Universidade de Aveiro
Ano 2020

Departamento de Química

Ana Sofia Aguiar Cachinho

Potencial enzimático de fungos filamentosos em pré-tratamento de borras de café para fermentação acidogénica

Enzymatic potential of filamentous fungi for pretreatment of spent coffee grounds for acidogenic fermentations

Universidade de Aveiro
Ano 2020

Departamento de Química

Ana Sofia Aguiar Cachinho

Potencial enzimático de fungos filamentosos em pré-tratamento de borras de café para fermentação acidogénica

Enzymatic potential of filamentous fungi for pretreatment of spent coffee grounds for acidogenic fermentations

Dissertação apresentada à Universidade de Aveiro para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Biotecnologia, realizada sobre a orientação científica da Prof. Ana Xavier, Professora Auxiliar do Departamento de Química, Universidade de Aveiro, e coorientação da Prof. Luísa Serafim, Professora Auxiliar do Departamento de Química, Universidade de Aveiro.

o júri

presidente

Professor Doutor João Filipe Colardelle da Luz Mano
Professor Catedrático do Departamento de Química, Universidade de Aveiro

Doutora Ana Paula Mora Tavares
Equiparada a Investigadora Auxiliar, Universidade de Aveiro

Professora Doutora Ana Maria Rebelo Barreto Xavier
Professora Auxiliar do Departamento de Química, Universidade de Aveiro

agradecimentos

Gostaria de agradecer à Professora Ana Xavier e à Professora Luísa Serafim por toda o simpatia, disponibilidade, orientação científica e apoio demonstrados ao longo do ano.

Gostaria de agradecer aos meus colegas do LEB pelo bom ambiente, pela entreajuda e amizade. Um agradecimento em especial à Joana e à Mariana por toda a paciência, disponibilidade e ajuda durante este ano.

Aos meus amigos, por me terem acompanhado nesta aventura, por todos os croissants com chocolate e por todas as memórias criadas.

À Scientific, especialmente à minha direção e aos meus meninos de RH, pela experiência enriquecedora.

Aos meus pais, irmão e irmã pelo apoio e por terem possibilitado a concretização desta etapa da minha vida.

A todos, muito obrigada!

palavras-chave

Borras de café, produção de enzimas, *Trametes versicolor, Paecilomyces variotii*, fermentação acidogénica, ácidos orgânicos de cadeia curta.

resumo

Com a depleção de recursos e o aumento da produção de resíduos, vários materiais lenhocelulósicos, têm sido estudados como potencias substratos para biorrefinarias. Pelas suas características, as borras de café (SCG), podem ser aplicadas em vários processos tais como a produção de ácidos orgânicos de cadeia curta (SCOA). Os SCOA são metabolitos versáteis e de elevado valor, que podem ser produzidos por fermentação acidogénica (AF) em alternativa à produção petroquímica. Para aumentar a competitividade da AF é necessário melhorar a taxa de hidrólise do substrato, seja ela química ou enzimática. A hidrólise enzimática é a escolha mais sustentável, no entanto, as enzimas estão associadas a elevados custos e, consequentemente, a hidrólise química é a opção preferida. Dado que os custos de produção de enzimas estão maioritariamente associados à matéria prima utilizados, têm sido estudadas novas estratégias. Uma delas é a utilização de resíduos orgânicos como substratos baratos com o intuito de reduzir os custos de produção e otimizar os processos. Neste trabalho, estudou-se o efeito individual de SCG (0.4 e 1.0% w/v) e o efeito combinado de SCG e casca de ananás (PP) (0.4 + 0.2% w/v e 1.0 + 0.4% w/v) para a produção de enzimas por *Trametes versicolor* e *Paecilomyces variotii*, tendo sido obtida atividade máxima de lacase 350 U/L no ensaio de *T. versicolor* com 0.4% (w/v) SCG. Posteriormente, os extratos enzimáticos obtidos foram utilizados no pré-tratamento de SCG. Realizou-se AF com SCG submetidas a diferentes pré-tratamentos físico-químicos e biológicos de modo a selecionar o melhor pré-tratamento para a produção de SCOA. De uma forma geral, os SCOA maioritariamente produzidos foram os ácidos acético e propiónico. A maior concentração de SCOA (2.52 g COD/L) foi obtida com hidrólise ácida, mas a fermentação submersa de *T. versicolor* levou a uma concentração similar de SCOA (2.44 g COD/L) e maior grau de acidificação (48.0%). Os resultados obtidos permitiram concluir que as SCG têm potencial como indutores e substratos para a produção de lacase e de SCOA, no entanto estes processos deverão ser otimizados para que se tornem mais competitivos.

