frequency (100 %, n = 8 samples) and concentration (up to 216 ng L^{-1}) in WWTP influents and effluents (Cui et al., 2020). Likewise, in China, Su et al. (2020) detected SDZ in river water with a mean concentration of 15 ng L^{-1} with a frequency of detection of 41 % (n = 125 samples), while Guo et al. (2019) detected concentrations of up to 4.5 ng L^{-1} in estuarine water with a detection rate of 83 % (n = 43 samples). Moreover, SDZ was also detected

in groundwater samples collected in China at concentrations of up to 3.10 ng L^{-1} (Qin et al., 2020). Few studies have been found in the literature dealing with SDZ analysis in environmental samples collected in Portugal. However, a study conducted by Palma et al. (2020) showed that SDZ was detected in the river Guadiana with a concentration of 75 ng L^{-1}, with a frequency of detection of 8 % (n = 12 samples). Other studies did not detect SDZ in the samples of river water, in WWTP influents and effluents collected in Portugal (Paíga et al., 2019, 2016). Apart from the associated spreading of bacterial resistance, the wide occurrence of SDZ, may have associated toxicity risks since, based on criterion issued by the Globally Harmonized System of Classification and Labelling of Chemicals (United Nations, 2011), it can be sorted as a very toxic organic contaminant (Duan et al., 2020).

Regarding the occurrence of OXA in environmental samples, few studies have been published. Tamtam et al. (2008) investigated the occurrence of 17 antibiotics in a river located in France. The authors detected an OXA concentration of 19 ng L^{-1} with a frequency of detection range from 10 % to 18 % (n = 44 samples). In another study that used samples collected in the same country, OXA concentration in estuarine samples, WWTP influents and effluents was below the limit of quantification (LOQ = 0.38 ng L^{-1}) (Miossec et al., 2019). Rodriguez-Mozaz et al. (2020) studied the occurrence of 17 antibiotics in treated effluent samples from 13 WWTPs located in 7 European countries (Portugal, Spain, Cyprus, Ireland, German, Finland, and Norway). The scientists have verified that the concentration of OXA was below the limit of detection (LOD = 18.83 ng L^{-1}) in all countries except in Ireland, where a value of 5.3 ng L^{-1} was detected (Rodriguez-Mozaz et al., 2020).

1.2.2. Occurrence of E1 and EE2 in the environment

Over the last decades, environmental monitoring activities reveal the presence of E1 and EE2 in water and soil. The occurrence of E1 and EE2 in aqueous environmental samples as in WWTP influents and effluents and in surface water is well documented (Tran et al., 2018). Nevertheless, most of the information available in the literature has exclusively focused on the occurrence in the dissolved phase of water samples, while information regarding the particulate phase (as sludge and biosolids) is still limited (Tran et al., 2018).

In surface waters in China, Tan et al. (2018) found E1 and EE2 concentrations of 1235 ng L^{-1} and 17 111.5 ng L^{-1}, respectively. Other studies reported much lower E1 and EE2 concentrations

(below 37.3 ng L^{-1} for E1 and below LOD (from 0.1 ng L^{-1} to 1.2 ng L^{-1}) for EE2) in surface waters in the same region (He et al., 2018; Zhang et al., 2014). In North America, E1 was detected in surface waters at concentrations of 5.2 ng L^{-1} (Singh et al., 2010). Whereas in Europe concentrations of E1 and EE2 of 17 ng L^{-1} and 0.4 ng L^{-1}, respectively, were revealed in surface waters (Esteban et al., 2014; Gorga et al., 2013; Hohenblum et al., 2004; Liu et al., 2004; Vethaak et al., 2005).

Regarding the presence of E1 and EE2 in WWTP influents and effluents, much higher concentrations were found by several authors. In Asian countries, such as China, Korea and Singapore, E1 and EE2 were detected in WWTP influents at concentrations below 154.3 ng L^{-1} and 42.3 ng L^{-1}, respectively (Chang et al., 2011; Huang et al., 2013; Tran and Gin, 2017). Whereas in WWTP effluents, E1 and EE2 were found at concentrations of 62.5 ng L^{-1} and 26 ng L^{-1}, respectively (Chang et al., 2011; Huang et al., 2013; Tran and Gin, 2017). In North America, the highest E1 concentrations of 52 ng L^{-1} and 54 ng L^{-1} were found in WWTP influents and effluents, respectively (Hedgespeth et al., 2012; Lee et al., 2005; Lishman et al., 2006). In the case of EE2, for the same regions, concentrations of 242 ng L^{-1} and below 10 ng L^{-1} were found in WWTP influents and effluents, respectively (Yang et al., 2011). In Brazil, Pessoa et al. (2014), quantified E1 and EE2 in WWTP influents and effluents and found much higher values. E1 concentrations of 3050 ng L^{-1} and 2080 ng L^{-1}, for influents and effluents were detected with a frequency of detection of 76 % and 48 % (n = 25 samples), respectively. For EE2, values of 3180 ng L^{-1} and 176 ng L^{-1} were detected for influents and effluents, with a frequency of detection of 52 % and 40 % (n = 25 samples), respectively (Pessoa et al., 2014).

In Europe, different concentrations in WWTP influents and effluents were found by some researchers. For example, a study conducted in France by Miège et al. (2009) showed that E1 was detected in WWTP influents and effluents at concentration levels of 670 ng L^{-1} and 95 ng L^{-1}, with a frequency of detection of 100 % (n = 109 samples) and 93 % (n = 79 samples), respectively. Whereas for EE2, the same authors detected concentrations of 70 ng L^{-1} and 5 ng L^{-1}, with a frequency of detection of 91 % (n = 70 samples) and 59 % (n = 33 samples), respectively (Miège et al., 2009). Likewise, Clara et al. (2005) investigated the occurrence of 9 pharmaceuticals in WWTP influents and effluents, in Austria, and observed similar E1 concentrations (670 ng L^{-1} and 72 ng L^{-1}, respectively), and EE2 concentrations (70 ng L^{-1} and 4 ng L^{-1}, respectively) to those reported by Miège et al. (2009) in France.

In Portugal, E1 and EE2 concentrations of 112.9 ng L^{-1} and 97.7 ng L^{-1}, respectively, were found in surface waters (Ribeiro et al., 2009). In WWTP influents and effluents E1 concentrations ranging from 189 ng L^{-1} to 2484 ng L^{-1} and of 25 ng L^{-1} were observed, with a frequency of detection

of 20 % (n = 10 samples) and 11 % (n = 9 samples), respectively (Salgado et al., 2010). In the case of EE2, lower values were reported, ranging from 103 ng L^{-1} to 106 ng L^{-1}, with a frequency of detection of 20 % (n = 10 samples) in WWTP influents and EE2 was not detected in WWTP effluents (Salgado et al., 2010).

As mentioned above, E1 and EE2 tend to heavily sorb onto sludge by adsorption process and are, therefore, mostly retained in the sludge (Tran et al., 2018). However, information on the quantification of E1 and EE2 in sludge is very scarce. Martín et al. (2015), in Spain, investigated the occurrence of pharmaceutically active compounds in sludge, and found E1 and EE2 concentrations of 887 ng g^{-1} and 483 ng g^{-1}, respectively. In Portugal, another study carried out by Salgado et al. (2010), indicated E1 concentration in sludge ranging from 8 ng g^{-1} to 181 ng g^{-1} with a frequency of detection of 22.2 % (n = 9 samples). For EE2, the authors found a concentration of 221 ng g^{-1} in sludge with a frequency of detection of 11.1 % (n = 9 samples) (Salgado et al., 2010).

The limitations of the method of analysis of E1 and EE2 in the solid phase of sludge explain the limited information on the determination of E1 and EE2 in sludge. For this reason, the development of an effective method for determining E1 and EE2 in the solid phase of sludge is of the greatest importance, as will be addressed in Chapter 3.

1.2.3. Removal processes for SDZ, OXA, E1 and EE2 in the environment

In order to mitigate the potential adverse effects of the presence of persistent substances in the environment, the removal efficiency of these pollutants in WWTPs should be assessed. The removal and transformation of pharmaceuticals during wastewater treatment is attributed to different biotic or abiotic processes. The biotic process consists of pharmaceuticals' biodegradation, mostly by bacteria and fungi, whereas the non-biotic or abiotic process can result from sorption, hydrolysis and photolysis of the pollutant (Michael et al., 2013).

In WWTPs, the conventional treatment of wastewater generally consists of a primary, secondary and sometimes tertiary stage. The main difference between the numerous WWTPs available in the world is the distinct biological and physical-chemical processes adopted for each treatment stage (Michael et al., 2013). The primary treatment aims to reduce the solids content of wastewater (oils and fats, greases, sand and sediments) (Tran et al., 2018). This stage, generally used at all WWTPs, is based on a mechanical process by filtration and sedimentation. The secondary treatment aims to remove organic matter and/or nutrients based on biological processes (i.e.

aerobic and anaerobic systems). In this step biological degradation is the key for the removal of several pollutants, leading to their mineralization or incomplete degradation (i.e. conversion into sub-products) (Luo et al., 2014). Different biological treatments such as conventional activated sludge (AS), membrane bioreactors (MBR), or biological filters (BF) can be applied (Michael et al., 2013). In the AS process, dissolved oxygen is applied to promote the growth of a biological floc that significantly removes organic matter and nitrogen (Michael et al., 2013). The tertiary treatment (TT) stage is generally used to remove phosphorus by precipitation and particles which are retained on a filter (Michael et al., 2013). In some cases, TT can be applied for effluent disinfection, generally using chlorination or ultraviolet (UV) irradiation.

Although different biological processes can interfere with the removal of pharmaceutical compounds in wastewater treatment, other factors have been highlighted. Outstanding among them are the existence and size of anoxic and anaerobic compartments, the biochemical oxygen demand (BOD_5), the suspended solids (SS) loading, the hydraulic retention time (HRT), the sludge retention time (SRT), the food-microorganism ratio (F/M ratio), the mixed liquor suspended solids (MLSS), the pH and temperature (Michael et al., 2013). It is noteworthy that SRT is linked to the growth rate of microorganisms (Michael et al., 2013). Therefore, higher SRTs can promote the degradation of pollutants due to the enrichment of slow-growing bacteria that provide a greater diversity of enzymes (Michael et al., 2013).

The performance (expressed as percentage of removal) of some WWTPs applying biological treatment for removing SDZ, OXA and E1 and EE2 as stated in the literature is summarized in Table 1.2 and Table 1.3, respectively. In the case of OXA, no data were found in the literature on the removal of this compound for the different treatment processes used in WWTP. The removal rate strongly depends on the compound, the country and treatment process adopted in WWTPs. For instance, for SDZ, in the conventional process of AS, distinct removals have been obtained for the different studies presented, showing in some cases from a negative removal up to 100 % (L. Gao et al., 2012; P. Gao et al., 2012; García-Galán et al., 2011; Kovalova et al., 2012; Li et al., 2009; Li and Zhang, 2011; Xu et al., 2007; Yan et al., 2014a). In the case of E1, similar results were found for the same treatment process (from a negative value of -61 % to more than 98 %) (Andersen et al., 2003; Baronti et al., 2000; Braga et al., 2005a, 2005b; Chang et al., 2011; D'Ascenzo et al., 2003; Fan et al., 2011; Johnson et al., 2000; Muller et al., 2008; Nakada et al., 2006; Shao and Ma, 2009; T. Ternes et al., 1999; Y. Zhou et al., 2012a). The negative removal of E1 during the sewage treatment process can be associated to the oxidation of the natural estrogen 17β-estradiol (E2) to E1 contributing to its increasing concentration in the treated effluent (Hashimoto and Murakami, 2009; Johnson and

Sumpter, 2001; Weber et al., 2005). For EE2, higher removal values (from 60.4 % to 98 %) were achieved during AS treatment according to some researchers (Andersen et al., 2003; Baronti et al., 2000; Johnson et al., 2000; Muller et al., 2008; Shao and Ma, 2009; H. Zhou et al., 2012). Since EE2 has a high affinity to solids, its removal during the activated sludge treatment may be due to adsorption processes (Clara et al., 2004; Ternes et al., 2004).

Table 1.2 SDZ and OXA removal in WWTPs using different treatment processes.

Country	Process [a]	Removal rate (%)		Reference
		SDZ	OXA	
China	AS	50	n/a	Xu et al. (2007)
	AS	~ - 5	n/a	Yan et al. (2014a)
	AS	63 to 73	n/a	L. Gao et al. (2012)
	MBR	93.8 to 99.7	n/a	Xia et al. (2012)
	A/AO/O	50 to 80	n/a	Ashfaq et al. (2017)
	A/AO/O	26 to 78	n/a	Cui et al. (2020)
	A/AO/O	51 to 79	n/a	L. Gao et al. (2012)
	A/AO/O	~30	n/a	Lin et al. (2018)
	Inv. A/AO/O	45 to 66	n/a	Cui et al. (2020)
	A/AO/AE	32.3	n/a	Yan et al. (2014b)
	A/AO/AE	<10	n/a	Yan et al. (2014a)
	AO/A/AO	<10	n/a	Yan et al. (2014a)
	AO/O	34	n/a	L. Gao et al. (2012)
	OD	64 to 75	n/a	Cui et al. (2020)
	OD	78	n/a	L. Gao et al. (2012)
	ET	71	n/a	L. Gao et al. (2012)
Japan	AS	72.8	n/a	Li et al. (2009)
	AS	87 to 100	n/a	Li and Zhang (2011)
UK	AS	22	n/a	P. Gao et al. (2012)
Brazil	MBR	33	n/a	Lastre-Acosta et al. (2020)
	MBR + O_3	100	n/a	Lastre-Acosta et al. (2020)
Switzerland	MBR	75 to 90	n/a	Abegglen et al. (2009)
	MBR	-23	n/a	Kovalova et al. (2012)
Spain	AS	0 to 100 [b]	n/a	García-Galán et al. (2011)
	BF	100	n/a	García-Galán et al. (2011)

[a] A/AO/AE, anaerobic/anoxic/aerobic process; A/AO/O, anaerobic/anoxic/oxic process; AO/A/AO, anoxic/anaerobic/anoxic process; AO/O, anoxic/oxic process; AS, activated sludge; BF, biological filter or trickle filter; ET, enhanced treatment; FB, fixed bed bioreactors; Inv. A/AO/O, inversed anaerobic/anoxic/oxic process; MBR, membrane bioreactor; OD, oxidation ditch.

[b] In some points the concentrations found in the effluent water are higher than concentrations in the influent waters.

n/a, not available.

Other researches evaluated the removal efficiency of the SDZ in MBR reactors and presented extremely different results, with high values in some cases and negative values in other cases (Abegglen et al., 2009; Kovalova et al., 2012; Lastre-Acosta et al., 2020; Xia et al., 2012). Moreover, García-Galán et al. (2011) evaluated the performance of BF on the removal of SDZ and

found a removal efficiency of 100 %, however, this process is much more expensive than the conventional process (Michael et al., 2013).

Table 1.3 E1 and EE2 removal in WWTPs using different treatment processes.

Country	Process [a]	Removal rate (%)		Reference
		E1	EE2	
China	AS	76	n/a	Chang et al. (2011)
	AS	78	n/a	Fan et al. (2011)
	AS	79.2	60.4	Shao and Ma (2009)
	AS	83.2	93.0	Zhou et al. (2012a)
	A/AO/O	75.4	>95	Nie et al. (2012)
	Inv. A/AO/O	82.7	80.2	Zhou et al. (2010)
	Hydr. Acid. CASS	79	67	Cui et al. (2006)
Japan	AS	86	n/a	Nakada et al. (2006)
UK	BF	30	n/a	Johnson et al. (2007)
Brazil	AS + BF	83	78	Ternes et al. (1999)
Canada	AS + LG	-50 to 98	n/a	Servos et al. (2005)
	AS + LG + TT	88.2	44.6	Cicek et al. (2007)
Australia	ET	14	n/a	Braga et al. (2005b, 2005a)
	AS	85	n/a	Braga et al. (2005b, 2005a)
The Netherlands	AS	66 to 98	77 to 98	Johnson et al. (2000)
Switzerland	AS + FB	49 to 99	69 to 94	Joss et al. (2004)
Germany	AS	98	90	Andersen et al. (2003)
	AS	~ 0	~ 0	Ternes et al. (1999)
Italy	AS	-61	85	Baronti et al. (2000)
	AS	61	n/a	D'Ascenzo et al. (2003)
	AS	74	n/a	Johnson et al. (2000)
France	AS	>96	61 to 87	Muller et al. (2008)
	AS + FB	44 to 59	34 to 45	Cargouët et al. (2004)
Spain	AS + TT	99	n.d.	Cases et al. (2011)
	MBR + FS	99	n.d.	Cases et al. (2011)
	MBR + HF	99	n.d.	Cases et al. (2011)
Portugal	AS, AS + TT, BF	87 to 99	~100	Salgado et al. (2010)

[a] A/AO/O, anaerobic/anoxic/oxic process; AS, activated sludge; BF, biological filter or trickle filter; ET, enhanced treatment; FB, fixed bed bioreactors; FS, flat sheet; HF, hollow fibre; Hydr. Acid. CASS, Hydrolytic - acidification cyclic activated sludge system; Inv. A/AO/O, inversed anaerobic/anoxic/oxic process; LG, lagoon; MBR, membrane bioreactor; TT, tertiary treatment. n/a, not available; n.d., not detected

Cases et al. (2011) investigated the removal of EE2 in a conventional AS plant connected with a TT and two membrane bioreactor pilot plants installed with flat sheet (FS) and hollow fibre (HF) modules and found no significant differences in the latter treatments compared to the conventional treatment. Other treatment processes were also assessed as the secondary biological treatment process for the removal of antibiotics and estrogens as depicted in Table 1.2 and Table 1.3. However, these processes provided results similar to conventional AS. In general, as biological

processes used in WWTPs are not an effective barrier for some of these recalcitrant compounds, additional treatment technologies are required (Ikehata et al., 2007).

In order to eliminate antibiotics and estrogens in the WWTPs before their disposal in the aquatic environment, other processes have been studied in the literature. The most prominent processes are membrane filtration (Bolong et al., 2009; Li et al., 2021; Nghiem et al., 2004; Wang et al., 2021; Xu et al., 2020), activated carbon adsorption (Grover et al., 2011, 2009; Liu et al., 2009; Snyder et al., 2007) and advanced oxidation processes (AOPs) (Bertoldi et al., 2019; Bian and Zhang, 2016; Conde-Cid et al., 2018; Liu et al., 2018; Miklos et al., 2018; Zhang and Li, 2014).

Membrane filtration and activated carbon are highly energetic processes and require intensive material (Ikehata et al., 2007). Moreover, these processes require the disposal of wastes such as membrane retentate and spent activated carbon and are appropriate for the treatment of relatively clean surface water and groundwater (Ikehata et al., 2007). Accordingly, AOPs seem to be a more appropriate treatment alternative for pharmaceutical compounds in wastewater.

1.2.3.1. ADVANCED OXIDATION PROCESSES

The AOPs are a variety of radical reactions that include combinations of chemical agents (e.g., ozone (O_3), hydrogen peroxide (H_2O_2), transition metals, and metal oxides) and auxiliary energy sources (e.g., ultraviolet-visible (UV-Vis) radiation, electronic current, g-radiation, and ultrasound). Examples of AOPs comprise O_3/H_2O_2, O_3/UV, O_3/H_2O_2/UV, H_2O_2/UV, Fenton (Fe^{2+}/H_2O_2), photo- and electro-Fenton, chelating agent-assisted Fenton/photo-Fenton, heterogeneous photooxidation using titanium dioxide ($TiO_2/h\nu$), g-radiolysis, and sonolysis (Ikehata et al., 2007).

Photochemical degradation belongs to AOP most applied for the removal of pollutants in aquatic environments. This process consists on the degradation of a substance by the action of sunlight or another source of light (Periša et al., 2013). Two types of photodegradation may occur in natural waters: direct photolysis and indirect photolysis (Oliveira et al., 2019; Periša et al., 2013). In direct photolysis the pollutant absorbs photons able to induce chemical transformations or transformation into other compounds, identified as photoproducts (Oliveira et al., 2019, 2016). In indirect photolysis, other compounds, called photosensitizers, adsorb the light, generating photoreactants such as hydroxyl radicals ($^{\bullet}OH$) and peroxyl radicals ($ROO^{\bullet}$) or singlet oxygen ($^1O_2$), that will then interact with the pollutant, resulting in its chemical transformation (Oliveira et al.,

2019, 2016). Alternatively, self-sensitization processes can also take place (Geng et al., 2020; Zhang et al., 2019, 2018). In this particular case, after the photon is absorbed directly by the pollutant, it reaches the triplet excited state and can transfer the energy to dissolved organic matter (DOM) or H_2O to form reactive oxygen species (ROS). Some photosensitizers may have a dual role, acting as photosensitizers or retarding photodegradation by screening sunlight or scavenging reactive species. The photodegradation of pharmaceutical substances is influenced by different factors such as the presence of humic substances (HS), pH of the media, salinity, dissolved oxygen (DO) and the presence of $^1O_2$ and $^{\bullet}OH$ scavengers. In this context, when assessing the photodegradation of pharmaceutical compounds in real matrices, these factors must be considered, as they may accelerate or retard the degradation of these pollutants.

HS are natural organic species obtained from biochemical and chemical reactions during the decay and transformation of plant and microbial remains (Gibson and Jong, 2018). These substances are heterogeneous mixtures of polydispersed organic materials, generally present in soils, sediments and natural waters (IHSS, 2007). In the aquatic environment, HS represent about 50 % of the DOM (IHSS, 2007). HS obtained from soils and sediments can be divided into three main fractions: humic acids (HA), fulvic acids (FA) and humin (IHSS, 2007). Usually, a suitable resin column was used to extract HA and FA from soil and other solid phase sources. In this process, the pH is adjusted to 2 and the components remain adsorbed into the column. Subsequently, HA and FA can be extracted from the resin with strong base. As HA are insoluble at low pH, to extract HA, the pH is adjusted to 1 by adding strong acid that, consequently, precipitates HA. The part that remains after the isolation of HA corresponds to the fraction of FA, with lowest molecular weight and soluble at all pH. Humin are not soluble at low or high pH values, and therefore, cannot be extracted with either a strong base or a strong acid (IHSS, 2007). Esteves et al. (1995) performed the extraction and isolation of the different fractions of HS using two columns, one of Amberlite XAD-8 [poly(methylmetacrylate)] and one of Amberlite XAD-4 (styrene divinylbenzene), connected in series. In the first column, HAs and FAs are retained, and in the second column a smaller fraction, known as XAD-4 fraction is retained. The properties and structure of HS samples differ according to the source of water or soil and the specific extraction process. However, the mean properties of HA, FA and humin from different sources are noticeably similar (IHSS, 2007).

In a study conducted by Bian and Zhang (2016) the photodegradation of SDZ was investigated at different pH values. Results showed that, under direct solar radiation, in ultrapure water, the SDZ degradation rate was 99 % in one hour with a half-life time ($t_{½}$) of 9.76 min (Bian and Zhang, 2016). The same authors obtained the lowest $t_{½}$ value (from 10.8 min to 12 min) for

higher pH (6.98 and 8.20) when compared to lower pH (4.52, $t_{½}$ = 15 min). The same trend of the pH effect on photodegradation of SDZ was recently also noticed by Conde-Cid et al. (2018). However, much higher $t_{½}$ values (from 13.2 h to 70.5 h) were presented by the researchers in ultrapure water at different pH tested (4, 5.5 and 7.2). Another study carried out by Sukul et al. (2008) showed an improvement in SDZ degradation in the presence of HS as HA ($t_{½}$ between 17.2 h and 31.4 h) and FA ($t_{½}$ between 12.6 h and 29.8 h), compared to ultrapure water ($t_{½}$ = 32 h). Few studies have been reported in the literature for OXA photodegradation. Orfanou et al. (2009) examined the photoinduced degradation of OXA under simulated solar irradiation and found $t_{½}$ between 18.3 h and 26.2 h at different OXA initial concentrations. Another study showed a much higher persistence of OXA in aquaculture waters ($t_{½}$ from 55.2 h to 115.2 h) under solar radiation (Lai and Lin, 2009). These results indicate that the different factors can interfere with the photodegradation of SDZ and OXA. Although there are some studies on this issue in literature, much remains to be done to understand the photodegradation of these pollutants in real samples.

The photodegradation of E1 and EE2 is well known (Atkinson et al., 2011; Bertoldi et al., 2019; Chowdhury et al., 2010; Ren et al., 2017; Silva et al., 2016a; Zhang and Li, 2014). Bertoldi et al. (2019) investigated the removal of E1 and EE2 in the absence and presence of natural organic matter (NOM) and FA in aqueous solutions. In the presence of NOM the authors found faster E1 degradation ($t_{½}$ = 29 min) than in ultrapure water solutions ($t_{½}$ = 51.72 min) and FA ($t_{½}$ = 39.61 min) to 2.5 mg L^{-1} of initial concentration (Bertoldi et al., 2019). In the case of EE2, no statistically difference between the absence and presence of organic matter was found ($t_{½}$ = 40.06 min, for 2.5 mg L^{-1} of initial concentration) (Bertoldi et al., 2019). Silva et al. (2016a, 2016b) investigated the photodegradation of E1 and EE2 under simulated solar radiation in the absence and presence of the different fractions of HA, FA and XAD-4 fraction. For E1, the authors reported a decrease of $t_{½}$ from 6.10 h in ultrapure water to 3.91 h, 3.57 h and 2.23 h in the presence of HA, FA and XAD- 4, respectively (Silva et al., 2016a). Likewise, for EE2 a decrease of $t_{½}$ from 46 h in ultrapure water to 6.4 h, 2.1 h and 2.7 h in the presence of HA, FA and XAD- 4, respectively was observed (Silva et al., 2016b).

At this point, it is worth mentioning that, although considerable information exists on the influence of different factors on E1 and EE2 photodegradation, only a little knowledge on the photodegradation of SDZ and OXA is available. For this reason, for SDZ and OXA photodegradation, different factors will be addressed in this thesis (chapter 2).

1.2.3.2. ADSORPTION

The adsorption process is strongly dependent on the physicochemical properties of the adsorbed compound, which can be characterized by the K_{ow}, solid-water distribution coefficient (K_d), pK_a, ionization state and the molecular structure (Silva et al., 2012; Tran et al., 2018). Indeed, pollutants with higher log K_{ow} (above 4.0) often exhibit high sorption potential onto the particulate phase, while compounds with lower log K_{ow} (below 2.5) tend to have a low sorption potential (Caliman and Gavrilescu, 2009; Luo et al., 2014). Moreover, K_d, the ratio between the adsorbed and the dissolved concentrations of the compound at the equilibrium, is commonly used to quantify the affinity of a compound for a sorbent (Silva et al., 2012). Pollutants with log K_d values lower than 2 are negligible for adsorption, while for compounds with values higher than 4 adsorption to the sludge is a major removal mechanism (Clara et al., 2004). Nevertheless, the properties of the adsorbent can significantly affect the pollutant adsorption (De Gisi et al., 2016; Silva, 2014).

Data in the literature indicates that SDZ and OXA have low affinity to solids probably due to their low log K_{ow} values (-0.09 and 0.68, respectively) (Table 1.1) (Lin et al., 2018; Yan et al., 2014b). Moreover, for E1 and EE2 log K_d values between 2.4 and 2.8 were reported in literature, indicating that adsorption is a relevant process in the removal of these compounds (Andersen et al., 2005; Clara et al., 2004; Ternes et al., 2004). Thus, these compounds tend to have a strong partition in the particle phase and, consequently, exhibit a high sorption potential in the order EE2> E1 (Jones-Lepp and Stevens, 2007). Moreover, these estrogens have very low vapor pressures ranging from 4.5×10^{-11} to 2.3×10^{-10} mm Hg, suggesting low volatility of these pollutants (Ying et al., 2002). As a result, in WWTPs, a large proportion of estrogens is removed from aqueous medium through adsorption processes onto sludge solid components (Johnson and Sumpter, 2001; Muller et al., 2010; Zeng et al., 2009b, 2009a) and only a small proportion is biodegraded during the treatment process, namely EE2 (Johnson and Sumpter, 2001). Usually, the contaminated sludge from WWTP is dehydrated and applied as fertilizer on agriculture fields, contributing to the indirect application of estrogens to soils and a concomitant increase in their occurrence in the environment (Ikehata et al., 2007; Martín et al., 2010). Taking into account the reasons aforementioned, the analysis of E1 and EE2 adsorption onto sludge is of high importance, as presented in chapters 4 and 5.

1.2.3.3. BIOLOGICAL PROCESSES

Biodegradation is one of the key elimination processes of several pollutants in both aqueous and particulate phases during biological wastewater and sludge treatment processes (Tran et al., 2018). Biodegradation can occur through two main mechanisms: metabolism and co-metabolism (Tran et al., 2018). In co-metabolism, the microorganisms are likely to degrade the compounds in the mandatory presence of growth substrate. Meanwhile, in metabolism microorganisms use the compounds as the sole energy and/or carbon source to sustain their biomass and produce the relevant enzymes and cofactors for their oxidation/reduction (Tran et al., 2018). Some authors pointed out that in WWTPs, co-metabolism is the process that occurs due to the toxicity/resistance of these compounds and the low concentrations detected in wastewaters, which, consequently, do not favour the growth of the microbial population (Tran et al., 2018).

The biodegradability of a compound has been often assessed by its kinetic constant k_{biol} (L g $MLSS^{-1}$ d^{-1}). As evidenced in previous studies, compounds with k_{biol} above 10 L g $MLSS^{-1}$ d^{-1} were easily biodegraded during biological wastewater treatment processes (removal efficiency >90 %); compounds with k_{biol} between 0.1 and 10 L g $MLSS^{-1}$ d^{-1} were moderately removed by biodegradation processes (removal efficiencies 20-90 %); and compounds with k_{biol} below 0.1 L g $MLSS^{-1}$ d^{-1} tend to be persistent during the biological wastewater treatment processes (removal efficiency <20 %) (Joss et al., 2006).

Some authors remarked that SDZ and OXA resist to biodegradation (Biošić et al., 2017; Lai and Lin, 2009; Li et al., 2020). In fact, low k_{biol} values (0.13 L g $MLSS^{-1}$ d^{-1} to 1.21 L g $MLSS^{-1}$ d^{-1}) have been pointed out for SDZ (Abegglen et al., 2009; Xu et al., 2019). With regard to the biodegradation of E1, moderate and high k_{biol} values (between 2 L g $MLSS^{-1}$ d^{-1} and 200 L g $MLSS^{-1}$ d^{-1}) were achieved by some authors, suggesting that these compounds are easily biodegraded in activated sludge systems (Abegglen et al., 2009; Alvarino et al., 2016; Joss et al., 2006, 2004). Whereas for EE2, lower k_{biol} values (between 0.02 L g $MLSS^{-1}$ d^{-1} and 20 L g $MLSS^{-1}$ d^{-1}) were found, indicating that this compound is recalcitrant to biodegradation processes (Abegglen et al., 2009; Joss et al., 2006; Su et al., 2015; Suarez et al., 2010).

The biodegradability of a compound can be strongly affected by different factors such as the potential redox of the medium (aerobic *vs.* anaerobic), temperature, pH, the adaptation of microorganisms to the compound (heterotrophs and autotrophs), the presence of primary substrates (i.e. ammonium, inorganic and organic carbon sources), and the physicochemical

properties of the compound (Tran et al., 2018). The influence of some of these parameters on the biodegradation of E1 and EE2 will be assessed in chapters 4 and 5.

1.2.3.3.1. ANAEROBIC DIGESTION

Anaerobic digestion is a biological process for the treatment of industrial or domestic effluent, in which organic substrates, and occasionally inorganic substrates, are degraded in the absence of oxygen to produce a renewable fuel gas, the biogas, consisting mainly of methane (CH_4) (60 %) and carbon dioxide (CO_2) (40 %) (Mir et al., 2016). Other trace components such as molecular hydrogen (H_2), hydrogen sulphide (H_2S) and nitrogen (N_2) can be present in the biogas (Mir et al., 2016; Srivastava et al., 2020).

Some benefits have been highlighted for the anaerobic digestion process such as energy production, low production of surplus biomass, low energy and space requirements, high efficiency of pathogen removal, and suitability of sludge for fertilization in agricultural fields (European Comission, 2017; Ward et al., 2008). In anaerobic digestion several compounds are degraded, which grouped together can be classified as primary substrates (present in the effluent to be treated), intermediate substrates and final products. The primary substrates can consist of oils, fats, carbohydrates and proteins. Intermediate substrates can be of a variety of compounds such as butyrate, propionate and ethanol. Final products usually consist of methane and carbon dioxide (Mir et al., 2016).

The process of anaerobic digestion can occur in four main steps: hydrolysis, acidogenesis or fermentation, acetogenesis and methanogenesis (Mir et al., 2016). These steps are ensured by a microbial consortium (members of the *Bacteria* and *Archaea* domains) which constitutes the methanogenic trophic chain (Angenent et al., 2004).

The anaerobic digestion process can be subdivided into seven main stages responsible for methane production, as shown in Figure 1.4, as described:

1. Hydrolysis of complex organic matter, namely proteins, carbohydrates and lipids, with conversion to their monomers, namely amino acids, sugars and long-chain fatty acids.
2. Fermentation of amino acids and sugars.
3. Anaerobic oxidation of long-chain fatty acids to intermediate products and hydrogen.

4. Anaerobic oxidation of intermediates (volatile acids, excluding acetate) to acetate and hydrogen.
5. Homoacetogenesis, with acetate production occurring from hydrogen and carbon dioxide.
6. Conversion of acetate into methane by the action of acetoclastic methanogenic microorganisms.
7. Conversion of hydrogen into methane by the action of methanogenic hydrogenotrophic microorganisms.

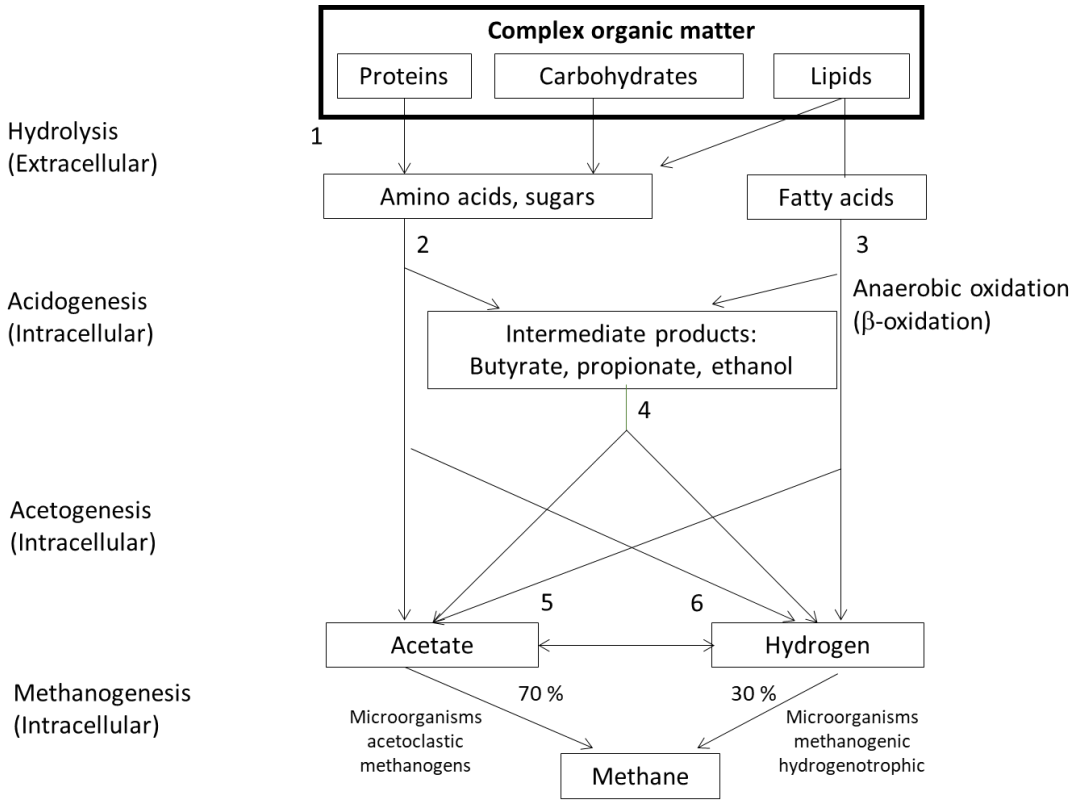


Figure 1.4 Process flow of the degradation of organic material through anaerobic digestion (adapted from Angenent et al. (2004) and Li et al.(2011)).

Due to the interactions between the different stages of the anaerobic process, a balanced relationship between the different trophic groups of microorganisms present in this process is fundamental (Angenent et al., 2004). The optimisation of anaerobic digestion processes is relevant as it allows the maximisation of the efficiencies of methanization, hydrogen production and volatile organic acids.

REFERENCES

Abegglen, C., Joss, A., McArdell, C.S., Fink, G., Schlüsener, M.P., Ternes, T.A., Siegrist, H., 2009. The fate of selected micropollutants in a single-house MBR. Water Res. 43, 2036–2046. doi:10.1016/j.watres.2009.02.005

Acosta-Rangel, A., Sánchez-Polo, M., Polo, A.M.S., Rivera-Utrilla, J., Berber-Mendoza, M.S., 2018. Sulfonamides degradation assisted by UV, UV/H2O2 and UV/K2S2O8: Efficiency, mechanism and byproducts cytotoxicity. J. Environ. Manage. 225, 224–231. doi:10.1016/J.JENVMAN.2018.06.097

Adeel, M., Song, X., Wang, Y., Francis, D., Yang, Y., 2017. Environmental impact of estrogens on human, animal and plant life: A critical review. Environ. Int. 99, 107–119. doi:10.1016/j.envint.2016.12.010

Al Aukidy, M., Verlicchi, P., Jelic, A., Petrovic, M., Barcelò, D., 2012. Monitoring release of pharmaceutical compounds: Occurrence and environmental risk assessment of two WWTP effluents and their receiving bodies in the Po Valley, Italy. Sci. Total Environ. 438, 15–25. doi:10.1016/J.SCITOTENV.2012.08.061

Alvarino, T., Suárez, S., Garrido, M., Lema, J.M., Omil, F., 2016. A UASB reactor coupled to a hybrid aerobic MBR as innovative plant configuration to enhance the removal of organic micropollutants. Chemosphere 144, 452–458. doi:10.1016/J.CHEMOSPHERE.2015.09.016

Andersen, H., Siegrist, H., Halling-Sørensen, B., Ternes, T.A., 2003. Fate of Estrogens in a Municipal Sewage Treatment Plant. Environ. Sci. Technol. 37, 4021–4026. doi:10.1021/es026192a

Andersen, H.R., Hansen, M., Kjølholt, J., Stuer-Lauridsen, F., Ternes, T., Halling-Sørensen, B., 2005. Assessment of the importance of sorption for steroid estrogens removal during activated sludge treatment. Chemosphere 61, 139–146. doi:10.1016/j.chemosphere.2005.02.088

Andreu, V., Blasco, C., Picó, Y., 2007. Analytical strategies to determine quinolone residues in food and the environment. TrAC Trends Anal. Chem. 26, 534–556. doi:10.1016/J.TRAC.2007.01.010

Angenent, L.T., Karim, K., Al-Dahhan, M.H., Wrenn, B.A., Domíguez-Espinosa, R., 2004. Production of bioenergy and biochemicals from industrial and agricultural wastewater. Trends Biotechnol. doi:10.1016/j.tibtech.2004.07.001

Aroh, K., 2018. Globalization, Industralization and Population Growth the Fundamental Drivers of Pollution in a Given Society (India). SSRN Electron. J. doi:10.2139/ssrn.3453740

Ashfaq, M., Li, Y., Wang, Y., Chen, W., Wang, H., Chen, X., Wu, W., Huang, Z., Yu, C.-P., Sun, Q., 2017. Occurrence, fate, and mass balance of different classes of pharmaceuticals and personal care products in an anaerobic-anoxic-oxic wastewater treatment plant in Xiamen, China. Water Res. 123, 655–667. doi:10.1016/J.WATRES.2017.07.014

Atkinson, S.K., Marlatt, V.L., Kimpe, L.E., Lean, D.R.S., Trudeau, V.L., Blais, J.M., 2012. The occurrence of steroidal estrogens in south-eastern Ontario wastewater treatment plants. Sci. Total Environ. 430, 119–125. doi:10.1016/j.scitotenv.2012.04.069

Atkinson, S.K., Marlatt, V.L., Kimpe, L.E., Lean, D.R.S., Trudeau, V.L., Blais, J.M., 2011. Environmental factors affecting ultraviolet photodegradation rates and estrogenicity of estrone and ethinylestradiol in natural waters. Arch. Environ. Contam. Toxicol. 60, 1–7.

doi:10.1007/s00244-010-9515-4

Baronti, C., Curini, R., D'Ascenzo, G., Di Corcia, A., Gentili, A., Samperi, R., 2000. Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. Environ. Sci. Technol. 34, 5059–5066. doi:10.1021/es001359q

Barreiros, L., Queiroz, J.F., Magalhães, L.M., Silva, A.M.T., Segundo, M.A., 2016. Analysis of 17-β-estradiol and 17-α-ethinylestradiol in biological and environmental matrices - A review. Microchem. J. 126, 243–262. doi:10.1016/j.microc.2015.12.003

Barry, A.L., Jones, R.N., Thornsberry, C., Ayers, L.W., Gerlach, E.H., Sommers, H.M., 1984. Antibacterial activities of ciprofloxacin, norfloxacin, oxolinic acid, cinoxacin, and nalidixic acid. Antimicrob. Agents Chemother. 25, 633–637. doi:10.1128/AAC.25.5.633

Batista, A.P.S., Pires, F.C.C., Teixeira, A.C.S.C., 2014. The role of reactive oxygen species in sulfamethazine degradation using UV-based technologies and products identification. J. Photochem. Photobiol. A Chem. 290, 77–85. doi:10.1016/J.JPHOTOCHEM.2014.06.006

Bertoldi, C., Rodrigues, A.G., Fernandes, A.N., 2019. Removal of endocrine disrupters in water under artificial light: the effect of organic matter. J. Water Process Eng. 27, 126–133. doi:10.1016/J.JWPE.2018.11.016

Bian, X., Zhang, J., 2016. Photodegradation of sulfadiazine in aqueous solution and the affecting factors. J. Chem. 2016, 1–5. doi:10.1155/2016/8358960

Biošić, M., Mitrevski, M., Babić, S., 2017. Environmental behavior of sulfadiazine, sulfamethazine, and their metabolites. Environ. Sci. Pollut. Res. 24, 9802–9812. doi:10.1007/s11356-017-8639-8

Bolong, N., Ismail, A.F., Salim, M.R., Matsuura, T., 2009. A review of the effects of emerging contaminants in wastewater and options for their removal. Desalination 239, 229–246. doi:10.1016/J.DESAL.2008.03.020

Boreen, A.L., Arnold, W.A., McNeill, K., 2005. Triplet-sensitized photodegradation of sulfa drugs containing six-membered heterocyclic groups: identification of an SO2 extrusion photoproduct. Environ. Sci. Technol. 39, 3630–3638. doi:10.1021/ES048331P

Boxall, A.B.A., 2004. The environmental side effects of medication. EMBO Rep. 5, 1110–1116. doi:10.1038/sj.embor.7400307

Braga, O., Smythe, G., Schafer, A., Feitz, A., 2005a. Steroid estrogens in primary and tertiary wastewater treatment plants. Water Sci. Technol. 52, 273–278.

Braga, O., Smythe, G., Schäfer, A., Feitz, A., 2005b. Fate of steroid estrogens in Australian inland and coastal wastewater treatment plants. Environ. Sci. Technol. 39, 3351–3358. doi:10.1021/es0501767

Brandt, E.M.F., de Queiroz, F.B., Afonso, R.J.C.F., Aquino, S.F., Chernicharo, C.A.L., 2013. Behaviour of pharmaceuticals and endocrine disrupting chemicals in simplified sewage treatment systems. J. Environ. Manage. 128, 718–726. doi:10.1016/j.jenvman.2013.06.003

Brooks, B.W., Maul, J.D., Belden, J.B., 2008. Antibiotics in aquatic and terrestrial ecosystems. Encycl. Ecol. 210–217. doi:10.1016/B978-008045405-4.00370-0

Caliman, F.A., Gavrilescu, M., 2009. Pharmaceuticals, personal care products and endocrine disrupting agents in the environment - a review. CLEAN - Soil, Air, Water 37, 277–303.

doi:10.1002/clen.200900038

Cargouët, M., Perdiz, D., Mouatassim-Souali, A., Tamisier-Karolak, S., Levi, Y., 2004. Assessment of river contamination by estrogenic compounds in Paris area (France). Sci. Total Environ. 324, 55–66. doi:10.1016/j.scitotenv.2003.10.035

Caruso, G., 2016. Antibiotic resistance in fish farming environments: a global concern. J. Fish. 10, 9–13.

Cases, V., Alonso, V., Argandoña, V., Rodriguez, M., Prats, D., 2011. Endocrine disrupting compounds: A comparison of removal between conventional activated sludge and membrane bioreactors. Desalination 272, 240–245. doi:10.1016/j.desal.2011.01.026

Chang, H., Wan, Y., Wu, S., Fan, Z., Hu, J., 2011. Occurrence of androgens and progestogens in wastewater treatment plants and receiving river waters: comparison to estrogens. Water Res. 45, 732–40. doi:10.1016/j.watres.2010.08.046

Chee-Sanford, J.C., Mackie, R.I., Koike, S., Krapac, I.G., Lin, Y.-F., Yannarell, A.C., Maxwell, S., Aminov, R.I., 2009. Fate and Transport of Antibiotic Residues and Antibiotic Resistance Genes following Land Application of Manure Waste. J. Environ. Qual. 38, 1086–1108. doi:10.2134/jeq2008.0128

Chen, X., Hu, J., 2010. Adsorption of natural estrogens and their conjugates by activated sludge. Water. Air. Soil Pollut. 206, 251–261. doi:10.1007/s11270-009-0102-0

Chowdhury, R.R., Charpentier, P., Ray, M.B., 2010. Photodegradation of estrone in solar irradiation. Ind. Eng. Chem. Res. 49, 6923–6930. doi:10.1021/ie901796x

Cicek, N., Londry, K., Oleszkiewicz, J.A., Wong, D., Lee, Y., 2007. Removal of selected natural and synthetic estrogenic compounds in a Canadian full-scale municipal wastewater treatment plant. Water Environ. Res. 79, 795–800. doi:10.2175/106143007X175744

Clara, M., Kreuzinger, N., Strenn, B., Gans, O., Kroiss, H., 2005. The solids retention time-a suitable design parameter to evaluate the capacity of wastewater treatment plants to remove micropollutants. Water Res. 39, 97–106. doi:10.1016/j.watres.2004.08.036

Clara, M., Strenn, B., Saracevic, E., Kreuzinger, N., 2004. Adsorption of bisphenol-A, 17β-estradiole and 17α- ethinylestradiole to sewage sludge. Chemosphere 56, 843–851. doi:10.1016/j.chemosphere.2004.04.048

Conde-Cid, M., Fernández-Calviño, D., Nóvoa-Muñoz, J.C., Arias-Estévez, M., Díaz-Raviña, M., Núñez-Delgado, A., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., 2018. Degradation of sulfadiazine, sulfachloropyridazine and sulfamethazine in aqueous media. J. Environ. Manage. 228, 239–248. doi:10.1016/J.JENVMAN.2018.09.025

Crini, G., Lichtfouse, E., Wilson, L.D., Morin-Crini, N., 2019. Conventional and non-conventional adsorbents for wastewater treatment. Environ. Chem. Lett. 17, 195–213. doi:10.1007/s10311-018-0786-8

Cui, C.W., Ji, S.L., Ren, H.Y., 2006. Determination of steroid estrogens in wastewater treatment plant of a controceptives producing factory. Environ. Monit. Assess. 121, 409–19. doi:10.1007/s10661-005-9139-8

Cui, J., Fu, L., Tang, B., Bin, L., Li, P., Huang, S., Fu, F., 2020. Occurrence, ecotoxicological risks of sulfonamides and their acetylated metabolites in the typical wastewater treatment plants and

receiving rivers at the Pearl River Delta. Sci. Total Environ. 709, 136192. doi:10.1016/J.SCITOTENV.2019.136192

Cui, J., Shen, Y., Li, R., 2013. Estrogen synthesis and signaling pathways during aging: From periphery to brain. Trends Mol. Med. doi:10.1016/j.molmed.2012.12.007

D'Ascenzo, G., Di Corcia, A., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M., Samperi, R., 2003. Fate of natural estrogen conjugates in municipal sewage transport and treatment facilities. Sci. Total Environ. 302, 199–209. doi:10.1016/S0048-9697(02)00342-X

De Gisi, S., Lofrano, G., Grassi, M., Notarnicola, M., 2016. Characteristics and adsorption capacities of low-cost sorbents for wastewater treatment: A review. Sustain. Mater. Technol. 9, 10–40. doi:10.1016/J.SUSMAT.2016.06.002

Duan, Y., Deng, L., Shi, Z., Liu, X., Zeng, H., Zhang, H., Crittenden, J., 2020. Efficient sulfadiazine degradation via in-situ epitaxial grow of Graphitic Carbon Nitride (g-C3N4) on carbon dots heterostructures under visible light irradiation: Synthesis, mechanisms and toxicity evaluation. J. Colloid Interface Sci. 561, 696–707. doi:10.1016/J.JCIS.2019.11.046

Duong, H.A., Pham, N.H., Nguyen, H.T., Hoang, T.T., Pham, H.V., Pham, V.C., Berg, M., Giger, W., Alder, A.C., 2008. Occurrence, fate and antibiotic resistance of fluoroquinolone antibacterials in hospital wastewaters in Hanoi, Vietnam. Chemosphere 72, 968–973. doi:10.1016/J.CHEMOSPHERE.2008.03.009

Esteban, S., Gorga, M., Petrovic, M., González-Alonso, S., Barceló, D., Valcárcel, Y., 2014. Analysis and occurrence of endocrine-disrupting compounds and estrogenic activity in the surface waters of Central Spain. Sci. Total Environ. 466–467, 939–951. doi:10.1016/j.scitotenv.2013.07.101

Esteves, V.I., Cordeiro, N.M.A., da Costa Duarte, A., 1995. Variation on the adsorption efficiency of humic substances from estuarine waters using XAD resins. Mar. Chem. 51, 61–66. doi:10.1016/0304-4203(95)00044-R

European Comission, 2017. Optimal use of biogas from waste streams [WWW Document].

European Union, 2000. Documents - Substances - Water - Environment - European Commission [WWW Document]. Water Framew. Dir. URL https://ec.europa.eu/environment/water/water-dangersub/lib_pri_substances.htm#prop_2011_docs (accessed 5.5.20).

Fan, Z., Wu, S., Chang, H., Hu, J., 2011. Behaviors of glucocorticoids, androgens and progestogens in a municipal sewage treatment plant: comparison to estrogens. Environ. Sci. Technol. 45, 2725–33. doi:10.1021/es103429c

Fekete, A., Schmitt-Kopplin, P., 2007. Capillary electrophoresis, in: Food Toxicants Analysis. Elsevier, pp. 561–597. doi:10.1016/B978-044452843-8/50016-X

Food and Agriculture Organization of the United Nations (FAO), 2020. Water pollution from agriculture: a global review [WWW Document]. URL http://www.fao.org/home/en/ (2020) (accessed 10.11.20).

Gabet-Giraud, V., Miège, C., Choubert, J.M., Ruel, S.M., Coquery, M., 2010. Occurrence and removal of estrogens and beta blockers by various processes in wastewater treatment plants. Sci. Total Environ. 408, 4257–69. doi:10.1016/j.scitotenv.2010.05.023

Gao, L., Shi, Y., Li, W., Niu, H., Liu, J., Cai, Y., 2012. Occurrence of antibiotics in eight sewage treatment plants in Beijing, China. Chemosphere 86, 665–671. doi:10.1016/j.chemosphere.2011.11.019

Gao, P., Ding, Y., Li, H., Xagoraraki, I., 2012. Occurrence of pharmaceuticals in a municipal wastewater treatment plant: Mass balance and removal processes. Chemosphere 88, 17–24. doi:10.1016/j.chemosphere.2012.02.017

García-Galán, M.J., Díaz-Cruz, M.S., Barceló, D., 2011. Occurrence of sulfonamide residues along the Ebro river basin. Removal in wastewater treatment plants and environmental impact assessment. Environ. Int. 37, 462–473. doi:10.1016/j.envint.2010.11.011

Geng, C., Liang, Z., Cui, F., Zhao, Z., Yuan, C., Du, J., Wang, C., 2020. Energy-saving photo-degradation of three fluoroquinolone antibiotics under VUV/UV irradiation: Kinetics, mechanism, and antibacterial activity reduction. Chem. Eng. J. 383, 123145. doi:10.1016/J.CEJ.2019.123145

Gibson, J.K., Jong, W.A., 2018. Experimental and Theoretical Approaches to Actinide Chemistry, Wiley.

Gorga, M., Petrovic, M., Barceló, D., 2013. Multi-residue analytical method for the determination of endocrine disruptors and related compounds in river and waste water using dual column liquid chromatography switching system coupled to mass spectrometry. J. Chromatogr. A 1295, 57–66. doi:10.1016/j.chroma.2013.04.028

Grover, D.P., Zhang, Z.L., Readman, J.W., Zhou, J.L., 2009. A comparison of three analytical techniques for the measurement of steroidal estrogens in environmental water samples. Talanta 78, 1204–10. doi:10.1016/j.talanta.2008.12.049

Grover, D.P., Zhou, J.L., Frickers, P.E., Readman, J.W., 2011. Improved removal of estrogenic and pharmaceutical compounds in sewage effluent by full scale granular activated carbon: Impact on receiving river water. J. Hazard. Mater. 185, 1005–1011. doi:10.1016/j.jhazmat.2010.10.005

Guo, X., Feng, C., Gu, E., Tian, C., Shen, Z., 2019. Spatial distribution, source apportionment and risk assessment of antibiotics in the surface water and sediments of the Yangtze Estuary. Sci. Total Environ. 671, 548–557. doi:10.1016/J.SCITOTENV.2019.03.393

Hagren, V., Peippo, P., Lövgren, T., 2005. Detecting and controlling veterinary drug residues in poultry, in: Food Safety Control in the Poultry Industry. Elsevier Ltd, pp. 44–82. doi:10.1533/9781845690236.44

Hamid, H., Eskicioglu, C., 2012. Fate of estrogenic hormones in wastewater and sludge treatment: A review of properties and analytical detection techniques in sludge matrix. Water Res. 46, 5813–5833. doi:10.1016/j.watres.2012.08.002

Hashimoto, T., Murakami, T., 2009. Removal and degradation characteristics of natural and synthetic estrogens by activated sludge in batch experiments. Water Res. 43, 573–582. doi:10.1016/j.watres.2008.10.051

He, S., Dong, D., Zhang, X., Sun, C., Wang, C., Hua, X., Zhang, L., Guo, Z., 2018. Occurrence and ecological risk assessment of 22 emerging contaminants in the Jilin Songhua River (Northeast China). Environ. Sci. Pollut. Res. 25, 24003–24012. doi:10.1007/s11356-018-2459-3

Hedgespeth, M.L., Sapozhnikova, Y., Pennington, P., Clum, A., Fairey, A., Wirth, E., 2012.

Pharmaceuticals and personal care products (PPCPs) in treated wastewater discharges into Charleston Harbor, South Carolina. Sci. Total Environ. 437, 1–9. doi:10.1016/J.SCITOTENV.2012.07.076

Hohenblum, P., Gans, O., Moche, W., Scharf, S., Lorbeer, G., 2004. Monitoring of selected estrogenic hormones and industrial chemicals in groundwaters and surface waters in Austria. Sci. Total Environ. 333, 185–193. doi:10.1016/j.scitotenv.2004.05.009

Huang, B., Wang, B., Ren, D., Jin, W., Liu, J., Peng, J., Pan, X., 2013. Occurrence, removal and bioaccumulation of steroid estrogens in Dianchi Lake catchment, China. Environ. Int. 59, 262–273. doi:10.1016/J.ENVINT.2013.06.018

Ikehata, K., Jodeiri Naghashkar, N., Gamal El-Din, M., 2007. Degradation of aqueous pharmaceuticals by ozonation and advanced oxidation processes: a review. Ozone Sci. Eng. 28, 353–414. doi:10.1080/01919510600985937

International Humic Substances Society, 2007. What are humic substances? [WWW Document]. URL https://humic-substances.org/what-are-humic-substances-2/ (accessed 12.15.20).

Janecko, N., Pokludova, L., Blahova, J., Svobodova, Z., Literak, I., 2016. Implications of fluoroquinolone contamination for the aquatic environment-A review. Environ. Toxicol. Chem. 35, 2647–2656. doi:10.1002/etc.3552

Jobling, S., Nolan, M., Tyler, C.R., Brighty, G., Sumpter, J.P., 1998. Widespread sexual disruption in wild fish. Environ. Sci. Technol. 32, 2498–2506. doi:10.1021/es9710870

Johnson, A., Belfroid, A., Di Corcia, A., 2000. Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent. Sci. Total Environ. 256, 163–173. doi:10.1016/S0048-9697(00)00481-2

Johnson, A., Sumpter, J., 2001. Removal of endocrine-disrupting chemicals in activated sludge treatment works. Environ. Sci. Technol. 35, 4697–4703. doi:10.1021/es010171j

Johnson, A., Williams, R., 2004. A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works. Environ. Sci. Technol. 38, 3649–3658. doi:10.1021/es035342u

Johnson, A., Williams, R., Simpson, P., Kanda, R., 2007. What difference might sewage treatment performance make to endocrine disruption in rivers? Environ. Pollut. 147, 194–202. doi:10.1016/j.envpol.2006.08.032

Jones-Lepp, T.L., Stevens, R., 2007. Pharmaceuticals and personal care products in biosolids/sewage sludge: the interface between analytical chemistry and regulation. Anal. Bioanal. Chem. 387, 1173–1183. doi:10.1007/s00216-006-0942-z

Jones, O.A.H., Voulvoulis, N., Lester, J.N., 2004. Potential ecological and human health risks associated with the presence of pharmaceutically active compounds in the aquatic environment. Crit. Rev. Toxicol. 34, 335–350. doi:10.1080/10408440490464697

Joss, A., Andersen, H., Ternes, T., Richle, P.R., Siegrist, H., 2004. Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization. Environ. Sci. Technol. 38, 3047–3055. doi:10.1021/es0351488

Joss, A., Zabczynski, S., Göbel, A., Hoffmann, B., Löffler, D., McArdell, C.S., Ternes, T.A., Thomsen, A., Siegrist, H., 2006. Biological degradation of pharmaceuticals in municipal wastewater

treatment: Proposing a classification scheme. Water Res. 40, 1686–1696. doi:10.1016/J.WATRES.2006.02.014

Kaur, H., Hippargi, G., Pophali, G.R., Bansiwal, A.K., 2019. Treatment methods for removal of pharmaceuticals and personal care products from domestic wastewater. Pharm. Pers. Care Prod. Waste Manag. Treat. Technol. 129–150. doi:10.1016/B978-0-12-816189-0.00006-8

Kidd, K.A., Blanchfield, P.J., Mills, K.H., Palace, V.P., Evans, R.E., Lazorchak, J.M., Flick, R.W., 2007. Collapse of a fish population after exposure to a synthetic estrogen. Proc. Natl. Acad. Sci. 104, 8897–8901. doi:10.1073/PNAS.0609568104

Kim, S., Aga, D.S., 2007. Potential ecological and human health impacts of antibiotics and antibiotic-resistant bacteria from wastewater treatment plants. J. Toxicol. Environ. Heal. Part B 10, 559–573. doi:10.1080/15287390600975137

Kokulnathan, T., Kumar, E.A., Wang, T.J., Cheng, I.C., 2021. Strontium tungstate-modified disposable strip for electrochemical detection of sulfadiazine in environmental samples. Ecotoxicol. Environ. Saf. 208, 111516. doi:10.1016/j.ecoenv.2020.111516

Kovalova, L., Siegrist, H., Singer, H., Wittmer, A., McArdell, C.S., 2012. Hospital wastewater treatment by membrane bioreactor: Performance and efficiency for organic micropollutant elimination. Environ. Sci. Technol. 46, 1536–1545. doi:10.1021/es203495d

Lai, H.-T., Lin, J.-J., 2009. Degradation of oxolinic acid and flumequine in aquaculture pond waters and sediments. Chemosphere 75, 462–468. doi:10.1016/J.CHEMOSPHERE.2008.12.060

Lastre-Acosta, A.M., Palharim, P.H., Barbosa, I.M., Mierzwa, J.C., Silva Costa Teixeira, A.C., 2020. Removal of sulfadiazine from simulated industrial wastewater by a membrane bioreactor and ozonation. J. Environ. Manage. 271, 111040. doi:10.1016/j.jenvman.2020.111040

Launay, M.A., Dittmer, U., Steinmetz, H., 2016. Organic micropollutants discharged by combined sewer overflows – Characterisation of pollutant sources and stormwater-related processes. Water Res. 104, 82–92. doi:10.1016/J.WATRES.2016.07.068

Lee, H.-B., Peart, T.E., Svoboda, M.L., 2005. Determination of endocrine-disrupting phenols, acidic pharmaceuticals, and personal-care products in sewage by solid-phase extraction and gas chromatography-mass spectrometry. J. Chromatogr. A 1094, 122–9. doi:10.1016/j.chroma.2005.07.070

Li, B., Zhang, T., 2011. Mass flows and removal of antibiotics in two municipal wastewater treatment plants. Chemosphere 83, 1284–1289. doi:10.1016/j.chemosphere.2011.03.002

Li, B., Zhang, T., Xu, Z., Fang, H.H.P., 2009. Rapid analysis of 21 antibiotics of multiple classes in municipal wastewater using ultra performance liquid chromatography-tandem mass spectrometry. Anal. Chim. Acta 645, 64–72. doi:10.1016/j.aca.2009.04.042

Li, H., Duan, L., Wang, H., Chen, Y., Wang, F., Zhang, S., 2020. Photolysis of sulfadiazine under UV radiation: Effects of the initial sulfadiazine concentration, pH, NO3− and Cd2+. Chem. Phys. Lett. 739, 136949. doi:10.1016/J.CPLETT.2019.136949

Li, Y., Park, S.Y., Zhu, J., 2011. Solid-state anaerobic digestion for methane production from organic waste. Renew. Sustain. Energy Rev. doi:10.1016/j.rser.2010.07.042

Li, Z., Dai, R., Yang, B., Chen, M., Wang, X., Wang, Z., 2021. An electrochemical membrane biofilm reactor for removing sulfonamides from wastewater and suppressing antibiotic resistance

development: Performance and mechanisms. J. Hazard. Mater. 404, 124198. doi:10.1016/j.jhazmat.2020.124198

Lian, J., Qiang, Z., Li, M., Bolton, J.R., Qu, J., 2015. UV photolysis kinetics of sulfonamides in aqueous solution based on optimized fluence quantification. Water Res. 75, 43–50. doi:10.1016/J.WATRES.2015.02.026

Lima, D.L.D., Schneider, R.J., Esteves, V.I., 2012. Sorption behavior of EE2 on soils subjected to different long-term organic amendments. Sci. Total Environ. 423, 120–124. doi:10.1016/J.SCITOTENV.2012.02.014

Lin, H., Li, H., Chen, L., Li, L., Yin, L., Lee, H., Yang, Z., 2018. Mass loading and emission of thirty-seven pharmaceuticals in a typical municipal wastewater treatment plant in Hunan Province, Southern China. Ecotoxicol. Environ. Saf. 147, 530–536. doi:10.1016/j.ecoenv.2017.08.052

Lin, J.-S., Pan, H.-Y., Liu, S.-M., Lai, H.-T., 2010. Effects of light and microbial activity on the degradation of two fluoroquinolone antibiotics in pond water and sediment. J. Environ. Sci. Heal. Part B 45, 456–465. doi:10.1080/03601231003800222

Lishman, L., Smyth, S.A., Sarafin, K., Kleywegt, S., Toito, J., Peart, T., Lee, B., Servos, M., Beland, M., Seto, P., 2006. Occurrence and reductions of pharmaceuticals and personal care products and estrogens by municipal wastewater treatment plants in Ontario, Canada. Sci. Total Environ. 367, 544–58. doi:10.1016/j.scitotenv.2006.03.021

Liu, M., Zhang, D., Han, J., Liu, C., Ding, Y., Wang, Z., Wang, A., 2020. Adsorption enhanced photocatalytic degradation sulfadiazine antibiotic using porous carbon nitride nanosheets with carbon vacancies. Chem. Eng. J. 382, 123017. doi:10.1016/J.CEJ.2019.123017

Liu, R., Zhou, J.L., Wilding, A., 2004. Simultaneous determination of endocrine disrupting phenolic compounds and steroids in water by solid-phase extraction-gas chromatography-mass spectrometry. J. Chromatogr. A 1022, 179–189. doi:10.1016/j.chroma.2003.09.035

Liu, X., Lu, S., Meng, W., Wang, W., 2018. Occurrence, source, and ecological risk of antibiotics in Dongting Lake, China. Environ. Sci. Pollut. Res. 25, 11063–11073. doi:10.1007/s11356-018-1290-1

Liu, Z., Kanjo, Y., Mizutani, S., 2009. Removal mechanisms for endocrine disrupting compounds (EDCs) in wastewater treatment — physical means, biodegradation, and chemical advanced oxidation: A review. Sci. Total Environ. 407, 731–748. doi:10.1016/j.scitotenv.2008.08.039

Lulijwa, R., Rupia, E.J., Alfaro, A.C., 2020. Antibiotic use in aquaculture, policies and regulation, health and environmental risks: a review of the top 15 major producers. Rev. Aquac. 12, 640–663. doi:10.1111/raq.12344

Luo, Y., Guo, W., Ngo, H.H., Nghiem, L.D., Hai, F.I., Zhang, J., Liang, S., Wang, X.C., 2014. A review on the occurrence of micropollutants in the aquatic environment and their fate and removal during wastewater treatment. Sci. Total Environ. 473–474, 619–641. doi:10.1016/J.SCITOTENV.2013.12.065

Lützhøft, H.-C.H., Vaes, W.H.., Freidig, A.P., Halling-Sørensen, B., Hermens, J.L.., 2000. 1-Octanol/water distribution coefficient of oxolinic acid: influence of pH and its relation to the interaction with dissolved organic carbon. Chemosphere 40, 711–714. doi:10.1016/S0045-6535(99)00441-5

Marti, E.J., Batista, J.R., 2014. Impact of secondary treatment types and sludge handling processes

on estrogen concentration in wastewater sludge. Sci. Total Environ. 470–471, 1056–1067. doi:10.1016/j.scitotenv.2013.10.070

Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2015. Pharmaceutically active compounds in sludge stabilization treatments: anaerobic and aerobic digestion, wastewater stabilization ponds and composting. Sci. Total Environ. 503–504, 97–104. doi:10.1016/j.scitotenv.2014.05.089

Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2010. Multi-residue method for the analysis of pharmaceutical compounds in sewage sludge, compost and sediments by sonication-assisted extraction and LC determination. J. Sep. Sci. 33, 1760–1766. doi:10.1002/jssc.200900873

Massé, D.I., Saady, N.M.C., Gilbert, Y., 2014. Potential of biological processes to eliminate antibiotics in livestock manure: an overview. Anim. an open access J. from MDPI 4, 146–63. doi:10.3390/ani4020146

Matthiessen, P., Gibbs, P.E., 1998. Critical appraisal of the evidence for tributyltin-mediated endocrine disruption in mollusks. Environ. Toxicol. Chem. 17, 37–43. doi:10.1002/etc.5620170106

Melo, S.A.S., Trovó, A.G., Bautitz, I.R., Nogueira, R.F.P., 2009. Degradação de fármacos residuais por processos oxidativos avançados. Quim. Nova 32, 188–197. doi:10.1590/S0100-40422009000100034

Michael, I., Rizzo, L., McArdell, C.S., Manaia, C.M., Merlin, C., Schwartz, T., Dagot, C., Fatta-Kassinos, D., 2013. Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. Water Res. doi:10.1016/j.watres.2012.11.027

Miège, C., Choubert, J.M., Ribeiro, L., Eusèbe, M., Coquery, M., 2009. Fate of pharmaceuticals and personal care products in wastewater treatment plants – Conception of a database and first results. Environ. Pollut. 157, 1721–1726. doi:10.1016/J.ENVPOL.2008.11.045

Miklos, D.B., Remy, C., Jekel, M., Linden, K.G., Drewes, J.E., Hübner, U., 2018. Evaluation of advanced oxidation processes for water and wastewater treatment – A critical review. Water Res. doi:10.1016/j.watres.2018.03.042

Miossec, C., Lanceleur, L., Monperrus, M., 2019. Multi-residue analysis of 44 pharmaceutical compounds in environmental water samples by solid-phase extraction coupled to liquid chromatography-tandem mass spectrometry. J. Sep. Sci. 42, 1853–1866. doi:10.1002/jssc.201801214

Mir, M.A., Hussain, A., Verma, C., 2016. Design considerations and operational performance of anaerobic digester: A review. Cogent Eng. 3, 1181696. doi:10.1080/23311916.2016.1181696

Moore, S.C., Matthews, C.E., Ou Shu, X., Yu, K., Gail, M.H., Xu, X., Ji, B.-T., Chow, W.-H., Cai, Q., Li, H., Yang, G., Ruggieri, D., Boyd-Morin, J., Rothman, N., Hoover, R.N., Gao, Y.-T., Zheng, W., Ziegler, R.G., 2016. Endogenous Estrogens, Estrogen Metabolites, and Breast Cancer Risk in Postmenopausal Chinese Women. J. Natl. Cancer Inst. 108, djw103. doi:10.1093/jnci/djw103

Muller, M., Combalbert, S., Delgenès, N., Bergheaud, V., Rocher, V., Benoît, P., Delgenès, J.-P., Patureau, D., Hernandez-Raquet, G., 2010. Occurrence of estrogens in sewage sludge and their fate during plant-scale anaerobic digestion. Chemosphere 81, 65–71. doi:10.1016/j.chemosphere.2010.06.062

Muller, M., Rabenoelina, F., Balaguer, P., Patureau, D., Lemenach, K., Budzinski, H., Barceló, D., de Alda, M.L., Kuster, M., Delgenès, J.-P., Hernandez-Raquet, G., 2008. Chemical and biological

analysis of endocrine-disrupting hormones and estrogenic activity in an advanced sewage treatment plant. Environ. Toxicol. Chem. 27, 1649. doi:10.1897/07-519.1

Nakada, N., Tanishima, T., Shinohara, H., Kiri, K., Takada, H., 2006. Pharmaceutical chemicals and endocrine disrupters in municipal wastewater in Tokyo and their removal during activated sludge treatment. Water Res. 40, 3297–303. doi:10.1016/j.watres.2006.06.039

Nelles, J.L., Hu, W.-Y., Prins, G.S., 2011. Estrogen action and prostate cancer. Expert Rev. Endocrinol. Metab. 6, 437–451. doi:10.1586/eem.11.20

Nghiem, L.D., Schäfer, A.I., Elimelech, M., 2004. Removal of Natural Hormones by Nanofiltration Membranes: Measurement, Modeling, and Mechanisms. Environ. Sci. Technol. 38, 1888–1896. doi:10.1021/es034952r

Nie, Y., Qiang, Z., Zhang, H., Ben, W., 2012. Fate and seasonal variation of endocrine-disrupting chemicals in a sewage treatment plant with A/A/O process. Sep. Purif. Technol. 84, 9–15. doi:10.1016/j.seppur.2011.01.030

Oliveira, C., Lima, D.L.D., Silva, C.P., Calisto, V., Otero, M., Esteves, V.I., 2019. Photodegradation of sulfamethoxazole in environmental samples: The role of pH, organic matter and salinity. Sci. Total Environ. 648, 1403–1410. doi:10.1016/j.scitotenv.2018.08.235

Oliveira, C., Lima, D.L.D., Silva, C.P., Otero, M., Esteves, V.I., 2016. Photodegradation behaviour of estriol: An insight on natural aquatic organic matter influence. Chemosphere 159, 545–551. doi:10.1016/j.chemosphere.2016.06.046

Orfanou, F., Michaleas, S., Benaki, D., Galanopoulou, O., Voulgari, A., Antoniadou-Vyza, E., 2009. Photostabilization of oxolinic acid in hydroxypropyl-β-cyclodextrins; implications for the effect of molecular self-assembly phenomena. J. Incl. Phenom. Macrocycl. Chem. 64, 289–297. doi:10.1007/s10847-009-9565-2

Paíga, P., Correia, M., Fernandes, M.J., Silva, A., Carvalho, M., Vieira, J., Jorge, S., Silva, J.G., Freire, C., Delerue-Matos, C., 2019. Assessment of 83 pharmaceuticals in WWTP influent and effluent samples by UHPLC-MS/MS: Hourly variation. Sci. Total Environ. 648, 582–600. doi:10.1016/j.scitotenv.2018.08.129

Paíga, P., Santos, L.H.M.L.M., Ramos, S., Jorge, S., Silva, J.G., Delerue-Matos, C., 2016. Presence of pharmaceuticals in the Lis river (Portugal): Sources, fate and seasonal variation. Sci. Total Environ. 573, 164–177. doi:10.1016/j.scitotenv.2016.08.089

Palma, P., Fialho, S., Lima, A., Novais, M.H., Costa, M.J., Montemurro, N., Pérez, S., de Alda, M.L., 2020. Pharmaceuticals in a Mediterranean Basin: The influence of temporal and hydrological patterns in environmental risk assessment. Sci. Total Environ. 709, 136205. doi:10.1016/j.scitotenv.2019.136205

Patel, M., Kumar, R., Kishor, K., Mlsna, T., Pittman, C.U., Mohan, D., 2019. Pharmaceuticals of emerging concern in aquatic systems: Chemistry, occurrence, effects, and removal methods. Chem. Rev. doi:10.1021/acs.chemrev.8b00299

Pedersen, J.A., Yeager, M.A., Suffet, I.H., 2003. Xenobiotic organic compounds in runoff from fields irrigated with treated wastewater. J. Agric. Food Chem. 51, 1360–72. doi:10.1021/jf025953q

Pérez-Lucas, G., Vela, N., El Aatik, A., Navarro, S., 2019. Environmental Risk of Groundwater Pollution by Pesticide Leaching through the Soil Profile, in: Pesticides - Use and Misuse and Their Impact in the Environment. IntechOpen. doi:10.5772/intechopen.82418

Periša, M., Babić, S., Škorić, I., Frömel, T., Knepper, T.P., 2013. Photodegradation of sulfonamides and their N 4-acetylated metabolites in water by simulated sunlight irradiation: kinetics and identification of photoproducts. Environ. Sci. Pollut. Res. 20, 8934–8946. doi:10.1007/s11356-013-1836-1

Pessoa, G.P., de Souza, N.C., Vidal, C.B., Alves, J.A.C., Firmino, P.I.M., Nascimento, R.F., dos Santos, A.B., 2014. Occurrence and removal of estrogens in Brazilian wastewater treatment plants. Sci. Total Environ. 490, 288–295. doi:10.1016/j.scitotenv.2014.05.008

Qin, L.-T., Pang, X.-R., Zeng, H.-H., Liang, Y.-P., Mo, L.-Y., Wang, D.-Q., Dai, J.-F., 2020. Ecological and human health risk of sulfonamides in surface water and groundwater of Huixian karst wetland in Guilin, China. Sci. Total Environ. 708, 134552. doi:10.1016/J.SCITOTENV.2019.134552

Queiroz, F.B., Brandt, E.M.F., Aquino, S.F., Chernicharo, C.A.L., Afonso, R.J.C.F., 2012. Occurrence of pharmaceuticals and endocrine disruptors in raw sewage and their behavior in UASB reactors operated at different hydraulic retention times. Water Sci. Technol. 66, 2562–9. doi:10.2166/wst.2012.482

Rasul, M.G., Majumdar, B.C., 2017. Abuse of antibiotics in aquaculture and it's effects on human, aquatic animal and environment. Haya Saudi J. Life Sci. 2, 81–88. doi:10.21276/haya

Ren, D., Huang, B., Xiong, D., He, H., Meng, X., Pan, X., 2017. Photodegradation of 17α-ethynylestradiol in dissolved humic substances solution: Kinetics, mechanism and estrogenicity variation. J. Environ. Sci. (China) 54, 196–205. doi:10.1016/j.jes.2016.03.002

Ribeiro, C., Tiritan, M.E., Rocha, E., Rocha, M.J., 2009. Seasonal and spatial distribution of several endocrine-disrupting compounds in the Douro River Estuary, Portugal. Arch. Environ. Contam. Toxicol. 56, 1–11. doi:10.1007/s00244-008-9158-x

Rodgers-Gray, T.P., Jobling, S., Kelly, C., Morris, S., Brighty, G., Waldock, M.J., Sumpter, J.P., Tyler, C.R., 2001. Exposure of juvenile roach (rutilus rutilus) to treated sewage effluent induces dose-dependent and persistent disruption in gonadal duct development. Environ. Sci. Technol. 35, 462–470. doi:10.1021/ES001225C

Rodriguez-Mozaz, S., Vaz-Moreira, I., Varela Della Giustina, S., Llorca, M., Barceló, D., Schubert, S., Berendonk, T.U., Michael-Kordatou, I., Fatta-Kassinos, D., Martinez, J.L., Elpers, C., Henriques, I., Jaeger, T., Schwartz, T., Paulshus, E., O'Sullivan, K., Pärnänen, K.M.M., Virta, M., Do, T.T., Walsh, F., Manaia, C.M., 2020. Antibiotic residues in final effluents of European wastewater treatment plants and their impact on the aquatic environment. Environ. Int. 140, 105733. doi:10.1016/j.envint.2020.105733

Rodríguez, E., Navarro-Villoslada, F., Benito-Peña, E., Marazuela, M.D., Moreno-Bondi, M.C., 2011. Multiresidue determination of ultratrace levels of fluoroquinolone antimicrobials in drinking and aquaculture water samples by automated online molecularly imprinted solid phase extraction and liquid chromatography. Anal. Chem. 83, 2046–2055. doi:10.1021/ac102839n

Romero, J., Feijoó, C.G., Navarrete, P., 2012. Antibiotics in aquaculture – use, abuse and alternatives, in: Health and Environment in Aquaculture. InTech, pp. 159–198. doi:10.5772/28157

Rose, E., Paczolt, K.A., Jones, A.G., 2013. The effects of synthetic estrogen exposure on premating and postmating episodes of selection in sex-role-reversed Gulf pipefish. Evol. Appl. 6, 1160–1170. doi:10.1111/eva.12093

Rosenfeld, P.E., Feng, L.G.H., Rosenfeld, P.E., Feng, L.G.H., 2011. Emerging Contaminants. Risks Hazard. Wastes 215–222. doi:10.1016/B978-1-4377-7842-7.00016-7

Salgado, R., Noronha, J.P., Oehmen, A., Carvalho, G., Reis, M.A.M., 2010. Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology. Water Sci. Technol. 62.

Samuelsen, O., Lunestad, B., Husevag, B., Hølleland, T., Ervik, A., 1992. Residues of oxolinic acid in wild fauna following medication in fish farms. Dis. Aquat. Organ. 12, 111–119. doi:10.3354/dao012111

Sarmah, A.K., Northcott, G.L., Leusch, F.D.L., Tremblay, L.A., 2006. A survey of endocrine disrupting chemicals (EDCs) in municipal sewage and animal waste effluents in the Waikato region of New Zealand. Sci. Total Environ. 355, 135–44. doi:10.1016/j.scitotenv.2005.02.027

Servos, M.R., Bennie, D.T., Burnison, B.K., Jurkovic, A., McInnis, R., Neheli, T., Schnell, A., Seto, P., Smyth, S.A., Ternes, T.A., 2005. Distribution of estrogens, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants. Sci. Total Environ. 336, 155–170. doi:10.1016/j.scitotenv.2004.05.025

Shao, X.-L., Ma, J., 2009. Fate and mass balance of 13 kinds of endocrine disrupting chemicals in a sewage treatment plant, in: 2009 3rd International Conference on Bioinformatics and Biomedical Engineering. IEEE, pp. 1–4. doi:10.1109/ICBBE.2009.5162850

Shrivastava, A., 2018. Polymerization, in: Introduction to Plastics Engineering. Elsevier, pp. 17–48. doi:10.1016/B978-0-323-39500-7.00002-2

Sica, D.A., 2008. Diuretics. Side Eff. Drugs Annu. 30, 252–261. doi:10.1016/S0378-6080(08)00021-4

Sidhu, J.P.S., Ahmed, W., Gernjak, W., Aryal, R., McCarthy, D., Palmer, A., Kolotelo, P., Toze, S., 2013. Sewage pollution in urban stormwater runoff as evident from the widespread presence of multiple microbial and chemical source tracking markers. Sci. Total Environ. 463–464, 488–496. doi:10.1016/J.SCITOTENV.2013.06.020

Silva, C.P., 2014. Occurrence and fate of estrogens and antibiotics in the invironment evaluated by low-cost analytical methodologies. PhD thesis, University of Aveiro, Portugal.

Silva, C.P., Lima, D.L.D., Groth, M.B., Otero, M., Esteves, V.I., 2016a. Effect of natural aquatic humic substances on the photodegradation of estrone. Chemosphere 145, 249–255. doi:10.1016/J.CHEMOSPHERE.2015.11.068

Silva, C.P., Lima, D.L.D., Otero, M., Esteves, V.I., 2016b. Photosensitized degradation of 17β-estradiol and 17α-ethinylestradiol: role of humic substances fractions. J. Environ. Qual. 45, 693–700. doi:10.2134/jeq2015.07.0396

Silva, C.P., Otero, M., Esteves, V., 2012. Processes for the elimination of estrogenic steroid hormones from water: a review. Environ. Pollut. 165, 38–58. doi:10.1016/j.envpol.2012.02.002

Singh, S.P., Azua, A., Chaudhary, A., Khan, S., Willett, K.L., Gardinali, P.R., 2010. Occurrence and distribution of steroids, hormones and selected pharmaceuticals in South Florida coastal environments. Ecotoxicology 19, 338–350. doi:10.1007/s10646-009-0416-0

Snyder, S.A., Adham, S., Redding, A.M., Cannon, F.S., DeCarolis, J., Oppenheimer, J., Wert, E.C., Yoon, Y., 2007. Role of membranes and activated carbon in the removal of endocrine

disruptors and pharmaceuticals. Desalination 202, 156–181. doi:10.1016/j.desal.2005.12.052

Srivastava, R.K., Shetti, N.P., Reddy, K.R., Aminabhavi, T.M., 2020. Sustainable energy from waste organic matters via efficient microbial processes. Sci. Total Environ. 722, 137927. doi:10.1016/j.scitotenv.2020.137927

Sturini, M., Speltini, A., Maraschi, F., Pretali, L., Profumo, A., Fasani, E., Albini, A., 2014. Environmental photochemistry of fluoroquinolones in soil and in aqueous soil suspensions under solar light. Environ. Sci. Pollut. Res. 21, 13215–13221. doi:10.1007/s11356-013-2124-9

Su, D., Ben, W., Strobel, B.W., Qiang, Z., 2020. Occurrence, source estimation and risk assessment of pharmaceuticals in the Chaobai River characterized by adjacent land use. Sci. Total Environ. 712, 134525. doi:10.1016/J.SCITOTENV.2019.134525

Su, L., Aga, D., Chandran, K., Khunjar, W.O., 2015. Factors impacting biotransformation kinetics of trace organic compounds in lab-scale activated sludge systems performing nitrification and denitrification. J. Hazard. Mater. 282, 116–124. doi:10.1016/j.jhazmat.2014.08.007

Suarez, S., Lema, J.M., Omil, F., 2010. Removal of pharmaceutical and personal care products (PPCPs) under nitrifying and denitrifying conditions. Water Res. 44, 3214–3224. doi:10.1016/j.watres.2010.02.040

Sukul, P., Lamshöft, M., Zühlke, S., Spiteller, M., 2008. Photolysis of 14C-sulfadiazine in water and manure. Chemosphere 71, 717–725. doi:10.1016/J.CHEMOSPHERE.2007.10.045

Sukul, P., Spiteller, M., 2006. Sulfonamides in the environment as veterinary drugs. Springer, New York, NY, pp. 67–101. doi:10.1007/0-387-32885-8_2

Sumpter, J.P., Jobling, S., 2013. The occurrence, causes, and consequences of estrogens in the aquatic environment. Environ. Toxicol. Chem. 32, 249–251. doi:10.1002/etc.2084

Tamtam, F., Mercier, F., Le Bot, B., Eurin, J., Tuc Dinh, Q., Clément, M., Chevreuil, M., 2008. Occurrence and fate of antibiotics in the Seine River in various hydrological conditions. Sci. Total Environ. 393, 84–95. doi:10.1016/J.SCITOTENV.2007.12.009

Tan, R., Liu, R., Li, B., Liu, X., Li, Z., 2018. Typical endocrine disrupting compounds in rivers of Northeast China: occurrence, partitioning, and risk assessment. Arch. Environ. Contam. Toxicol. 75, 213–223. doi:10.1007/s00244-017-0482-x

Ternes, T., Kreckel, P., Mueller, J., 1999. Behaviour and occurrence of estrogens in municipal sewage treatment plants — II. Aerobic batch experiments with activated sludge. Sci. Total Environ. 225, 91–99. doi:10.1016/S0048-9697(98)00335-0

Ternes, TA, Stumpf, M., Mueller, J., Haberer, K., Wilken, R., Servos, M., 1999. Behavior and occurence of estrogen in municipal sewage treatment plants - I. investigation in Germany, Canada and Brazil. Sci. Total environtment 225, 81–90.

Ternes, T.A., Herrmann, N., Bonerz, M., Knacker, T., Siegrist, H., Joss, A., 2004. A rapid method to measure the solid-water distribution coefficient (K d) for pharmaceuticals and musk fragrances in sewage sludge. Water Res. 38, 4075–4084. doi:10.1016/j.watres.2004.07.015

Toledo-Neira, C., Bravo, M.A., Álvarez-Lueje, A., 2016. New approach for determination of flumequine and oxolinic acid in aqueous samples by ionic liquid-based dispersive liquid-liquid microextraction and high-performance liquid chromatography-fluorescence detection. J. Chil. Chem. Soc. 61, 3262–3266. doi:10.4067/S0717-97072016000400022

Tran, N.H., Gin, K.Y.-H., 2017. Occurrence and removal of pharmaceuticals, hormones, personal care products, and endocrine disrupters in a full-scale water reclamation plant. Sci. Total Environ. 599–600, 1503–1516. doi:10.1016/J.SCITOTENV.2017.05.097

Tran, N.H., Reinhard, M., Gin, K.Y.-H., 2018. Occurrence and fate of emerging contaminants in municipal wastewater treatment plants from different geographical regions-a review. Water Res. 133, 182–207. doi:10.1016/J.WATRES.2017.12.029

Van der Aa, N.G.F.M., Kommer, G.J., Van Montfoort, J.E., Versteegh, J.F.M., 2011. Demographic projections of future pharmaceutical consumption in the Netherlands. Water Sci. Technol. 63, 825–831. doi:10.2166/wst.2011.120

Verlicchi, P., Al Aukidy, M., Zambello, E., 2012. Occurrence of pharmaceutical compounds in urban wastewater: Removal, mass load and environmental risk after a secondary treatment—A review. Sci. Total Environ. 429, 123–155. doi:10.1016/J.SCITOTENV.2012.04.028

Verlicchi, P., Galletti, A., Petrovic, M., Barceló, D., 2010. Hospital effluents as a source of emerging pollutants: An overview of micropollutants and sustainable treatment options. J. Hydrol. 389, 416–428. doi:10.1016/J.JHYDROL.2010.06.005

Vethaak, A.D., Lahr, J., Schrap, S.M., Belfroid, A.C., Rijs, G.B.J., Gerritsen, A., De Boer, J., Bulder, A.S., Grinwis, G.C.M., Kuiper, R. V., Legler, J., Murk, T.A.J., Peijnenburg, W., Verhaar, H.J.M., De Voogt, P., 2005. An integrated assessment of estrogenic contamination and biological effects in the aquatic environment of The Netherlands. Chemosphere 59, 511–524. doi:10.1016/j.chemosphere.2004.12.053

Wagman, A.S., Wentland, M.P., 2007. Quinolone antibacterial agents, in: Comprehensive Medicinal Chemistry II. Elsevier Ltd., pp. 667–696. doi:10.1016/b0-08-045044-x/00220-0

Wang, B., Li, H., Liu, T., Guo, J., 2021. Enhanced removal of cephalexin and sulfadiazine in nitrifying membrane-aerated biofilm reactors. Chemosphere 263, 128224. doi:10.1016/j.chemosphere.2020.128224

Ward, A.J., Hobbs, P.J., Holliman, P.J., Jones, D.L., 2008. Optimisation of the anaerobic digestion of agricultural resources. Bioresour. Technol. doi:10.1016/j.biortech.2008.02.044

Weber, S., Leuschner, P., Kämpfer, P., Dott, W., Hollender, J., 2005. Degradation of estradiol and ethinyl estradiol by activated sludge and by a defined mixed culture. Appl. Microbiol. Biotechnol. 67, 106–112. doi:10.1007/s00253-004-1693-4

WHO, 2020. World Health Organization [WWW Document]. URL https://www.who.int/ (accessed 8.12.20).