keywords

Spent coffee grounds, enzyme production, *Trametes versicolor*, *Paecilomyces variotii*, acidogenic fermentation, short-chain organic acids.

abstract

With resource depletion and increasing waste production, lignocellulosic biomass has been studied as biorefinery substrates. Due to its characteristics, spent coffee grounds (SCG) can be applied in numerous bioprocesses, such as the production of short-chain organic acids (SCOA). SCOA are versatile and high valued metabolites that can be produced by Acidogenic Fermentation (AF) as an alternative to the petrochemical process. To enhance AF competitiveness is necessary to improve the hydrolysis rate which can be achieved by enzymatic or chemical hydrolysis. Enzymatic hydrolysis is considered a more sustainable alternative. However, enzymes are costly, and consequently, chemical hydrolysis is the preferred option. Since the used substrate represents a huge cost in enzyme production, new strategies are necessary. One possibility is the use of organic wastes. These cheap substrates can contribute to reducing production costs. In this work, the sole effect of SCG (0.4 and 1.0% w/v) and the combined effect of SCG and pineapple peel (PP) (0.4 + 0.2% w/v and 1.0 + 0.4% w/v) were studied as inducers for enzyme production by *Trametes versicolor* and *Paecilomyces variotii*. In the assay with 0.4% SCG, a laccase activity in *T. versicolor* of 350 U/L was recorded. Posteriorly, the enzymatic extracts obtained were used for SCG pretreatment. To select the best pretreatment for SCOA production, AF was performed with SCG submitted to different physicochemical and biological pretreatments. Generally, the main SCOA produced were acetic, and propionic acids. Acid hydrolysis led to the highest SCOA concentration (2.52 g COD/L) but submerged fermentation by *T. versicolor* led to a similar SCOA concentration (2.44 g COD/L) and higher acidification degree (48.0%). The obtained results showed the great potential of *T. versicolor* enzymes for SCG pretreatment. The experiments performed allowed us to conclude that SCG can be potentially used as a laccase inducer and as a substrate for SCOA production, however, these processes must be optimized to become more competitive.

List of Contents

List of Abbreviations

ABTS - 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid

AF – Acidogenic fermentation

AH – Spent coffee grounds pretreated with acid hydrolysis

AnD - Anaerobic digestion

BH - Spent coffee grounds pretreated with alkaline hydrolysis

C/N - Carbon-to-nitrogen

CGA - Chlorogenic acids

COD - Chemical oxygen demand

FIC - First intermediate compound

HPLC - High-performance liquid chromatography

HRT - Hydraulic retention time

Lac - Laccase

LDA - Lignin-degrading auxiliary

LiP – Lignin Peroxidase

LME - Lignin-modifying enzymes

MC- Moisture content

MMC – Mixed microbial cultures

MnP – Manganese Peroxidase

ORL - Organic loading rate

PHA – Polyhydroxyalkanoates

PP – Pineapple peel

PvM - Spent coffee grounds pretreated with the enzymatic extract obtained from *P. variotii* without inducers

PvP - Spent coffee grounds pretreated with the enzymatic extract obtained from *P. variotii* with SCG and PP as inducers

PvS - Spent coffee grounds pretreated with the enzymatic extract obtained from *P. variotii* with SCG as an inducer

PvSmF – Spent coffee grounds pretreated with submerged fermentation by *P. variotii*

PvSSF – Spent coffee grounds pretreated with solid-state fermentation by *P. variotii*

SCG - Spent coffee grounds

SCOA – Short-chain organic acids

SCP - Single Cell Protein

SE - Spent coffee grounds pretreated with supercritical extraction

SE+ BH - Spent coffee grounds pretreated with supercritical extraction and alkaline hydrolysis