Wolf, L., Zwiener, C., Zemann, M., 2012. Tracking artificial sweeteners and pharmaceuticals introduced into urban groundwater by leaking sewer networks. Sci. Total Environ. 430, 8–19. doi:10.1016/J.SCITOTENV.2012.04.059

Xia, S., Jia, R., Feng, F., Xie, K., Li, H., Jing, D., Xu, X., 2012. Effect of solids retention time on antibiotics removal performance and microbial communities in an A/O-MBR process. Bioresour. Technol. 106, 36–43. doi:10.1016/j.biortech.2011.11.112

Xu, N., Xu, Y.F., Xu, S., Li, J., Tao, H.C., 2012. Removal of estrogens in municipal wastewater treatment plants: A Chinese perspective. Environ. Pollut. 165, 215–224. doi:10.1016/j.envpol.2011.12.025

Xu, R., Qin, W., Tian, Z., He, Y., Wang, X., Wen, X., 2020. Enhanced micropollutants removal by nanofiltration and their environmental risks in wastewater reclamation: A pilot-scale study. Sci. Total Environ. 744, 140954. doi:10.1016/j.scitotenv.2020.140954

Xu, R., Wu, Z., Zhou, Z., Meng, F., 2019. Removal of sulfadiazine and tetracycline in membrane bioreactors: linking pathway to microbial community shift. Environ. Technol. 40, 134–143. doi:10.1080/09593330.2017.1380714

Xu, W., Zhang, G., Li, X., Zou, S., Li, P., Hu, Z., Li, J., 2007. Occurrence and elimination of antibiotics at four sewage treatment plants in the Pearl River Delta (PRD), South China. Water Res. 41, 4526–4534. doi:10.1016/j.watres.2007.06.023

Yadav, S., Jadeja, N.B., Dafale, N.A., Purohit, H.J., 2019. Pharmaceuticals and personal care products mediated antimicrobial resistance: future challenges. Pharm. Pers. Care Prod. Waste Manag. Treat. Technol. 409–428. doi:10.1016/B978-0-12-816189-0.00017-2

Yan, Q., Gao, X., Chen, Y.P., Peng, X.Y., Zhang, Y.X., Gan, X.M., Zi, C.F., Guo, J.S., 2014a. Occurrence, fate and ecotoxicological assessment of pharmaceutically active compounds in wastewater and sludge from wastewater treatment plants in Chongqing, the Three Gorges Reservoir Area. Sci. Total Environ. 470–471, 618–630. doi:10.1016/j.scitotenv.2013.09.032

Yan, Q., Gao, X., Huang, L., Gan, X.M., Zhang, Y.X., Chen, Y.P., Peng, X.Y., Guo, J.S., 2014b. Occurrence and fate of pharmaceutically active compounds in the largest municipal wastewater treatment plant in Southwest China: Mass balance analysis and consumption back-calculated model. Chemosphere 99, 160–170. doi:10.1016/j.chemosphere.2013.10.062

Yang, X., Flowers, R.C., Weinberg, H.S., Singer, P.C., 2011. Occurrence and removal of pharmaceuticals and personal care products (PPCPs) in an advanced wastewater reclamation plant. Water Res. 45, 5218–5228. doi:10.1016/J.WATRES.2011.07.026

Yao, C., Li, T., Twu, P., Pitner, W.R., Anderson, J.L., 2011. Selective extraction of emerging contaminants from water samples by dispersive liquid–liquid microextraction using functionalized ionic liquids. J. Chromatogr. A 1218, 1556–1566. doi:10.1016/J.CHROMA.2011.01.035

Yi, X., Tran, N.H., Yin, T., He, Y., Gin, K.Y.-H., 2017. Removal of selected PPCPs, EDCs, and antibiotic resistance genes in landfill leachate by a full-scale constructed wetlands system. Water Res. 121, 46–60. doi:10.1016/J.WATRES.2017.05.008

Ying, G.-G., Kookana, R.S., Ru, Y.-J., 2002. Occurrence and fate of hormone steroids in the environment. Environ. Int. 28, 545–551. doi:10.1016/S0160-4120(02)00075-2

Yu, Z., Xiao, B., Huang, W., Peng, P., 2004. Sorption of steroid estrogens to soils and sediments. Environ. Toxicol. Chem. 23, 531. doi:10.1897/03-192

Zeghioud, H., Kamagate, M., Coulibaly, L.S., Rtimi, S., Assadi, A.A., 2019. Photocatalytic degradation of binary and ternary mixtures of antibiotics: reactive species investigation in pilot scale. Chem. Eng. Res. Des. 144, 300–309. doi:10.1016/J.CHERD.2019.02.015

Zeng, Q., Li, Y., Gu, G., 2009a. Nitrate-dependent degradation of 17α-ethinylestradiol by acclimated activated sludge under anaerobic conditions. J. Chem. Technol. Biotechnol. 84, 1841–1847. doi:10.1002/jctb.2255

Zeng, Q., Li, Y., Gu, G., Zhao, J., Zhang, C., Luan, J., 2009b. Sorption and biodegradation of 17β-Estradiol by acclimated aerobic activated sludge and isolation of the bacterial strain. Environ.

Eng. Sci. 26, 783–790. doi:10.1089/ees.2008.0116

Zhang, A., Li, Y., 2014. Removal of phenolic endocrine disrupting compounds from waste activated sludge using UV, H2O2, and UV/H2O2 oxidation processes: Effects of reaction conditions and sludge matrix. Sci. Total Environ. 493, 307–323. doi:10.1016/J.SCITOTENV.2014.05.149

Zhang, M., Mao, Q., Feng, J., Yuan, S., Wang, Q., Huang, D., Zhang, J., 2016. Validation and application of an analytical method for the determination of selected acidic pharmaceuticals and estrogenic hormones in wastewater and sludge. J. Environ. Sci. Heal. Part A 51, 914–920. doi:10.1080/10934529.2016.1191304

Zhang, Z., Gao, P., Cheng, J., Liu, G., Zhang, X., Feng, Y., 2018. Enhancing anaerobic digestion and methane production of tetracycline wastewater in EGSB reactor with GAC/NZVI mediator. Water Res. 136, 54–63. doi:10.1016/J.WATRES.2018.02.025

Zhang, Z., Ren, N., Kannan, K., Nan, J., Liu, L., Ma, W., Qi, H., Li, Y., 2014. Occurrence of endocrine-disrupting phenols and estrogens in water and sediment of the Songhua River, Northeastern China. Arch. Environ. Contam. Toxicol. 66, 361–369. doi:10.1007/s00244-014-9998-5

Zhang, Z., Xie, X., Yu, Z., Cheng, H., 2019. Influence of chemical speciation on photochemical transformation of three fluoroquinolones (FQs) in water: Kinetics, mechanism, and toxicity of photolysis products. Water Res. 148, 19–29. doi:10.1016/J.WATRES.2018.10.027

Zhou, H., Huang, X., Wang, X., Zhi, X., Yang, C., Wen, X., Wang, Q., Tsuno, H., Tanaka, H., 2010. Behaviour of selected endocrine-disrupting chemicals in three sewage treatment plants of Beijing, China. Environ. Monit. Assess. 161, 107–21. doi:10.1007/s10661-008-0731-6

Zhou, H., Zhou, Y., Li, H., Wang, F., 2012. Fate and removal of selected endocrine-disrupting compounds in sewage using activated sludge treatment. Water Environ. J. 26, 435–444. doi:10.1111/j.1747-6593.2011.00302.x

Zhou, Y., Zha, J., Wang, Z., 2012a. Occurrence and fate of steroid estrogens in the largest wastewater treatment plant in Beijing, China. Environ. Monit. Assess. 184, 6799–813. doi:10.1007/s10661-011-2459-y

Zhou, Y., Zha, J., Xu, Y., Lei, B., Wang, Z., 2012b. Occurrences of six steroid estrogens from different effluents in Beijing, China. Environ. Monit. Assess. 184, 1719–29. doi:10.1007/s10661-011-2073-z

PART I

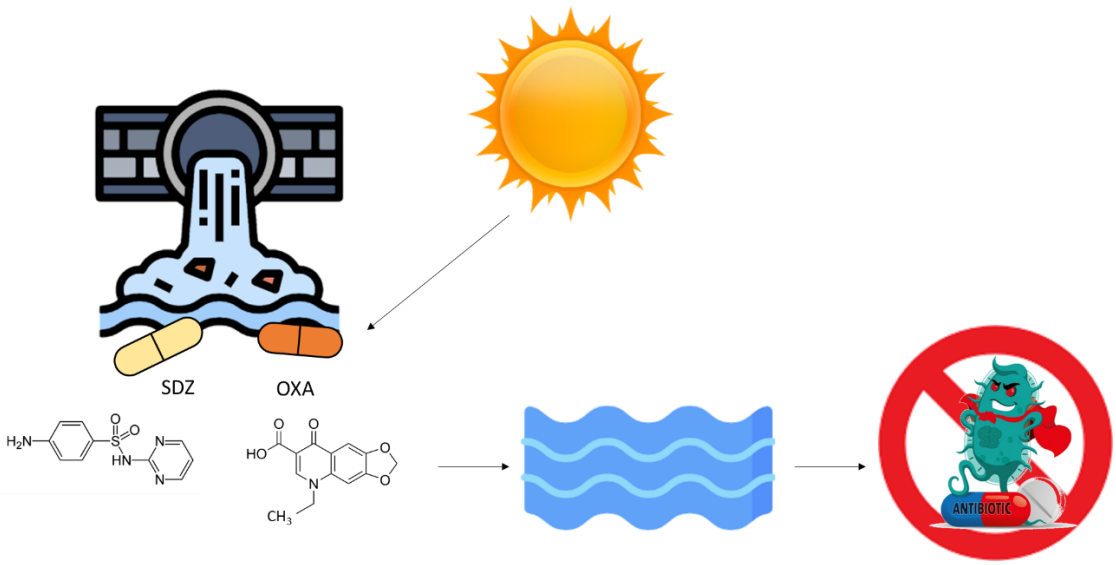
EVALUATION OF THE REMOVAL OF ANTIBIOTICS IN THE AQUATIC ENVIRONMENT

CHAPTER 2

PHOTOSENSITIZED DEGRADATION OF SULFADIAZINE AND OXOLINIC ACID IN WATER

The work presented and discussed in this chapter resulted in the following publications:

1. Louros, V. L., Silva, C. P., Nadais, H., Otero, M., Esteves, V. I., & Lima, D. L. D. (2020a). Photodegradation of sulfadiazine in different aquatic environments – Evaluation of influencing factors. Environmental Research, 188, 109730. https://doi.org/10.1016/J.ENVRES.2020.109730
2. Louros, V. L., Silva, C. P., Nadais, H., Otero, M., Esteves, V. I., & Lima, D. L. D. (2020b). Oxolinic acid in aquaculture waters: Can natural attenuation through photodegradation decrease its concentration? Science of The Total Environment, 749, 141661. https://doi.org/10.1016/J.SCITOTENV.2020.141661


SDZ
OXA
ANTIBIOTIC

SUMMARY

Direct and indirect photodegradation of sulfadiazine (SDZ) and oxolinic acid (OXA) using solar radiation, and influencing factors concerning antibiotics photodegradation in different aquatic environments were evaluated in order to have a better knowledge about their persistence.

The results hereby presented showed a faster photodegradation for OXA in ultrapure water (97 % to 99 %, after 4 h) than for SDZ (24 % to 30 %, after 4 h). Photodegradation of SDZ was found to be more efficient at higher pH ($t_{½}$ = 6.76 h ± 0.07 h, at pH = 7.3; $t_{½}$ = 12.19 h ± 0.03 h, at pH = 6.3), in presence of humic substances (HS) ($t_{½}$ between 1.76 h ± 0.04 h and 2.42 h ± 0.04 h), as well as in presence of NaCl ($t_{½}$ = 1.00 h ± 0.06 h) or synthetic sea salts ($t_{½}$ = 0.78 h ± 0.06 h). Using hydroxyl radical ($^{\bullet}OH$) and singlet oxygen ($^1O_2$) scavengers, it was possible to infer that direct photolysis was the main pathway for SDZ photodegradation in ultrapure water. Furthermore, results under N_2 purging confirmed that $^1O_2$ could not be too much relevant in the phototransformation of SDZ. The referred observations were used for the interpretation of results obtained in environmental matrices, namely the final effluent of a wastewater treatment plant (WWTP), fresh and brackish water, in which the SDZ photodegradation ($t_{½}$ between 2.32 h ± 0.05 h and 3.48 h ± 0.07 h) was found to be much faster than in ultrapure water ($t_{½}$ = 6.76 h ± 0.07 h).

Regarding OXA, a remarkable decrease on its photodegradation was achieved in solutions containing HS ($t_{½}$ between 1.70 h ± 0.05 h and 2.38 h ± 0.07 h) and synthetic sea salts ($t_{½}$ = 4.25 h ± 0.04 h), in comparison with ultrapure water ($t_{½}$ = 0.99 h ± 0.04 h). The presence of NaCl appeared not to affect the OXA photodegradation ($t_{½}$ = 0.74 h ± 0.02 h). Moreover, under solar radiation, the use of an $^1O_2$ scavenger led to a pronounced retardation of OXA decay, suggesting that $^1O_2$ plays an important role in OXA photodegradation process. In environmental matrices, a very sharp decrease of OXA photodegradation was evident, being more pronounced in brackish water ($t_{½}$ = 4.03 h ± 0.04 h).

Consequently, the different factors investigated exhibited a crucial importance on the photodegradation of both SDZ and OXA that can justify the results obtained in real environmental matrices.

CONTENTS

2.1. CONTEXTUALIZATION

Pharmaceuticals' environmental persistence usually depends, to a large extent, on the activity of microorganisms with the capability to degrade them (Caracciolo et al., 2015). However, since antibiotics may resist microbial degradation, alternative processes, such as photolysis, may be more influential on their persistence and effective in their degradation than biological processes (Pouliquen et al., 2007). Photolysis has, in fact, been considered as an effective way to decrease antibiotics' concentration in the environment (Oliveira et al., 2019; Silva et al., 2020). As previously mentioned, photolysis can happen by one of two ways: directly or indirectly (Figure 2.1).

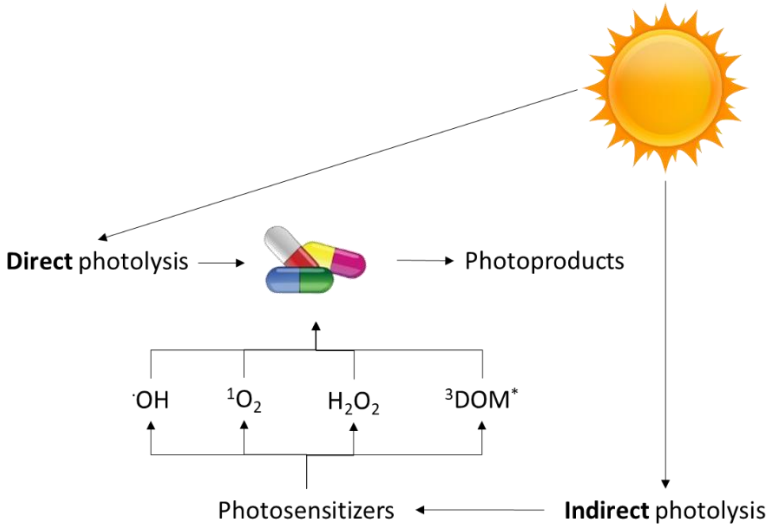


Figure 2.1 Direct and indirect photoprocesses occurring in the aquatic environment. Legend: •OH, hydroxyl radicals; $^1O_2$, singlet oxygen; ^{3}DOM*, dissolved organic matter in the triplet state.

In direct photodegradation, photon absorption by the compound itself promotes electrons from its initial ground-state to produce electronically excited species in the singlet state or triplet state that then decompose into photo- products. On the other hand, triplet state can transfer the energy to dissolved oxygen (DO) or H_2O to form reactive oxygen species (ROS) such as hydroxyl radicals (•OH) or singlet oxygen ($^1O_2$), which subsequently cause the self-sensitized pollutant' photooxidation. In indirect photodegradation, other substances present in the medium (photosensitizers) absorb the radiation and generate radicals (e.g. •OH, $^1O_2$, peroxyl radicals (ROO•) and excited triplet states of dissolved organic matter (^{3}DOM*)) (Carlos et al., 2012; Silva et al., 2016a; Yi et al., 2018; Zhao et al., 2019) capable of causing the phototransformation of the

pollutant. In any case, these processes can be noticeably impacted by the characteristics of the aquatic medium and, therefore, for an appropriate assessment of the environmental fate and risks of antibiotics, namely sulfadiazine (SDZ) and oxolinic acid (OXA), it is necessary to understand the effects of the matrix properties on their photodegradation.

Different researchers have evaluated the effect that some environmental factors may have on the photodegradation of different antibiotics (Table 2.1). Even though photodegradation has been studied for a wide range of antibiotics, information on the influence of different factors on SDZ and OXA photodegradation is very scarce. OXA photodegradation studies in literature are limited, however, for other quinolone antibiotics (QAs) there are some published results (Table 2.1). Pseudo-first order rate constants can vary a lot depending on the QA under study. Turiel et al. (2005) have studied the evolution of degradation and photoproducts of OXA in river water samples and reported that 80 % of OXA remained unaltered, after five months stored at room temperature under direct sunlight, pointing to a recalcitrant behaviour of OXA in the aquatic environment. Pouliquen et al. (2007) also observed a low contribution (10 %) of photolysis on OXA degradation in water, being more stable to photolysis than other QAs, which was considered to presage a higher environmental impact of OXA comparatively with other antibiotics from the same class. On the other hand, TiO_2-catalysed photodegradation was proven to be 100 % efficient in OXA degradation (Pereira et al., 2013). Lai and Lin (2009) investigated the fate of OXA (C_i = 20 mg L^{-1}) in waters from aquaculture ponds using natural light and found $t_{1/2}$ values between 55.2 h and 115.2 h.

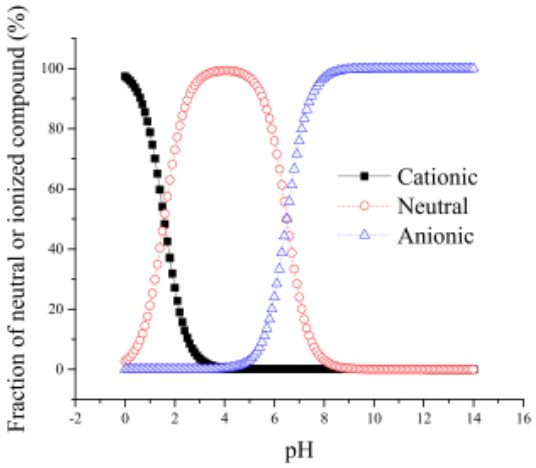
In the case of sulfonamides (SAs) photodegradation, in particular for SDZ, some studies have been presented in literature (Bian and Zhang, 2016; Conde-Cid et al., 2018; Liu et al., 2018; Periša et al., 2013; Sukul et al., 2008). Bahnmüller et al. (2014) investigated the phototransformation of SDZ in sunlit surface water and achieved $t_{1/2}$ values between 5.33 h and 11.55 h in river samples (pH = 8.5) and between 3.15 h and 7.70 h in wastewater samples (pH = 8.0 to 8.3). Some studies emphasize that pH significantly influences SDZ speciation in the aquatic environment (Lian et al., 2015; Liu et al., 2016). Figure 2.2 present the diagram of pH influence on the SDZ speciation in aqueous solution and the different structures of this molecule according to its dissociation constant (pK_a). Three types of species can be formed by the dissociation of SDZ: for pH < 1.8, SDZ exists mainly in the cationic form (SDZ^+), for pH between 1.8 and 6.5, the main form is neutral (SDZ^0), and for pH > 6.5, SDZ exists in its anionic form (SDZ^-) (Li et al., 2020). Thus, SDZ has two pK_a values (p$K_{a,1}$ = 1.57 to 2.14 and p$K_{a,2}$ = 6.34 to 6.99) and acts as a weak acid that may occur in two main forms - HSDZ (undissociated form) and SDZ^- (deprotonated form) (Acosta-Rangel et al., 2018; Batista et al., 2014; Boreen et al., 2005; Lian et al., 2015).

Table 2.1 Pseudo-first order rate constants (k (h^{-1})) found in literature for the photodegradation of SAs and QAs antibiotics under natural or simulated solar irradiation.

Antibiotic [a]	Matrix	pH	Conductivity (mS cm^{-1})	k (h^{-1})	$t_{1/2}$ (h)	Reference
CIP	Freshwater	7.7	0.23	13.2	0.05	Sturini et al. (2012)
	Ultrapure water	9		1.7	0.4	Vasconcelos et al. (2009)
		5		0.9	0.8	
DAN	Freshwater	7.7	0.23	39.6	0.02	Sturini et al. (2012)
DIF	Ultrapure water	5.6		0.8	0.87	Prabhakaran et al. (2009)
	HA (10 mg L^{-1})	4.3		0.4	1.73	
	FA (10 mg L^{-1})	4.3		0.5	1.39	
ENR	Freshwater	7.7	0.23	14.4	0.05	Sturini et al. (2012)
		8	0.38	9	0.08	Sturini et al. (2010)
FLU	Eel pond water	8.4	0.91	0.01	55.2	Lai and Lin (2009)
	Shrimp pond water	8.1	18	0.02	45.6	
LEV	Freshwater	7.7	0.23	11.4	0.06	Sturini et al. (2012)
MAR	Freshwater	7.7	0.23	3.7	0.19	Sturini et al. (2012)
		8	0.38	3.6	0.19	Sturini et al. (2010)
MOX	Freshwater	7.7	0.23	20.4	0.03	Sturini et al. (2012)
OXA	Eel pond water	8.4	0.91	0.013	55.2	Lai and Lin (2009)
	Shrimp pond water	8.1	18	0.006	115.2	
SARA	Ultrapure water	5.6		0.3	2.31	Prabhakaran et al. (2009)
	HA (10 mg L^{-1})	4.6		0.2	3.47	
	FA (10 mg L^{-1})	4.6		0.2	3.47	
SDZ	Ultrapure water	4.52		2.76	0.26	Bian and Zhang (2016)
		6.98		3.9	0.18	
		8.20		3.42	0.20	
	Ultrapure water with N_2 instead of O_2			2.70	0.26	
	Ultrapure water	4.0		0.010	70.5	Conde-Cid et al. (2018)
		5.5		0.036	19.1	
		7.2		0.052	13.2	
	Ultrapure water			1.27	0.54	Liu et al. (2018)
	C_3H_8O (50 mg L^{-1})			0.72	0.88	
	NaN_3 (50 mg L^{-1})			0.65	1.06	
	Ultrapure water	4		0.0871	7.96	Periša et al. (2013)
		8		0.3650	1.90	
	Ultrapure water	7		0.022	32	Sukul et al. (2008)
	HA (5 mg L^{-1}, 10 mg L^{-1}, 50 mg L^{-1})	7		0.022 to 0.040	17.2 to 31.4	
	FA (5 mg L^{-1}, 10 mg L^{-1}, 50 mg L^{-1})	7		0.023 to 0.055	12.6 to 29.8	

[a] CIP: ciprofloxacin; DAN: danofloxacin; DIF: difloxacin; ENR: enrofloxacin; FLU: flumequine; LEV: levofloxacin; MAR: marbofloxacin; MOX: moxifloxacin; OXA: oxolinic acid; SARA: sarafloxacin; SDZ, sulfadiazine.

(a)



(b)

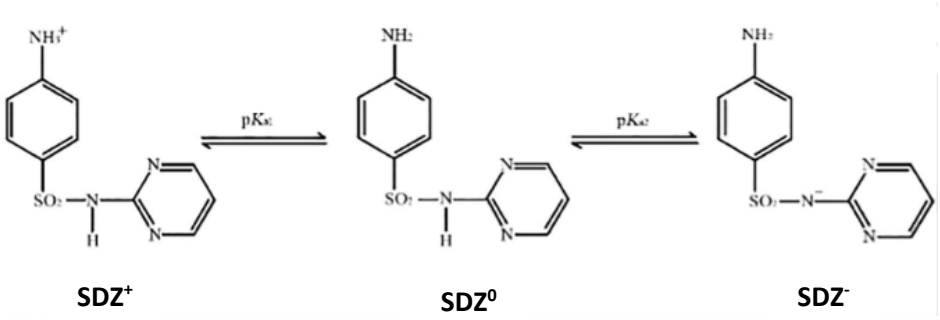


Figure 2.2 SDZ speciation in aqueous solution: (a) diagram of pH influence on the SDZ speciation, and (b) different chemical structures of SDZ according to its pK_a values (Li et al., 2020; Liu et al., 2016)**.**

Vione and Koehler (2018) pointed out that direct photolysis is more important for SDZ^- than for HSDZ, whilst in the indirect process, both species are oxidised fast by ^{3}DOM* and their reaction with $^1O_2$ is barely meaningful. On the other hand, SDZ is among the compounds known to undergo the back-reduction process and both SDZ^- and HSDZ may be reduced back to the initial compounds by the phenolic moieties contained in dissolved organic matter (DOM) (Vione and Koehler, 2018). Yet, the type and concentration of DOM have been shown to largely affect the phototransformation of SDZ (Bahnmüller et al., 2014). Still, most of the studies about the effect of DOM on SDZ photodegradation have been carried out using freshwater and effluent DOM (Bahnmüller et al., 2014; Sukul et al., 2008; Wang et al., 2018). However, populations usually concentrate along the coasts and, therefore, the discharge of antibiotics in coastal waters and the effects of seawater DOM on their photodegradation cannot be disregarded (Zhou et al., 2020). Indeed, seawater DOM

has already shown to have higher reactivity than freshwater DOM on the photodegradation of SAs (Wang et al., 2018). Along with DOM, halide ions were found to be responsible for a SDZ photodegradation larger in estuarine than in freshwater by Zhao et al. (2019), who highlighted that antibiotics photodegradation from freshwater downstream into seawater is largely unknown. Contrarily, Conde-Cid et al. (2018) did not find a significant influence of ionic species (including the halide Cl^-) on the photodegradation of SDZ. These authors (Conde-Cid et al., 2018) highlighted the increase of SDZ photodegradation (Table 2.1) with pH (between 4 and 7.2), which they related with the increase in the anionic form (SDZ^-).

Regarding the presence of humic substances (HS), Sukul et al. (2008) reported an improvement in SDZ photodegradation rate, in particular in presence of HA ($t_{½}$ between 17.2 h to 31.4 h) and FA ($t_{½}$ between 12.6 h to 29.8 h) compared to ultrapure water ($t_{½}$ = 32 h). Differently, other authors verified that HS acted as light barriers by attenuating the light intensity (filter effect) for other pharmaceuticals as difloxacin (DIF, C_i = 10 mg L^{-1}), sarafloxacin (SAR, C_i = 10 mg L^{-1}) or sulfamethoxazole (SMX, C_i = 100 µg L^{-1}) (Oliveira et al., 2019; Prabhakaran et al., 2009). For OXA, no studies have been found in literature regarding the importance of the presence of HS on its photodegradation.

DO is another factor that may affect antibiotics photodegradation, since the generation rate of ROS increases with increasing DO concentration (Saadati et al., 2016). Among ROS, Ge et al. (2019) indicated that $^1O_2$ oxidation can be a central factor in determining the fate of SAs (especially in waters with a pH > 7), with oxidation by $^•OH$ also contributing to phototransformation relative to direct photolysis. Moreover, Liu et al. (2018) studied the contribution of ROS on the photodegradation of SDZ. In particular, the authors (Liu et al., 2018) used C_3H_8O and NaN_3 (50 mg L^{-1}) to capture two of the main ROS in the catalytic reaction process, namely $^•OH$ and $^1O_2$. The authors underlined the key role of $^•OH$ and $^1O_2$ in the photocatalytic process of SDZ and pointed out contribution rates of 43.4 % and 5.19 %, respectively (Liu et al., 2018).

As shown above, among the few works available in literature on the influence of environmental factors on SDZ and OXA photodegradation, either antibiotics concentrations used are rather high (in the mg L^{-1} range), or contradictory results were obtained. Therefore, knowledge is needed for appropriate risk assessment and management regarding the discharge of antibiotics. In order to contribute to a better understanding of the fate and persistence of SDZ and OXA in natural waters, this work aimed: (i) to determine the effects of influencing factors such as the presence of several fractions of HS (humic acids (HA), fulvic acids (FA), and XAD-4 fraction), pH, salinity, DO and the presence of $^1O_2$ and $^•OH$ scavengers on the photodegradation of SDZ and OXA;

and (ii) to find out if these effects (or their absence) can be related with the photodegradation behaviour of SDZ and OXA in real matrices, namely fresh, brackish and wastewater.

Apart from this approach, another main novelty of this work was studying the effect of HS fractions extracted from estuarine waters on the photodegradation of SDZ and OXA, including for the very first time the XAD-4 fraction in the case of SDZ and HA, FA and XAD-4 in the case of OXA. Since the extraction of HS from estuarine waters is especially challenging due to the highly diluted and saline aqueous medium, the obtained results constitute a relevant contribution.

2.2. EXPERIMENTAL SECTION

2.2.1. REAGENTS AND STANDARDS

OXA (Fisher Scientific, 98 %) stock solution (10 mg L^{-1}) was prepared by dissolving the compound in 0.03 mol L^{-1} sodium hydroxide solution with the aid of sonication for 60 min. SDZ (TCI Europe, > 99 %) stock solution (10 mg L^{-1}) was prepared by dissolving the compound in ultrapure water sonicating for 60 min. SDZ and OXA stock solutions were prepared using 0.001 mol L^{-1} phosphate buffer. Sodium dihydrogen phosphate dihydrate (Fluka, Biochemika, ≥ 99.5 %) and di-sodium hydrogen phosphate dihydrate (Fluka, Biochemika, ≥ 99 %) were used to prepare a stock solution of phosphate buffer 0.1 mol L^{-1}, which was diluted to 0.001 mol L^{-1}. The pH of this solution was adjusted using hydrochloric acid (2 mol L^{-1}) (NormaPur, 37 %) to both 6.3 and 7.3 for SDZ stock solution and 7.3 for OXA stock solution. HS (HA, FA, and XAD-4) used in the photodegradation experiments were extracted and isolated from estuarine water (Ria de Aveiro, Aveiro, Portugal) by Santos et al (1994) and Esteves (1995). Synthetic sea salt solution (21 ‰) was prepared dissolving Red Sea Salt (Red Sea Europe) in phosphate buffer solution (0.001 mol L^{-1}, pH 7.3). Sodium chloride solution 21 ‰ (Fluka, 99.5 %) was also prepared in phosphate buffer solution (0.001 mol L^{-1}, pH 7.3). A stock solution of sodium azide (NaN_3, Riedel-de Haën, 99 %) was prepared at a concentration of 0.023 mol L^{-1} and isopropanol (C_3H_8O, Sigma-Aldrich, 99.8 %) stock solution was prepared at a concentration of 1 mol L^{-1}, both in ultrapure water (Milli-Q plus 185, Millipore). For high-performance liquid chromatography with a UV–vis detector (HPLC-UV) analysis, methanol (Fisher Scientific, HPLC grade) and formic acid (Sigma-Aldrich, > 98 %) were used.

2.2.2. SDZ AND OXA ANALYSIS

SDZ and OXA quantification was performed using HPLC-UV. The device consisted of a Waters Alliance 2695 Separations Module equipped with a Waters 2487 Dual Absorbance detector. Separation was carried out at 25 °C using a 150 mm × 4.6 mm i.d. ACE® C18 column-PFP (5 µm particle size) connected to a 4.6 mm i.d. ACE® 5 C18 guard column. For SDZ and OXA analysis, the mobile phase consisted of methanol: 0.1 % formic acid, 20:80 (*v*/*v*) and 45:55 (*v*/*v*), respectively, both at a flow rate of 0.9 mL min^{-1}. An injection volume of 20 µL was used and detection was

performed at 270 nm wavelength. Methanol and 0.1 % formic acid aqueous solutions were filtered through a 0.2 µm polyamide membrane filter (Whatman) before use as mobile phase.

2.2.3. DISSOLVED ORGANIC CARBON ANALYSIS

Dissolved organic carbon (DOC) was measured using a Total Organic Carbon analyser, TOC-V_{CPH}, from Shimadzu. Samples were acidified with 2 % (*v*/*v*) of HCl 2 mol L^{-1}, previously to the analysis. A stock solution of 1000 mg L^{-1} potassium hydrogen phthalate ($KHC_8H_4O_4$) was prepared in ultrapure water. The calibration curve was performed using standard solutions of $KHC_8H_4O_4$ (0.0–10.0 mg L^{-1}), by dilution of proper amounts of the stock solution in ultrapure water. The correlation coefficient (*r*) and limit of detection (LOD) for the obtained calibration curve were 0.9992 and 0.613 mg L^{-1}, respectively. To confirm the stability of the calibration curve, a newly prepared standard solution of 5.0 mg L^{-1} of $KHC_8H_4O_4$ was analysed daily prior to the samples.

2.2.4. PHOTODEGRADATION EXPERIMENTS

For the irradiation experiments, a solar radiation simulator Solarbox 1500 (Co.fo.me.gra, Italy) was used. This instrument is equipped with a xenon arc lamp (1500 W) and UV filters that limit the transmission of light below 290 nm. All experiments were performed with constant irradiation of 55 W m^{-2} (290–400 nm), which corresponds to 550 W m^{-2} in the spectral range, according to the manufacturer. The level of irradiance and temperature was monitored by a multimeter (Co.fo.me.gra, Italy) equipped with a UV 290–400 nm band sensor and a black standard temperature sensor. A parabolic reflection system was used to ensure irradiation uniformity in the chamber, which was kept refrigerated by an air-cooling system.

SDZ and OXA aqueous solutions (20 mL) were irradiated, in triplicate, in quartz tubes (internal diameter × height = 1.8 × 20 cm) covered with Parafilm M®. Each set of experiments was accompanied by dark controls prepared in the corresponding matrix, which were maintained inside the solar simulator during the same time as the irradiated solutions and under identical conditions apart from the irradiation (quartz tubes covered by aluminium foil), in order to prove the absence of any thermal and/or microbiological degradation. Aliquots (500 µL) of irradiated SDZ and OXA solutions and dark controls were withdrawn from quartz tubes throughout time, stored in the dark

at 4 °C and analysed within 24 h. The remaining concentration of SDZ and OXA in irradiated solutions (*C*) was compared with that in the respective dark control (C_0) for determining the percentage of degradation at each irradiation time (*t*, h). GraphPad Prism 5 was used to determine the fittings of experimental data to the following pseudo-first-order kinetic equation:

$$\frac{C}{C_0} = e^{-kt} \qquad \textbf{(Eq. 2.1)}$$

Where *k* is the pseudo-first-order degradation rate constant (h^{-1}). Also, SDZ and OXA half-life times ($t_{1/2}$) were calculated as ln2/*k*.

2.2.4.1. Effect of pH, humic substances and salinity

Photodegradation was studied using an initial SDZ and OXA concentrations of 500 µg L^{-1}, and 100 µg L^{-1} and 250 µgL^{-1}, respectively. The concentrations of SDZ and OXA used was chosen according to the LOD of the HPLC-UV and in order to follow more than 95 % of SDZ photodegradation.

Since SDZ presents two pK_a values, pH greatly affects the antibiotic speciation in solution, and thus its photodegradation behaviour. On the other hand, the pH of most natural waters is between 6 and 8.7, which comprises the p$K_{a,2}$ of SDZ. Therefore, the effect of pH at two different values, namely below and above p$K_{a,2}$, was here assessed. For this purpose, the working SDZ solution was prepared using a 0.001 mol L^{-1} phosphate buffer, adjusting pH to 6.3 or 7.3. The working OXA solution was prepared using a 0.001 mol L^{-1} phosphate buffer, adjusting pH to 7.3.

To evaluate the influence of DOM in SDZ and OXA photodegradation, HS (HA, FA and XAD-4 fraction (the most hydrophilic fraction of HS)) were used as model of the most abundant aquatic organic matter. SDZ and OXA solutions with each of the HS fractions (20 mg L^{-1}) were prepared in 0.001 mol L^{-1} phosphate buffer with pH adjusted to 7.3. The concentration of HS fractions (20 mg L^{-1}) was selected considering that freshwater DOC concentration is usually between 2 mg L^{-1} and 10 mg L^{-1} (Leech et al., 2009) and that DOC of HS is about 50 % of their concentration (Esteves et al., 1995; Santos et al., 1994). Characterization of these purified fractions by elemental analysis, solid-state ^{13}C cross polarization magic angle spinning nuclear magnetic resonance (CPMAS-NMR) and UV–- visible spectrophotometry was performed and deeply discussed in Esteves et al. (2009).

Salinity of brackish water can range between 0.5 ‰ and 35 ‰, depending on the type of estuaries, tides, weather, or other factors (Levinton, 1995). Thus, the effect of salinity was evaluated using SDZ or OXA solutions prepared in 0.001 mol L^{-1} phosphate buffer with pH adjusted to 7.3, containing 21 ‰ NaCl or 21 ‰ synthetic sea salts.

2.2.4.2. EFFECT OF SCAVENGERS AND DISSOLVED OXYGEN

In order to qualitatively investigate the role of ROS, SDZ and OXA photodegradation was evaluated in presence of two different scavengers: NaN_3 (as $^1O_2$ scavenger) and C_3H_8O (as $^{\bullet}OH$ scavenger) (Batista et al., 2014; Silva, 2014). SDZ and OXA standard solutions were prepared in 0.001 mol L^{-1} phosphate buffer with pH adjusted to 7.3, containing 5 mmol L^{-1} of NaN_3 or 20 mmol L^{-1} of C_3H_8O and subjected to irradiation. NaN_3 concentration was chosen accordingly to Thermofisher online information (Thermofisher, 2020). For C_3H_8O, concentrations between 10 and 100 mM have been used in literature (Acero et al., 2019; Zhang et al., 2016), thus an intermediate concentration was chosen. The role of DO on antibiotics photodegradation was evaluated purging SDZ or OXA solution (in 0.001 mol L^{-1} phosphate buffer; pH 7.3) with N_2 for 5 min. Deoxygenation of solutions was used to explore the role of triplet excited states, since oxygen (a ground state triplet because of its unpaired electrons) is a quencher of triplet excited states.

2.2.4.3. EFFECT OF NATURAL WATER MATRIX

In order to evaluate the influence of natural water matrices in SDZ and OXA photodegradation, fresh, brackish and wastewater samples were used. Freshwater was collected from a river located in Aveiro, while brackish water was collected from Ria de Aveiro (Aveiro, Portugal) (Figure 2.3). Wastewater was sampled from one of the Aveiro's WWTPs, after secondary treatment, corresponding to the final effluent (Figure 2.3). Immediately after collection, all samples were filtered through 0.45 µm nitrocellulose membrane filters (Millipore) and stored at 4 °C until use, within 7 days. Salinity, conductivity and pH of the samples were measured using a Multi 3320 m from WTW. DOC was measured as described previously (section 2.2.3).

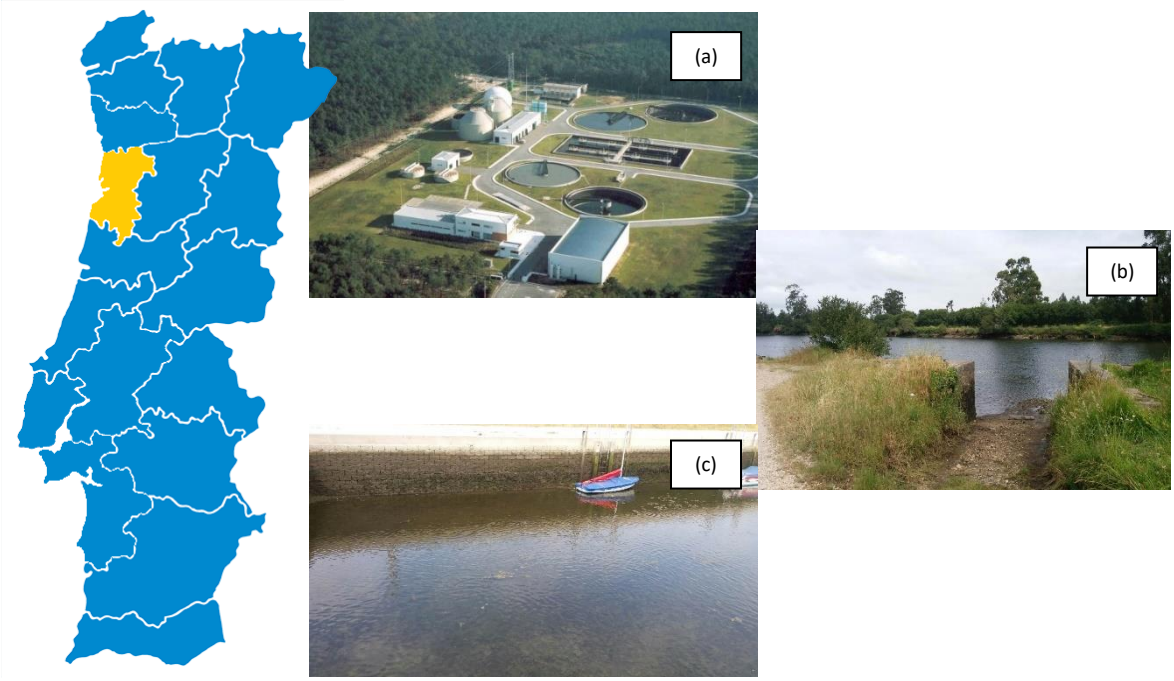


Figure 2.3 Sampling points of (a) wastewater samples – WWTP, (b) freshwater samples from *Novo do Príncipe* river and (c) estuarine samples from *Ria de Aveiro*.

2.3. RESULTS AND DISCUSSION

2.3.1. CHARACTERIZATION OF HS

In this work were used estuarine HS which were isolated, purified and whose fractions were fully characterized by Esteves (1995). In what respects elemental analysis (results depicted in Table 2.2). It must take in account that the elemental oxygen was determined independently from the other elements. The sum of C, H, N, S, and O is lower than 100 % possibly due the existence of other elements. It may be seen that the XAD-4 fraction has a lower carbon content than HA and FA; contrarily, the oxygen content of XAD-4 is higher. This indicates greater content of oxygen functional groups on XAD-4 fraction than in the other two fractions used. According to the data in literature thermogravimetric analysis indicated that HA are the most thermoresistant and are decomposed at temperatures above 300 °C, thus it may be concluded that HA constitute a fraction with higher aromaticity and lower functional and aliphatic groups content when compared with FA and XAD-4 fractions (Oliveira et al., 2019).

Table 2.2 Elemental analysis of HS fractions used. Results are corrected for humidity at 60°C and ashes at 750°C (adapted from Esteves (1995)). C, H, N and S were determined simultaneously, and O was determined independently in another batch.

Sample	Elemental analysis (%)				
	C	H	N	S	O
HA	54.8	4.0	3.6	1.8	32.4
FA	54.5	4.7	1.9	1.0	34.9
XAD-4	48.8	4.2	3.1	1.2	40.5

The UV spectra of 20 mg L^{-1} of the three HS fractions, performed by Oliveira et al. (2019), in the range 200 nm to 600 nm showed the decrease of absorbance values for all HS isolates (Figure 2.4). The differences in absorbance intensities are most likely caused by the difference in chromophore concentrations. The greater content of chromophores allows for more light absorption, which can be confirmed by the UV spectra. Through the investigated range of absorbance, the highest values were obtained for HA, while the lowest for XAD-4.

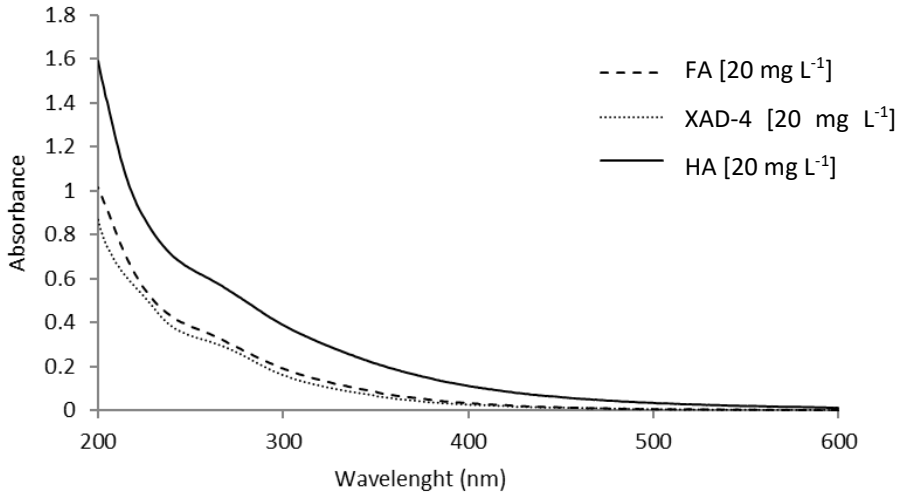


Figure 2.4 UV-visible spectra of the three fractions of HS (adapted from Oliveira et al. (2019)).

2.3.2. CHARACTERIZATION OF WATER SAMPLES

The properties of collected water samples are presented in Table 2.3. pH of water samples varied between 7.3 ± 0.3 and 8.7 ± 0.4. As expected, the highest salinity values were obtained in brackish water (33.20 ‰), while in wastewater or freshwater samples it was much lower (0 % to 1.40 ‰). Also as expected, the wastewater was the one with the highest DOC value (24.0 mg C L^{-1} ± 0.1 mg C L^{-1}), followed by brackish (9.8 mg C L^{-1} ± 0.3 mg C L^{-1}) and fresh waters (8.3 mg C L^{-1} ± 0.4 mg C L^{-1}).

Table 2.3 Physico-chemical characteristics of environmental water samples.

Sample	pH	Conductivity (mS cm^{-1})	Salinity (‰)	TDS [a] (g L^{-1})	DOC [b] (mg C L^{-1})
Freshwater	7.3 ± 0.3	0.26	0.00	0.26	8.3 ± 0.4
Brackish water	8.6 ± 0.3	50.70	33.20	50.70	9.8 ± 0.3
Wastewater	8.7 ± 0.4	3.03	1.40	2.75	24.0 ± 0.1

[a] TDS, total dissolved solids.

[b] DOC, dissolved organic carbon.

2.3.3. PERFORMANCE OF THE CHROMATOGRAPHIC ANALYTICAL METHOD

Under optimized conditions, the performance of the method was evaluated using the *r*, LOD and linearity (Lin (%) = 100 % – RSD_b, where RSD_b is the relative standard deviation of the slope). LOD was calculated from the calibration curve as $a + 3s_{y/x}$, where a is the intercept of the regression line and $s_{y/x}$ is the statistical parameter that estimates the random errors in the y-axis (signal). The linear range was 25 µg L^{-1} to 500 µg L^{-1} and 10 µg L^{-1} to 500 µg L^{-1}, while LOD was 21.2 µg L^{-1} and 12.2 µg L^{-1}, for SDZ and OXA, respectively. SDZ and OXA calibration curve presented a good correlation coefficient (*r* above 0.998) and good linearity values (above 98.4 %) for the concentration range used in this study (Table 2.4).

Table 2.4 Quantitative parameters for SDZ and OXA analytical curves obtained by HPLC – UV.

Analyte	Linear range (µg L^{-1})	Correlation coefficient (*r*)	Linearity (LIN) (%)	Limit of detection (LOD) (µg L^{-1})
SDZ	25 to 500	0.9987	98.41	21.2
OXA	10 to 500	0.9996	98.97	12.2

2.3.4. PHOTODEGRADATION KINETICS OF SDZ

Results on the effect of pH, HS, salinity, NaN_3 and C_3H_8O scavengers and N_2 purging on the photodegradation of SDZ are depicted in Figure 2.5, which represents C/C_0 *versus* irradiation time under the different conditions described in section 2.2.4.

2.3.4.1. EFFECT OF pH AND HUMIC SUBSTANCES

Regarding photodegradation of SDZ at pH 6.3 and 7.3, results are shown in Figure 2.5 (a). Complete photodegradation of SDZ at pH 7.3 occurred within 36 h, while at pH 6.3 it took more than 48 h to eliminate SDZ from aqueous solution. The constant *k* obtained at pH 7.3 was 0.103 h^{-1} ± 0.003 h^{-1}, while at pH 6.3 was 0.0567 h^{-1} ± 0.0005 h^{-1} (Table 2.5).

At pH 6.3, 83 % of SDZ is in the neutral form (HSDZ), while at pH 7.3, 67 % of SDZ is in its anionic form (SDZ^-). As it was already shown that the value of *k* of SDZ at pH 7.3 is almost twice the value obtained at pH 6.3 (Table 2.5). From these results, it may be concluded that photodegradation is more efficient for SDZ^- than for HSDZ. Since natural water usually has a pH higher than 7.3 (Oliveira et al., 2019), the SDZ photodegradation would be favoured under the pH of most of

environmental waters. However, Vione et al. (2018) observed that in natural waters (with DOC contents between 6 mg C L^{-1} and 13 mg C L^{-1}), both species were oxidised fast by the photogenerated ^{3}DOM*, but SDZ^- underwent back reactions at a larger extent than HSDZ, thus reducing the photodegradation efficiency. Results of the photodegradation of SDZ in ultrapure water (pH 6.3) demonstrated that after 32 h, 84 % of SDZ was eliminated from the aqueous solution. On the other hand, DOC results shown that 20 % of SDZ was completely mineralized into CO_2 and H_2O at pH 7.3.

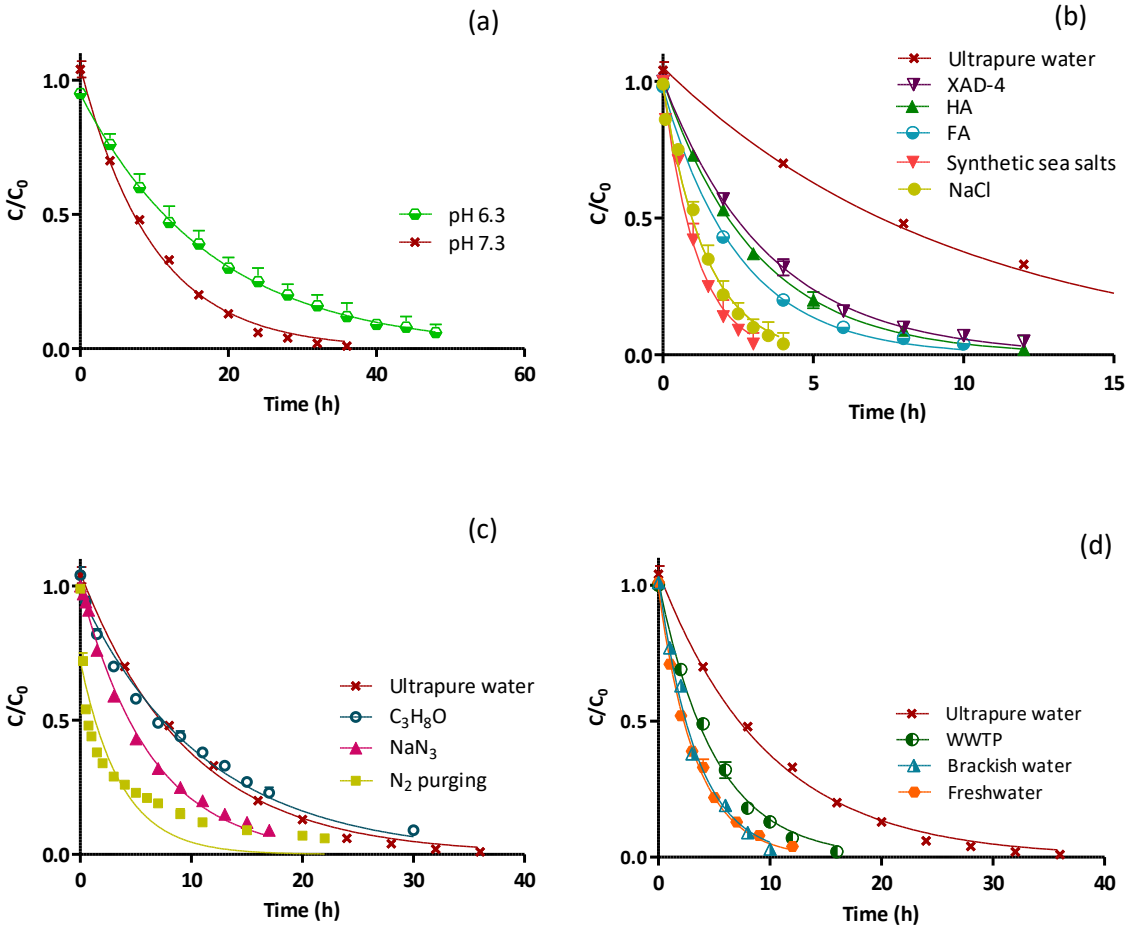


Figure 2.5 Experimental results on SDZ photodegradation (C_i = 500 µg L^{-1}) throughout time together with fittings to the pseudo-first-order kinetic model obtained in (a) ultrapure water at different pH values; (b) different HS fractions (20 mg L^{-1}), synthetic seawater (21 ‰) and NaCl (21 ‰) at pH= 7.3; (c) presence of C_3H_8O, NaN_3 and with N_2 purging at pH = 7.3; and, (d) three different environmental water matrices at natural pH values. Nonlinear regression analysis with GraphPad Prism 5 was used for curve fitting. Note that, for most of the experimental points, error bars are too small to be visible in the figure.

Regarding the photodegradation of SDZ in presence of different HS fractions (at pH 7.3), results are presented in Figure 2.5 (b) and Table 2.5. The influence of organic matter will be a

balance of opposite contributions: photosensitizing effect (through formation of reactive species) and inhibitory effects (inner filter effect, scavenging/quenching of reactive species and/or back-reductions) (Oliveira et al., 2019). A retardation of photodegradation by organic matter has been observed for some pharmaceuticals (Santoke and Cooper, 2017). Different effects by different fractions have also been described in literature; for example, Batista et al. (2016) found that HA slightly increased photodegradation of sulfamerazine, while FA significantly decreased its photodegradation. However, in this work, the photodegradation of SDZ accelerated under the presence of the three HS fractions (Figure 2.5 (b)). Indeed, the $t_{1/2}$ in ultrapure water was 6.76 h ± 0.07 h, while in presence of HS it was in the range 1.76 h ± 0.04 h to 2.42 h ± 0.04 h. These results showed that photodegradation of SDZ is predominantly enhanced by ^{3}DOM*, rather than inhibited by back-reductions, inner filter effect or scavenging/ quenching of reactive species.

Table 2.5 Data on SDZ (C_0 = 500 μg L^{-1}) pseudo-first-order rate constants (k (h^{-1})), determination coefficient (R^2), and half-life ($t_{1/2}$ (h)) obtained for different matrices under simulated solar radiation. (SD stands for standard deviation, n = 3).

Sample	k ± SD (h^{-1})	R^2	$t_{1/2}$ ± SD (h)
Ultrapure water, pH = 6.3	0.0567 ± 0.0005	0.9996	12.19 ± 0.03
Ultrapure water, pH = 7.3	0.103 ± 0.003	0.9973	6.76 ± 0.07
HA	0.321 ± 0.005	0.9995	2.16 ± 0.02
FA	0.39 ± 0.01	0.9984	1.76 ± 0.04
XAD-4	0.286 ± 0.007	0.9988	2.42 ± 0.04
Sea salts (21‰)	0.89 ± 0.06	0.9882	0.78 ± 0.06
NaCl (21‰)	0.70 ± 0.04	0.9897	1.00 ± 0.06
NaN_3	0.157 ± 0.005	0.9963	4.41 ± 0.06
C_3H_8O	0.090 ± 0.004	0.9874	7.7 ± 0.1
N_2	0.28 ± 0.05	0.8335	2.5 ± 0.3
Freshwater	0.30 ± 0.01	0.9963	2.32 ± 0.05
Brackish water	0.29 ± 0.02	0.9919	2.4 ± 0.1
WWTP	0.199 ± 0.007	0.9960	3.48 ± 0.07

HS used in this study were extracted and isolated from coastal estuarine water (Esteves et al., 2009). These HS, compared with HS from freshwater, are known to have lower absorbance, due to a lesser influence from terrestrial organic matter (Esteves et al., 2009). Moreover, these HS generally present smaller adsorption coefficients (a_λ, m^{-1}) than those from terrestrial origin, thus lower a_{350} can be related to a lower aromatic carbon concentration, in agreement with the dominant role of aromatic chromophores in coloured DOM. Esteves et al. (2009) also reported that FA and XAD-4 fraction from estuarine coastal water are generally more aliphatic fractions than

fractions from terrestrial origin, with higher lignin-derived structures. Comparing results obtained using different HS fractions, it was possible to observe that $t_{1/2}$ obtained for FA fraction was 1.76 h ± 0.04 h. In fact, this fraction is known to be the most photo-chemically active fraction of DOM (Li and Sun, 2014). On the other hand, HA presented a higher $t_{1/2}$ (2.16 h ± 0.02 h) and XAD-4 fraction the highest $t_{1/2}$value (2.42 h ± 0.04 h). Results demonstrated that FA are more efficient as natural photosensitizers than HA and XAD-4. This may be related to the fact that FA are less hydrophobic and less enriched in aromatic groups than HA (Oliveira et al., 2019) and thus, with less ability to act as light filter (inner filter effect). In fact, the spectra of the three fractions of HS (Oliveira et al., 2019) show that, within the studied wavelength range, the absorbance followed the order XAD-4 < FA < HA. Because of the structural heterogeneity of HS, they do not produce a well-resolved spectrum and the absorbance increases monotonously as the wavelength decreases. The fact that HA present a higher ability to absorb radiation may explain, at least partially, the lower SDZ photodegradation in their presence. As mentioned before, HS from estuarine coastal water present a lower aromatic carbon concentration, thus, a higher photodegradation rate of SDZ in this type of water samples is expected.

Also, a correlation between the aromatic degree of DOM and the antioxidant activity has been mentioned, and therefore, HS with lower aromatic degree (as FA) have lower antioxidant activity (lower inhibition by back-reductions) (Vione et al., 2018). Moreover, DOM from aquatic origin (such as estuarine water), with low aromaticity, was shown to be a softer inhibitor than DOM from terrestrial origin, with high aromaticity, and thus, likely to act less as an antioxidant. Overall, presence of DOM, as simulated by HS, has an enhancement effect on the photodegradation of SDZ, which due to the characteristics of coastal estuarine samples will be favoured in this type of HS, rather than in terrestrial DOM.

2.3.4.2. EFFECT OF SALINITY

Concerning the effect of salinity, the obtained results (Figure 2.5 (b)) demonstrated the photosensitizer effect that salinity has on the SDZ photodegradation. Fitted parameters of the pseudo-first-order kinetic equation, which are depicted in Table 2.5, confirmed the higher k (0.70 h^{-1} ± 0.04 h^{-1}) in presence of NaCl compared to that obtained in ultrapure water (0.103 h^{-1} ± 0.003 h^{-1}), resulting in a $t_{1/2}$ almost 7 times lower. The photosensitizing effect of NaCl can be attributed to the presence of chloride. Halides have been only considered as scavengers of

OH during advanced oxidation process (AOP), especially in saline waters, where AOP would be anticipated to be ineffective. However, •OH scavenging by halides simply converts •OH to more selective oxidants, being AOP treatment in saline waters highly contaminant-specific (Grebel et al., 2010). Halides, such as chloride, can be converted into radical and non-radical reactive halogen species (RHS) by sensitized photolysis and by reactions with secondary ROS produced through sunlight-initiated reactions in water and atmospheric aerosols, such as •OH, ozone, and nitrate radical (Yang and Pignatello, 2017). For the chloride concentration used, chloride atoms will quickly react with the anions to form dihalide radical anions ($Cl_2^{\bullet-}$) (Pinto et al., 2018). According to Pinto et al. (2018), both direct and sensitized photolysis can be used to produce chloride atoms in aqueous solutions; these react rapidly with excess of chloride ion to produce the corresponding $Cl_2^{\bullet-}$ as the dominant species. Chloride ions are also oxidised by •OH, with initial reversible formation of the $ClOH^{\bullet-}$ radical anion, which subsequently reacts with chloride ions to produce $Cl_2^{\bullet-}$ (Pinto et al., 2018). Halide scavenging may dramatically reduce the treatment efficiency of electron-poor contaminants that react slowly with RHS, but the extent of the reduction with electron-rich contaminants may be less remarkable (Grebel et al., 2010). Zhao et al. (2019) demonstrated that RHS were largely responsible for the halide-specific enhancement in the SDZ photodegradation, rather than other reactive species, such as ^{3}DOM* and •OH. These findings are in agreement with those observed in this work but different from those obtained by other authors, which pointed out to the absence of significant effects of ionic species (Conde-Cid et al., 2018) or the inhibitory effect of salinity on the photocatalytic degradation of SAs antibiotics (Oliveira et al., 2019; Yang et al., 2015). When comparing the results that were obtained in this work using NaCl and synthetic sea salts at the same concentration, *k*, determined in synthetic sea salts (0.89 h^{-1} ± 0.06 h^{-1}) was higher than in NaCl (0.70 h^{-1} ± 0.04 h^{-1}). Sodium and chloride ions represent about 91% of all seawater ions, even though there are lower quantities of other ions in seawaters (e.g., K^+, Mg^{2+}, or SO_4^{2-}) (Pinto et al., 2018). The synthetic sea salts from Red Sea Salt used in this study contain ions like calcium, magnesium, and carbonates, being free of synthetic additives, nitrates, phosphates or heavy metals (Red Sea Salt, 2019). Therefore, in synthetic sea salts, carbonates, such as bicarbonate (HCO_3^-), may have favoured SDZ photodegradation since they can act as an •OH scavenger and produce selective carbonate radicals ($CO_3^{\bullet-}$) (Li et al., 2018), responsible for the degradation of electron-rich organic pollutants. Studies of pollutants' photodegradation behaviour using coastal waters are extremely important, since due to the global climate change, coastal lagoons are likely to experience high salinity in the coming decades (Pinto et al., 2018).

2.3.4.3. EFFECT OF SCAVENGERS AND DISSOLVED OXYGEN

C_3H_8O and NaN_3 were used to evaluate the influence of $^{\bullet}OH$ and $^1O_2$, respectively, on SDZ photodegradation (Figure 2.5 (c) and Table 2.5). C_3H_8O is a relatively selective and routinely used $^{\bullet}OH$ scavenger. The mechanism of the reaction between these two species is shown in the following equation:

$$^{\bullet}OH + (CH_3)_2\,CHOH \rightarrow H_2O + (CH_3)_2C^{\bullet}OH \quad \textbf{(Eq. 2.2)}$$

Besides that, the mechanism of the reaction between NaN_3 and $^1O_2$ is:

$$N_3^- + {}^1O_2 \rightarrow N_3^{\bullet} + O_2^{\bullet-} \quad \textbf{(Eq. 2.3)}$$

As may be seen in Table 2.5 and Figure 2.5 (c), in presence of C_3H_8O, *k* presented no evident decline after adding this $^{\bullet}OH$ scavenger (*k* decreased from 0.103 h^{-1} ± 0.003 h^{-1} (in ultrapure water) to 0.090 h^{-1} ± 0.004 h^{-1} (Table 2.5), suggesting that direct photolysis was the main pathway for SDZ photodegradation behaviour in ultrapure water. Bahnmüller et al. (2014) also reported that, under the presence of C_3H_8O, a slight decrease in the SDZ *k* occurred in ultrapure water, which confirmed the importance of direct photodegradation in this matrix. In presence of NaN_3 (Figure 2.5 (c)), Table 2.5 evidence that *k* increased significantly from 0.103 h^{-1} ± 0.003 h^{-1} (in ultrapure water) to 0.157 h^{-1} ± 0.005 h^{-1}. NaN_3 in presence of $^1O_2$ originates azidyl radical ($N_3^{\bullet}$) and $O_2^{\bullet-}$ (Eq. 2.3), thus the increase in *k* may be explained by the formation of $^1O_2$ whose action is intensified by the presence of azide (Trawiński and Skibiński, 2019). In order to evaluate the influence of DO in SDZ photodegradation, solutions were purged with N_2. Results (Figure 2.5 (c) and Table 2.5) demonstrated a higher *k* under N_2 purging (0.28 h^{-1} ± 0.05 h^{-1}) than the obtained without purging (0.103 h^{-1} ± 0.003 h^{-1}) (Table 2.5). The triplet species of ground-state of oxygen can absorb energy from triplet sensitizers, given rise to oxygen in singlet state. SDZ can be included into the sulfa-drugs with varying five-membered heterocyclic substituents, such as sulfamethoxazole, sulfamethizole and sulfamoxole. Zhou and Moore (1997) demonstrated that sulfamethoxazole exhibits a negligible oxygen uptake when irradiated by itself, but a significant capacity to photosensitize the oxidation of histidine (type II photosensitized oxidation reaction generating singlet oxygen) (Baptista et al., 2017; Zhou and Moore, 1997). Also, Zhou and Moore (1997) suggested that the reactions sensitized

by sulfamethoxazole can be inhibited by molecular oxygen acting as a quencher. In this context we can put the hypothesis that SDZ has the same behaviour as sulfamethoxazole when in a poor oxygen environment justifying the increase of *k* from 0.103 h^{-1} ± 0.003 h^{-1} (in ultrapure water) to 0.28 h^{-1} ± 0.05 h^{-1} in N_2 purged.

Therefore, the obtained results under N_2 purging further confirm that $^1O_2$ could not be too much relevant in the transformation of SDZ observed in this work. These results are in agreement with those by Vione and Koehler (2018), who also reported that SDZ reaction with $^1O_2$ was barely meaningful and that the main SDZ phototransformation process was direct photolysis (predominant species at pH 7.3). Contrarily, $^1O_2$ oxidation was suggested by Ge et al. (2019) as a determinant process in determining the fate of SAs in the aquatic medium, especially at pH higher than 7. Contradictory results may be related to the fact that oxygen can have opposite effects: the increased yields of ROS accelerates the self-sensitized photolysis of SDZ, while the greater quenching of excited SDZ inhibits its direct photodegradation. With the increase in DO level, the increase in self-sensitized photodegradation is outweighed by the reduction in direct photolysis.

2.3.4.4. EFFECT OF NATURAL WATER MATRICES

In order to understand the photodegradation behaviour of SDZ in different aquatic media, three water samples with different characteristics were used (Table 2.5). These experiments were conducted at the natural pH of each water sample. The photodegradation rate of SDZ in these samples was found to be much higher than the obtained in ultrapure water (Figure 2.5 (d)). Kinetic parameters from SDZ photodegradation are depicted in Table 2.5, which shows that *k* in ultrapure water (0.103 h^{-1} ± 0.003 h^{-1}) was quite lower than that of natural water samples (between 0.199 h^{-1} and 0.30 h^{-1}). Amongst the natural water samples here considered, the lowest *k* was obtained in the WWTP effluent. Meanwhile, SDZ photodegradation experiments in fresh and brackish water samples presented similar results. Since both ultrapure water and freshwater samples had the same pH value, differences in the photodegradation rate cannot be attributed to SDZ speciation. However, freshwater sample presented a DOC of 8.3 mg C L^{-1} and as it was already shown in this work, enhancement of SDZ photodegradation by ^{3}DOM* was dominant over other DOM effects such as back-reductions, inner filter effect or scavenging/quenching of reactive species. On the other hand, in the wastewater sample, with a pH of 8.7, SDZ is present in its anionic form, which is more susceptible to photodegradation (Vione et al., 2018). Nonetheless, wastewater sample has a

large DOC of 24.0 mg C L^{-1} and, therefore, the slower SDZ photodegradation could be related to the inhibition of ^{3}DOM*-mediated phototransformation by the back reactions (Vione and Koehler, 2018) and by inner filter effect. In what concerns the brackish water, the pH 8.6 favours the presence of SDZ entirely in the negative form, which is more susceptible to photodegradation. The remarkably higher salinity of the brackish water (33.20 ‰) also helps to justify the larger photodegradation rate compared to that observed in ultrapure water. Indeed, the formation of RHS is responsible for the halide-specific enhancement in the SDZ photodegradation in brackish water. However, other factors such as DOM content may affect the photodegradation of SDZ in brackish water. The DOC content of the brackish water sample is 9.8 mg C L^{-1}, which means that DOM will have both photosensitizing (through formation of reactive species) and inhibitory (inner filter effect, scavenging/ quenching of reactive species and/or back-reductions) effects.

2.3.5. PHOTODEGRADATION KINETICS OF OXA

Results on the effect of OXA concentration, HS, salinity, NaN_3 and C_3H_8O scavengers on the photodegradation of OXA are depicted in Figure 2.6, which represents C/C_0 *versus* irradiation time under the different conditions described in section 2.2.4.

2.3.5.1. EFFECT OF INITIAL CONCENTRATION AND HUMIC SUBSTANCES

As may be seen in Figure 2.6 (a), OXA photodegradation occurs faster at 100 µg L^{-1} than at 250 µg L^{-1}. Indeed, concentration has already been shown to influence degradation since photolysis can be decreased due to photon limitation occurring at higher initial concentrations (Chowdhury et al., 2011). This effect has been confirmed in this work, since k of 1.24 h^{-1} ± 0.02 h^{-1} and 0.70 h^{-1} ± 0.02 h^{-1} and $t_{1/2}$ of 0.56 h ± 0.01 h and 0.99 h ± 0.04 h (Table 2.6) were determined at OXA concentrations of 100 µg L^{-1} and 250 µg L^{-1}, respectively. In order to follow the degradation of OXA until to its complete photodegradation using the analytical method presented above, OXA was spiked into different water matrices at final concentration of 250 µg L^{-1}.

As previously highlighted, there are few published results on OXA photodegradation, and those available were performed at initial OXA concentrations of 1 mg L^{-1} to 20 mg L^{-1} (Lai and Lin, 2009; Turiel et al., 2005; Zeghioud et al., 2019). Such concentrations are higher than those used in this work, so, in agreement with the concentration effect here determined, resulted in $t_{1/2}$ values

much higher than those observed in this study. For instance, Lai and Lin (2009) used 20 mg L^{-1} of OXA and obtained $t_{1/2}$ that varied between 55.2 h and 115.2 h, depending on the matrix (Table 2.1).

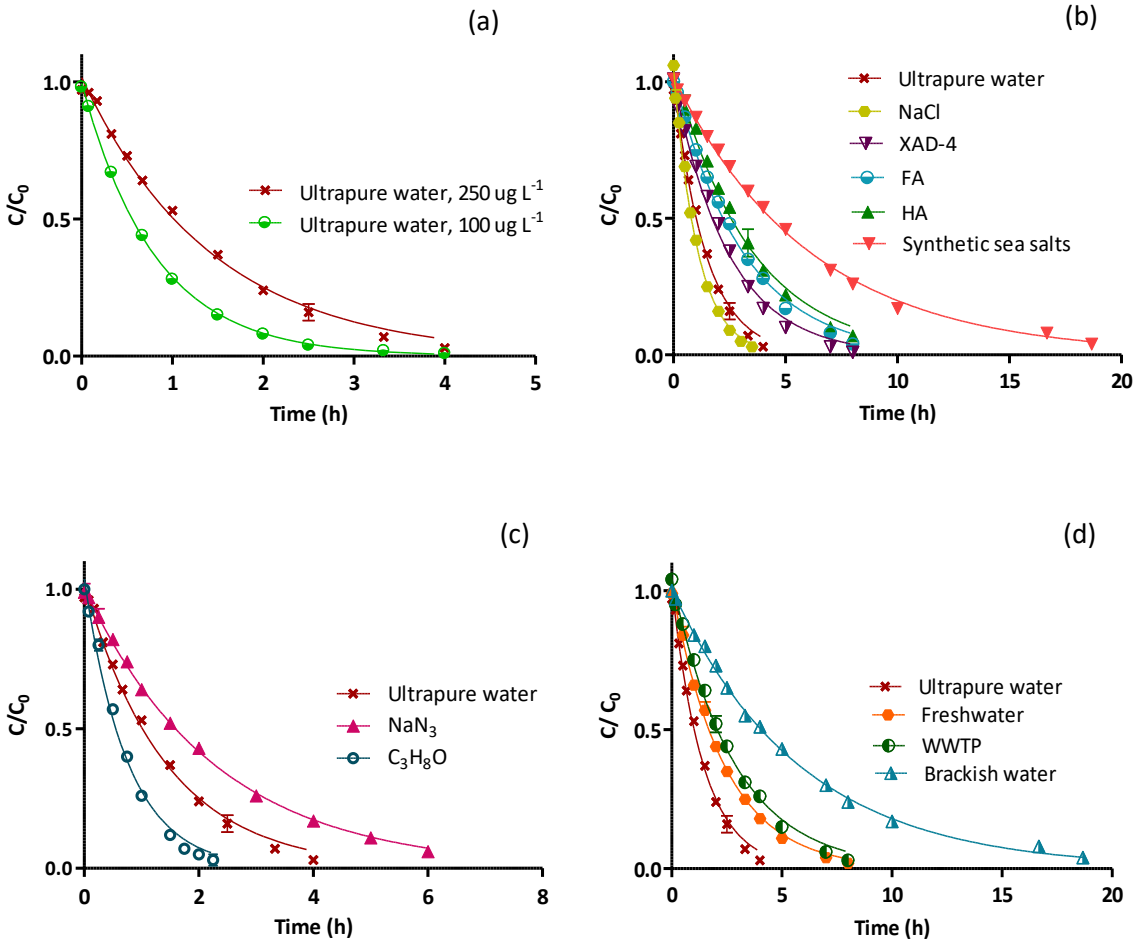


Figure 2.6 Experimental results on OXA photodegradation throughout time together with fittings to the pseudo-first-order kinetic model obtained in (a) ultrapure water at different OXA initial concentration; (b) different HS fractions (20 mg L^{-1}), synthetic seawater (21 ‰) and NaCl (21 ‰) at pH= 7.3; (c) presence of C_3H_8O and NaN_3; and, (d) three different environmental water matrices at natural pH values. Nonlinear regression analysis with GraphPad Prism 5 was used for curve fitting. Note that, for most of the experimental points, error bars are too small to be visible in the figure.

Organic matter may result in opposite effects on the photodegradation rate (Oliveira et al., 2019; Silva et al., 2016a, 2016b). If on one side, organic matter can act as a photosensitizer (enhancing the photodegradation by being promoted to a transient excited state, which may react directly with the pollutant or react with oxygen present in solution, forming ROS); on the other side, it can also have an inhibitory effect (generally, by acting as a filter (inner filter effect), decreasing the radiation available for the pollutant). Thus, the global effect of organic matter will be a balance

between these two opposite contributions. Results obtained for OXA photodegradation under the presence of HS, namely HA, FA and XAD-4 fraction, are shown in Figure 2.6 (b), which evidences a decrease in photodegradation rate in presence of HS. This is confirmed by the corresponding *k* and $t_{1/2}$ values in Table 2.6.

As may be observed, OXA $t_{1/2}$ varied between 1.70 h ± 0.05 h and 2.38 h ± 0.07 h in presence of the different fractions of HS, which are higher than the value obtained in ultrapure water (0.99 h ± 0.04 h). Thus, in this case, the inhibitory effect of HS prevailed in relation to their photosensitizing effect. DOM inhibition has already been observed for the photodegradation of antibiotics, such as SAs (Oliveira et al., 2019; Wenk and Canonica, 2012). Also, Prabhakaran et al. (2009) reported an inhibition of QA antibiotics photodegradation rate in presence of HA and FA (Table 2.1). Apart from the inner filter effect, inhibition can also derive from other processes as the scavenging/quenching of reactive species and back-reductions to the parent compound by ^{3}DOM*. Results obtained in this work (Figure 2.6 (b)) using different HS fractions demonstrated a higher inhibition in presence of HA than in presence of FA and XAD-4 (Table 2.6).

Table 2.6 Data on OXA pseudo-first-order rate constants (*k* (h^{-1})), determination coefficient (R^2), and half-life ($t_{1/2}$ (h)) obtained for different matrices under simulated solar radiation. (SD stands for standard deviation, *n* = 3).

Sample	C_0 ($\mu g\ L^{-1}$)	*k* ± SD (h^{-1})	R^2	$t_{1/2}$ ± SD (h)
Ultrapure water	100	1.24 ± 0.02	0.9995	0.56 ± 0.01
Ultrapure water	250	0.70 ± 0.02	0.9955	0.99 ± 0.04
HA	250	0.29 ± 0.01	0.9905	2.38 ± 0.07
FA	250	0.32 ± 0.01	0.9957	2.15 ± 0.05
XAD-4	250	0.41 ± 0.01	0.9947	1.70 ± 0.05
Sea salts (21‰)	250	0.163 ± 0.003	0.9982	4.25 ± 0.04
NaCl (21‰)	250	0.93 ± 0.02	0.9978	0.74 ± 0.02
NaN_3	250	0.438 ± 0.007	0.9991	1.58 ± 0.02
C_3H_8O	250	1.32 ± 0.06	0.9930	0.53 ± 0.04
Freshwater	250	0.42 ± 0.01	0.9976	1.65 ± 0.03
Brackish water	250	0.172 ± 0.003	0.9984	4.03 ± 0.04
WWTP	250	0.36 ± 0.01	0.9964	1.95 ± 0.04

Considering the characterization of the HS fractions, HA is the most hydrophobic fraction, being more enriched in aromatic and/or chromophoric groups, followed by FA and XAD-4. Also, XAD-4 fraction is the richest fraction in oxygen functional groups with lower carbon but higher oxygen content than HA and FA. Results suggest an inverse correlation between the HS aromaticity

and their photosensitizing effect, which is coincident with the observations by Oliveira et al. (2019) and Silva et al. (2016b). HA fraction, presenting higher aromaticity, has a higher ability to absorb light and thus, a higher inner filter effect, which would explain the lower OXA photodegradation rate in presence of this fraction.

2.3.5.2. EFFECT OF SALINITY

Salinity has been shown to have a different influence on the photodegradation behaviour of organic pollutants. Amongst SA antibiotics, salinity was considered to inhibit photodegradation in the case of SMX (Oliveira et al., 2019), but an opposite effect was observed for SDZ in this study and by other authors (Zhao et al., 2019), with RHS being considered responsible for the enhancement of SDZ photodegradation observed in saline waters. Results represented in Figure 2.6 (b) demonstrate small differences between photodegradation of OXA in ultrapure water and in presence of 21 ‰ NaCl solution. However, using 21 ‰ synthetic sea salts solution, the decrease in OXA photodegradation was very sharp, with $t_{1/2}$ increasing from 0.99 h ± 0.04 h, in ultrapure water, to 4.25 ± 0.04 h, in synthetic sea salts. Indeed, the $t_{1/2}$ of OXA in synthetic sea salts was just slightly higher than that observed in brackish water ($t_{1/2}$ = 4.03 h ± 0.04 h). The increase in $t_{1/2}$ observed in synthetic sea salts, as compared with NaCl, may be attributed to OXA stabilization due to chelate formation between the QA and divalent cations in the synthetic sea salts solution, since one of the main properties of QA is the chelate binding of metal cations to the carbonyl ring and one of the carboxylic oxygens (Rigos et al., 2004; Turel, 2002). Moreover, carbonates present in the synthetic sea salt solution, such as bicarbonate (HCO_3^-), could inhibit OXA photodegradation since they can act as $^{\bullet}OH$ scavengers (Acero et al., 2019; Bian and Zhang, 2016), by the main mechanism as follows (Bian and Zhang, 2016):

$$HCO_3^- + {}^{\bullet}OH \rightarrow CO_3^{-\bullet} + H_2O \qquad \textbf{(Eq. 2.4)}$$

In fact, indirect photolysis mediated by $^{\bullet}OH$ has already been pointed out to have an important role in the photodegradation of some fluoroquinolones (Zhang et al., 2019). $^{\bullet}OH$ may result from the reduction of superoxide ($O_2^{\bullet-}$), which is produced from the one-electron reduction of O_2, and which is the first step in the formation of ROS (Xiao et al., 2020).

2.3.5.3. EFFECT OF SCAVENGERS

The influence of $^{\bullet}OH$ and $^{1}O_2$ on OXA photodegradation was assessed by using C_3H_8O and NaN_3, respectively. $^{\bullet}OH$ has been pointed out to have an important role in the photodegradation of some fluoroquinolones. Surprisingly, and as may be seen in Figure 2.6 (c), OXA photodegradation was faster in presence of C_3H_8O, than in ultrapure water. In agreement, *k* was higher in presence of this $^{\bullet}OH$ scavenger (*k* increased from 0.70 h^{-1} ± 0.02 h^{-1} (in ultrapure water) to 1.32 h^{-1} ± 0.06 h^{-1} (in presence of the scavenger)) (Table 2.6). The increase in the photodegradation rate indicates that C_3H_8O increases OXA photodegradation. The presence of C_3H_8O (20 mmol L^{-1}) alters the properties of the aqueous solvent and it is known that changes in the solvent may induce changes in triplet state (mainly when involving carbonyl groups) affecting the rate of photodegradation (Cowan and Drisko, 1976), in this case, increasing OXA photodegradation rate. Notwithstanding, these results do not allow to make definite conclusions on the role of $^{\bullet}OH$ in OXA photodegradation.

The N_3^- ions are relatively selective quenchers of $^{1}O_2$ (Eq. 2.3), thus, if these species are involved in the process, inhibition of OXA photodegradation should be observed under the presence of NaN_3. In presence of NaN_3, *k* presented an evident decline from 0.70 h^{-1} ± 0.02 h^{-1} (in ultrapure water) to 0.438 h^{-1} ± 0.007 h^{-1} (Table 2.6). The pronounced retardation of OXA decay under solar radiation in presence of this scavenger allows inferring that the self-sensitized photo-oxidation processes of $^{1}O_2$ generation was involved in the OXA photodegradation. To the best of our knowledge, the participation of $^{1}O_2$ in the photodegradation of OXA was here assessed for the very first time. Nonetheless, several authors have already highlighted that $^{1}O_2$ plays an important role in the photodegradation of other fluoroquinolones (Ge et al., 2010; Geng et al., 2020; Niu et al., 2016).

2.3.5.4. EFFECT OF NATURAL WATER MATRICES

The obtained results on OXA photodegradation in natural water samples are depicted in Figure 2.6 (d). It must be highlighted that photodegradation experiments in these matrices were conducted at the natural pH of each water sample (in the range 7.3 to 8.6). It is known that pH influences the speciation of OXA (pK_a = 5.94 ± 0.20), and thus its photodegradation behaviour.

However, for the pH of the aqueous matrices used in this work, OXA is in its negative form and, therefore, pH effects on speciation may be neglected. Results in Figure 2.6 (d) make evident that OXA photodegradation in wastewater, freshwater and, especially, in brackish water is slower than in ultrapure water. This is confirmed by the corresponding k and $t_{1/2}$ presented in Table 2.6. As for the latter, the value determined in ultrapure water was 0.99 h ± 0.04 h, while in wastewater, freshwater and in brackish water $t_{1/2}$ was 1.95 h ± 0.04 h, 1.65 h ± 0.03 h and 4.03 h ± 0.04 h, respectively. These results demonstrate that the OXA photodegradation rate is slower in the natural matrices, particularly in brackish water, with a $t_{1/2}$ four times higher than the observed in ultrapure water. Consequently, as compared with ultrapure water, it may be expected a larger OXA persistence in the aquatic environment, along with potential risks, such as enhancing microbial resistance through prolonged exposure of microorganisms to OXA.

Wenk and Canonica (2012) also found a correlation between the DOM aromatic degree and the antioxidant activity. DOM of terrestrial origin, with high aromaticity, was shown to have an inhibition effect higher than DOM of aquatic origin, with low aromaticity, and thus more likely to act as antioxidant (Wenk and Canonica, 2012). The inhibitory effect of DOM may explain, at least partially, the slower OXA photodegradation observed in freshwater, brackish water and wastewater as compared with ultrapure water (Figure 2.6 (d)). In fact, apart from other influencing factors in these complex matrices, the larger inhibitory effect observed in brackish water (9.8 mg C L^{-1} DOM, k = 0.172 h^{-1} ± 0.003 h^{-1}) than in freshwater (8.3 mg C L^{-1} DOM, k = 0.42 h^{-1} ± 0.01 h^{-1}), may be related to some extent with DOM concentration. Although the DOC of fresh and brackish water samples was very similar, salinity was a main difference between them. For that reason, the high salinity observed in brackish water (33.2 ‰) compared to other natural samples (below 1.4 ‰), may have significantly contributed to the inhibition of photodegradation of OXA.

2.4. CONCLUSIONS

The main purpose of this work was to understand the SDZ and OXA photodegradation behaviour in natural water samples and gather information about the influence of water composition, such as organic matter and salinity.

SDZ photodegradation was shown to be notably affected by pH, the presence of HS and salinity. Thus, in ultrapure water, the $t_{1/2}$ determined for SDZ was 6.8 h, at pH = 7.3 and 12.2 h, at pH = 6.3. Under the presence of HA, FA or XAD-4 fractions of estuarine HS, SDZ photodegradation was favoured, with $t_{1/2}$ of 2.2 h, 1.8 h and 2.4 h, respectively. On the other hand, salinity exhibited a photosensitizing effect, with $t_{1/2}$ of 1.0 h and 0.8 h in NaCl and sea salts (both 21 ‰), respectively. Studies in the presence of scavengers, namely C_3H_8O and NaN_3, and under N_2 purging, allowed to verify that $^1O_2$ and $^{\bullet}OH$ could not be too much relevant in the transformation of SDZ. All these effects were related to photodegradation results in real matrices, namely wastewater, freshwater and brackish water, where the determined $t_{1/2}$ were 3.5 h, 2.3 h and 2.4 h, respectively. In wastewater, the relatively high pH (8.7) favoured SDZ photodegradation but the very high DOC concentration probably lessened it by back reactions inhibition of ^{3}DOM*-mediated phototransformation, inner filter effects and/or scavenging/quenching of reactive species. Differently, ^{3}DOM*-mediated phototransformation was probably dominant and had an important role in freshwater while halide-specific enhancement of SDZ photodegradation may have occurred in saline brackish water.

On the other hand, in the case of OXA, results allowed to conclude that the persistence of this pollutant is much higher in environmental water samples than in ultrapure water ($t_{1/2}$ = 0.99 h), especially when present in matrices with high salinity values, such as brackish water ($t_{1/2}$ = 4.03 h). Organic matter also has a negative effect on OXA photodegradation rate, which might partially explain the decrease of the photodegradation rate observed in the freshwater sample ($t_{1/2}$ = 1.65 h). In what concerns the species that may be involved on the photodegradation of OXA in ultrapure water, results suggest that self-sensitized photo-oxidation processes of $^1O_2$ generation were involved in this process.

REFERENCES

Acero, J.L., Real, F.J., Javier Benitez, F., Matamoros, E., 2019. Degradation of neonicotinoids by UV irradiation: Kinetics and effect of real water constituents. Sep. Purif. Technol. 211, 218–226. doi:10.1016/J.SEPPUR.2018.09.076

Acosta-Rangel, A., Sánchez-Polo, M., Polo, A.M.S., Rivera-Utrilla, J., Berber-Mendoza, M.S., 2018. Sulfonamides degradation assisted by UV, UV/H2O2 and UV/K2S2O8: Efficiency, mechanism and byproducts cytotoxicity. J. Environ. Manage. 225, 224–231. doi:10.1016/J.JENVMAN.2018.06.097

Bahnmüller, S., von Gunten, U., Canonica, S., 2014. Sunlight-induced transformation of sulfadiazine and sulfamethoxazole in surface waters and wastewater effluents. Water Res. 57, 183–192. doi:10.1016/J.WATRES.2014.03.019

Baptista, M.S., Cadet, J., Di Mascio, P., Ghogare, A.A., Greer, A., Hamblin, M.R., Lorente, C., Nunez, S.C., Ribeiro, M.S., Thomas, A.H., Vignoni, M., Yoshimura, T.M., 2017. Type I and Type II Photosensitized Oxidation Reactions: Guidelines and Mechanistic Pathways. Photochem. Photobiol. doi:10.1111/php.12716

Batista, A.P.S., Pires, F.C.C., Teixeira, A.C.S.C., 2014. The role of reactive oxygen species in sulfamethazine degradation using UV-based technologies and products identification. J. Photochem. Photobiol. A Chem. 290, 77–85. doi:10.1016/J.JPHOTOCHEM.2014.06.006

Batista, A.P.S., Teixeira, A.C.S.C., Cooper, W.J., Cottrell, B.A., 2016. Correlating the chemical and spectroscopic characteristics of natural organic matter with the photodegradation of sulfamerazine. Water Res. 93, 20–29. doi:10.1016/J.WATRES.2015.11.036

Bian, X., Zhang, J., 2016. Photodegradation of sulfadiazine in aqueous solution and the affecting factors. J. Chem. 2016, 1–5. doi:10.1155/2016/8358960

Boreen, A.L., Arnold, W.A., McNeill, K., 2005. Triplet-sensitized photodegradation of sulfa drugs containing six-membered heterocyclic groups: identification of an SO2 extrusion photoproduct. Environ. Sci. Technol. 39, 3630–3638. doi:10.1021/ES048331P

Caracciolo, A.B., Topp, E., Grenni, P., 2015. Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities. A review. J. Pharm. Biomed. Anal. 106, 25–36. doi:10.1016/J.JPBA.2014.11.040

Carlos, L., Mártire, D.O., Gonzalez, M.C., Gomis, J., Bernabeu, A., Amat, A.M., Arques, A., 2012. Photochemical fate of a mixture of emerging pollutants in the presence of humic substances. Water Res. 46, 4732–4740. doi:10.1016/J.WATRES.2012.06.022

Chowdhury, R.R., Charpentier, P.A., Ray, M.B., 2011. Photodegradation of 17β-estradiol in aquatic solution under solar irradiation: Kinetics and influencing water parameters. J. Photochem. Photobiol. A Chem. 219, 67–75. doi:10.1016/J.JPHOTOCHEM.2011.01.019

Conde-Cid, M., Fernández-Calviño, D., Nóvoa-Muñoz, J.C., Arias-Estévez, M., Díaz-Raviña, M., Núñez-Delgado, A., Fernández-Sanjurjo, M.J., Álvarez-Rodríguez, E., 2018. Degradation of sulfadiazine, sulfachloropyridazine and sulfamethazine in aqueous media. J. Environ. Manage. 228, 239–248. doi:10.1016/J.JENVMAN.2018.09.025

Cowan, D.O., Drisko, R.L., 1976. Photochemical techniques and the photochemistry of ketones, in:

Elements of Organic Photochemistry. Springer US, Boston, MA, pp. 75–133. doi:10.1007/978-1-4684-2130-9_3

Esteves, V.I., 1995. Extracção e caracterização de substâncias húmicas de diferentes ambientes aquáticos. PhD thesis, University of Aveiro, Portugal.