SE+AH - Spent coffee grounds pretreated with supercritical extraction and acid hydrolysis

SIC – Secondary intermediate compound

SmF - Submerged fermentation

SSF - Solid-state fermentation

TDM - *Trametes* defined medium

TvP - Spent coffee grounds pretreated with the enzymatic extract obtained from *T. versicolor* with SCG and PP as inducers

TvS - Spent coffee grounds pretreated with the enzymatic extract obtained from *T. versicolor* with SCG as an inducer

TvSmF - Spent coffee grounds pretreated with submerged fermentation by *T. versicolor*

TvSSF - Spent coffee grounds pretreated with solid-state fermentation by *T. versicolor*

UV - Ultraviolet

VSS - Volatile suspended solids

WWTP- Wastewater treatment plant

WWTT - Wastewater treatment tanks

List of Tables

List of Figures

List of equations

1. Introduction

Coffee is one of the most traded commodities in the world and its production and processing are associated with large amounts of by-products, such as spent coffee grounds (SCG)[1]. Like other organic wastes, SCG majorly ends in landfills, are used as compost, or are burned for energy production. The current waste management strategies are inadequate since SCG are produced in high volumes and their high content in phenolic compounds is associated with environmental problems[2].

To solve these problems, governmental institutions have been advocating the concept of the Circular Economy and encouraging biorefineries implementation[3]. Organic wastes are no longer seen as residues but as profitable opportunities and as a potential feedstock for bioprocesses. In this sense, several studies explored SCG bio-based applications[4], such as the production of short-chain organic acids (SCOA)[5].

Being a complex material, SCG needs to be submitted to hydrolysis to obtain simpler molecules for further bioprocessing[6]. Enzymatic hydrolysis is more sustainable than chemical hydrolysis, however, enzyme production is still highly costly due to equipment, installation, and the raw materials used, which has an impact on bioprocess cost and competitivity. Regarding costs decrease, the utilization of cheap and easily available biowaste as a substrate, and the integration of enzyme production on a biorefinery plan have been studied[7].

As decomposers, filamentous fungi *Trametes versicolor* and *Paecylomyces variotii* possess powerful secretomes rich in hydrolytic and oxidative enzymes which have been explored for years[8–10]. To improve the industrial process and enzyme's commercial value, alternative strategies such as using organic waste for enzyme production have been studied. From a Circular Economy perspective a more environmental-friendly and profitable waste management strategy must be implemented in future following United Nations sustainable development goals.

Therefore, this work starts to study the fungus *Trametes versicolor* and *Paecilomyces variotii* for selecting the best conditions for some enzymes production. After that it proceeds evaluating biological and physicochemical pretreatments efficiency for short-chain organic acids production by acidogenic fermentation of spent coffee grounds.

2. State of the art

2.1. Waste production and environmental problematic

Population increase, industrial progress, economic development, and urbanization degree are the main factors with implications on natural resources consumption and waste generation. Globally, it is estimated an annual waste production of 4.0 billion tons, including municipal, industrial, and hazardous waste[11]. By itself, municipal solid waste represents 2.0 billion annual tons, of which, 70% is disposed in landfills or open dumping. If there is no improvement in management strategies, it is anticipated that, by the year 2050, global waste reach 2.6 billion tons[12]. Furthermore, the most common waste management strategies such as landfills represent an environmental problem, since they are associated with ecosystem damage and contaminations, and others[13].

Even though prevention strategies are demonstrated to be the most effective with fewer costs associated[3], reducing waste production can be challenging owing to society's industrialization degree and global population growth [14]. The actual energy and material flux Linear Economy model has been criticized due to its environmental, social, and economic impact. The consumption pattern relies on raw material extraction, product manufacture, use, and, finally, disposal. Therefore, natural reserves will not be able to guarantee the future generation's needs[15].