Esteves, V.I., Cordeiro, N.M.A., da Costa Duarte, A., 1995. Variation on the adsorption efficiency of humic substances from estuarine waters using XAD resins. Mar. Chem. 51, 61–66. doi:10.1016/0304-4203(95)00044-R

Esteves, V.I., Otero, M., Duarte, A.C., 2009. Comparative characterization of humic substances from the open ocean, estuarine water and fresh water. Org. Geochem. 40, 942–950. doi:10.1016/J.ORGGEOCHEM.2009.06.006

Ge, L., Chen, J., Wei, X., Zhang, S., Qiao, X., Cai, X., Xie, Q., 2010. Aquatic photochemistry of fluoroquinolone antibiotics: kinetics, pathways, and multivariate effects of main water constituents. Environ. Sci. Technol. 44, 2400–2405. doi:10.1021/es902852v

Ge, L., Zhang, P., Halsall, C., Li, Y., Chen, C.-E., Li, J., Sun, H., Yao, Z., 2019. The importance of reactive oxygen species on the aqueous phototransformation of sulfonamide antibiotics: kinetics, pathways, and comparisons with direct photolysis. Water Res. 149, 243–250. doi:10.1016/J.WATRES.2018.11.009

Geng, C., Liang, Z., Cui, F., Zhao, Z., Yuan, C., Du, J., Wang, C., 2020. Energy-saving photo-degradation of three fluoroquinolone antibiotics under VUV/UV irradiation: Kinetics, mechanism, and antibacterial activity reduction. Chem. Eng. J. 383, 123145. doi:10.1016/J.CEJ.2019.123145

Grebel, J.E., Pignatello, J.J., Mitch, W.A., 2010. Effect of halide ions and carbonates on organic contaminant degradation by hydroxyl radical-based advanced oxidation processes in saline waters. Environ. Sci. Technol. 44, 6822–6828. doi:10.1021/es1010225

Lai, H.-T., Lin, J.-J., 2009. Degradation of oxolinic acid and flumequine in aquaculture pond waters and sediments. Chemosphere 75, 462–468. doi:10.1016/J.CHEMOSPHERE.2008.12.060

Leech, D.M., Snyder, M.T., Wetzel, R.G., 2009. Natural organic matter and sunlight accelerate the degradation of 17ß-estradiol in water. Sci. Total Environ. 407, 2087–2092. doi:10.1016/J.SCITOTENV.2008.11.018

Levinton, J.S., 1995. Marine Biology. Function, Biodiversity, Ecology. Oxford University Press, New York.

Li, C., Zhang, D., Peng, J., Li, X., 2018. The effect of pH, nitrate, iron (III) and bicarbonate on photodegradation of oxytetracycline in aqueous solution. J. Photochem. Photobiol. A Chem. 356, 239–247. doi:10.1016/J.JPHOTOCHEM.2018.01.004

Li, H., Duan, L., Wang, H., Chen, Y., Wang, F., Zhang, S., 2020. Photolysis of sulfadiazine under UV radiation: Effects of the initial sulfadiazine concentration, pH, NO3− and Cd2+. Chem. Phys. Lett. 739, 136949. doi:10.1016/J.CPLETT.2019.136949

Li, S., Sun, W., 2014. Photocatalytic degradation of 17α-ethinylestradiol in mono- and binary systems of fulvic acid and Fe(III): Application of fluorescence excitation/emission matrixes. Chem. Eng. J. 237, 101–108. doi:10.1016/J.CEJ.2013.10.002

Lian, J., Qiang, Z., Li, M., Bolton, J.R., Qu, J., 2015. UV photolysis kinetics of sulfonamides in aqueous

solution based on optimized fluence quantification. Water Res. 75, 43–50. doi:10.1016/J.WATRES.2015.02.026

Liu, K., Xu, S., Zhang, M., Kou, Y., Zhou, X., Luo, K., Hu, L., Liu, X., Liu, M., Bai, L., 2016. Estimation of the toxicity of sulfadiazine to Daphnia magna using negligible depletion hollow-fiber liquid-phase microextraction independent of ambient pH. Sci. Rep. 6, 39798. doi:10.1038/srep39798

Liu, X., Liu, Y., Lu, S., Guo, W., Xi, B., 2018. Performance and mechanism into TiO2/Zeolite composites for sulfadiazine adsorption and photodegradation. Chem. Eng. J. 350, 131–147. doi:10.1016/J.CEJ.2018.05.141

Niu, X.-Z., Busetti, F., Langsa, M., Croué, J.-P., 2016. Roles of singlet oxygen and dissolved organic matter in self-sensitized photo-oxidation of antibiotic norfloxacin under sunlight irradiation. Water Res. 106, 214–222. doi:10.1016/J.WATRES.2016.10.002

Oliveira, C., Lima, D.L.D., Silva, C.P., Calisto, V., Otero, M., Esteves, V.I., 2019. Photodegradation of sulfamethoxazole in environmental samples: The role of pH, organic matter and salinity. Sci. Total Environ. 648, 1403–1410. doi:10.1016/j.scitotenv.2018.08.235

Pereira, J.H.O.S., Reis, A.C., Queirós, D., Nunes, O.C., Borges, M.T., Vilar, V.J.P., Boaventura, R.A.R., 2013. Insights into solar TiO2-assisted photocatalytic oxidation of two antibiotics employed in aquatic animal production, oxolinic acid and oxytetracycline. Sci. Total Environ. 463–464, 274–283. doi:10.1016/J.SCITOTENV.2013.05.098

Periša, M., Babić, S., Škorić, I., Frömel, T., Knepper, T.P., 2013. Photodegradation of sulfonamides and their N 4-acetylated metabolites in water by simulated sunlight irradiation: kinetics and identification of photoproducts. Environ. Sci. Pollut. Res. 20, 8934–8946. doi:10.1007/s11356-013-1836-1

Pinto, M.I., Salgado, R., Laia, C.A.T., Cooper, W.J., Sontag, G., Burrows, H.D., Branco, L., Vale, C., Noronha, J.P., 2018. The effect of chloride ions and organic matter on the photodegradation of acetamiprid in saline waters. J. Photochem. Photobiol. A Chem. 360, 117–124. doi:10.1016/J.JPHOTOCHEM.2018.04.020

Pouliquen, H., Delépée, R., Larhantec-Verdier, M., Morvan, M.-L., Le Bris, H., 2007. Comparative hydrolysis and photolysis of four antibacterial agents (oxytetracycline oxolinic acid, flumequine and florfenicol) in deionised water, freshwater and seawater under abiotic conditions. Aquaculture 262, 23–28. doi:10.1016/J.AQUACULTURE.2006.10.014

Prabhakaran, D., Sukul, P., Lamshöft, M., Maheswari, M.A., Zühlke, S., Spiteller, M., 2009. Photolysis of difloxacin and sarafloxacin in aqueous systems. Chemosphere 77, 739–746. doi:10.1016/J.CHEMOSPHERE.2009.08.031

Red Sea Salt [WWW Document], 2019. URL https://www.redseafish.com/red-sea-salts/red-sea-salt/ (accessed 8.12.20).

Rigos, G., Nengas, I., Alexis, M., Troisi, G.M., 2004. Potential drug (oxytetracycline and oxolinic acid) pollution from Mediterranean sparid fish farms. Aquat. Toxicol. 69, 281–288. doi:10.1016/J.AQUATOX.2004.05.009

Saadati, F., Keramati, N., Ghazi, M.M., 2016. Influence of parameters on the photocatalytic degradation of tetracycline in wastewater: A review. Crit. Rev. Environ. Sci. Technol. 46, 757–782. doi:10.1080/10643389.2016.1159093

Santoke, H., Cooper, W.J., 2017. Environmental photochemical fate of selected pharmaceutical

compounds in natural and reconstituted Suwannee River water: Role of reactive species in indirect photolysis. Sci. Total Environ. 580, 626–631. doi:10.1016/J.SCITOTENV.2016.12.008

Santos, M.E., Esteves, V.I., Amado, F.L., Duarte, A.C., 1994. Humic Substances in the Global Environment and Implications on Human Health, in: Senesi N. and Miano T.M. (Eds.), Elsevier, Amsterdam. pp. 877–882.

Silva, C.P., 2014. Occurrence and fate of estrogens and antibiotics in the environment evaluated by low-cost analytical methodologies. PhD thesis, University of Aveiro, Portugal.

Silva, C.P., Lima, D.L.D., Groth, M.B., Otero, M., Esteves, V.I., 2016a. Effect of natural aquatic humic substances on the photodegradation of estrone. Chemosphere 145, 249–255. doi:10.1016/J.CHEMOSPHERE.2015.11.068

Silva, C.P., Lima, D.L.D., Otero, M., Esteves, V.I., 2016b. Photosensitized degradation of 17β-estradiol and 17α-ethinylestradiol: role of humic substances fractions. J. Environ. Qual. 45, 693–700. doi:10.2134/jeq2015.07.0396

Silva, C.P., Oliveira, C., Ribeiro, A., Osório, N., Otero, M., Esteves, V.I., Lima, D.L.D., 2020. Sulfamethoxazole exposure to simulated solar radiation under continuous flow mode: Degradation and antibacterial activity. Chemosphere 238, 124613. doi:10.1016/J.CHEMOSPHERE.2019.124613

Sturini, M., Speltini, A., Maraschi, F., Profumo, A., Pretali, L., Fasani, E., Albini, A., 2010. Photochemical degradation of marbofloxacin and enrofloxacin in natural waters. Environ. Sci. Technol. 44, 4564–4569. doi:10.1021/es100278n

Sturini, M., Speltini, A., Maraschi, F., Profumo, A., Pretali, L., Irastorza, E.A., Fasani, E., Albini, A., 2012. Photolytic and photocatalytic degradation of fluoroquinolones in untreated river water under natural sunlight. Appl. Catal. B Environ. 119–120, 32–39. doi:10.1016/J.APCATB.2012.02.008

Sukul, P., Lamshöft, M., Zühlke, S., Spiteller, M., 2008. Photolysis of 14C-sulfadiazine in water and manure. Chemosphere 71, 717–725. doi:10.1016/J.CHEMOSPHERE.2007.10.045

Trawiński, J., Skibiński, R., 2019. Rapid degradation of clozapine by heterogeneous photocatalysis. Comparison with direct photolysis, kinetics, identification of transformation products and scavenger study. Sci. Total Environ. 665, 557–567. doi:10.1016/J.SCITOTENV.2019.02.124

Turel, I., 2002. The interactions of metal ions with quinolone antibacterial agents. Coord. Chem. Rev. 232, 27–47. doi:10.1016/S0010-8545(02)00027-9

Turiel, E., Bordin, G., Rodríguez, A.R., 2005. Study of the evolution and degradation products of ciprofloxacin and oxolinic acid in river water samples by HPLC-UV/MS/MS-MS. J. Environ. Monit. 7, 189–195. doi:10.1039/B413506G

Vasconcelos, T.G., Henriques, D.M., König, A., Martins, A.F., Kümmerer, K., 2009. Photo-degradation of the antimicrobial ciprofloxacin at high pH: Identification and biodegradability assessment of the primary by-products. Chemosphere 76, 487–493. doi:10.1016/J.CHEMOSPHERE.2009.03.022

Vione, D., Fabbri, D., Minella, M., Canonica, S., 2018. Effects of the antioxidant moieties of dissolved organic matter on triplet-sensitized phototransformation processes: Implications for the photochemical modeling of sulfadiazine. Water Res. 128, 38–48. doi:10.1016/J.WATRES.2017.10.020

Vione, D., Koehler, B., 2018. Modelled phototransformation kinetics of the antibiotic sulfadiazine in organic matter-rich lakes. Sci. Total Environ. 645, 1465–1473. doi:10.1016/J.SCITOTENV.2018.07.206

Wang, J., Chen, J., Qiao, X., Wang, Y., Cai, X., Zhou, C., Zhang, Y., Ding, G., 2018. DOM from mariculture ponds exhibits higher reactivity on photodegradation of sulfonamide antibiotics than from offshore seawaters. Water Res. 144, 365–372. doi:10.1016/J.WATRES.2018.07.043

Wenk, J., Canonica, S., 2012. Phenolic Antioxidants Inhibit the Triplet-Induced Transformation of Anilines and Sulfonamide Antibiotics in Aqueous Solution. Environ. Sci. Technol. 46, 5455–5462. doi:10.1021/es300485u

Xiao, Y., Carena, L., Näsi, M.-T., Vähätalo, A. V., 2020. Superoxide-driven autocatalytic dark production of hydroxyl radicals in the presence of complexes of natural dissolved organic matter and iron. Water Res. 177, 115782. doi:10.1016/J.WATRES.2020.115782

Yang, C.-C., Huang, C.-L., Cheng, T.-C., Lai, H.-T., 2015. Inhibitory effect of salinity on the photocatalytic degradation of three sulfonamide antibiotics. Int. Biodeterior. Biodegradation 102, 116–125. doi:10.1016/J.IBIOD.2015.01.015

Yang, Y., Pignatello, J., 2017. Participation of the Halogens in Photochemical Reactions in Natural and Treated Waters. Molecules 22, 1684. doi:10.3390/molecules22101684

Yi, Z., Wang, J., Tang, Q., Jiang, T., 2018. Photolysis of sulfamethazine using UV irradiation in an aqueous medium. RSC Adv. 8, 1427–1435. doi:10.1039/C7RA09564C

Zeghioud, H., Kamagate, M., Coulibaly, L.S., Rtimi, S., Assadi, A.A., 2019. Photocatalytic degradation of binary and ternary mixtures of antibiotics: reactive species investigation in pilot scale. Chem. Eng. Res. Des. 144, 300–309. doi:10.1016/J.CHERD.2019.02.015

Zhang, Y., Li, J., Zhou, L., Wang, G., Feng, Y., Wang, Z., Yang, X., 2016. Aqueous photodegradation of antibiotic florfenicol: kinetics and degradation pathway studies. Environ. Sci. Pollut. Res. 23, 6982–6989. doi:10.1007/s11356-015-5897-1

Zhang, Z., Xie, X., Yu, Z., Cheng, H., 2019. Influence of chemical speciation on photochemical transformation of three fluoroquinolones (FQs) in water: Kinetics, mechanism, and toxicity of photolysis products. Water Res. 148, 19–29. doi:10.1016/J.WATRES.2018.10.027

Zhao, Q., Fang, Q., Liu, H., Li, Y., Cui, H., Zhang, B., Tian, S., 2019. Halide-specific enhancement of photodegradation for sulfadiazine in estuarine waters: Roles of halogen radicals and main water constituents. Water Res. 160, 209–216. doi:10.1016/J.WATRES.2019.05.061

Zhou, C., Xie, Q., Wang, J., Chen, X., Niu, J., Chen, J., 2020. Effects of dissolved organic matter derived from freshwater and seawater on photodegradation of three antiviral drugs. Environ. Pollut. 258, 113700. doi:10.1016/J.ENVPOL.2019.113700

Zhou, W., Moore, D.E., 1997. Photosensitizing activity of the anti-bacterial drugs sulfamethoxazole and trimethoprim. J. Photochem. Photobiol. B Biol. 39, 63–72. doi:10.1016/S1011-1344(96)07468-4

PART II

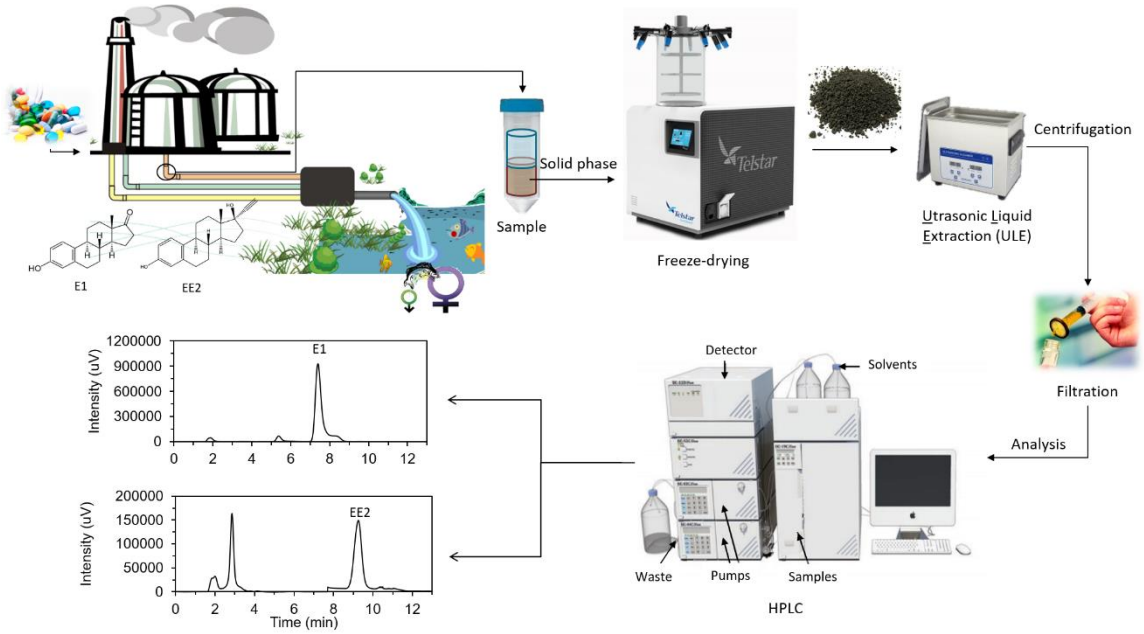
EVALUATION OF THE REMOVAL OF ESTROGENS IN AQUEOUS AND SLUDGE SAMPLES

CHAPTER 3

DETERMINATION OF ESTRONE AND 17α-ETHINYLESTRADIOL IN DIGESTED SLUDGE BY ULTRASONIC LIQUID EXTRACTION AND HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY WITH FLUORESCENCE DETECTION

The work presented and discussed in this chapter resulted in the following publication:

1. Louros, V. L., Lima, D. L. D., Leitão, J. H., Esteves, V. I., & Nadais, H. G. (2019). Determination of estrone and 17α-ethinylestradiol in digested sludge by ultrasonic liquid extraction and high-performance liquid chromatography with fluorescence detection. Journal of Separation Science, 42(8), 1585–1592. https://doi.org/10.1002/jssc.201801114


E1
EE2
Sample
Solid phase
Freeze-drying
Utrasonic Liquid Extraction (ULE)
Centrifugation
Filtration
Analysis
Detector
Solvents
Waste
Pumps
Samples
HPLC
Intensity (uV)
E1
EE2
Time (min)

SUMMARY

Estrogens estrone (E1) and 17α-ethinylestradiol (EE2) are increasingly recognised as important micropollutants to be monitored in wastewater treatment plants (WWTPs). These chemicals are retained onto sludge due to their high adsorption. Since sludge is largely used in land applications, the monitoring of those chemicals in sludge samples is of great importance. This study describes a method for the determination of E1 and EE2 in fresh sludge samples. After spiking fresh digested sludge with E1 an EE2 and maintaining contact during 5 min, 30 min 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 (HPLC) with fluorescence detection (FLD). The average recoveries obtained for E1 and EE2 using the different contact times were 103 % ± 3 % and 97 % ± 4 %, respectively. Fresh sludge samples from one wastewater treatment plant located in Portugal were analysed and estrone was detected in primary fresh sludge, anaerobic digested sludge and dehydrated sludge at a concentration in the range from 1 μg g^{-1} to 4.8 μg g^{-1}. The method here used does not require any sample clean-up, being fast and simple, reliable and inexpensive, making possible its application for monitoring the contamination of sludge with these estrogens.

CONTENTS

3.1. CONTEXTUALIZATION

The disposal of sludge generated during wastewater treatment is one of the main environmental issues nowadays. Sewage sludge generated as a by-product in wastewater treatment plant (WWTP) is usually partially dehydrated and applied as fertilizer (Martín et al., 2015, 2010). The widespread application of sludge onto soil is mainly due to its advantageous properties such as organic, nitrogen and phosphorus content (Martín et al., 2015). According to Kelessidis and Stasinakis (2012), 53 % of sludge generated in the European Union (EU) is used in agriculture land directly or after composting. Nonetheless, the main concern is that sewage sludge tends to concentrate potential contaminants from wastewater such as estrogens and heavy metals (Martín et al., 2015). This has led the European Union to include some pollutants in the monitoring list, in which estrone (E1) and 17α-ethinylestradiol (EE2) are included (European Union, 2020; Martín et al., 2015). However, currently, there is no regulation to control their presence in sewage sludge in European Union legislation (Kelessidis and Stasinakis, 2012).

As highlighted, the estrogens E1 and EE2 are hydrophobic compounds (Ying et al., 2002) and easily adsorbed in the order EE2 > E1 (Jones-Lepp and Stevens, 2007). In WWTP, a large proportion of these estrogens is removed from aqueous medium through adsorption processes onto sludge solid components (Johnson and Sumpter, 2001; Muller et al., 2010; Zeng et al., 2009a, 2009b). For this reason, concentrations of E1 and EE2 ranging 8 ng g^{-1} to 887 ng g^{-1} and 1.85 ng g^{-1} to 483 ng g^{-1}, respectively, have been found in sludge solid samples (Martín et al., 2015, 2010; Salgado et al., 2010; Zhu et al., 2015). Due to estrogens sorption onto sludge, it is crucial to develop a simple analytical methodology able to determine these compounds adsorbed onto sludge after the wastewater treatment. In the last years, a significant number of studies described and proposed different analytical methodologies with different extraction processes to detect and/or quantify E1 and EE2 mostly in environmental water samples, being the quantification of estrogens retained onto sludge usually neglected (Barreiros et al., 2016). Some of the exceptions are shown in Table 3.1.

Generally, the estrogens' quantification in sludge requires a highly efficient sample pre-treatment procedure, due to the complexity of the sample, and also high sensitive and selective analytical instrumentation. Several extraction strategies have been applied for sludge samples, including the ultrasonic liquid extraction (ULE) (Hashimoto and Murakami, 2009; Martín et al., 2010; Nie et al., 2009; Salgado et al., 2010; Ternes et al., 2002; Zeng et al., 2009a; Zhou et al., 2012) and accelerated solvent extraction (ASE) (Muller et al., 2010; Ye et al., 2012), both using organic

solvents. The ASE and ULE methods have been reported as presenting good and similar recovery rates for E1 and EE2. However, ULE has been usually chosen due to the use of commonly available equipment's, requirement of small volumes of extracting solvents, and fast extraction procedures, resulting in lower degradation of target compounds (Martín et al., 2010).

For the analytical determination of estrogens, gas chromatography (GC) and liquid chromatography (LC) coupled to mass spectrometry (MS) or preferably tandem mass spectrometry (MS/MS) detection have attracted great interest, not only due to the increased availability of this type of detector worldwide, but most probably to its high sensitivity and selectivity. Nevertheless, GC and LC coupled to MS detection can present some drawbacks, such as the requirement of sample clean-up, as well as sometimes derivatization procedures (Barreiros et al., 2016), high maintenance cost associated to the sophisticated equipment and highly trained specialists. High-performance liquid chromatography (HPLC) coupled to diode array (DAD) and/or fluorescence detector (FLD) have also been proposed for the determination of estrogens in influents, effluents, and solid matrices (Lima et al., 2013; Martín et al., 2010). For instance, Martín et al. (2010) used ULE, followed by a clean-up using solid phase extraction (SPE) and HPLC with DAD and FLD detectors, to detect pharmaceutical compounds in primary, secondary, and anaerobically digested dehydrated sewage sludge. The authors reported satisfactory recoveries of E1 and EE2, in the range from 65.1 % to 88.8 % and 89.8 % to 105 %, respectively; and low limit of quantification (LOQ) for E1 (14.8 ng g^{-1} to 30.4 ng g^{-1}) and EE2 (2.30 ng g^{-1} to 3.84 ng g^{-1}) (Table 3.1) (Martín et al., 2010). However, reports of extraction efficiencies considering fresh sludge spiked with standard estrogens are limited; most of them referred that standards are spiked onto freeze-dried sludge (Martín et al., 2010; Muller et al., 2010; Nie et al., 2009; Nieto et al., 2008; Ternes et al., 2002; Zhang et al., 2016), which do not mimic the natural adsorption process that estrogens are subjected to in naturally contaminated samples. On the other hand, the clean-up procedure used in most of the studies reported in the literature is not only expensive, but can also contribute to possible biodegradation of estrogens due to longer sample preparation time. 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 a known amount of compound 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. Finally, the method was applied to determine the concentration levels of E1 and EE2 in real sludge, collected from an operating WWTP located in Portugal.

Table 3.1 Characteristics of the methods described in literature for the determination of the selected estrogens in sludge samples.

WWTP Location	Sample	Compounds	Method[a]	Sample amount (g)	Extraction solvents[b]	Recovery (%)	LOD (ng g^{-1})	Reference
China	Activated sludge from two WWTPs	E1 E2 EE2	ULE-SPE-GC-MS	1.0	MeOH/ ACE (1:1, *v/v*) (3 mL × 5 mL)	72.8 to 120.8 71.3 to 104.2 81.2 to 128.7	0.45 0.36 3	Nie et al. (2009)
	Sludge samples from one WWTP	E1 E2 EE2	ULE-SPE-UHPLC-MS/MS	1.0	MeOH/ McIlvaine buffer (1:1, *v/v*) (3 mL × 5 mL)	85.8 to 106.2 94.4 to 96.2 83.5 to 94.4	0.15 0.57 1.02	Zhu et al. (2015)
	Activated sludge from ten WWTPs	E1 E2 EE2	ASE – SPE – GC-MS	0.5	3 cycles of MeOHl/ ACE (1:1, *v/v*)	77.2 to 118.3	0.09 to 0.6	Ye et al. (2012)
	Discharged sludge samples from one WWTP	E1 E2 EE2	ULE – SPE-GC-MS	0.5	3 × (15 mL Phosphate buffer solution + 20 mL acetonitrile)	94.4 to 133 89.7 to 131 121 to 126	4.3 3.4 2.9	Zhang et al. (2016)
	Activated sludge from one WWTP	E1 E2 EE2	ASE-LLE-Florisil-AAE- HLB-LC-MS/ MS	0.5	2 cycles of MeOH/ ACE (1:1, *v/v*)	75.3 87.7 87.9	-	Chen et al. (2012)
	Sewage sludge from one WWTP	E1 E2 EE2	ULE-SPE-UHPLC-MS/MS	0.1	Acetonitrile/ultra-pure water (5:3, *v/v*) (8 mL + 8 mL)	82.1 to 90.3 96.2 to 124.1 87.9 to 102.1	0.1 0.2 0.2	Yu et al. (2011)
Australia	Dewatered sludge from one WWTP	E1 E2 EE2	ULE-SPE-GC-MS	-[c]	ACE/ hexane (1:1, *v/v*) (100 mL)	75 87 95	-	Braga et al. (2005)
Germany	Activated sludge and digested sludge from two WWTPs	E1 E2 EE2	ULE-GPC-Silica gel-GC-MS/MS	0.5	MeOH, MeOH, ACE, ACE (4 mL +3 mL+3 mL +3 mL)	77 to 104 66 to 73 57 to 99	0.6 0.6 1.2	Ternes et al. (2002)
Italy	Sediment samples from three lakes	E1 E2 EE2	ULE-SPE-GCB-UHPLC-MS/MS	2	Water/MeOH/ACE (1:2:1, *v/v*) (15 mL)	96 to 108 91 to 101 93 to 104	0.64 0.38 2.1	Cavaliere et al. (2016)
Spain	Primary, secondary and digested sludge from one WWTP	E1 E2 EE2	ULE-SPE-HPLC-FLD/DAD	1.0 (primary and secondary sludge) 1.5 (digested sludge)	MeOH, MeOH, ACE (5 mL+2 mL+2 mL)	65.1 to 88.8 94.5 to 106 89.8 to 105	4.4 to 9.1 0.8 to 1.2 0.7 to 1.2	Martín et al. (2010)
	Sewage sludge from two WWTPs	E1 E2 EE2	ASE-HPLC-MS/MS	1	2 cycles of MeOH/ ACE (1:1, *v/v*), 2 cycles of water (pH 7)/ MeOH (1:1, *v/v*)	88 92 88	11 150 150	Nieto et al. (2008)
Portugal	Secondary sludge from five WWTPs	E1 E2 EE2	ULE – SPME – HPLC-DAD – MS/ MS	2.0[d]	MeOH, MeOH, ACE, ACE (4 mL +2 mL + 2 mL +2 mL)	104[e] - -	-	Salgado et al. (2010)

[a] AAE, aqueous alkali extraction; ACE, acetone; ASE, accelerated solvent extraction; DAD, diode array detector; FLD, fluorescence detection; GC, gas chromatography; GCB, graphitized carbon black; GPC, gel permeation chromatography; HLB, hydrophilic–lipophilic balance; HPLC, high-performance liquid chromatography; LLE, liquid-liquid extraction; MS, mass spectrometry; MS/MS, tandem mass spectrometry; SPE, solid-phase extraction; SPME, solid-phase microextraction; UHPLC, ultra-high-performance liquid chromatography; ULE, ultrasonic liquid extraction.

[b] MeOH, methanol.

[c] 50 mL of fresh sludge sample.

[d] Mass of centrifuged sludge.

[e] Recoveries were obtained with WWTP influent. The value presented is the minimum value obtained from 5 WWTPs.

3.2. EXPERIMENTAL SECTION

3.2.1. REAGENTS AND STANDARDS

Steroid hormones E1 (purity ≥ 99 %) and EE2 (purity ≥ 98 %), were supplied by Sigma-Aldrich. HPLC grade methanol, acetone and acetonitrile, were from Fischer Chemical, Carlo Erba and VWR (Prolabo), respectively. Ultrapure water was obtained from a Milli-Q Millipore system (Milli-Q plus 185). E1 and EE2 individual standard stock solutions were prepared in acetonitrile at a concentration of 1000 mg L^{-1} and further diluted to working concentrations with acetonitrile. All stock and working solutions were stored at 4 °C in the dark prior to use.

3.2.2. E1 AND EE2 ANALYSIS

E1 and EE2 analysis were performed on a 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-PFP (5 µm particle size) connected to a 4.6 mm i.d. ACE® 5 C18 guard column was used for the separation. The isocratic mobile phase used to determine E1 and EE2 consisted of ultrapure water: acetonitrile mixtures of (50:50, *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 (Lima et al., 2013). Both column and cell temperatures were maintained at 25°C. Before their use as mobile phase, water and acetonitrile were filtered through a 0.2 µm polyamide membrane filter (Whatman).

3.2.3. SAMPLE COLLECTION

Primary sludge, secondary sludge, anaerobic digested sludge, and dehydrated sludge samples were collected from a WWTP (39 278 m^3 effluent per day), located in Aveiro, Portugal (Figure 3.1). After collection, samples were sealed and placed on ice in a sampling box and immediately shipped back to the laboratory. Total suspended solids (TSS) and volatile suspended

solids (VSS) concentration were analysed according to the Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

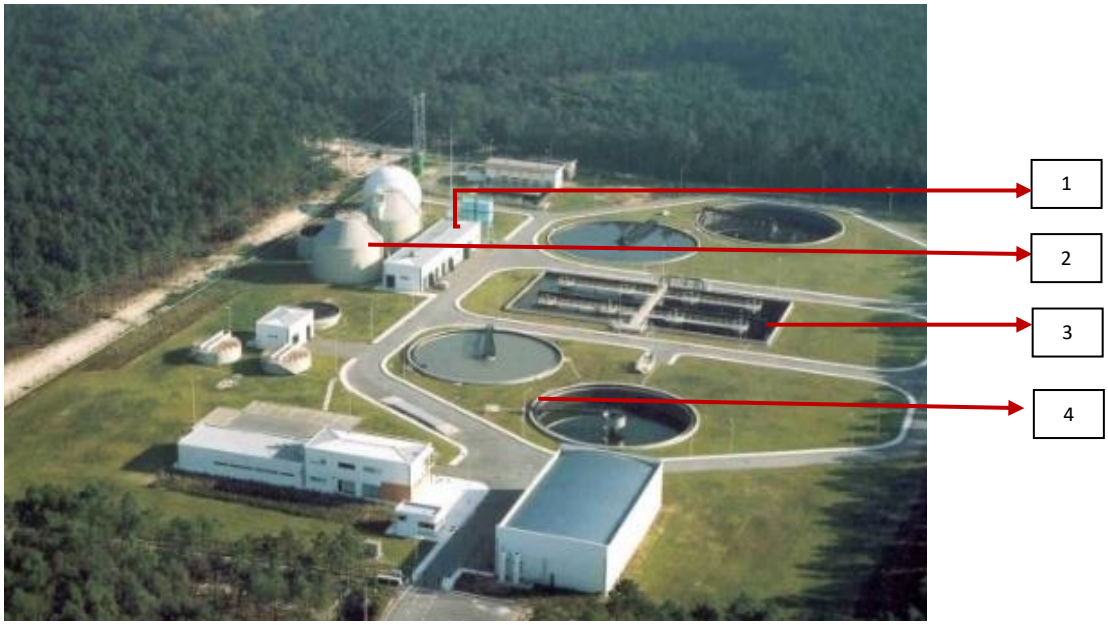


Figure 3.1 Sampling points of wastewater samples – WWTP. Legend: (1) dehydrated sludge; (2) anaerobic digested sludge; (3) secondary sludge and (4) primary sludge.

3.2.4. SAMPLE TREATMENT AND EXTRACTION

3.2.4.1. EVALUATION OF EXTRACTION PROCEDURE

Digested sludge (maintained under continuous stirring at room temperature) samples were subjected to the experimental procedure illustrated in Figure 3.2. Briefly, 50 mL sludge samples, corresponding to approximately 0.51 g ± 0.01 g and 0.62 g ± 0.02 g of dried sample for E1 and EE2 analysis, respectively, were placed into teflon tubes (VWR) and centrifuged at 4000 rpm (2576 × *g*) for 10 min using a Mega Star 600 centrifuge (VWR). Sample supernatant was collected, immediately filtered with 0.2 µm PVDF filter, stored at 4°C and analysed within the next 24 h. The sedimented solid phase was first stored at –80°C for 24 h and then freeze-dried at –86°C in a LyoQuest Freeze Dryer (Telstar) for 48 h. The resulting dried solid samples were ground with a pestle and mortar, weighed, and used within 48 h.

The freeze-dried and ground sludge samples (0.51 g ± 0.01 g and 0.62 g ± 0.02 g used to extract E1 and EE2, respectively) 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, samples were vigorously vortexed (Velp Scientifica) during 1 min and ultrasonicated in an Ultrasonic Cleaner USC-T ultrasonic bath (VWR) for 1 h. The slurry was then centrifuged (Mega Star 600, VWR) at 4000 rpm (2576 × *g*) for 10 min and the supernatant of each extraction was collected. Each of the extraction supernatants was filtered using PVDF 0.2 µm filters and analysed individually by HPLC-FLD.

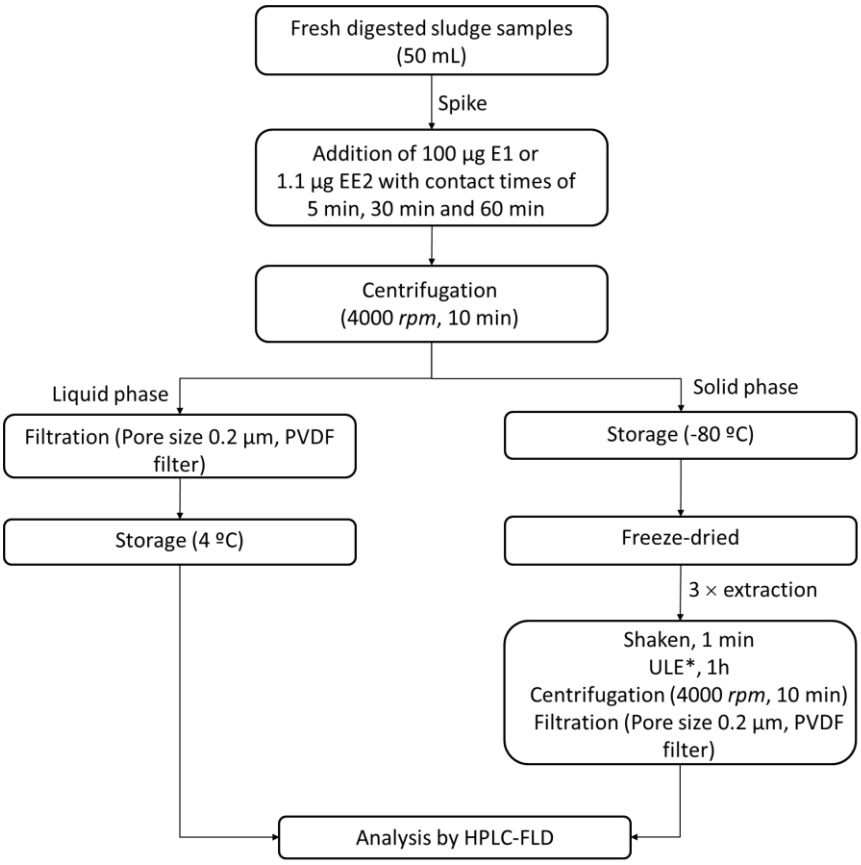


Figure 3.2 Schematic diagram for the evaluation of the extraction process used to quantify E1 and EE2 in liquid and solid phases of sludge. *Ultrasonic liquid extraction (ULE) was carried out with 9 mL of methanol, 4.5 mL of methanol and 4.5 mL of acetone per gram of dried sludge.

To determine the recovery efficiency of the ULE described method, 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, 30 and 60 min was used to evaluate sorption interactions of the estrogens with the sludge under continuous agitation. Triplicate samples were prepared and analysed following the flowchart presented in Figure 3.2.

The method reproducibility was evaluated based on the relative standard deviation (RSD) of the obtained recovery rates. E1 and EE2 mass in fresh digested sludge samples before spiking was also determined and denominated as blank sample. The recovery rate was calculated as the ratio between the estrogens mass determined in fresh digested sludge after spiking with E1 or EE2 and the total estimated mass in the sample (spiked plus the determined E1 or EE2 in the fresh digested sludge – blank sample). The total E1 and EE2 individual mass was determined as the sum of the masses quantified in the sludge samples liquid and solid phases.

3.2.4.2. SAMPLE ANALYSIS

To quantify E1 and EE2 in fresh digested sludge by the ULE method, 150 mL of primary sludge, 200 mL of secondary sludge and anaerobic digested sludge, and 3 g of dried dehydrated sludge were used (the sample volume used was chosen in order to obtain a similar mass of freeze-dried and ground sludge). The addition of 9.0 mL and 4.5 mL of methanol and 4.5 mL of acetone per g of dry sample allowed to extract E1 and EE2 from these samples. Organic supernatants (methanol plus acetone), were combined and evaporated under a nitrogen stream in order to concentrate them four times, filtered (0.2 μm PVDF filter) and analysed by HPLC-FLD. Samples were extracted and analysed in triplicate.

3.3. RESULTS AND DISCUSSION

3.3.1. PERFORMANCE OF THE CHROMATOGRAPHIC ANALYTIC METHOD AND EXTRACTION EFFICIENCY

For E1 and EE2 analysis, calibration curves with nine and six points, respectively, were established, and LOD and linearity were calculated. LOD was calculated from each calibration curve as $a + 3s_{y/x}$, where a is the intercept of the regression line and $s_{y/x}$ is the statistical parameter which estimates the random errors in the y-axis (signal). The linear range was between 50 µg L^{-1} and 3500 µg L^{-1} for E1 and between 10 µg L^{-1} and 500 µg L^{-1} for EE2. The LOD, in terms of mass of estrogen per mass of dry sludge was calculated according to the following equation:

$$\text{LOD } (\mu g\, g^{-1}) = \frac{\text{LOD } (\mu g\, L^{-1}) \times \text{volume of extractive solvent (L)}}{\text{dry sludge mass (g)}} \quad \textbf{(Eq. 3.1)}$$

LOD values were below 0.305 µg g^{-1} ± 0.003 µg g^{-1} for E1 and 0.0516 µg g^{-1} ± 0.0006 µg g^{-1} for EE2 (for LOD method determination in the solid samples, the dry sludge mass of 0.766 ± 0.005 g was considered). E1 and EE2 presented a good correlation coefficient (above 0.999) and good linearity values (between 99.6 % and 99.5 %, respectively) for the concentration range used in this study (Table 3.2).

Table 3.2 Quantitative parameters for E1 and EE2 analytical curves obtained by HPLC – FLD.

Analyte	Linear range	Correlation coefficient (*r*)	Linearity (LIN)	Limit of detection (LOD)	
				Liquid phase	Solid phase
	(µg L^{-1})		(%)	(µg L^{-1})	(µg g^{-1})
E1	50 to 3500	0.9999	99.56	41.3	0.305
EE2	10 to 500	0.9999	99.45	7.0	0.0516

To evaluate the developed ULE method the recovery rates, after each ULE extraction step using different contact times, were determined and are presented in Table 3.3. Also, HPLC-FLD chromatograms of estrogen standard solutions, liquid phase of the digested sludge and extracts obtained from the extraction of the solid phase of the digested sludge sample, before and after being spiked with E1 and EE2 are presented in Figure 3.3. Results showed that at contact time of 5 min, 71 %, 17 % and 12.4 % of E1 and 72 %, 18.6 % and 9 % of EE2 were extracted in the first, second and third extraction, respectively (Table 3.3).

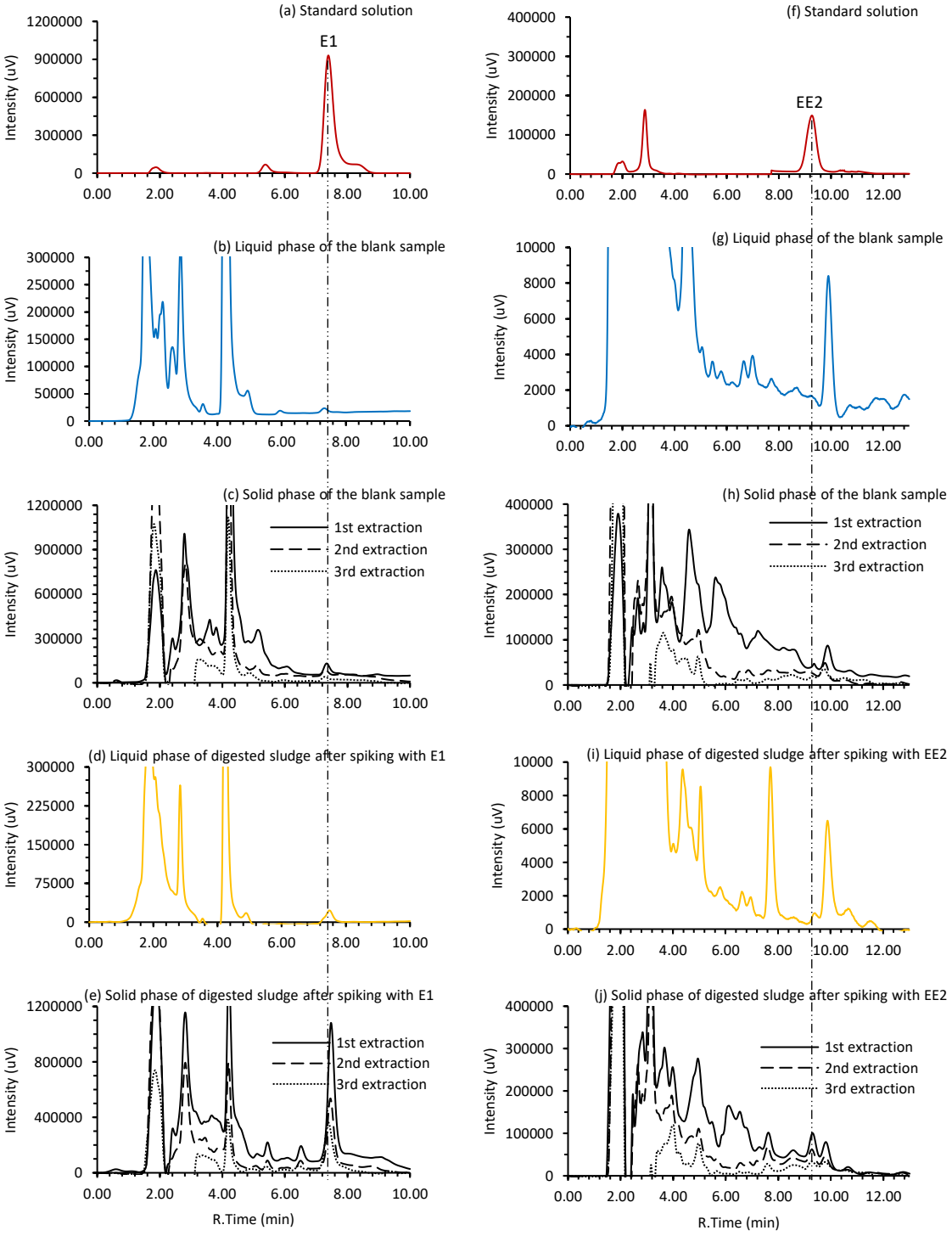


Figure 3.3 HPLC-FLD chromatograms of E1 in (a) standard solution; (b) liquid phase of the blank sample; (c) solid phase of blank sample; (d) liquid phase of digested sludge after spiking with E1; (e) solid phase of digested sludge after spiking with E1; and, HPLC-FLD chromatograms of EE2 in (f) standard solution; (g) liquid phase of the blank sample; (h) solid phase of blank sample; (i) liquid phase of digested sludge after spiking with EE2; (j) solid phase of digested sludge after spiking with of EE2.