With the crescent environmental concerns, the United Nations defined goals and principles to ensure a more sustainable resource use and waste management conscience, focusing on reducing waste environmental impact by 2030[16,17]. To achieve a more sustainable economy, it is important to change from the traditional view of waste management, where short-term approaches and cost avoidance strategies were followed, to a long-term strategy, with value creation as the focus. To do this, it is important not only treating waste as potential feedstock but also, creating new processes and technologies to enable waste minimization on upstream processes, upgrade separating strategies, and implementing tools to enhance process efficiency and increase value recuperation[3].

Circular Economy (CE) seeks to implement a closed system, like what happens in nature and reducing, reusing, recycling, and recovering materials. By mitigate (extending product life), close (implementing ways to give product and its wastes a new life), and narrow

(using optimized processes requiring fewer resources) products life cycle loop. Consequently, CE could be used as a useful device for LE substitution, by promoting natural resources preservation, while creating economic and social value[18]. In the last years, interest in CE has aroused especially from politicians and entrepreneurs, and the European Union is putting great effort into its implementation[19,20].

The utilization of waste has been studied as a potential biorefinery feedstock[21]. The biorefinery is analogous to the petrochemical refinery, where biomass is submitted to physical, chemical, and biological processing to co-produce energy, chemical products, and materials. In this process substrate and respective by-products are entirely used, being a way of waste management and allowing its valorization[2]. Organic waste represents an appealing feedstock not only because it is cheap, available in a high quantity, and a sustainable option, but also, its complex composition allows different final products[22].

2.2. Spent coffee grounds

2.2.1. Coffee Industry

Coffee is one of the most popular and traded beverages involving a huge commercial network[23]. It presented an estimated production of 10,140 million kg in 2018/19 and projected production of 10,044 million kg in 2019/20, majorly from Arabica (*Coffea Arabica*) and Robusta (*Coffea Canephora*) varieties. Although the production decreased from 2018 to 2019, coffee popularity is continuously increasing and the global consumption is foreseen to grow from 9,918 million kg in 2018/19 to 10,074 million kg in 2019/20[24].

Coffee cherry (Figure 1A) is constituted by four layers (silverskin, parchment, pulp, and epicarp) surrounding two coffee beans[2]. After harvesting, coffee cherries must pass an extent pathway until their roasted form (Figure 1B)[23].

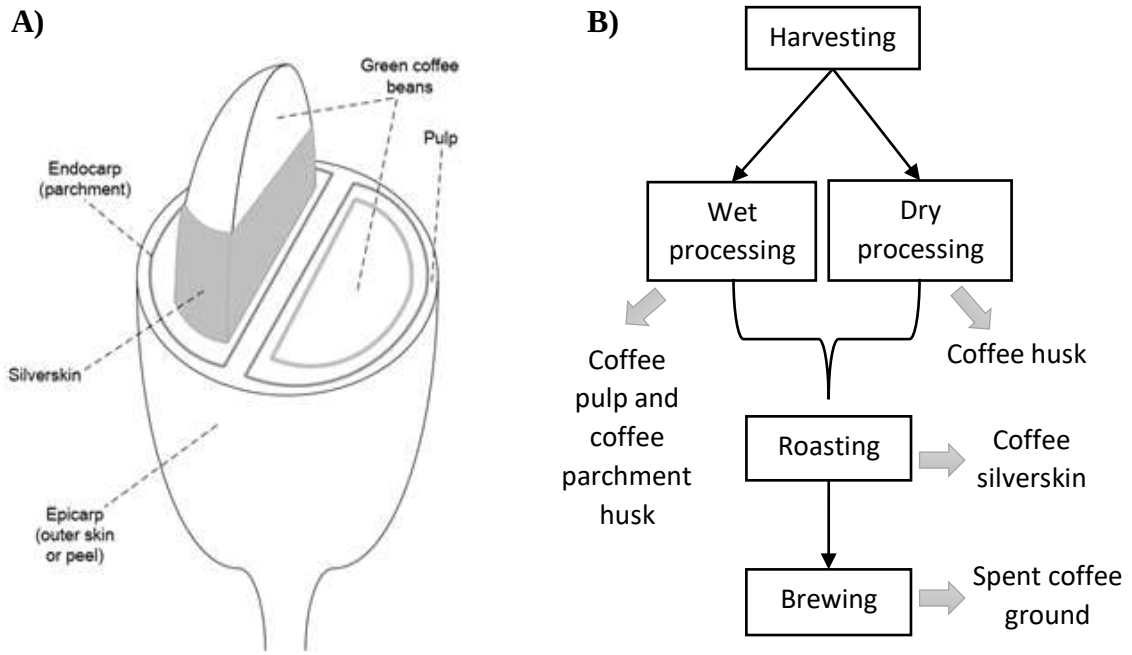

Figure 1. A) Coffeeberry. B) Coffee processing steps and respective by-products (adapted[21]).