Table 3.3 Recovery results of E1 and EE2 at different contact times after each extraction step.

Analyte	Extraction step	Mass compound (µg) [a]			Recovery rate (%) [a, b]		
		5 min	30 min	60 min	5 min	30 min	60 min
E1	First extraction (9.0 mL of methanol per g dry sample)	69 ± 3	69 ± 2	65.1 ± 0.6	71 ± 2	69 ± 2	67.6 ± 0.6
	Second extraction (4.5 mL of methanol per g dry sample)	16 ± 1	17 ± 2	19 ± 1	17 ± 2	17 ± 2	19 ± 1
	Third extraction (4.5 mL of acetone per g dry sample)	12.0 ± 0.3	13.3 ± 0.4	14 ± 1	12.4 ± 0.2	13.3 ± 0.2	13.3 ± 0.5
	Sum	97 ± 3	99 ± 3	98 ± 2			
EE2	First extraction (9.0 mL of methanol per g dry sample)	0.74 ± 0.07	0.71 ± 0.01	0.72 ± 0.05	72 ± 2	71 ± 2	68 ± 2
	Second extraction (4.5 mL of methanol per g dry sample)	0.192 ± 0.009	0.21 ± 0.02	0.214 ± 0.005	18.6 ± 0.7	20 ± 1	20 ± 1
	Third extraction (4.5 mL of acetone per g dry sample)	0.09 ± 0.01	0.09 ± 0.01	0.123 ± 0.008	9 ± 1	9 ± 1	11.7 ± 0.8
	Sum	1.02 ± 0.07	1.01 ± 0.02	1.06 ± 0.05			

[a] Mean value ± standard deviation (n = 3).

[b] Relative to mass adsorbed onto sludge.

Figure 3.4 shows the mass profiles of E1 and EE2, in batch experiments with different contact times using fresh digested sludge samples, spiked with 100 µg E1 and 1.1 µg EE2. In the blank sample, 12 µg of E1 was detected in the liquid phase and 8 µg in the solid phase, while for EE2, 0.1 µg was detected in the solid phase. Estrogens mass immediately after spiking (contact time of 0 min) were estimated as the sum of the estrogen mass measured immediately before spiking and the spiked mass (100 µg E1 and 1.1 µg EE2). After the different contact times used (5, 30 and 60 min), more than 96.8 µg of E1 and 1.0 µg of EE2 were adsorbed onto sludge. The patterns of extraction of E1 and EE2 were quite similar, regardless of the contact times used. In this study, it was observed that not only both E1 and EE2 are highly adsorbed onto sludge, but also that this adsorption process is almost instantaneous.

The precision and accuracy of the developed ULE method can be assessed by the average of the recovery extraction shown in Table 3.4. Results indicated good recovery efficiencies of the overall method, which ranged from 103 % for E1 to 97 % for EE2, showing good accuracy. Also, recoveries obtained were slightly higher than those referred in literature (Martín et al., 2010; Nie et al., 2009; Ternes et al., 2002); a good precision was also observed with RSD values below 4 % (U.S. EPA recommend RSD values lower than 20 %) (Table 3.4).

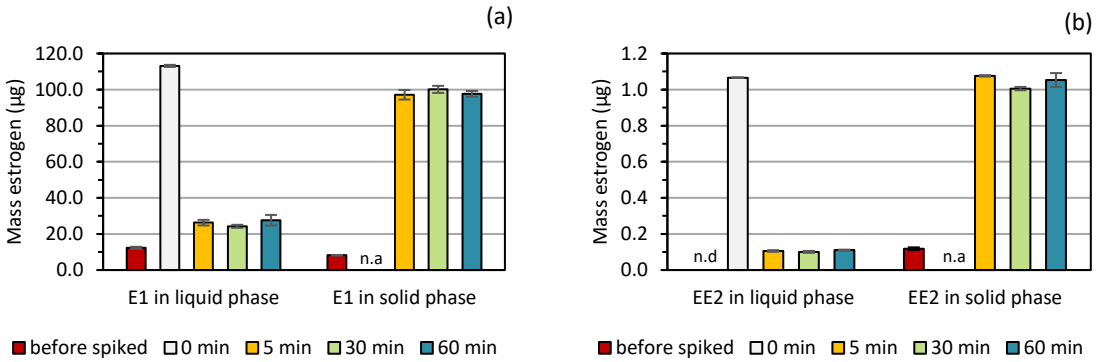


Figure 3.4 Mass profiles of E1 (a) and EE2 (b) using the spike of 100 µg E1 and 1.1 µg EE2 in fresh digested sludge samples. n.a, not analyzed. n.d, not detected.

Table 3.4 E1 and EE2 recovery results.

Analyte	Extraction recovery[a] (%)			
	5 min[b]	30 min[b]	60 min[b]	Average[c]
E1	102 ± 4	103 ± 2	104 ± 4	103 ± 3
EE2	100.5 ± 0.2	93.4 ± 0.5	99 ± 3	97 ± 4

[a] Values obtained for a mass spike of 100 µg E1 and 1.1 µg EE2 into fresh sludge.

[b] Mean value ± standard deviation (n = 3).

[c] Mean value ± standard deviation (n = 9).

Martín et al. (2010) observed that estrogens were efficiently extracted from sediments using two portions of methanol followed by acetone (5 mL + 2 mL + 2 mL). These authors also showed that a higher sonication time (15 min) improved estrogens extraction recoveries from sediment samples. In our study, a higher volume of extracting solvents and a total ultrasonication of 1 h were used to improve the extraction capacity.

In most studies reported in the literature, the recovery rates were obtained by spiking the compounds under study in freeze-dried sludge (Martín et al., 2010; Muller et al., 2010; Nie et al., 2009; Ternes et al., 2002), which do not mimic the adsorption behaviour that occurs in fresh sludge from WWTP. Braga et al. (2005) added E1 and EE2 standards to a 50 mL of fresh sludge samples but could only achieve satisfactory recovery rates of 75 % and 95 %, respectively, using ULE-SPE followed by ultra-high-performance liquid chromatography (UHPLC)-MS/MS. Lower LOD (between 0.6 ng g^{-1} and 1.2 ng g^{-1}) was obtained by Ternes et al. (2002), however, the clean-up steps adopted, and sample derivatization needed for GC-MS analysis increases the analysis time and cost. Nieto et al. (2008) presented an ASE-LC-MS/MS method to determine a group of estrogens and conjugated

estrogens in sewage sludge but obtained lower recovery rates (88 %) for both E1 and EE2, as well as higher LOD (0.150 μg g^{-1}) for EE2, compared with the one obtained in our study. Several works used LC coupled to MS detection to quantify estrogens in these type of samples (Cavaliere et al., 2016; Chen et al., 2012; Yu et al., 2011; Zhu et al., 2015). However, as mentioned before, several disadvantages can be listed, such as the requirement of sample clean-up using SPE, which is expensive and time-consuming, as well as high maintenance cost associated to the sophisticated equipment and the need of highly trained specialists. In summary, the ULE-HPLC-FLD method hereby developed presents the following advantages: high extraction recoveries, simplicity, low cost, no need of derivatization, which avoids the additional operation and reduces analysis time, thus preventing possible biodegradation of estrogens during sample preparation.

3.3.2. Determination of E1 and EE2 in WWTP aqueous and sludge samples

The developed method was applied for the determination of E1 and EE2 concentration in sludge samples (primary sludge, secondary sludge, anaerobic digested sludge and dehydrated sludge) collected from one WWTP in September and December 2017. The physical-chemical properties of sludge samples used in this study can be found in Table 3.5 and the estrogens concentrations detected in those samples in Table 3.6. Figure 3.3 shows the HPLC-FLD chromatogram of one of the sludge samples analysed.

Table 3.5 Chemical characterization of the sludge samples used in this study.

Parameter [a]	Sample					
	September 2017			December 2017		
	Primary sludge	Secondary sludge	Digested sludge	Primary sludge	Secondary sludge	Digested sludge
TSS (g L^{-1})	24.0 ± 0.5	19.1 ± 0.5	16.2 ± 0.6	26 ± 2	4.4 ± 0.1	19.1 ± 0.4
VSS (g L^{-1})	19.0 ± 0.4	15.3 ± 0.6	12.2 ± 0.6	22 ± 2	3.47 ± 0.09	14.3 ± 0.4
pH	6.87	6.67	7.36	6.18	7.09	7.24
ALK (g $CaCO_3$ L^{-1})	1.96	0.98	2.65	1.08	0.46	3.17
Conductivity (mS cm^{-1})	11.20	6.33	13.29	3.13	2.17	7.19
TDS (g L^{-1})	6.20	3.44	7.39	1.7	1.17	3.84
Salinity	6.00	3.50	7.70	1.60	1.10	3.9

[a] ALK, Alkalinity; TDS, Total Dissolved Solids; TSS, Total Suspended Solids; VSS, Volatile Suspended Solids. For suspended solids, triplicate analyses were performed for each sample.

To date, limited data are available worldwide about the concentration levels of steroid estrogens adsorbed onto WWTP sludge. Martín et al. (2015) investigated the occurrence of pharmaceutical compounds in different types of sludge (primary sludge, secondary sludge,

anaerobically-digested dehydrated sludge, composted sludge, mixed sludge, aerobically-digested dehydrated sludge and lagoon sludge) and concluded that for estrogens, the most contaminated samples were from primary sludge. In our study, the concentrations of E1 and EE2 were analysed in both solid and liquid phase of sludge samples. 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 dehydrated sludge (1.00 μg g^{-1}). The concentrations of E1 detected in the samples collected in December 2017 were higher than in those collected in September 2017. Zeng et al. (2009a) reported that higher temperature improves biodegradation rate of estrogens, thus these results can be explained by the lower environmental temperature observed in December, resulting in a slower E1 degradation. In the secondary sludge samples analysed, E1 and EE2 concentration were below the LOD (0.305 μg g^{-1} for E1 and 0.052 μg g^{-1} for EE2).

E1 concentration levels obtained in this study were slightly higher than those reported in the literature (between 0.008 μg g^{-1} and 0.887 μg g^{-1}) (Martín et al., 2015; Salgado et al., 2010). However, Martín et al. (2015) detected similar E1 concentration levels (up 0.887 μg g^{-1}) in primary sludge samples, compared to our study.

Primary sludge presents a high hydrophobicity, which might explain the higher concentration detected in these types of samples, since hormones are hydrophobic contaminants and tend to adsorb onto these materials. The E1 concentration values detected in the solid phase of primary sludge suggest adsorption as an important removal mechanism of hormones from wastewater. Moreover, since sludge in WWTP adsorbs a significant number of hydrophobic compounds, such as estrogens, appropriate treatment is mandatory for waste sludge prior to its discharge into the environment.

Table 3.6 Results of E1 and EE2 concentrations detected in different samples of WWTP.

Sample	Date of analysis	Estrogen concentration			
		Solid phase [a]		Total (liquid phase + solid phase) [b]	
		E1	EE2	E1	EE2
		(μg g^{-1})		(μg L^{-1})	
Primary sludge	04/09/2017	3.61 ± 0.05	<LOD	81.3 ± 0.6	<LOD
	12/12/2017	4.8 ± 0.1	<LOD	164 ± 1	<LOD
Secondary sludge	04/09/2017	<LOD	<LOD	<LOD	<LOD
	12/12/2017	<LOD	<LOD	<LOD	<LOD
Digested sludge	04/09/2017	<LOD	<LOD	<LOD	<LOD
	12/12/2017	1.84 ± 0.07	<LOD	<LOD	<LOD
Dehydrated sludge	04/09/2017	<LOD	<LOD	-	-
	12/12/2017	1.00 ± 0.05	<LOD	-	-

[a] LOD below 0.305 μg g^{-1} for E1 and 0.052 μg g^{-1} for EE2

[b] LOD of 41.3 μg L^{-1} for E1 and 7.0 μg L^{-1} for EE2.

3.4. CONCLUSIONS

This work presents the development of a fast and simple analytical method for the determination of E1 and EE2 in liquid and solid phases of sludge samples collected in WWTP. The methodology is based on ultrasonic liquid extraction 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 the existing analytical methodologies, the main advantages of the proposed method are good extraction recoveries, set-up easiness, simplicity, cost-effectiveness and reduced sample preparation time, preventing possible degradation of E1 and EE2 during sample preparation.

The analytical methodology developed in this study was applied for E1 and EE2 monitoring in sludge samples (primary sludge, secondary sludge, anaerobic digested sludge and dehydrated sludge) collected from an operating WWTP. Results indicated that E1 was detected in fresh primary sludge, anaerobic digested sludge and dehydrated sludge at a concentration in the range from 1.00 μg g^{-1} to 4.8 μg g^{-1}. In the other samples analysed, the concentration values of both 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 WWTP sludge samples.

REFERENCES

American Public Health Association (APHA), 2005. Standard methods for the examination of water & wastewater, the 21st edition, american public health association, washington, DC, USA.

Barreiros, L., Queiroz, J.F., Magalhães, L.M., Silva, A.M.T., Segundo, M.A., 2016. Analysis of 17-β-estradiol and 17-α-ethinylestradiol in biological and environmental matrices - A review. Microchem. J. 126, 243–262. doi:10.1016/j.microc.2015.12.003

Braga, O., Smythe, G., Schäfer, A., Feitz, A., 2005. Fate of steroid estrogens in Australian inland and coastal wastewater treatment plants. Environ. Sci. Technol. 39, 3351–3358. doi:10.1021/es0501767

Cavaliere, C., Capriotti, A.L., Ferraris, F., Foglia, P., Samperi, R., Ventura, S., Laganà, A., 2016. Multiresidue analysis of endocrine-disrupting compounds and perfluorinated sulfates and carboxylic acids in sediments by ultra-high-performance liquid chromatography–tandem mass spectrometry. J. Chromatogr. A 1438, 133–142. doi:10.1016/j.chroma.2016.02.022

Chen, Q., Shi, J., Wu, W., Liu, X., Zhang, H., 2012. A new pretreatment and improved method for determination of selected estrogens in high matrix solid sewage samples by liquid chromatography mass spectrometry. Microchem. J. 104, 49–55. doi:10.1016/j.microc.2012.04.008

European Union (EU), 2020. Selection of substances for the 3rd Watch List under the Water Framework Directive [WWW Document]. Eur. Med. Agency. URL https://www.ema.europa.eu/en/human-regulatory/post-authorisation/pharmacovigilance/medicines-under-additional-monitoring/list-medicines-under-additional-monitoring (accessed 12.21.20).

Hashimoto, T., Murakami, T., 2009. Removal and degradation characteristics of natural and synthetic estrogens by activated sludge in batch experiments. Water Res. 43, 573–582. doi:10.1016/j.watres.2008.10.051

Johnson, A., Sumpter, J., 2001. Removal of endocrine-disrupting chemicals in activated sludge treatment works. Environ. Sci. Technol. 35, 4697–4703. doi:10.1021/es010171j

Jones-Lepp, T.L., Stevens, R., 2007. Pharmaceuticals and personal care products in biosolids/sewage sludge: the interface between analytical chemistry and regulation. Anal. Bioanal. Chem. 387, 1173–1183. doi:10.1007/s00216-006-0942-z

Kelessidis, A., Stasinakis, A.S., 2012. Comparative study of the methods used for treatment and final disposal of sewage sludge in European countries. Waste Manag. 32, 1186–1195. doi:10.1016/j.wasman.2012.01.012

Lima, D.L.D., Silva, C.P., Otero, M., Esteves, V.I., 2013. Low cost methodology for estrogens monitoring in water samples using dispersive liquid-liquid microextraction and HPLC with fluorescence detection. Talanta 115, 980–985. doi:10.1016/j.talanta.2013.07.007

Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2015. Pharmaceutically active compounds in sludge stabilization treatments: anaerobic and aerobic digestion, wastewater stabilization ponds and composting. Sci. Total Environ. 503–504, 97–104. doi:10.1016/j.scitotenv.2014.05.089

Martín, J., Santos, J.L., Aparicio, I., Alonso, E., 2010. Multi-residue method for the analysis of

pharmaceutical compounds in sewage sludge, compost and sediments by sonication-assisted extraction and LC determination. J. Sep. Sci. 33, 1760–1766. doi:10.1002/jssc.200900873

Muller, M., Combalbert, S., Delgenès, N., Bergheaud, V., Rocher, V., Benoît, P., Delgenès, J.-P., Patureau, D., Hernandez-Raquet, G., 2010. Occurrence of estrogens in sewage sludge and their fate during plant-scale anaerobic digestion. Chemosphere 81, 65–71. doi:10.1016/j.chemosphere.2010.06.062

Nie, Y., Qiang, Z., Zhang, H., Adams, C., 2009. Determination of endocrine-disrupting chemicals in the liquid and solid phases of activated sludge by solid phase extraction and gas chromatography-mass spectrometry. J. Chromatogr. A 1216, 7071–7080. doi:10.1016/j.chroma.2009.08.064

Nieto, A., Borrull, F., Pocurull, E., Marcé, R.M., 2008. Determination of natural and synthetic estrogens and their conjugates in sewage sludge by pressurized liquid extraction and liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1213, 224–230. doi:10.1016/j.chroma.2008.10.043

Salgado, R., Noronha, J.P., Oehmen, A., Carvalho, G., Reis, M.A.M., 2010. Analysis of 65 pharmaceuticals and personal care products in 5 wastewater treatment plants in Portugal using a simplified analytical methodology. Water Sci. Technol. 62.

Ternes, T.A., Andersen, H., Gilberg, D., Bonerz, M., 2002. Determination of estrogens in sludge and sediments by liquid extraction and GC/MS/MS. Anal. Chem. 74, 3498–3504. doi:10.1021/ac015717z

Ye, X., Guo, X., Cui, X., Zhang, X., Zhang, H., Wang, M.K., Qiu, L., Chen, S., 2012. Occurrence and removal of endocrine-disrupting chemicals in wastewater treatment plants in the Three Gorges Reservoir area, Chongqing, China. J. Environ. Monit. 14, 2204–2211. doi:10.1039/c2em30258f

Ying, G.-G., Kookana, R.S., Ru, Y.-J., 2002. Occurrence and fate of hormone steroids in the environment. Environ. Int. 28, 545–551. doi:10.1016/S0160-4120(02)00075-2

Yu, Y., Huang, Q., Cui, J., Zhang, K., Tang, C., Peng, X., 2011. Determination of pharmaceuticals, steroid hormones, and endocrine-disrupting personal care products in sewage sludge by ultra-high-performance liquid chromatography–tandem mass spectrometry. Anal. Bioanal. Chem. 399, 891–902. doi:10.1007/s00216-010-4295-2

Zeng, Q., Li, Y., Gu, G., 2009a. Nitrate-dependent degradation of 17α-ethinylestradiol by acclimated activated sludge under anaerobic conditions. J. Chem. Technol. Biotechnol. 84, 1841–1847. doi:10.1002/jctb.2255

Zeng, Q., Li, Y., Gu, G., Zhao, J., Zhang, C., Luan, J., 2009b. Sorption and biodegradation of 17β-Estradiol by acclimated aerobic activated sludge and isolation of the bacterial strain. Environ. Eng. Sci. 26, 783–790. doi:10.1089/ees.2008.0116

Zhang, M., Mao, Q., Feng, J., Yuan, S., Wang, Q., Huang, D., Zhang, J., 2016. Validation and application of an analytical method for the determination of selected acidic pharmaceuticals and estrogenic hormones in wastewater and sludge. J. Environ. Sci. Heal. Part A 51, 914–920. doi:10.1080/10934529.2016.1191304

Zhou, H., Zhou, Y., Li, H., Wang, F., 2012. Fate and removal of selected endocrine-disrupting compounds in sewage using activated sludge treatment. Water Environ. J. 26, 435–444.

doi:10.1111/j.1747-6593.2011.00302.x

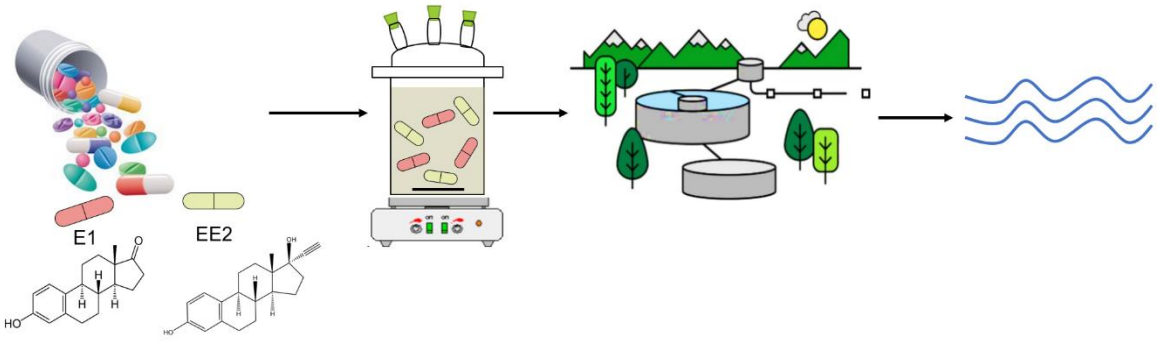
Zhu, B., Ben, W., Yuan, X., Zhang, Y., Yang, M., Qiang, Z., 2015. Simultaneous detection of endocrine disrupting chemicals including conjugates in municipal wastewater and sludge with enhanced sample pretreatment and UPLC-MS/MS. Environ. Sci. Process. Impacts 17, 1377–1385. doi:10.1039/C5EM00139K

CHAPTER 4

REMOVAL OF ESTRONE AND 17α-ETHINYLESTRADIOL BY DIGESTED SLUDGE USING BATCH EXPERIMENTS

The work presented and discussed in this chapter resulted in following submitted article:

1. Louros, V.L., Sousa, A.F., Lima, D.L.D., Santos, P., Leitão, J.H., Esteves, V.I. & Nadais, H.G. Estrogens in wastewaters: can different operating conditions improve their removal by digested sludge?


E1
EE2

SUMMARY

The removal of estrone (E1) and 17α-ethinylestradiol (EE2) from synthetic wastewater using active sludge (non-sterilised sludge) under anaerobic conditions was assessed in this work by a series of batch experiments. Different operating conditions were used, including three temperatures (20 °C, 25 °C, and 34 °C), three mixed liquor suspended solid (MLSS) contents (2 g L^{-1}, 3 g L^{-1} and 5 g L^{-1}), and the addition of nitrate as a supplement. In order to use environmental relevant concentrations and to overcome the limit of detection associated to high-performance liquid chromatography (HPLC), the quantification of estrogens was made in the sludge solid phase and in the supernatant of the reactor. However, as expected, E1 and EE2 were not detected in the supernatant of the reactor, probably due to their rapid adsorption onto the sludge solid phase. Results indicated that the increase in temperature favoured E1 removal from the reactor mixture. However, in the case of EE2, temperature does not seem to affect removal. The lowest MLSS concentrations promoted a higher removal of E1 and EE2. Moreover, the presence of nitrate does not seem to have influence E1 and EE2 removal. Complete E1 removal from the reactor mixture occurred at MLSS concentration of 2 g L^{-1} within 25 h, at 25 °C. However, 265 h were not enough for EE2 elimination at 25 °C, and 34 °C. Our results indicate that in biological processes for estrogens' removal both adsorption and biodegradation can occur simultaneously, and although adsorption is the fastest process, biodegradation is responsible for the elimination of the compounds from the environment and not only from the aqueous phase.

CONTENTS

4.1. CONTEXTUALIZATION

In wastewater treatment plants (WWTP), conventional and current systems applied for wastewater treatment are not adequate for the efficient removal of estrogens. The removal of estrogens using sewage sludge is mainly due to adsorption and biodegradation phenomena, which can occur simultaneously, although the adsorption process appears to be faster (Johnson and Sumpter, 2001; Zeng et al., 2009). Currently, no applicable legislation exists about effluents discharge or the use of sludge contaminated with estrogens and/or other organic contaminants in land application (Bernardelli et al., 2015). Therefore, it is urgent to find efficient methods for the removal of estrogens from contaminated wastewater and sludge, by exploring mechanisms and factors that influence estrogens removal from wastewater by biological processes. Some progress has been achieved related to estrogens removal by biological processes using batch experiments. However, several analytical difficulties have arisen in the identification and quantification of these contaminants not only adsorbed onto sludge solid phase (due to the extraction procedure needed), but also in the wastewater (due to low concentrations expected). For instance, Paterakis et al. (2012) examined the fate and behaviour of estrogens during the anaerobic digestion using primary and mixed sewage sludge (primary sludge plus waste activated sludge). Batch experiments were carried out at laboratory scale using both mesophilic (35 °C) and thermophilic (55 °C) temperatures, at estrogens' concentrations mimicking those released in the environment. Researchers observed a positive effect on 17α-ethinylestradiol (EE2) removal efficiency (with an increase of 11 %) when increasing the temperature from mesophilic to thermophilic for the different sludge types tested. Additionally, the same authors observed an increase of estrone (E1) removal (20 %) with primary sludge using thermophilic temperatures, but no significant changes were observed with mixed sludge. Zeng et al. (2009) also investigated the effect of temperature on EE2 removal using acclimated sludge with synthetic wastewater. These authors observed that higher temperature (30 °C *vs.* 10 °C to 20 °C) improved biodegradation rate, but reduced the adsorption of EE2 onto sludge. Bernardelli et al. (2015) evaluated the degradation of estrogens using activated sludge under aerobic conditions at mixed liquor volatile suspended solids (MLVSS) contents of 1 g L^{-1}, 2 g L^{-1} and 3 g L^{-1}. These authors observed that the lower MLVSS concentration did not favour estrogens' removal. In addition, for MLVSS concentrations of 2 g L^{-1} and 3 g L^{-1}, no significant removal efficiencies were observed for both E1 and EE2, after 8 h of contact with sludge. Other researchers have shown that the presence of nitrate is critical for EE2 biodegradation (Zeng et al., 2009). Zeng et al. (2009) referred that in the absence of nitrate, the removal of EE2 was attributed to adsorption

onto sludge, while in the presence of nitrate, biodegradation was the dominant process for EE2 removal.

In most of the batch experiments reported so far, estrogens were spiked onto sludge solid phase after freeze-drying (Nieto et al., 2008; Ternes et al., 2002; Zhang et al., 2016). Other studies determined estrogens' concentration only in the sludge supernatant (liquid phase) (Bernardelli et al., 2015; Ren et al., 2007a) or used sterilized sludge in order to inhibit microbial activity, being the amount of estrogens adsorbed to the sludge solid component obtained as an estimate (Bernardelli et al., 2015). Thus, the laboratory-scale results reported in the literature do not represent the natural adsorption interactions that can occur between estrogens and sludge in naturally contaminated samples.

This study used synthetic wastewater effluent contaminated with estrogens and aimed to evaluate the influence of temperature (20 °C, 25 °C, and 34 °C), mixed liquor suspended solids (MLSS) content (2 g L^{-1}, 3 g L^{-1}, and 5 g L^{-1}), and nitrate supplementation on the removal of E1 and EE2 from sludge. For that, a series of batch experiments were conducted using digested sludge samples collected from a local WWTP and synthetic wastewater spiked with E1 or EE2. Our study is justified because reports on batch experiments under anaerobic conditions, considering active sludge (non-sterilised sludge) spiked with estrogens solution are limited. In addition, studies where estrogens' concentration were determined in the sludge solid phase using extraction procedures are also scarce.

4.2. EXPERIMENTAL SECTION

4.2.1. REAGENTS AND STANDARDS

Steroid hormones E1 (purity ≥ 99 %) and EE2 (purity ≥ 98 %), were supplied by Sigma-Aldrich. HPLC grade methanol, acetone and acetonitrile, were from Fischer Chemical, Carlo Erba and VWR (Prolabo), respectively. Ultrapure water was obtained from a Milli-Q Millipore system (Milli-Q plus 185). Individual standard stock solutions of E1 and EE2 were prepared in acetonitrile at a concentration of 1000 mg L^{-1} and further diluted to working concentrations with ultrapure water. Prior to use all the stock and working solutions were stored at 4 °C in the dark.

In order to simulate the wastewater from the WWTP, under controlled conditions, a stock solution of synthetic wastewater was prepared according to Hashimoto and Murakami (2009) with minor modifications and was composed of peptone (6 g L^{-1}), meat extract (4 g L^{-1}), urea (1 g L^{-1}), NaCl (0.3 g L^{-1}), KH_2PO_4 (1 g L^{-1}), KCl (0.14 g L^{-1}), $CaCl_2$ (0.14 g L^{-1}), and $MgSO_4 \cdot 7H_2O$ (0.1 g L^{-1}). The synthetic wastewater stock solution was stored in the dark at 4 °C for up to one week. The average total chemical oxygen demand (COD) of the synthetic wastewater stock solution was 226 $\pm$ 20 mg L^{-1}.

Samples of digested sludge used in the present work consisted of a mixture of primary sludge plus waste activated sludge collected from a WWTP treating 39 278 m^3 wastewater per day and located in Aveiro, Portugal (as presented in chapter 3). After collection, the samples were sealed and placed on ice in a storing box for immediate shipment back to our laboratory.

4.2.2. E1 AND EE2 ANALYSIS

The extraction and quantification and of E1 and EE2 were based on the method described in chapter 3, with minor modifications. Briefly, the aliquot samples (200 mL) were immediately separated, after sampling, into the liquid and solid phases by centrifugation (4000 rpm (2576 × *g*), 10 min). The supernatant (liquid phase) sample was immediately filtered with 0.2 µm PVDF filter, stored at 4 °C and analysed by high-performance liquid chromatography with a fluorescence detector (HPLC-FLD) within the next 24 h. The solid phase of the mixture was freeze-dried, grounded, and then successively extracted with 18 mL and 9 mL of methanol, followed by 9 mL of acetone per g dry sample. In each extraction step, the samples were vigorously vortexed (Velp

Scientifica) during 1 min and subjected to ultrasonic liquid extraction (ULE) using an Ultrasonic Cleaner USC-T ultrasonic bath (VWR) for 1 h. After ULE, the three solvent fractions were combined, filtered and analysed by HPLC-FLD.

For E1 and EE2 analysis by HPLC-FLD, a DGU-20ASR degasser, a LC-30AD pump, a CTO-20AC column oven, and a SIL-30AC autosampler were used. A 150 mm × 4.6 mm internal diameter (i.d.) ACE® C18 column-PFP (pentafluorophenyl; 5 µm particle size) connected to a 4.6 mm i.d. ACE® 5 C18 guard column was used for the separation. The isocratic mobile phase used to determine E1 and EE2 consisted of ultrapure water: acetonitrile mixtures of 50:50, *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 (as described in chapter 3). The temperature of both the column and the cell was maintained at 25 °C. Before being used as mobile phase, water and acetonitrile were filtered through a 0.2 µm polyamide membrane filter (Whatman).

The spiked mass was determined considering that all the estrogen added to the aqueous solution adsorbed immediately onto the sludge solid phase (as indicated by previous studies presented in chapter 3). Moreover, the estrogens quantity present in the collected sludge was determined previously to their use. The adsorption percentage of each estrogen onto the solid phase of the mixture was calculated considering the spiked mass (per mass of solid phase) and the mass quantified in the solid phase.

4.2.3. BATCH EXPERIMENTS

Batch reactors (Figure 4.1) with a working volume of 5 L were inoculated with an adequate amount of sludge sample diluted with distilled water to obtain the desired MLSS concentration (Table 4.1). Then, synthetic wastewater solution (2 %, *v*/*v*) (Hashimoto and Murakami, 2009) and a stock solution of target estrogens (E1 or EE2) dissolved in ultrapure water were added to the reactor. In all experiments, E1 and EE2 initial concentrations were 80 µg L^{-1} and 4 µg L^{-1}, respectively.

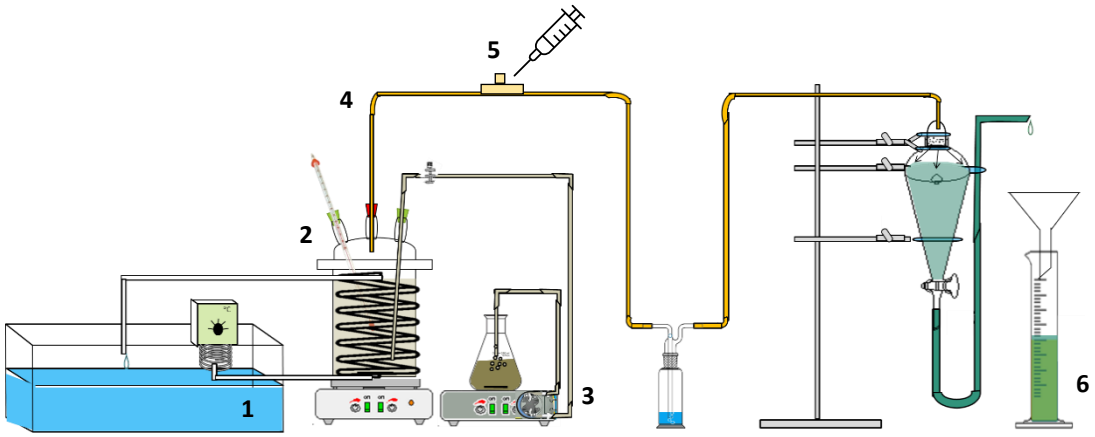


Figure 4.1 Laboratory-scale scheme of batch experiments used in this study. (1) thermostatic bath; (2) batch reactor; (3) peristaltic pump; (4) biogas outlet; (5) biogas sampling septum; (6) water displacement method for biogas measuring.

As shown in Table 4.1, four series of batch experiments were performed under different conditions. The first series (R1-R6) was intended to examine the influence of temperature (20 °C, 25 °C and 34 °C) on the concentration of E1 and EE2 adsorbed onto active solid sludge, and was carried out using a MLSS concentration of 2 g L^{-1}. The second series (R7-R12) was intended to examine the influence of sludge sterilization with different MLSS content (2 g L^{-1}, 3 g L^{-1} and 5 g L^{-1}). In order to obtain non-active sludge, the sludge and synthetic wastewater mixture was autoclaved (Vanlock, LDZM – 80KCS-III) at 120 °C for 30 min (Bernardelli et al., 2015; Chen and Hu, 2010; Li et al., 2005). Also, irradiation using a cobalt source was performed as a comparison method for the sludge inactivation. For that purpose, 900 mL of sludge and synthetic wastewater mixture contained in a 1 L Schott flask were subject to total radiation of 38 kGy. The third series (R13-R18) was intended to examine the influence of the MLSS content (2 g L^{-1}, 3 g L^{-1} and 5 g L^{-1}) on the concentration of E1 and EE2 adsorbed onto active solid sludge. The fourth series (R19-R20) was intended to examine the influence of nitrate supplementation on the concentration of E1 and EE2 adsorbed onto active solid sludge and was carried out using a MLSS concentration of 2 g L^{-1}; this evaluation was performed by adding $NaNO_3$ in order to obtain the concentration of 25 mg NO_3^-N L^{-1} according to Zeng et al. (2009).

Table 4.1 Experimental conditions used in the batch experiments.

Series	Reactor	Spiked estrogen	Sludge condition	Temperature (°C)	MLSS (g TSS L^{-1})
1	R1	E1	Active sludge (non-sterilised sludge) + synthetic wastewater+ distilled water	Room temperature ≈ 20 °C	1.8 ± 0.2
	R2			24.9 ± 0.3	1.61 ± 0.07
	R3			34.2 ± 0.4	1.98 ± 0.03
	R4	EE2		Room temperature ≈ 20 °C	1.8 ± 0.2
	R5			25 ± 1	1.61 ± 0.09
	R6			34.4 ± 0.5	1.83 ± 0.08
2	R7	E1	Non-active sludge (sterilised) + synthetic wastewater+ distilled water (autoclave at 120°C/ 30 min)	25.5 ± 0.9	1.57 ± 0.07
	R8			24.5 ± 0.7	2.8 ± 0.1
	R9			25.5 ± 0.7	4.72 ± 0.01
	R10	EE2		25.8 ± 0.4	1.7 ± 0.1
	R11			24.8 ± 0.4	2.7 ± 0.2
	R12			25.0 ± 0.1	4.6 ± 0.1
3	R13	E1	Active sludge (non-sterilised sludge) + synthetic wastewater+ distilled water	25.1 ± 0.6	2.06 ± 0.05
	R14			25.1 ± 0.4	3.00 ± 0.08
	R15			24.5 ± 1.4	4.9 ± 0.1
	R16	EE2		25 ± 2	1.9 ± 0.1
	R17			24 ± 2	2.7 ± 0.01
	R18			24 ± 1	4.9 ± 0.2
4	R19	E1	Active sludge (non-sterilised sludge) + synthetic wastewater+ distilled water + nitrate suppl. (25 mg NO_3-N L^{-1})	26 ± 2	1.94 ± 0.07
	R20	EE2		26 ± 3	1.8 ± 0.1

The reactors were prepared under anaerobic conditions with continuous agitation using a magnetic stirrer. In order to obtain the anaerobic conditions, each reactor was purged with nitrogen for 15 min and capped. The desired temperature was maintained by the recirculation of water through the external jacket of the reactor connected to a thermostatic bath. In the top layer of each reactor, a layer of fibreglass and aluminium foil was applied to prevent heat loss and exposure to sunlight. The produced biogas was measured by the water displacement method. The concentrations of total suspended solids (TSS), volatile suspended solids (VSS), alkalinity, and chemical oxygen demand (COD) of the sludge samples were determined at the beginning and at the end of each batch assay, according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The pH, conductivity, and total dissolved solids (TDS) were measured with a Consort C861.

Sampling was carried out using a peristaltic pump (Watson Marlow, 101U/R) and a 200 mL aliquot for each replicate was collected from the reactor at predefined intervals. Three replicates were collected for each sampling time. The batch reactors were monitored in terms of E1 and EE2 concentration in the supernatant of the reactor and adsorbed onto the solid sludge.

4.3. RESULTS AND DISCUSSION

4.3.1. START-UP SLUDGE CHARACTERIZATION

The digested sludge collected was washed 3 times with tap water (Ren et al., 2007a). Between each washing, the sludge was allowed to settle for one day and the supernatant was removed. Table 4.2 shows the main characteristics of digested sludge used in batch experiments.

Table 4.2 Main characteristics of digested sludge used in batch experiments.

Parameter [a]	Digested sludge sample
TSS (g L^{-1})	19.1 ± 0.4
VSS (g L^{-1})	14.3 ± 0.4
pH	7.24
ALK (g $CaC0_3$ L^{-1})	3.17
Conductivity (mS cm^{-1})	7.19
TDS (g L^{-1})	3.84
Salinity	3.90
DOC (mg L^{-1})	125 ± 2
OC (%)	38.8 ± 0.6
IC (%)	< LOD

[a] ALK, alkalinity; DOC, dissolved organic carbon; IC, inorganic carbon; LOD, limit of detection; OC, organic carbon; TDS, total dissolved solids; TSS, total suspended solids; VSS, volatile suspended solids.

4.3.2. EFFECT OF TEMPERATURE ON E1 AND EE2 REMOVAL

Figure 4.2 shows the mass of E1 and EE2 adsorbed onto the sludge in the first series of batch experiments (R1-R6) carried out at the indicated temperatures. The pH of the mixture samples taken from all reactors ranged from 7.2 to 7.9.

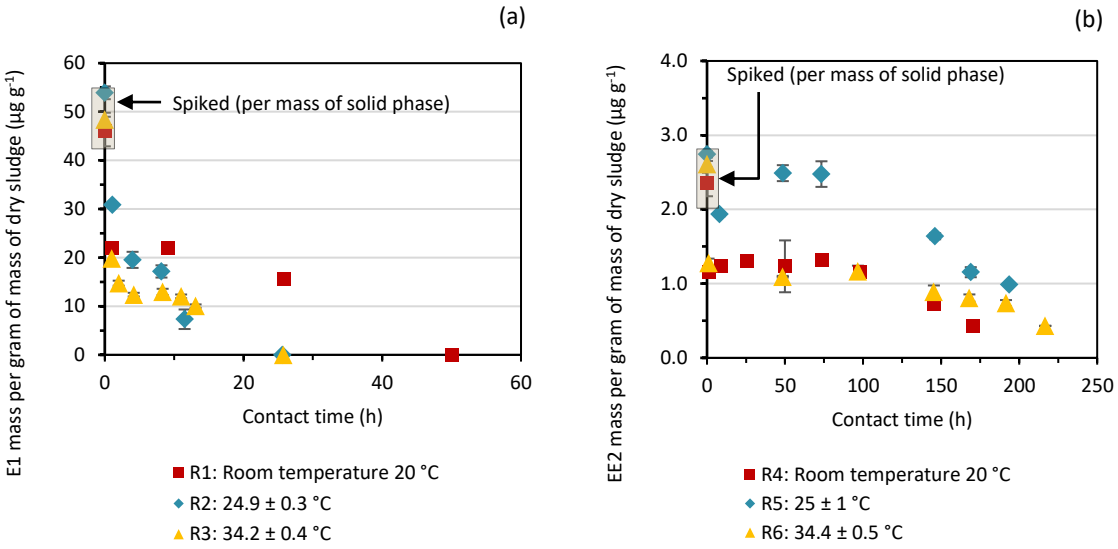


Figure 4.2 Concentration profiles of E1 (a) and EE2 (b) in solid phase of a mixture of active sludge (non-sterilised sludge) and synthetic wastewater (MLSS concentration of 2 g L^{-1}) at 20 °C, 25 °C and 34 °C.

For all tested times, E1 and EE2 concentrations in the supernatant of the reactor (liquid phase) were below the limit of detection (LOD of 41.3 µg L^{-1} and 7.0 µg L^{-1} for E1 and EE2, respectively). As expected, these results indicate that both estrogens under study tend to adsorb very quickly onto the solid phase of the mixture at all temperatures tested, as also reported by previous studies (Hashimoto and Murakami, 2009). At contact time of 1 h, similar E1 adsorption percentages were observed at 20 °C and 34.2 °C ± 0.4 °C (between 41 % and 48 %), while at 25 °C a higher adsorption value was obtained (57 %). However, after 8 h of contact time with active sludge, similar E1 adsorption values in solid phase of the mixture at 24.9 °C ± 0.3 °C and 34.2 °C ± 0.4 °C were obtained (between 27 % and 32 %). After 25 h of contact, E1 was completely removed from the solid phase of the mixture at 24.9 °C ± 0.3 °C and 34.2 °C ± 0.4 °C, which indicated that biodegradation occurred. At approximately 20 °C, after 25 h of contact with sludge, 34 % of the E1 added was still detected in the solid phase and more than 48 h were needed to observe its complete removal. Although similar results for E1 removal were obtained at both 24.9 °C ± 0.3 °C and 34.2 °C ± 0.4 °C, the energy required to maintain the reactors at higher temperatures, such as 34.2 °C ± 0.4 °C, must be a factor to be considered when choosing the best operation conditions.

For EE2, similar estrogen mass was determined adsorbed onto sludge at approximately 20 °C and 34.4 °C ± 0.5 °C, with adsorption values around 49 % after 1 h and adsorption values between 31 % and 34 % for 150 h. After 168 h a lower estrogen mass was detected at approximately

20 °C (with an adsorption value of 18 %). Results obtained allowed us to conclude that temperature does not seem to have a marked effect on EE2 behaviour, which is in agreement with the results obtained by Carballa et al. (2007). Moreover, it is possible to observe in Figure 4.2 (b), that more than 216 h were necessary to remove more than 83 % of EE2 adsorbed onto the sludge solid phase at 25 °C ± 1 °C and 34.4 °C ± 0.5 °C.

Until now, research on the effect of temperature on estrogens removal by active sludge under anaerobic conditions is fairly limited. Carballa et al. (2007) studied the removal of different pharmaceutical compounds under anaerobic conditions in batch experiments at different temperatures (37 °C and 55 °C) using a spiked estrogen concentration of 4 μg L^{-1}. Researchers achieved estrogens' removal efficiencies between 40 % and 90 % and concluded that sludge adaptation to the substrate (between 6 d and 30 d) was crucial for removing these compounds, but no temperature effect was observed. Another study conducted by Zeng et al. (2009), showed that after two months' of sludge acclimation with a spiked estrogen concentration of 5 μg L^{-1}, the higher EE2 removal rate was achieved at the temperature of 30 °C (98 %). The lower estrogens' removal efficiency obtained in our study, when compared with other studies, can be explained by the use of non-acclimated sludge in contrast to the use of adapted biomass cited in the literature.

4.3.3. Effect of MLSS content on E1 and EE2 removal

To examine the influence of the MLSS content on the concentration profiles of E1 and EE2 onto the mixture of sludge and synthetic wastewater, three tests with different MLSS content (approximately 2 g L^{-1}, 3 g L^{-1} and 5 g L^{-1}) were conducted. The second assay (R7-R12) was performed to gain further insight on the adsorption contribution (without biodegradation) on the removal of estrogens using non-active sludge (sterilised sludge). The third series of batch experiments (R13-R18) was performed to assess the influence of active sludge (non-sterilised sludge) on the removal of E1 and EE2 by adsorption and biodegradation.

4.3.3.1. E1 AND EE2 ADSORPTION ONTO NON-ACTIVE SLUDGE (STERILISED SLUDGE)

Some authors reported that the active sludge properties can be altered when subjected to autoclaving (denatured), potentially modifying physical properties of the sludge flocs, which could significantly affect their adsorption capacity (Bernardelli et al., 2015; Suzuki and Maruyama, 2006). For that reason, a batch experiment with concentrated sludge sterilized by irradiation with a cobalt source was conducted in our study for comparison. Non-active sludge (sterilised sludge) with MLSS concentration of 2 g L^{-1} was spiked with EE2, and the EE2 adsorption was similar to that obtained with non-active sludge by autoclaving (33.5 % *vs.* 34.0 % by irradiation with a cobalt source). Thus, due to the availability and easier access to an autoclave, the thermal sterilization procedure was adopted in the batch experiments (R7-R12) conducted using non-active sludge samples with different MLSS content (2 g L^{-1}, 3 g L^{-1} and 5 g L^{-1}) (Figure 4.3). The time needed to process collected samples for E1 and EE2 determination in the sludge solid phase (extraction procedure) was approximately 1 h. Thus, 1 h was the time used to investigate the adsorption of each compound onto the non-active sludge.

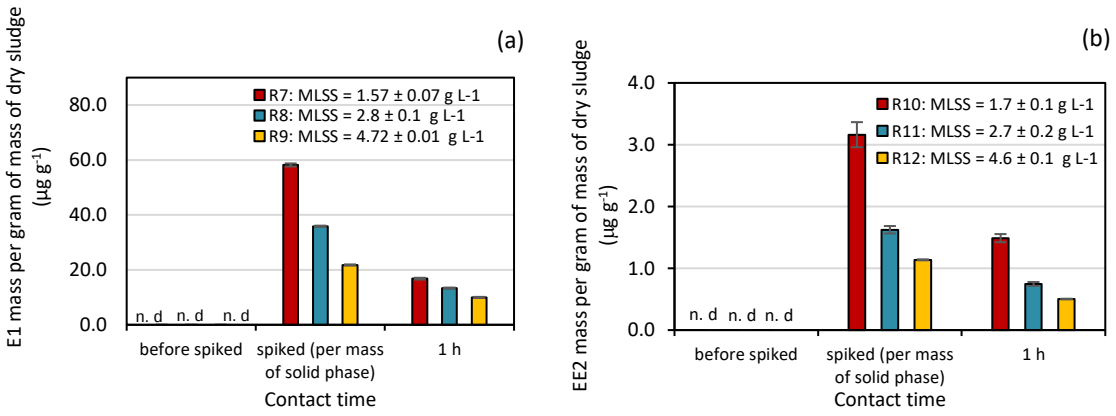


Figure 4.3 Concentration profiles of E1 (a) and EE2 (b) in the solid phase of a mixture of non-active sludge (sterile sludge autoclaved at 120 °C for 30 min) and synthetic wastewater, at 25 °C and MLSS concentration of 2 g L^{-1}, 3 g L^{-1} and 5 g L^{-1}. n.d, not detected.

Results of the analysed E1 concentration adsorbed onto non-active sludge (sterilised sludge) showed that about 16.8 µg g^{-1} ± 0.3 µg g^{-1}, 13.4 µg g^{-1} ± 0.2 µg g^{-1} and 10.0 µg g^{-1} ± 0.2 µg g^{-1} of E1 were detected in the solid phase of the mixture using the spike concentrations of 58.2 µg g^{-1} ± 0.6, 35.9 µg g^{-1} ± 0.2 µg g^{-1}, and 21.8 µg g^{-1} ± 0.2 µg g^{-1}, with MLSS concentration of 1.57 g L^{-1} ±

0.07 g L^{-1}, 2.8 g L^{-1} ± 0.1 g L^{-1}, and 4.72 g L^{-1} ± 0.01 g L^{-1}, respectively. Whereas in the case of EE2, 1.49 μg g^{-1} ± 0.07 μg g^{-1}, 0.75 μg g^{-1} ± 0.03 μg g^{-1}, and 0.50 μg g^{-1} ± 0.01 μg g^{-1} were detected in the solid phase of the mixture using the spike concentrations of 3.2 μg g^{-1} ± 0.2 μg g^{-1}, 1.62 μg g^{-1} ± 0.06 μg g^{-1}, and 1.14 μg g^{-1} ± 0.01 μg g^{-1}, with MLSS concentration of 1.7 g L^{-1} ± 0.1 g L^{-1}, 2.7 g L^{-1} ± 0.2 g L^{-1} and 4.6 g L^{-1} ± 0.1 g L^{-1}, respectively. Thus, taking these values and calculating the percentage of adsorption, after 1 h, 29 %, 37 % and 46 % of the amount of E1 spiked were adsorbed onto the non-active sludge (sterilised sludge) with MLSS concentration of 1.57 g L^{-1} ± 0.07 g L^{-1}, 2.8 g L^{-1} ± 0.1 g L^{-1}, and 4.72 g L^{-1} ± 0.01 g L^{-1}, respectively. Thus, the higher MLSS concentration led to a higher E1 adsorption value onto solid phase of the non-active sludge. For EE2, after 1 h, similar adsorption results (between 44 % and 47 %) were obtained for the different MLSS concentrations tested. Presenting the results in percentage of adsorption divided by MLSS content, for E1, values of 18.5 % g^{-1} L^{-1}, 13.2 % g^{-1} L^{-1} and 9.8 % g^{-1} L^{-1} were obtained with MLSS concentration of 1.57 g L^{-1} ± 0.07 g L^{-1}, 2.8 g L^{-1} ± 0.1 g L^{-1}, and 4.72 g L^{-1} ± 0.01 g L^{-1}, respectively. For EE2, values of 27.6 % g^{-1} L^{-1}, 17.1 % g^{-1} L^{-1} and 9.5 % g^{-1} L^{-1} were achieved with MLSS concentration of 1.7 g L^{-1} ± 0.1 g L^{-1}, 2.7 g L^{-1} ± 0.2 g L^{-1} and 4.6 g L^{-1} ± 0.1 g L^{-1}. These results suggest that the highest estrogen adsorption onto sludge occurred for the more diluted sludge.

Bernardelli et al. (2015) analysed the adsorption of estrogens onto non-active sludge and synthetic wastewater using a spiked estrogen concentration of 100 μg L^{-1}. These authors obtained adsorption values of 45 % for E1 and 90 % for EE2 (at initial contact time). For E1, researchers obtained adsorption results similar to those obtained in the present study using non-active sludge. However, for EE2, lower adsorption values were obtained in our work. This difference may result from distinct physical properties of floc particles present in non-active sludge which would have different effects on their affinity as sorbents, especially for EE2 (Bernardelli et al., 2015; Johnson et al., 2000).

4.3.3.2. E1 AND EE2 REMOVAL FROM THE MIXTURE OF ACTIVE SLUDGE (NON-STERILISED SLUDGE) AND SYNTHETIC WASTEWATER

The concentration profiles of E1 and EE2 in the third series of batch experiments (R13-R18), conducted using the mixture of active sludge (non-sterilised sludge) and synthetic wastewater with different MLSS content (2 g L^{-1}, 3 g L^{-1} and 5 g L^{-1}), are shown in Figure 4.4. In order to investigate the adsorption without the biodegradation process for each MLSS tested, the initial concentration

of each compound adsorbed onto the solid phase of the mixture was estimated. To calculate this value, the adsorption results obtained using the non-active sludge (sterilized sludge) were considered. Due to the low spiked estrogen concentrations used in this work, the concentration of E1 and EE2 in the supernatant of the reactor (liquid phase) of the mixture analysed was always lower than the LOD, as expected.

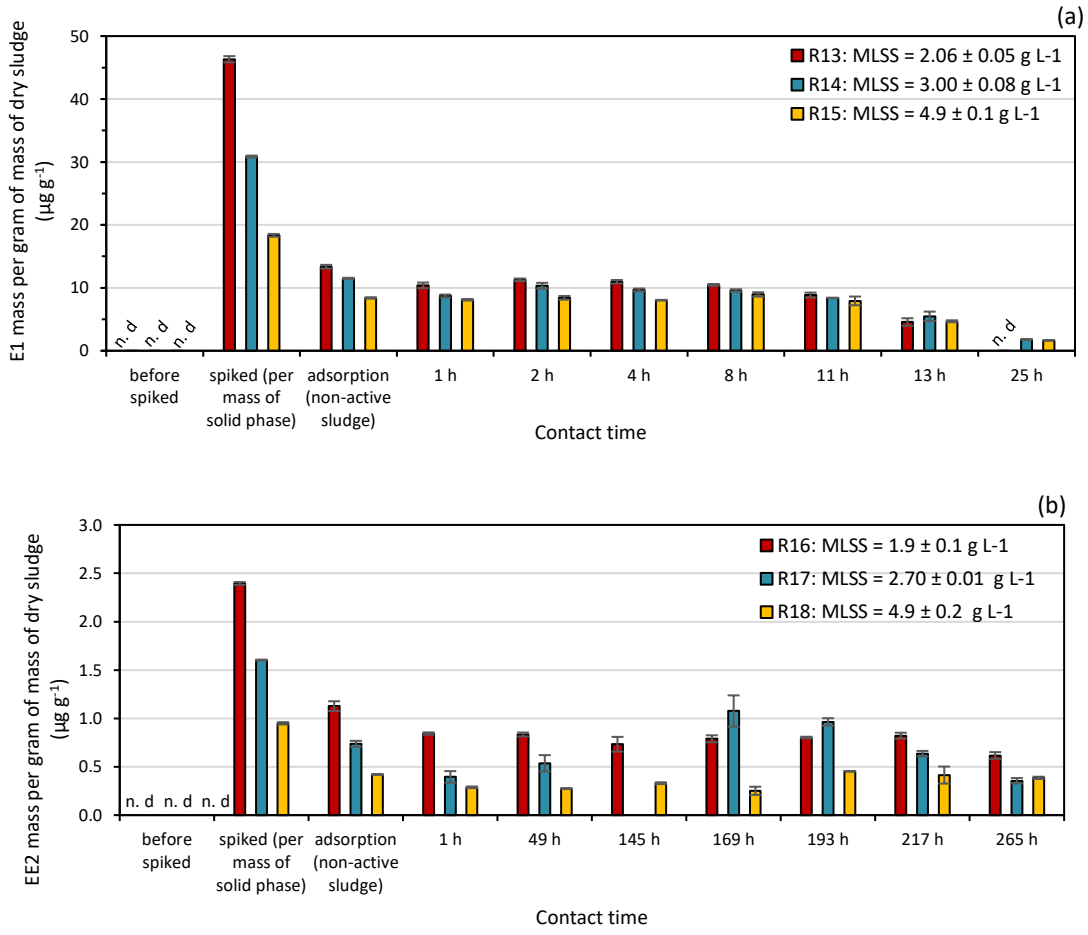


Figure 4.4 Concentration profiles of E1 (a) and EE2 (b) in the solid phase of a mixture of non-active (sterilised sludge) or active sludge (non-sterilised sludge) and synthetic wastewater, with MLSS concentration of 2 g L^{-1}, 3 g L^{-1} and 5 g L^{-1}. n.d, not detected.

In the experiments with different MLSS content, the E1 concentration in the solid phase of the mixture was almost constant between 2 h and 11 h. After 25 h, E1 was completely removed from the solid phase using 2.06 g L^{-1} ± 0.05 g L^{-1} MLSS concentration. At MLSS concentrations of 3.00 g L^{-1} ± 0.08 g L^{-1} and 4.9 g L^{-1} ± 0.1 g L^{-1}, 6 % to 9 % of the initially E1 spiked mass remained in the solid sludge. Since for all contact times tested the amount of E1 in the reactor supernatant

(liquid phase) was lower than the LOD and the amount detected in the solid phase was very low, the results indicate that biodegradation has occurred. For EE2, our results showed that the mass adsorbed onto the solid phase was almost constant until 217 h, probably due to sludge saturation. After 265 h of contact time, similar EE2 adsorption values were obtained in solid phase for the MLSS concentrations of 1.9 g L^{-1} ± 0.1 g L^{-1} and 2.70 g L^{-1} ± 0.01 g L^{-1} (22 % to 26 %), while for the 4.9 g L^{-1} ± 0.2 g L^{-1}, a higher percentage (41 %) of the compound was detected in solid sludge phase. Taking into account the results of adsorption in non-active sludge (sterilised sludge), and the amount of EE2 detected in the solid phase of the reactor using active sludge (non-sterile sludge), the results indicate that biodegradation has occurred, particularly after 265 h.

Hashimoto and Murakami (2009) investigated the fate of estrogens E1 and EE2 in batch experiments using active sludge (non-sterilised sludge) with 2 g L^{-1} MLSS concentration which was spiked with 1 µg L^{-1} of estrogen. These authors observed that after 4 h of contact time with the sludge more than 79 % of the spiked E1 was removed from liquid and solid phases under aerobic conditions and 15 % remained in the mixture solid phase. In our study, 24 % of the E1 amount added was detected in solid phase after 4 h of contact time under anaerobic conditions using the same MLSS concentration. For EE2, Hashimoto and Murakami (2009) observed that under aerobic conditions and with a MLSS concentration of 2 g L^{-1}, approximately 85 % of the initial spiked EE2 was degraded after 8 h of contact with sludge, and EE2 was completely removed after 24 h. In our study using different MLSS contents, 29 % to 35 % of the spiked EE2 was detected in solid phase under anaerobic conditions after 49 h of contact time, but for the complete EE2 removal from solid phase more than 265 h were required. According to some authors, the adsorption and biodegradation of complex substrates such as estrogens, strongly depends on the presence in the sludge of an adapted microbial consortium (Hashimoto and Murakami, 2009; Nadais et al., 2003). Thus, the difference of the removal profile of EE2 (which includes biodegradation) at different MLSS contents observed in our work may result from the use of non-adapted sludge. In addition, the aeration conditions, aerobic or anaerobic, may result in different estrogens biodegradation profiles by the sludge. The lower EE2 spiked concentration (1 µg L^{-1}) used in batch experiments reported in the literature (Hashimoto and Murakami, 2009) can also contribute to the higher removal of this compound from solid phase, contrasting with the lower removal achieved in this work when spiking higher concentrations of EE2 (4 µg L^{-1}).

4.3.4. EFFECT OF THE PRESENCE OF NITRATE ON E1 AND EE2 REMOVAL

Figure 4.5 presents the results of E1 and EE2 concentration in the third (R13 and R16) and in the fourth (R19 and R20) series of batch experiments obtained in absence of nitrate and with nitrate supplementation, respectively. The batch experiments were conducted using active sludge (non-sterilised sludge) samples with a MLSS content of approximately 2 g L^{-1}. In the solid phase of the mixture, similar E1 adsorption values were obtained (approximately 10 %) in the presence and absence of nitrate after 13 h of contact time. For EE2, a similar trend was observed in the presence or absence of nitrate, with adsorption values ranging from 22 % to 26 % after 265 h of contact time with sludge. According to some authors, nitrification consists in the conversion of nitrogen from a reduced form (ammonia) to an oxidized form (nitrate) due to the presence of denitrifying microorganisms (Grandclément et al., 2017; Koh et al., 2008). These microorganisms, in particular ammonia-oxidizing bacteria (AOB), can oxidize micropollutants co-metabolically with their ammonia monooxygenase, thus enhancing their removal (Margot et al., 2016). In fact, previous batch experiments reported in literature have shown that the degradation of estrogens strongly depends on the presence of nitrate (Ren et al., 2007b; Shi et al., 2004; Zeng et al., 2009). For example, Zeng et al. (2009) investigated the attenuation of EE2 using activated sludge collected from an anaerobic system with and without the presence of nitrate (25 mg NO_3^- N L^{-1}). These researchers observed that in the absence of nitrate, the EE2 removal is due to sludge adsorption and not to biodegradation. Conversely, in the presence of nitrate, an overall removal rate of EE2 greater than 96 % was observed after 72 h. In contrast, another study carried out by Margot et al. (2016) revealed that the degradation of micropollutants is strongly influenced by the presence of heterotrophic organisms, while AOB did not play a significant role in their removal. On the other hand, Paetkau (2011) studied the EE2 removal in a conventional membrane bioreactor and a simultaneous nitrification-denitrification membrane bioreactor and the results showed no significant differences in the removal efficiency. In our study, the supplementation with nitrate did not affect the degradation of E1 and EE2 adsorbed onto sludge, most probably due to the use of sludge not acclimated to the denitrification process (Hai et al., 2014).

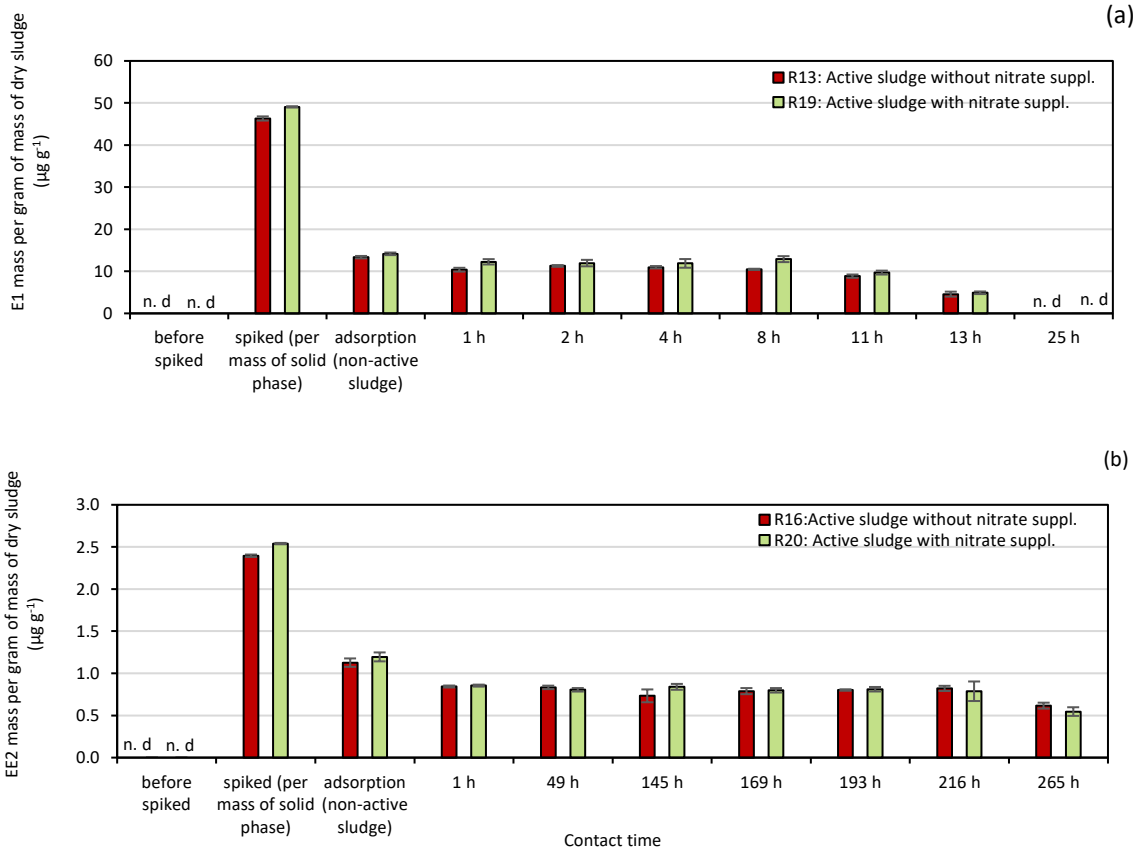


Figure 4.5 Concentration profiles of E1 (a) and EE2 (b) in the solid phase of a mixture of active sludge (non-sterilised sludge) or non-active sludge (sterilised sludge) and synthetic wastewater, without nitrate and with nitrate supplement with MLSS concentration of 2 g L^{-1}.

4.4. CONCLUSIONS

In this study, the effects of temperature, MLSS content and presence of nitrate on the removal of E1 and EE2 from synthetic wastewater was investigated using anaerobic batch experiments. When in contact with sludge, E1 and EE2 were quickly adsorbed onto the solid phase of the mixture of sludge and synthetic wastewater. Temperature seems to be a major factor influencing the removal of E1 from sludge. However, no temperature effect was observed for EE2 removal. The results with the non-active sludge (sterilised sludge) showed that a higher MLSS concentration leads to a higher concentration value of E1 adsorbed onto the solid phase of the mixture of non-active sludge and synthetic wastewater (29 % to 49 %), while in the case of EE2, the MLSS seems to not affect the adsorption of this compound onto the non-active sludge (44 % to 47 %). In the experiments with active sludge (non-sterilised sludge), the lower MLSS content tested (2 g L^{-1}) favoured E1 removal. For EE2, the lower adsorption values were obtained with the lower MLSS contents (2 g L^{-1} and 3 g L^{-1}). The presence of nitrate does not seem to affect the removal of E1 or EE2 from the mixture, probably due to the non-adaptation of sludge to the denitrification process. EE2 proved to be the most persistent under these conditions.

REFERENCES

American Public Health Association (APHA), 2005. Standard methods for the examination of water & wastewater, the 21st edition, american public health association, washington, DC, USA.

Bernardelli, J.K., Liz, M. V., Belli, T.J., Lobo-Recio, M.A., Lapolli, F.R., 2015. Removal of estrogens by activated sludge under different conditions using batch experiments. Brazilian J. Chem. Eng. 32, 421–432. doi:10.1590/0104-6632.20150322s00003667

Carballa, M., Omil, F., Ternes, T., Lema, J.M., 2007. Fate of pharmaceutical and personal care products (PPCPs) during anaerobic digestion of sewage sludge. Water Res. 41, 2139–2150. doi:10.1016/j.watres.2007.02.012

Chen, X., Hu, J., 2010. Adsorption of natural estrogens and their conjugates by activated sludge. Water. Air. Soil Pollut. 206, 251–261. doi:10.1007/s11270-009-0102-0

Grandclément, C., Seyssiecq, I., Piram, A., Wong-Wah-Chung, P., Vanot, G., Tiliacos, N., Roche, N., Doumenq, P., 2017. From the conventional biological wastewater treatment to hybrid processes, the evaluation of organic micropollutant removal: A review. Water Res. 111, 297–317. doi:10.1016/J.WATRES.2017.01.005

Hai, F., Yamamoto, K., Lee, C.-H., 2014. Membrane biological reactors: theory, modeling, design, management and applications to wastewater reuse.

Hashimoto, T., Murakami, T., 2009. Removal and degradation characteristics of natural and synthetic estrogens by activated sludge in batch experiments. Water Res. 43, 573–582. doi:10.1016/j.watres.2008.10.051

Johnson, A., Belfroid, A., Di Corcia, A., 2000. Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent. Sci. Total Environ. 256, 163–173. doi:10.1016/S0048-9697(00)00481-2

Johnson, A., Sumpter, J., 2001. Removal of endocrine-disrupting chemicals in activated sludge treatment works. Environ. Sci. Technol. 35, 4697–4703. doi:10.1021/es010171j

Koh, Y.K.K., Chiu, T.Y., Boobis, A., Cartmell, E., Scrimshaw, M.D., Lester, J.N., 2008. Treatment and removal strategies for estrogens from watewater. Environ. Technol. 29, 245–267. doi:10.1080/09593330802099122

Li, F., Yuasa, A., Obara, A., Mathews, A.P., 2005. Aerobic batch degradation of 17-beta estradiol (E2) by activated sludge: effects of spiking E2 concentrations, MLVSS and temperatures. Water Res. 39, 2065–75. doi:10.1016/j.watres.2005.02.009

Margot, J., Lochmatter, S., Barry, D.A., Holliger, C., 2016. Role of ammonia-oxidizing bacteria in micropollutant removal from wastewater with aerobic granular sludge. Water Sci. Technol. 73, 564–575. doi:10.2166/wst.2015.514

Nadais, M.H., Capela, M.I., Arroja, L.M., Duarte, A.C., 2003. Biosorption of Milk Substrates onto Anaerobic Flocculent and Granular Sludge. Biotechnol. Prog. 19, 1053–1055. doi:10.1021/bp0255356

Nieto, A., Borrull, F., Pocurull, E., Marcé, R.M., 2008. Determination of natural and synthetic estrogens and their conjugates in sewage sludge by pressurized liquid extraction and liquid chromatography-tandem mass spectrometry. J. Chromatogr. A 1213, 224–230.

doi:10.1016/j.chroma.2008.10.043

Paetkau, M., 2011. Comparison of ethinylestradiol and nitrogen removal in a conventional and simultaneous nitrification-denitrification membrane bioreactor.

Paterakis, N., Chiu, T.Y., Koh, Y.K.K., Lester, J.N., McAdam, E.J., Scrimshaw, M.D., Soares, A., Cartmell, E., 2012. The effectiveness of anaerobic digestion in removing estrogens and nonylphenol ethoxylates. J. Hazard. Mater. 199–200, 88–95. doi:10.1016/j.jhazmat.2011.10.075

Ren, Y.-X., Nakano, K., Nomura, M., Chiba, N., Nishimura, O., 2007a. A thermodynamic analysis on adsorption of estrogens in activated sludge process. Water Res. 41, 2341–2348. doi:10.1016/j.watres.2007.01.058

Ren, Y.-X., Nakano, K., Nomura, M., Chiba, N., Nishimura, O., 2007b. Effects of bacterial activity on estrogen removal in nitrifying activated sludge. Water Res. 41, 3089–3096. doi:10.1016/j.watres.2007.04.028

Shi, J., Fujisawa, S., Nakai, S., Hosomi, M., 2004. Biodegradation of natural and synthetic estrogens by nitrifying activated sludge and ammonia-oxidizing bacterium Nitrosomonas europaea. Water Res. 38, 2322–9. doi:10.1016/j.watres.2004.02.022

Suzuki, Y., Maruyama, T., 2006. Fate of natural estrogens in batch mixing experiments using municipal sewage and activated sludge. Water Res. 40, 1061–1069. doi:10.1016/j.watres.2005.12.043

Ternes, T.A., Andersen, H., Gilberg, D., Bonerz, M., 2002. Determination of estrogens in sludge and sediments by liquid extraction and GC/MS/MS. Anal. Chem. 74, 3498–3504. doi:10.1021/ac015717z

Zeng, Q., Li, Y., Gu, G., 2009. Nitrate-dependent degradation of 17α-ethinylestradiol by acclimated activated sludge under anaerobic conditions. J. Chem. Technol. Biotechnol. 84, 1841–1847. doi:10.1002/jctb.2255

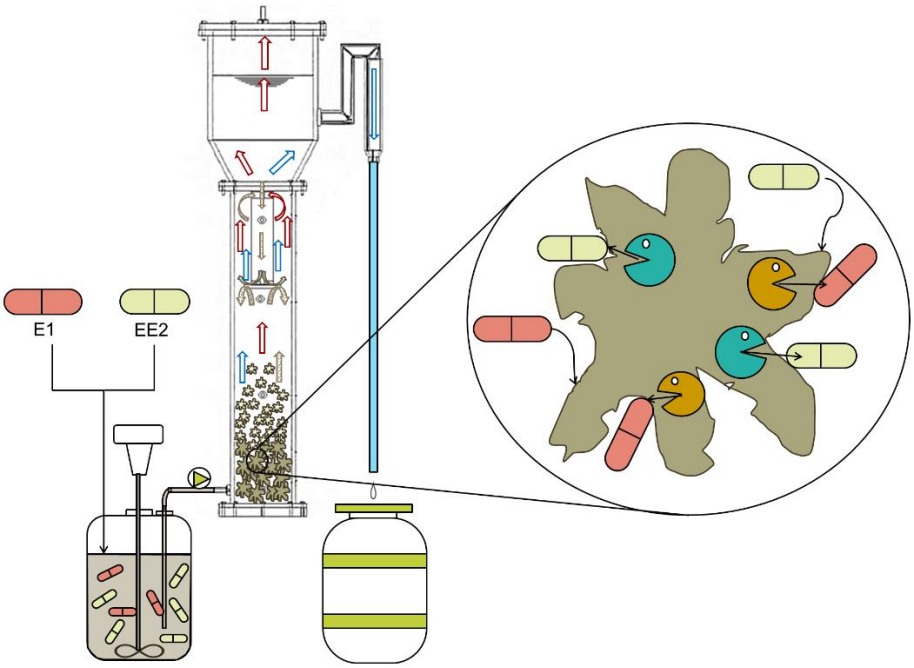
Zhang, M., Mao, Q., Feng, J., Yuan, S., Wang, Q., Huang, D., Zhang, J., 2016. Validation and application of an analytical method for the determination of selected acidic pharmaceuticals and estrogenic hormones in wastewater and sludge. J. Environ. Sci. Heal. Part A 51, 914–920. doi:10.1080/10934529.2016.1191304

CHAPTER 5

REMOVAL OF ESTRONE AND 17α-ETHINYLESTRADIOL IN UPFLOW ANAEROBIC SLUDGE BLANKET REACTORS USING DIFFERENT OPERATION MODES

The work presented and discussed in this chapter resulted in following article:

1. Louros, V.L., Lima, D.L.D., Leitão, J.H., Esteves, V.I. & Nadais, H.G. (2021) Impact of UASB reactors operation mode on the removal of estrone and 17α-ethinylestradiol from wastewaters. Science of The Total Environment, 764, 144291. https://doi.org/10.1016/j.scitotenv.2020.144291.


E1
EE2

SUMMARY

This work aims to compare the performance of the continuous operation (CO) and intermittent operation (IO) of upflow anaerobic sludge blanket (UASB) reactors for the removal of estrone (E1) and 17α-ethinylestradiol (EE2) from wastewaters. Results suggest that the IO contribute to the improvement of the overall removal of estrogens (above 95 % for E1 and EE2) when compared to CO (49 % for E1 and 39 % for EE2). For both CO and IO, biodegradation was the main removal mechanism for E1, while for EE2, adsorption onto the biological sludge was the major removal pathway. Moreover, a higher biodegradation of estrogens was obtained with the IO compared to CO (69.4 % *vs.* 43.3 % for E1 and 21.8 % *vs.* 8.0 % for EE2). The favourable effect of IO can be justified by effluent recirculation during the feedless period which promotes the adaptation of microbial biomass to estrogens' biodegradation.

CONTENTS

5.1. CONTEXTUALIZATION

Biological processes are usually the most cost-effective on the removal of organic contaminants from wastewaters. The removal of estrogens in aerobic systems is well documented (Bernardelli et al., 2015). Previous batch experiments conducted under aerobic conditions have shown that approximately 94 % of spiked estrone (E1) was removed from the liquid phase (15 % by adsorption and 79 % by biodegradation), after 4 h of contact and using the initial estrogen concentration of 1 mg L^{-1} (Hashimoto and Murakami, 2009). In the case of 17α-ethinylestradiol (EE2), the authors observed that this compound was more resistant to removal and after 4 h of contact with sludge approximately 75 % of spiked concentration was removed from the liquid phase (~25 % by adsorption and ~50 % by biodegradation) (Hashimoto and Murakami, 2009).

Despite high estrogen removals reported in the literature using aerobic processes, by adsorption and/or biodegradation, these processes often generate persistent metabolites or produce conjugated compounds which may also suffer deconjugation in the environment, recovering their initial toxicity (Khunjar et al., 2011). Some studies described that the substrate present in the raw influent competitively inhibits the degradation of estrogens (Joss et al., 2004). Nevertheless, estrogens are present in wastewater treatment plants (WWTPs) at low concentrations, usually in the range of ng L^{-1} to µg L^{-1} (Fleming et al., 2016; Pessoa et al., 2014). Consequently, the insufficient amount of these compounds cannot be used as a carbon source to support the growth of the microorganisms, and they are mostly removed by co-metabolism instead of primary metabolism (Li et al., 2020).

In the last decades, important developments were achieved in anaerobic treatment systems owing to the advantages that these processes present comparatively to conventional aerobic systems. Although intensive research efforts have been undertaken to better understand the estrogens' removal from wastewaters under anaerobic conditions, there is contradictory evidence in the literature. Some authors have observed estrogens biodegradation efficiencies above 95 % (Zeng et al., 2009), while others have not detected biological degradation, even after long incubation periods (Alvarino et al., 2016; Arias et al., 2018; Vassalle et al., 2020). Moreover, some researchers showed that in anaerobic systems some substrates are easily metabolized while others, such as estrogens, are not immediately metabolized and may be adsorbed onto the microbial aggregates (Xu et al., 2014; Zeng et al., 2009).

Upflow anaerobic sludge blanket (UASB) reactors are considered as very competitive anaerobic systems due to the production of high-quality effluents, lower energy requirements,

minimization of sludge production, and production of higher methane content in biogas (Alvarino et al., 2016, 2014). The intermittent operation (IO) of UASB reactors has been recommended to enhance the performance of the treatment process for the biodegradation of complex substrates (Nadais et al., 2005). The advantage effect of IO is attributable to the improved ability to develop microbial consortia well adapted to the biodegradation of a wide variety of substrates (Couras et al., 2014). IO consists of a periodical interruption of the reactor feed, in an alternating sequence of feed and feedless periods. During feeding periods simple substrates are readily degraded while complex substrates are adsorbed onto the biomass aggregates. During feedless periods, the microbial population is deprived of simple substrates, forcing the metabolism of complex substrates that are retained in the sludge.

Some studies are available on the treatment of wastewaters contaminated with estrogens using UASB reactors. However, a combination of a UASB reactor as pre-treatment followed by aerobic treatment was the most widespread combination applied (Alvarino et al., 2019, 2016; Moya-Llamas et al., 2018; Vassalle et al., 2020) and a detailed study restricted to the UASB reactor has been poorly documented. Furthermore, E1 and EE2 are hydrophobic substances easily adsorbed to the sludge (Ying et al., 2002) and most surveys indicated the removal of estrogens only from the liquid phase, neglecting the amount of compound adsorbed in the sludge during the treatment process (Moya-Llamas et al., 2018; Vassalle et al., 2020), and, consequently, a complete mass balance is seldom accomplished.

To the best of the authors' knowledge, there are no research studies dealing with the IO of UASB reactors for the treatment of wastewaters contaminated with estrogens. The aim of the present study was to compare the performance of the IO and continuous operation (CO) of UASB reactors on the removal of the estrogens E1 and EE2 from wastewaters, operated under the same initial conditions (initial sludge, feed organic loads and temperature). The overall removal efficiency of estrogens was evaluated for both systems considering the main removal mechanisms, biodegradation and adsorption, and the mass balances of estrogens in the liquid (LP) and solid phases (SP) were determined.

5.2. EXPERIMENTAL SECTION

5.2.1. REAGENTS AND STANDARDS

E1 (99 %) and EE2 (98 %) were provided by Sigma-Aldrich. HPLC grade methanol, acetone and acetonitrile, were from Fischer Chemical, Carlo Erba and VWR (Prolabo), respectively. Ultrapure water was obtained from a Milli-Q Millipore system (Milli-Q plus 185). Individual standard stock solutions of estrogens were prepared by dissolving the compounds in acetonitrile at a concentration of 1000 mg L^{-1}, sonicated for 60 min and stored at 4 °C in the dark until their use.

5.2.2. E1 AND EE2 ANALYSIS

Quantification of E1 and EE2 in the LP of the influent and treated effluent, as well as in LP and SP of the sludge, was based on the methods described in chapter 3 with minor modifications. Briefly, immediately after collection the LP samples were filtered with a 0.2 µm PVDF filter, stored at 4 °C and analysed by high-performance liquid chromatography with a fluorescence detector (HPLC-FLD) within 24 h. For the sludge samples, the aliquots sampled were immediately centrifugated (4000 rpm (2576 × *g*), 10 min) separating the LP from SP. The supernatant LP sample was immediately filtered, stored at 4 °C and analysed by HPLC-FLD within 24 h. The sludge SP was freeze-dried, grounded, and then successively extracted with 18 mL and 9 mL of methanol, followed by 9 mL of acetone per g dry sample. In each extraction step, the samples were vigorously vortexed (Velp Scientifica) during 1 min and subjected to ultrasonic liquid extraction (ULE) using an Ultrasonic Cleaner USC-T ultrasonic bath (VWR) for 1 h. After ULE, the three solvent fractions were combined, filtered, and analysed by HPLC-FLD.

5.2.3. SEED SLUDGE AND SYNTHETIC WASTEWATER

Samples of digested sludge used in this work contained a mixture of primary sludge and waste activated sludge, collected from a WWTP treating 39 278 m^3 wastewater per day and located in Aveiro, Portugal (as presented in chapter 3). The sludge collected was washed 3 times with tap

water (Ren et al., 2007), prior to use. Between each wash, sludge was allowed to settle for one day and the supernatant was removed.

A synthetic wastewater stock solution was prepared according to Hashimoto and Murakami (2009) with minor modifications, containing peptone (6 g L^{-1}), meat extract (4 g L^{-1}), urea (1 g L^{-1}), NaCl (0.3 g L^{-1}), KH_2PO_4 (1 g L^{-1}), KCl (0.14 g L^{-1}), $CaCl_2$ (0.14 g L^{-1}), and $MgSO_4 \cdot 7H_2O$ (0.1 g L^{-1}). Immediately after preparation, the solution was stored up to one week in the dark at 4 °C.

5.2.4. EXPERIMENTAL SET-UP AND OPERATION

Four lab-scale UASB reactors were operated during 21 days with an upflow velocity (ν_{up}) of 0.03 m h^{-1}. Two UASB reactors were used for the removal experiments with E1 (R1 and R2) and the other two with EE2 (R3 and R4). For each estrogen studied, one UASB reactor was operated in the IO and the other in the CO (Table 5.1 and Table 5.2). UASB reactors were operated with a hydraulic retention time (HRT) of 30 h (considering the feeding period for the IO) (Table 5.1). A schematic diagram of the UASB reactor used in this work is shown in Figure 5.1. The UASB reactors with a working volume of 6 L were inoculated with approximately 4 L of flocculent sludge and topped with three-phase separators (Couras et al., 2014). The IO reactors were operated for 6 cycles (I to VI), each with 12 h of feeding period, followed by 72 h of feedless period. The feedless period consisted of the recirculation of the treated effluent collected during the feeding period, while the CO reactors were fed continuously during all the assay (Table 5.2).

For the CO experiments, the estrogens were spiked to the influent at initial concentrations of 1000 μg L^{-1} and 500 μg L^{-1}, for E1 and EE2, respectively. For the IO experiments, the estrogen concentration in the feed was seven times higher than the feed concentration applied for the CO reactors, so that in each cycle (total period of 84 h) the total mass of estrogen and the chemical oxygen demand (COD) feed concentration admitted to the reactor were identical (Table 5.1). The feed consisted of diluted synthetic wastewater (2 %, *v/v*) (Hashimoto and Murakami, 2009) in distilled water, spiked with the target estrogen (E1 or EE2).

To obtain the anaerobic conditions, the sludge placed inside of each reactor was purged with nitrogen for 15 min and capped. The desired temperature (between 20 °C and 25 °C) (Alvarino et al., 2019, 2016, 2014; Buntner et al., 2013; Vassalle et al., 2020) was maintained by the recirculation of water through the external jacket of the reactor connected to a thermostatic bath.

In the top layer of each UASB reactor, a layer of fibreglass and aluminium foil was used to prevent heat loss and exposure to sunlight.

During the whole experiment, samples of biogas, influent and effluent were collected from the UASB reactors at the beginning and at the end of each cycle. For the IO reactors, the effluent was also collected at the end of the feeding period. To determine the amount of estrogens adsorbed onto sludge solid sample, the initial estrogens mass in the collected sludge was quantified. The estrogens' concentration adsorbed onto solid sludge phase at the end of the experiment was also determined. Due to the considerable volume of sludge needed for the E1 or EE2 analysis (about 600 mL) and in order to preserve the quantity of microorganisms inside of the UASB reactors, estrogens concentration adsorbed onto the sludge was not determined along the assays, but only at the end of the experiment.

Table 5.1 Operating conditions of UASB reactors.

Reactor	Operation mode	Compound	Spiked concentration [a] ($\mu g\ L^{-1}$)	Average spiked mass per cycle [b] (μg)	Flow ($L\ h^{-1}$)	HRT [a,c] (h)	v_{up} [d] ($m\ h^{-1}$)	SRT [e] (d)	COD feed [a] ($g\ COD\ L^{-1}$)	OLR [a,f] ($g\ COD\ L^{-1}\ d^{-1}$)	pH feed	T (°C)	Feed period per cycle (d)	Feedless period per cycle (d)	Total period of operation (d)	Number of cycles
R1	Continuous	E1	1000	16,800	0.2	30	0.03	92.5	1.4 ± 0.2	1.1 ± 0.2	7.4 ± 0.5	25.8 ± 0.6	3.5	0	21	-
R2	Intermittent	E1	7000	16,800	0.2	30	0.03	92.3	9.8 ± 1.6	7.8 ± 1.3	6.5 ± 0.1	25.6 ± 0.7	0.5	3	21	VI
R3	Continuous	EE2	500	8400	0.2	30	0.03	278.9	0.87 ± 0.08	0.70 ± 0.07	7.1 ± 0.8	22.2 ± 0.4	3.5	0	21	-
R4	Intermittent	EE2	3500	8400	0.2	30	0.03	82.0	6.1 ± 0.6	4.9 ± 0.5	6.5 ± 0.1	19.7 ± 1.3	0.5	3	21	VI

a Operating conditions in the feed period.

b Average spiked mass = total fed mass during one cycle times the number of feeding days.

c HRT, a hydraulic retention time.

d v_{up}, upflow velocity.

e SRT, solids retention time.

f OLR, organic loading rate.

Table 5.2 Feeding schedule for each UASB reactor.

Reactor	Cycle																				
	I				II			III				IV			V				VI		
	Day																				
	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
R1	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
R2	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□
R3	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■
R4	■	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□	□

Legend:
■ = 12 hours feed with HRT of 30 h.
□ = 72 hours feedless with HRT of 30 h.

Biogas production was measured by the water displacement method and its composition was monitored using an SRI® 8610 C gas chromatograph equipped with a Haysep® Q (2.5 m × 2.1 mm) column and a Thermal Conductivity Detector (T = 100 °C). Injection temperature was 61 °C and Helium was used as carrier gas (Flow = 10 mL min^{-1}). The sludge samples concentrations of total suspended solids (TSS), volatile suspended solids (VSS), and COD were determined according to Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The pH was measured with a Consort C861.

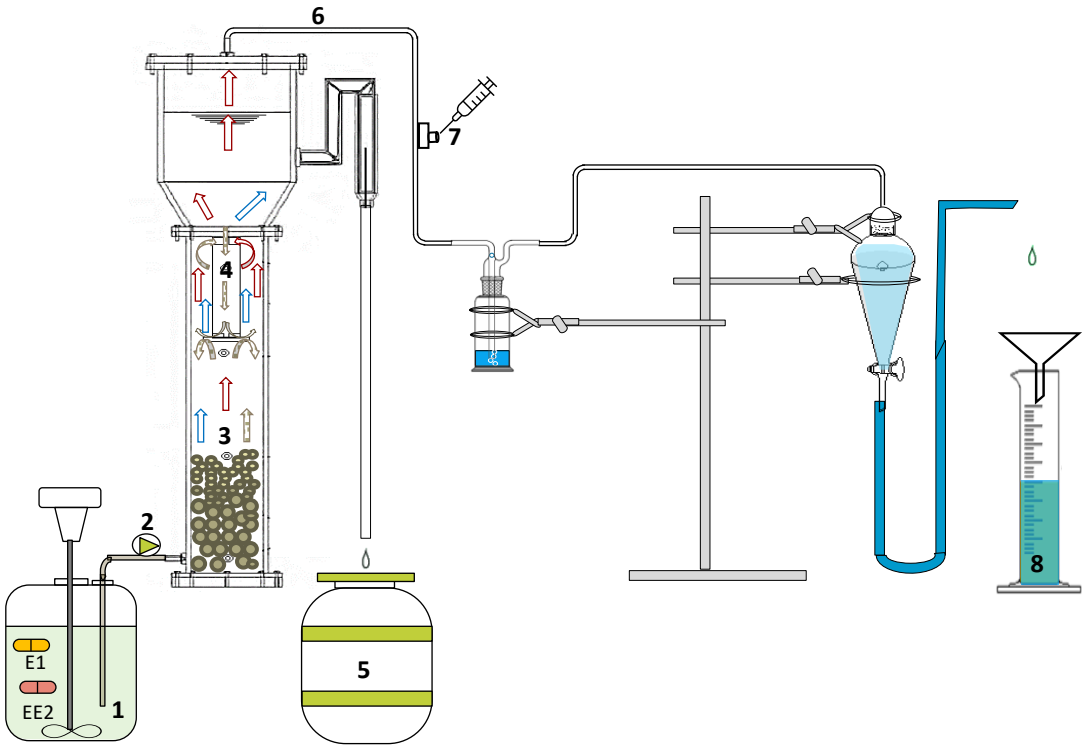


Figure 5.1 Schematic diagram of UASB reactors. Legend: (1) feed tank; (2) feed pump; (3) UASB reactor; (4) gas-liquid-solid separator; (5) treated effluent tank; (6) biogas outlet; (7) biogas sampling septum; (8) water displacement method for biogas measuring.

The percentage of COD removal (COD_R) was determined according to equation 5.1 (Couras et al., 2014):

$$COD_R\ (\%) = \frac{COD_{T,feed} - COD_{s,effluent}}{COD_{T,feed}} \times 100\% \qquad \textbf{(Eq. 5.1)}$$

Where $COD_{T,feed}$ is the total COD of the feed (g COD L^{-1}) and $COD_{s,effluent}$ is the soluble COD of treated effluent (g COD L^{-1}).

The methanization percentage was calculated using equation 5.2 (Couras et al., 2014):

$$Methanization\ (\%) = \frac{COD\text{-}CH_4}{COD_R} \times 100\% \quad \textbf{(Eq. 5.2)}$$

Where $COD\text{-}CH_4$ is the mass of COD converted to methane (g).

5.2.5. ESTROGENS MASS BALANCE AND KINETICS

A mass balance of E1 and EE2 was performed to assess the adsorbed and biodegraded amounts for the CO and IO of UASB reactors and were quantified using equation 5.3 (Alvarino et al., 2014):

$$F_{biod} = F_{inf} - (F_{eff} + F_{sor}) \quad \textbf{(Eq. 5.3)}$$

where F_{inf}, F_{eff}, F_{sor} and F_{biod} correspond to the mass flows (in µg) of the influent, effluent (analysed both in a dissolved fraction of LP), and adsorbed onto sludge, respectively. Thus, the amount of biodegraded E1 and EE2 (F_{biod}) can be determined.

Previous studies have reported that biodegradation constant (k_{biol}) could be determined considering the pseudo-first-order kinetics (Alvarino et al., 2014) reported in equation 5.4:

$$F_{biod} = k_{biol} \cdot X_{VSS} \cdot C_{eff} \cdot V \quad \textbf{(Eq. 5.4)}$$

Where k_{biol} is the first-order-rate constant (L g_{VSS}^{-1} d^{-1}), X_{VSS} is the volatile suspended solids concentration (g_{VSS} L^{-1}) inside the reactor, C_{eff} is the final compound concentration in the LP of treated effluent (µg L^{-1}), and V is the reactor volume (L).

5.3. RESULTS AND DISCUSSION

5.3.1. START-UP SLUDGE CHARACTERIZATION

The main characteristics determined for the digested sludge used as inoculum for the UASB reactors are shown in Table 5.3. In the experiments with E1, the average pH values of treated effluent were 5.6 ± 0.6 and 7.4 ± 0.6, for the CO and IO, respectively. For the EE2 assays, the average pH values of treated effluent were 7.2 ± 0.4 and 6.7 ± 0.9, for the CO and IO, respectively. The VSS obtained in treated effluent were below 0.15 g SSV L^{-1} in the assays with E1, while in the experiments with EE2, VSS values were below 0.18 g SSV L^{-1}.

Table 5.3 Main characteristics of sludge from UASB reactors.

Parameter [a]	Reactor							
	R1: E1 continuous operation		R2: E1 intermittent operation		R3: EE2 continuous operation		R4: EE2 intermittent operation	
	Initial [b]	Final [c]	Initial [b]	Final [c]	Initial [b]	Final [c]	Initial [b]	Final [c]
TSS (g L^{-1})	20.4 ± 0.7	3.82 ± 0.07	23 ± 1	6 ± 0.1	29 ± 2	5 ± 1	25 ± 2	6.4 ± 0.2
VSS (g L^{-1})	16.1 ± 0.5	3.1 ± 0.2	17.5 ± 0.7	4.6 ± 0.1	21 ± 2	4.1 ± 0.7	19 ± 1	5.1 ± 0.2
pH	5.62	6.10	5.35	7.49	5.53	6.90	5.03	7.00
OC (%)	41.9 ± 0.8	42 ± 1	42.3 ± 0.8	40.9 ± 0.1	42 ± 1	41 ± 0.6	44.3 ± 0.4	44.8 ± 0.2
IC (%)	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD	<LOD

[a] IC, inorganic carbon; LOD, limit of detection; OC, organic carbon; TSS, total suspended solids; VSS, volatile suspended solids.
[b] Real sludge before inoculation.
[c] Mixture of sludge and effluent collected inside of the UASB reactor.

5.3.2. REACTORS PERFORMANCE

The biogas composition from experiments with E1 and EE2 was analysed. In E1 experiments, the average concentration of methane in the biogas was 81 % and 72 % for the CO and IO, respectively. In EE2 experiments, the methane content was 64 % and 96 % for the CO and IO, respectively. Under IO conditions, a higher average biogas flow rate was achieved compared to CO (0.16 L d^{-1} *vs.* 0.08 L d^{-1} for E1 and 0.16 L d^{-1} *vs.* 0.13 L d^{-1} for EE2). These results confirmed the high quality of the biogas produced, in agreement with the findings attained by other authors, with values ranging from 65 % to 80 % for the treatment of wastewaters in UASB reactors (Alvarino et al., 2016, 2014; Arias et al., 2018; Buntner et al., 2013; Moya-Llamas et al., 2018). However, higher values of average biogas production (between 46 L d^{-1} and 100 L d^{-1}) have been reported, which can be justified by the substrate used (dairy wastewater), the higher UASB reactor volume (>120 L), the

lower HRT (between 12 h and 13 h) and the higher time of operation (between 150 days and 292 days) (Alvarino et al., 2016; Buntner et al., 2013). Instead, Moya-Llamas et al. (2018) obtained much lower results for this parameter (below 5 L d^{-1}) using synthetic wastewater as substrate and higher HRT (37 h).

Results of COD removal and methanization in UASB reactors are shown in Figure 5.2.

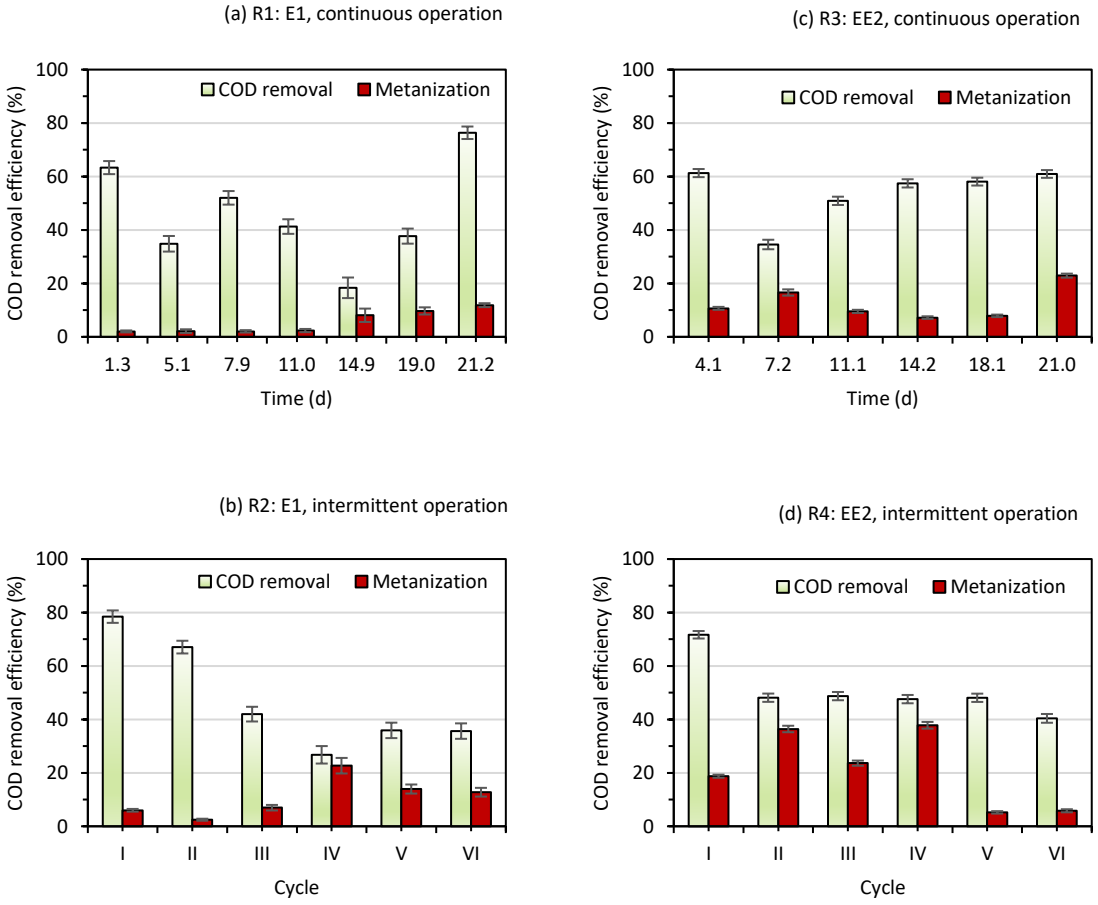


Figure 5.2 Results of COD removal and methanization in UASB reactor (a) R1: fed with synthetic wastewater spiked with E1 using CO; (b) R2: fed with synthetic wastewater spiked with E1 using IO; (c) R3: fed with synthetic wastewater spiked with EE2 using CO; (d) R4: fed with synthetic wastewater spiked with EE2 using IO. (Note that, for some of the experimental points, error bars are too small to be visible in the figure).

For the IO, higher COD removal efficiencies were achieved (ranging from 26.8 % to 78.5 % for E1 and from 40.4 % to 71.7 % for EE2) than those for the CO (ranging from 18.3 % to 76.4 % for E1 and from 34.5 % to 61.3 % for EE2) (Figure 5.2). Using CO, the methanization of COD ranged from 2.0 % to 11.8 % and 7.2 % to 22.9 % in the experiments with E1 and EE2, respectively. When using

IO, the methanization of COD ranged from 2.5 % to 22.7 % and from 5.2 % to 37.8 % in experiments with E1 and EE2, respectively.

Alvarino et al. (2016) pointed out higher COD removal values (above 95%) under anaerobic conditions treating dairy wastewater. Other authors have reported COD removal values ranging 40 % and 86 % when treating synthetic wastewaters and raw sewage (Moya-Llamas et al., 2018; Vassalle et al., 2020). Higher COD methanization values have also been pointed in the literature (45 % to 74 %) when treating dairy wastewater (Arias et al., 2018; Buntner et al., 2013), most probably as the result of a highly adapted microbial population present in the sludge.

5.3.3. E1 AND EE2 REMOVAL

For the IO, higher removal efficiencies of E1 and EE2 from the LP were obtained at the end (after feed and feedless periods) of all cycles analysed (above 90 %) compared to CO (ranging 37 % to 80 % for E1 and 16 % to 94 % for EE2) at the same HRT of 30 h (Figure 5.3).

Under CO, an initial decrease of E1 removal from the LP of treated effluent was observed until 10 h, suggesting that E1 was removed mainly by an adsorption process, which decreases probably due to sludge saturation. After this period, an increase in the removal of E1 from the LP of the treated effluent was registered until approximately 20 h. This increase, maybe related to the increase in biodegradation, which will increase the number of binding sites available for adsorption. Following this time, the E1 removal remained constant, suggesting that an equilibrium between adsorption and biodegradation is reached. In the case of EE2, for CO, there was a significant decrease in the removal of this compound from LP of the treated effluent until approximately 10 h. This fact can be a result of sludge saturation, followed by a constant EE2 removal from LP, indicating an equilibrium between biodegradation and adsorption. The results obtained for the removal of E1 and EE2 in the liquid phase of treated effluent can be justified by the initially fast estrogen adsorption onto the surface of the sludge, which was faster and not followed by an immediate biodegradation, and consequently, a lower specific surface was available for the continuous estrogens adsorption.

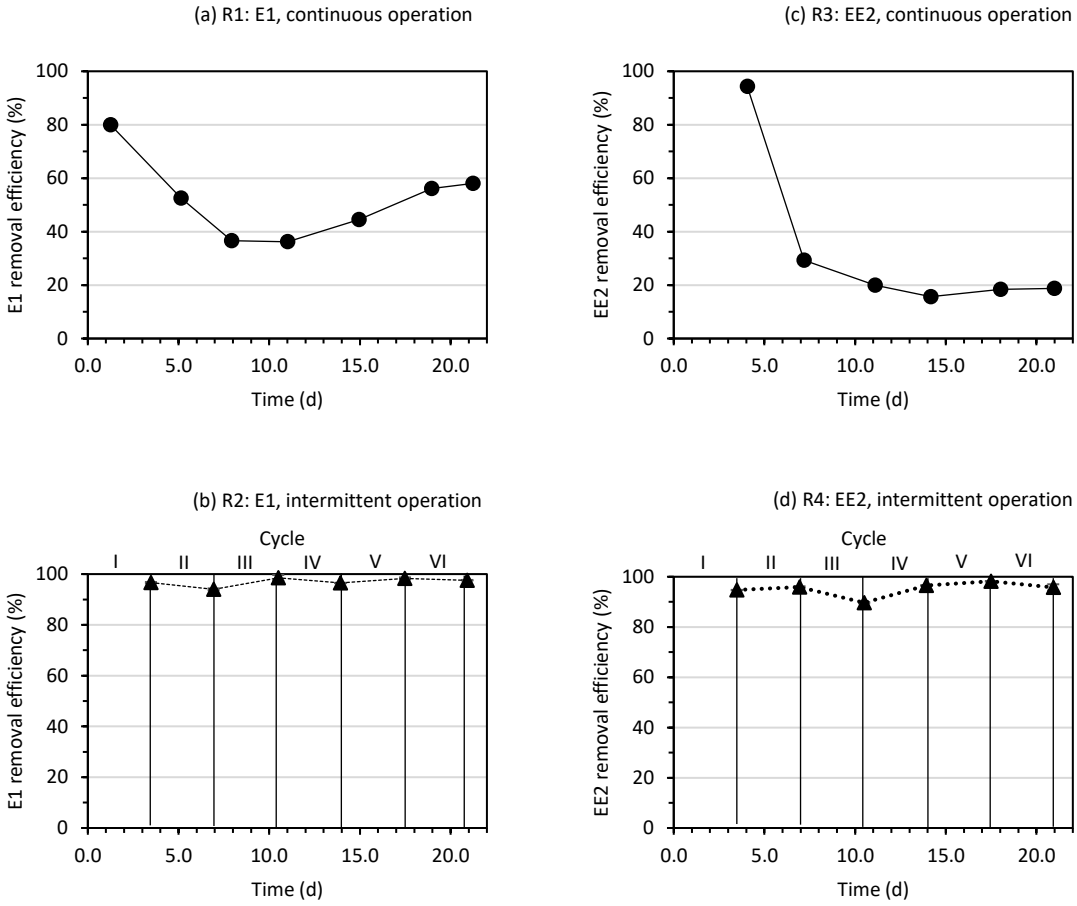


Figure 5.3 Results of E1 and EE2 removal from the LP in UASB reactor (a) R1: fed with synthetic wastewater spiked with E1 using CO; (b) R2: fed with synthetic wastewater spiked with E1 using IO; (c) R3: fed with synthetic wastewater spiked with EE2 using CO; (d) R4: fed with synthetic wastewater spiked with EE2 using IO. (Note that, for most of the experimental points, error bars are too small to be visible in the figure).

In the IO, the clearly higher removal efficiencies of E1 and EE2 from LP obtained may have resulted from a better contact between the compounds and the biomass, which has provided a higher available surface for adsorption and an improved mass transfer from the LP to the SP, due to the recirculation of treated effluent. Moreover, the IO contributed to a quick and constant adsorption of estrogens in the SP of sludge throughout the six cycles, while for the CO their removal from the LP of treated effluent was significantly lower indicating a lower adsorption and biodegradation.

Similar estrogens removal efficiencies from LP have been pointed out by previous studies using the CO of UASB reactors. Vassalle et al. (2020) reported an E1 removal efficiency of 40 % when treating raw sewage, using similar temperatures (23.5 °C) and solids retention time (SRT) of 20 days

but a lower HRT (7 h). Arias et al. (2018) obtained EE2 removal rates in the range from 20 % to 25 % when treating dairy wastewaters in a UASB reactor after more than 4 months of operation using similar temperatures (21 °C), lower HRT (20 h), and higher sludge concentration (30 g SST L^{-1}). Another study conducted by Moya-Llamas et al. (2018) reported higher removal rates for E1 (ranging from 40 % to 85 %) and EE2 (ranging from 70 % to 95 %) after 172 days of operation of an UASB system for the treatment of wastewaters operated at higher temperatures (above 26 °C), SRT (90 days), and HRT (37 h).

5.3.4. E1 AND EE2 ADSORPTION AND BIODEGRADATION

The adsorption and biodegradation percentages for E1 and EE2 removal using the CO and IO modes were established through a mass balance, which includes measurement of estrogens in the LP of the influent and the treated effluent, as well as in SP and LP of the sludge (Figure 5.4). For the IO, only 4.1 % of the spiked amount of E1 and 5.5 % of the spiked amount of EE2 remained in the treated effluent, while for the CO, 51.0 % of the spiked amount of E1 and 61.0 % of the spiked amount of EE2 were detected in the treated effluent. Adsorption values of E1 and EE2 onto SP of sludge were 4.7 and 2.3 times higher using the IO compared to CO, respectively. The high estrogens adsorption values (between 5.7 % and 26.5 % for E1 and between 31.0 % and 72.7 % for EE2) obtained in this study can be justified by the hydrophobic nature of these compounds, more evident in the case of EE2. These results indicate that IO may have contributed to improve the contact between estrogens and sludge.

The mass balance for E1 and EE2 suggests that the IO caused an increase in E1 and EE2 biodegradation, with values of 69.4 % and 21.8 %, respectively. For the CO the biodegradation estimated values were 43.3 % for E1 and 8.0 % for EE2. The resistance of EE2 to biodegradation using IO and CO can be linked to the ethynyl group in the 17-position, which blocks the potential formation of a ketone and stereotypically hinders access to the hydroxyl group in the 17-position. These features justify the higher persistence of EE2 when compared to E1 (Czajka and Londry, 2006).

Published information about estrogens removal mechanisms (involving biodegradation and adsorption) using UASB reactors is limited. Some studies have been undertaken using the UASB reactor followed by an aerobic process (Alvarino et al., 2016). Nevertheless, there is no data on the removal of estrogens using the IO of UASB reactors. Alvarino et al. (2016) investigated the

behaviour of E1 and EE2 using the CO in a UASB reactor coupled to a hybrid aerobic membrane bioreactor (MBR). The authors reported that no biodegradation or adsorption was detected for both E1 and EE2. In another work carried out by Alvarino et al. (2014), the researchers found values similar to those reported in the present work for E1 adsorption onto sludge (~4 %) and E1 biodegradation rate (~34 %) using UASB units under CO for the treatment of dairy wastewater. In the experiments with EE2, the authors obtained a much lower adsorption value (~2 %) and higher biodegradation rate (~47 %) (Alvarino et al., 2014), as compared to the work hereby presented (31 % of adsorption and 8 % of biodegradation). The distinct biodegradation efficiencies observed can be attributed to different factors, such as the distinct HRT, the absence of nitrifying conditions, the COD concentration in the inlet, and the initial EE2 concentration. Moreover, in the work reported in the literature, the sludge used for the inoculum of the UASB reactor was granular sludge obtained from a full-scale reactor treating brewery wastewaters, whereas in our study, flocculent anaerobic sludge was used (Alvarino et al., 2014). Flocculent sludge exhibited much lower dimensions and, consequently, a higher specific surface is available for estrogens adsorption (Alvarino et al., 2016). Consequently, in our study, higher amounts of E1 and EE2 could adsorb onto the UASB reactor sludge.

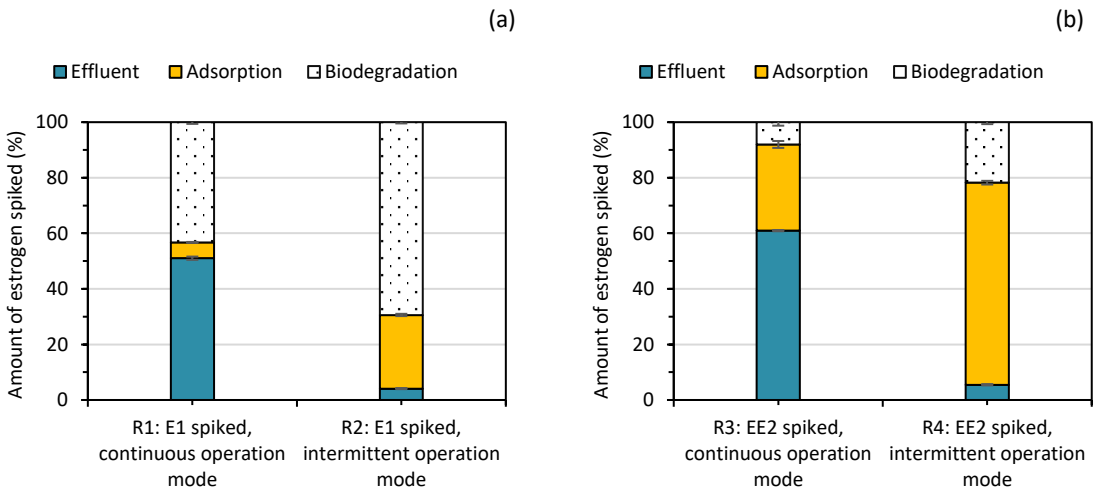


Figure 5.4 Mass balance for the CO and IO of UASB reactors for the treatment of synthetic wastewater contaminated with (a) E1 and (b) EE2. (Note that, for some of the experimental points, error bars are too small to be visible in the figure).

Adsorption and biodegradation results obtained in our work may be related to the short time of operation of UASB reactors used (21 days). Operating times applied in the literature varied from several weeks to a few months (Alvarino et al., 2019, 2016, 2014; Arias et al., 2018; Moya-Llamas et al., 2018; Vassalle et al., 2020). A study carried out by Chan et al.(2018) has investigated the anaerobic co-digestion of a mixture of food waste and domestic wastewater using an UASB reactor operated during 10 days. Larcher and Yargeau (2013) investigated the biodegradation of EE2 by heterotrophic bacteria and observed that this compound can be efficiently removed after 48 h in the presence of *Rhodococcus rhodochrous*, a bacterial species commonly present in activated sludge. With the combination of bacterial cultures, the authors found about 43% of EE2 removal after 300 h. On the other hand, Yu et al. (2007) investigated the influence of the presence of bacteria (strains KC1-14) isolated from activated sludge on E1 degradation and remarked that E1 can be removed after 5 days. Thus, the operation time of UASB reactors proposed in this work can be considered suitable for comparison of the two operation modes.

In the presented study, higher values of k_{biol} coefficients determined at the end of the assay (after 21 days) were achieved using the IO (0.80 L g_{VSS}^{-1} d^{-1} ± 0.02 L g_{VSS}^{-1} d^{-1} for E1 and 0.10 L g_{VSS}^{-1} d^{-1} ± 0.01 L g_{VSS}^{-1} d^{-1} for EE2) compared to the CO (0.31 L g_{VSS}^{-1} d^{-1} ± 0.03 L g_{VSS}^{-1} d^{-1} for E1 and 0.015 L g_{VSS}^{-1} d^{-1} ± 0.004 L g_{VSS}^{-1} d^{-1} for EE2). These findings indicate that the IO favours the E1 and EE2 biodegradation in wastewater using UASB reactors. The beneficial effect of IO can be justified by the feedless periods, during which the microbial population present in the sludge is deprived of easily degradable substrates present in the feed and are forced to degrade complex substrates, such as estrogens (Couras et al., 2014). The comparison of the results of k_{biol} coefficients obtained in the present with previous results reported by Alvarino et al. (2016, 2014) (between 0.01 L g_{VSS}^{-1} d^{-1} and 0.04 L g_{VSS}^{-1} d^{-1} for E1 and between 0.02 L g_{VSS}^{-1} d^{-1} and 0.04 L g_{VSS}^{-1} d^{-1} for EE2) evidence that higher values for E1 and similar values for EE2 were achieved in the present study using the CO of UASB reactor. It is important to highlight that in the present study the estrogens concentration entering the UASB reactors were higher than the concentrations reported in the literature (between 1 g L^{-1} and 10 μg L^{-1}). This may influence the biodegradation kinetics since the pseudo-first order kinetics are driven by the concentration of estrogens in the reactor (Alvarino et al., 2016, 2014; Arias et al., 2018; Moya-Llamas et al., 2018).

5.4. CONCLUSIONS

In the present research, an innovative process for wastewater treatment contaminated with estrogens was proposed based on the IO of UASB reactors. In this context, the performance of the CO and IO of UASB reactors was investigated regarding the removal of E1 and EE2 from synthetic wastewater. Higher E1 and EE2 removal efficiencies by biodegradation and adsorption were achieved (above 95 %) under the IO compared to the CO (ranging from 39 % to 49 %). Additionally, the IO of the UASB reactor enhanced 2.6 and 5.0 times the values of biodegradation kinetic coefficient, k_{biol}, for E1 and EE2, respectively. The improvements of the IO compared to the CO are attributed to the adaptation of the microbial population to complex substances that are retained in the sludge, as estrogens.

Results attained in this investigation indicated that the IO of UASB reactors can be a promising, sustainable, and robust alternative to aerobic processes coupled to UASB reactors for the removal of estrogens from wastewaters. It is expected that the increase of the feedless period for the IO mode allows the development of a microbial population involved in the biodegradation of E1 and EE2. Thus, the studies presented in this research represent a step forward in the knowledge about the performance of UASB reactors on the removal of two estrogens, E1 and EE2.

REFERENCES

Alvarino, T., Allegue, T., Fernandez-Gonzalez, N., Suarez, S., Lema, J.M., Garrido, J.M., Omil, F., 2019. Minimization of dissolved methane, nitrogen and organic micropollutants emissions of effluents from a methanogenic reactor by using a preanoxic MBR post-treatment system. Sci. Total Environ. 671, 165–174. doi:10.1016/J.SCITOTENV.2019.03.169

Alvarino, T., Suárez, S., Garrido, M., Lema, J.M., Omil, F., 2016. A UASB reactor coupled to a hybrid aerobic MBR as innovative plant configuration to enhance the removal of organic micropollutants. Chemosphere 144, 452–458. doi:10.1016/J.CHEMOSPHERE.2015.09.016

Alvarino, T., Suarez, S., Lema, J.M., Omil, F., 2014. Understanding the removal mechanisms of PPCPs and the influence of main technological parameters in anaerobic UASB and aerobic CAS reactors. J. Hazard. Mater. 278, 506–513. doi:10.1016/J.JHAZMAT.2014.06.031

American Public Health Association (APHA), 2005. Standard methods for the examination of water & wastewater, the 21st edition, american public health association, washington, DC, USA.

Arias, A., Alvarino, T., Allegue, T., Suárez, S., Garrido, J.M., Omil, F., 2018. An innovative wastewater treatment technology based on UASB and IFAS for cost-efficient macro and micropollutant removal. J. Hazard. Mater. 359, 113–120. doi:10.1016/J.JHAZMAT.2018.07.042

Bernardelli, J.K., Liz, M. V., Belli, T.J., Lobo-Recio, M.A., Lapolli, F.R., 2015. Removal of estrogens by activated sludge under different conditions using batch experiments. Brazilian J. Chem. Eng. 32, 421–432. doi:10.1590/0104-6632.20150322s00003667

Buntner, D., Sánchez, A., Garrido, J.M., 2013. Feasibility of combined UASB and MBR system in dairy wastewater treatment at ambient temperatures. Chem. Eng. J. 230, 475–481. doi:10.1016/J.CEJ.2013.06.043

Chan, P.C., de Toledo, R.A., Shim, H., 2018. Anaerobic co-digestion of food waste and domestic wastewater – effect of intermittent feeding on short and long chain fatty acids accumulation. Renew. Energy 124, 129–135. doi:10.1016/J.RENENE.2017.07.029

Couras, C.S., Louros, V.L., Grilo, A.M., Leitão, J.H., Capela, M.I., Arroja, L.M., Nadais, M.H., 2014. Effects of operational shocks on key microbial populations for biogas production in UASB (Upflow Anaerobic Sludge Blanket) reactors. Energy 73, 866–874. doi:10.1016/j.energy.2014.06.098

Czajka, C.P., Londry, K.L., 2006. Anaerobic biotransformation of estrogens. Sci. Total Environ. 367, 932–941. doi:10.1016/j.scitotenv.2006.01.021

Fleming, M., Achari, G., Hassan, Q.K., 2016. Modeling the loading and fate of estrogens in wastewater treatment plants. Cogent Environ. Sci. 2. doi:10.1080/23311843.2016.1222690

Hashimoto, T., Murakami, T., 2009. Removal and degradation characteristics of natural and synthetic estrogens by activated sludge in batch experiments. Water Res. 43, 573–582. doi:10.1016/j.watres.2008.10.051

Joss, A., Andersen, H., Ternes, T., Richle, P.R., Siegrist, H., 2004. Removal of estrogens in municipal wastewater treatment under aerobic and anaerobic conditions: consequences for plant optimization. Environ. Sci. Technol. 38, 3047–3055. doi:10.1021/es0351488

Khunjar, W.O., MacKintosh, S.A., Skotnicka-Pitak, J., Baik, S., Aga, D.S., Love, N.G., 2011. Elucidating

the relative roles of ammonia oxidizing and heterotrophic bacteria during the biotransformation of 17α-ethinylestradiol and trimethoprim. Environ. Sci. Technol. 45, 3605–3612. doi:10.1021/es1037035

Larcher, S., Yargeau, V., 2013. Biodegradation of 17α-ethinylestradiol by heterotrophic bacteria. Environ. Pollut. 173, 17–22. doi:10.1016/j.envpol.2012.10.028

Li, C., Kong, X., Lan, L., Tadda, M.A., Liu, D., 2020. Effects of carbon sources on 17 beta-estradiol degradation by: Sphingomonas sp. and the analysis of the involved intracellular metabolomics. Environ. Sci. Process. Impacts 22, 197–206. doi:10.1039/c9em00438f

Moya-Llamas, M.J., Trapote, A., Prats, D., 2018. Removal of micropollutants from urban wastewater using a UASB reactor coupled to a MBR at different organic loading rates. Urban Water J. 15, 437–444. doi:10.1080/1573062X.2018.1508599

Nadais, H., Capela, I., Arroja, L., Duarte, A., 2005. Optimum cycle time for intermittent UASB reactors treating dairy wastewater. Water Res. 39, 1511–1518. doi:10.1016/J.WATRES.2005.01.020

Pessoa, G.P., de Souza, N.C., Vidal, C.B., Alves, J.A.C., Firmino, P.I.M., Nascimento, R.F., dos Santos, A.B., 2014. Occurrence and removal of estrogens in Brazilian wastewater treatment plants. Sci. Total Environ. 490, 288–295. doi:10.1016/j.scitotenv.2014.05.008

Ren, Y.-X., Nakano, K., Nomura, M., Chiba, N., Nishimura, O., 2007. A thermodynamic analysis on adsorption of estrogens in activated sludge process. Water Res. 41, 2341–2348. doi:10.1016/j.watres.2007.01.058

Vassalle, L., García-Galán, M.J., Aquino, S.F., Afonso, R.J. de C.F., Ferrer, I., Passos, F., Mota, C.R., 2020. Can high rate algal ponds be used as post-treatment of UASB reactors to remove micropollutants? Chemosphere 248, 125969. doi:10.1016/J.CHEMOSPHERE.2020.125969

Xu, Y., Xu, N., Llewellyn, N.R., Tao, H., 2014. Occurrence and removal of free and conjugated estrogens in wastewater and sludge in five sewage treatment plants. Environ. Sci. Process. Impacts 16, 262–270. doi:10.1039/C3EM00199G

Ying, G.-G., Kookana, R.S., Ru, Y.-J., 2002. Occurrence and fate of hormone steroids in the environment. Environ. Int. 28, 545–551. doi:10.1016/S0160-4120(02)00075-2

Yu, C.-P., Roh, H., Chu, K.-H., 2007. 17β-Estradiol-degrading bacteria isolated from activated sludge. Environ. Sci. Technol. 41, 486–492. doi:10.1021/es060923f

Zeng, Q., Li, Y., Gu, G., 2009. Nitrate-dependent degradation of 17α-ethinylestradiol by acclimated activated sludge under anaerobic conditions. J. Chem. Technol. Biotechnol. 84, 1841–1847. doi:10.1002/jctb.2255

FINAL REMARKS

CONCLUSIONS AND FUTURE WORK

CONCLUSIONS

Antibiotics and estrogens are recognised as important micropollutants to be monitored in WWTPs due to their continuous input and persistence in the environment. Although antibiotics and estrogens appear at relatively low concentrations ranging between ng L^{-1} and µg L^{-1} levels, they may pose serious effects on the environment and human health. WWTPs represent an important source of environmental contamination not only from wastewater effluents discharged in aquatic media, but also from sludge application in agriculture. A lot of research has been undertaken by the scientific community concerning the occurrence, fate and behaviour of antibiotics, and estrogens in the environment. Despite these contributions, much remains to be done on the elimination of these pollutants. Thus, the main aim of this work was to do research on possible suitable strategies to mitigate the presence of two antibiotics, SDZ and OXA and two estrogens, E1 and EE2 in the environment. Due to the resistance of SDZ and OXA to biodegradation and their persistence in the aqueous phase, their photodegradation was searched in this work, and described in the first part of this thesis. On the other hand, as E1 and EE2 have high affinity to the solid phase, the biodegradation and adsorption of both estrogens were evaluated and described in the second part of this thesis.

Photodegradation is considered as one of the most important naturally occurring processes for the transformation and/or elimination of contaminants in the aquatic environment. Different environmental factors can affect the photodegradation of pollutants, acting as photosensitizers, thus accelerating the photodegradation of the compound, or having a filter effect retarding the photodegradation by screening sunlight or scavenging reactive species. Therefore, photodegradation may largely affect the fate of antibiotics (like SDZ and OXA) in surface waters, being the assessment of the effect of the matrix characteristics essential for understanding SDZ and OXA photodegradation behaviour. The study of direct and indirect photodegradation of SDZ and OXA using simulated solar radiation was presented in chapter 2 and the main conclusions were the following:

- OXA was rapidly photodegraded in ultrapure water, while SDZ was hardly photodegraded: for 4 h of irradiation, OXA degraded 97 % and SDZ degraded less than 30 %;
- The pH increase favoured the photodegradation of SDZ ($t_{½}$ = 6.76 h, at pH = 7.3; $t_{½}$ = 12.19 h, at pH = 6.3), revealing that its photodegradation is more efficient for

the anionic form (SDZ^-), which is the dominant species at the common pH values of natural waters, than for neutral form (HSDZ);

- SDZ photodegradation was notably enhanced under the presence of the three fractions of HS namely HA, FA, and XAD-4 fraction: $t_{½}$ ranged from 1.76 h to 2.42 h in comparison with ultrapure water with $t_{½}$ = 6.76 h. This increase was attributed to the photosensitizing effect of DOM;
- Salinity had a marked photosensitizing effect on the photodegradation of SDZ ($t_{½}$ ranged from 0.78 h to 1.00 h), which was not solely related to the presence of NaCl but also of other salts, such as bicarbonates;
- The presence of NaN_3, as $^1O_2$ scavenger, induced the photodegradation of SDZ (decreasing the $t_{½}$ from 6.76 h to 4.41 h), whilst in the presence of C_3H_8O, as $^{\bullet}OH$ scavenger, a decrease on its photodegradation was observed ($t_{½}$ = 7.7 h). Thus, it was possible to infer that the increase on SDZ photodegradation by the presence of NaN_3 could be explained by the possible formation of $^1O_2$ whose action is intensified by the presence of azide;
- N_2 purging also induced the SDZ photodegradation ($t_{½}$ = 2.5 h), confirming that the presence of molecular oxygen can also act as a quencher of SDZ photodegradation;
- These effects were related with the larger SDZ photodegradation observed in real matrices, namely in wastewater, brackish water and freshwater ($t_{½}$ between 2.32 h and 3.48 h) in comparison with that occurring in ultrapure water ($t_{½}$ =6.76 h);
- Contrarily to what was observed for SDZ, a decrease on OXA photodegradation was observed in presence of HS: $t_{½}$ ranged from 1.70 h to 2.38 h, in comparison with ultrapure water, with $t_{½}$ of 0.99 h. This inhibitory effect may result from the presence of DOM, overcoming the photosensitizing effect;
- In comparison with ultrapure water ($t_{½}$ = 0.99 h), the presence of NaCl seemed not to affect the OXA photodegradation ($t_{½}$ = 0.74 h), while the presence of synthetic sea salts negatively affected its photodegradation ($t_{½}$ = 4.25 h). These results suggested that carbonates present in the synthetic sea salts' solution could inhibit OXA photodegradation since is known that they can act as $^{\bullet}OH$ scavengers;
- $^1O_2$ scavenger led to a pronounced retardation of OXA decay ($t_{½}$ = 1.58 h), indicating that $^1O_2$ plays an important role in OXA photodegradation process suggesting that the self-sensitized photo-oxidation processes of $^1O_2$ generation was engaged in OXA photodegradation. $^{\bullet}OH$ scavenger resulted in faster

photodegradation of OXA ($t_{½}$ = 0.53 h) than in ultrapure water ($t_{½}$ = 0.99 h); however, no conclusions could be drawn from this scavenger's action on OXA photodegradation.

- In environmental matrices, a very sharp decrease of OXA photodegradation was evident, being more pronounced in brackish water ($t_{½}$ = 4.03 h).

These findings constitute a relevant contribution and point to photodegradation as an important loss mechanism for SDZ and OXA in surface waters. The factors studied and their effects are extremely important and must be taken into account in the design of low-cost and low-energy alternatives to conventional tertiary wastewater treatments, such as constructed wetlands or stabilization lagoons.

Regarding the estrogens, some research has been reported in the literature concerning their occurrence and fate in the liquid phase of wastewater. However, it is notable that these pollutants are removed from wastewater by the adsorption process onto the sludge, being mostly retained in the particulate phase of sludge. In this sense, studies concerning the occurrence and fate of estrogens in solid phase of sludge are scarce. A possible justification for the limited knowledge on the occurrence of estrogens in solid phase of sludge are the analytical difficulties in the identification and quantification (as the extraction procedure needed). Thus, the second part of this thesis starts by describing a development of a fast, simple, inexpensive and effective method for determining E1 and EE2 in the solid phase of sludge without any sample pre-treatment (chapter 3). In the developed method, the freeze-dried samples were subjected to ULE, with methanol and acetone, and analysed by HPLC with FLD. The method developed was applied for the determination of E1 and EE2 in sludge samples (primary sludge, secondary sludge, anaerobic digested sludge and dehydrated sludge) collected from an operating WWTP in two different seasons (summer and winter).

Detailed accomplishments and conclusions of chapter 3 were as follows:

- Recovery rates of 103 % and 97 % were obtained in sludge samples for E1 and EE2, respectively, revealing that the developed method can be applied to monitor the contamination of sludge with these estrogens;
- 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 dehydrated sludge (1.00 μg g^{-1});
- EE2 was not detected in any of the sludge samples analysed.

Estrogens removal by adsorption and biodegradation has been a major concern for the scientific community. Different factors can influence the removal of E1 and EE2 from wastewater. Therefore, chapter 4 presented the influence of temperature, MLSS content, and nitrate supplementation on the removal of E1 and EE2 in anaerobic processes. Detailed accomplishments and conclusions of chapter 4 were as follows:

- A rapid adsorption of E1 and EE2 onto the sludge solid phase was observed;
- Higher temperatures (25 °C and 34 °C *vs.* 20 °C) favoured E1 removal from the solid phase but did not influence the removal of EE2;
- The lowest MLSS concentrations promoted a higher removal of E1 and EE2 from the solid phase of sludge (2 g L^{-1} and 3 g L^{-1});
- The presence of nitrate does not appear to affect the removal of both E1 and EE2 from the solid phase of sludge;
- EE2 is much more persistent than E1 in sludge.

In order to improve the removal of E1 and EE2 from wastewater and sludge, the performance of the CO and IO of UASB reactors was investigated and presented in chapter 5. UASB reactors are considered as very competitive anaerobic systems due to the production of high-quality effluents, lower energy requirements, minimization of sludge production, and production of higher methane content in biogas. IO consists of a periodical interruption of the reactor feed formed by a sequence of alternate feed and feedless periods. Feedless periods improve the growth of key microbial groups that trigger the degradation of recalcitrant compounds as estrogens. Biodegradation and adsorption were the main mechanisms considered for the removal of E1 and EE2. For this purpose, the mass balances of estrogens in the LP and SP were determined. Detailed accomplishments and conclusions of chapter 5 were as follows:

- The IO resulted in a significant increase of the overall removal of E1 and EE2 (above 95% for E1 and EE2) when compared to CO (49% for E1 and 39% for EE2);
- An improvement on biodegradation rate of E1 and EE2 (69.4 % for E1 and 21.8 % for EE2) was obtained in comparison with CO (43.3 % for E1 and 8.0 % for EE2);
- IO enhanced the adsorption of E1 and EE2 onto the solid phase (26.5 % for E1 and 72.7 % for EE2) when compared to CO (5.7 % for E1 and 31.0% for EE2).

Therefore, the IO of UASB reactors can be a promising, sustainable, and robust alternative for the removal of estrogens from wastewater. Despite the contribution of this work, much more

needs to be investigated in order to maximize the biodegradation of these compounds in the solid phase of sludge. For instance, perhaps the increase of the feedless period for the IO mode can promote a microbial population growth which in turn promotes biodegradation of E1 and EE2.

FUTURE WORK

In order to gain a deeper understanding on SDZ and OXA photochemical fate, further research is to be carried out on these antibiotics photodegradation pathways and products in different matrices, assessing the toxicity of the resulting photoproducts. Also, the quantification of the concentration of the reactive species, namely ROS and DOM in the different aqueous matrices, should be determined to ascertain their relative importance in SDZ and OXA photodegradation. Such information, together with the degree of mineralization that occurs along photodegradation, is essential for the implementation of wastewater alternative treatments that allow for the removal of antibiotics. On the other hand, in view of the large influence of DOM in SDZ and OXA photochemical degradation, research is to be undertaken on the effects of the HS fractions (HA, FA and XAD-4) from waters of different origin. Moreover, to improve the photodegradation rate of SDZ and OXA, the presence of green materials as photocatalysts must be studied. Carbon dots (CDs), obtained by green synthesis methods, have very interesting properties as photocatalysts and can be a promising solution. Thus, an interesting future work will be to synthesize these materials and to evaluate the photodegradation of SDZ and OXA in their presence and primarily in ultrapure water. Moreover, materials should also be used in different environmental samples as brackish water, fresh water and wastewater, and results compared with those obtained for materials in ultrapure water.

On the other hand, with regard to the removal of estrogens (by adsorption and biodegradation), further research should be undertaken on the performance for a longer period of IO of UASB. In this context, different operating cycles of IO of UASB reactors and different feeding and feedless periods should be studied. In addition, the adaptation of biomass to estrogens should be assessed, for instance through the characterization of the microbial community composition by 16S amplicon sequencing. It is important to underline that in this work an additional study was carried out to investigate the microbial species that play a key role on the degradation of E1 and EE2. However, these results were not obtained in time to be included and discussed in this thesis.

The high concentration of estrogens also influences their removal by microorganisms, which might be due to primary metabolism instead of co-metabolism. Thus, in order to assess the removal of estrogens by the co-metabolism of microorganisms, studies using initial concentrations similar to those found in WWTP samples should be taken into account. Furthermore, the study of the influence of different organic loads, as well as different hydraulic retention times, on the removal of E1 and EE2 in UASB reactors is of extreme importance.

Regarding the end-of-life of the saturated sludge, this destination must be carefully analysed. In the case of incineration, the emissions gases should be monitored, and the gaseous products must be identified. Alternatively, if this destination is the landfill disposal, additional studies should be carried out to evaluate the harmful effects of the disposal of this sludge.

In terms of full-scale implementation of the strategies proposed in this work for the removal of antibiotics and estrogens, the economic feasibility study should be carried out.