Over this complex process, 50% of the coffee berry results in huge amounts of lignocellulosic organic waste, usually discarded in landfills, or burned to generate energy[25]. These strategies could be problematic since caffeine and phenolic compounds negatively affect the environment. However, their composition is rich in polysaccharides, proteins, and fat, making coffee by-products an interesting feedstock for chemical and bio-based processes resulting in high-value products that can be applied in pharmaceutics, cosmetics, and food industries[26]. Although there is no coffee production in European countries, Europe has a strong coffee industry and is the major coffee consumer[24], therefore, coffee by-products such as SCG, are relevant for the biotechnology process.

2.2.2. Composition of SCG

During the coffee brewing or preparation of industrial soluble coffee, the hot water passes through the grounded beans and extracts the soluble compounds from the remaining lignocellulosic residue, the SCG[2]. By itself, the coffee industry generates approximately six

million tons of SCG by year[27], whereas each ton of green coffee beans produces 650 kg of SCG[23]. Like the major waste, SCG are disposed in landfills, burned to produce energy, or even used as adulterated roasted and grounds coffee. These strategies are not only associated with environmental impact, due to, caffeine, tannins, and polyphenols present on SCG and the volatile organic compounds generated, but also to economic problems[28,29].

SCG composition (Figure 2) could vary depending on plant varieties, growing conditions, processing, and extraction methods used. The SCG are mainly composed of an interwoven network of cellulose, hemicelluloses, and lignin[30–32]. Other components with significant amounts are proteins and fat[2] and also can be found some phenolic compounds, caffeine, tannins, minerals, and ashes[26].

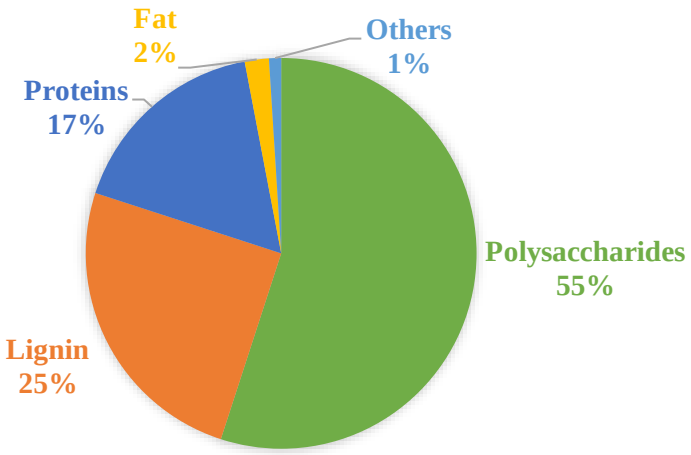

Figure 2. Spent coffee grounds composition.

2.2.2.1. Lignocellulosic fraction

Contrary to other lignocellulosic residues, SCG has a higher content of hemicelluloses (30-40 wt%) prior to cellulose (about 10 wt%)[6] and the monosaccharides composition ranges from 21-57% mannose, 14-30% galactose, 9-19% glucose, and 2-6% arabinose[1].

Cellulose is a D-anhydroglucose linear homopolysaccharide[6], formed by 1,000 to 15,000 units with three hydroxyl groups and linked by (1,4)-β-glycosidic linkages. Each chain contains a reducing end with an aliphatic and a carbonyl group; and a non-reducing end with a closed ring structure. This polysaccharide can present crystalline (more resistant) and

amorphous (less resistant) microfibrils, depending on intra and inter-molecular hydrogen bonds established, that influence molecule solubility and reactivity. As the key structural polysaccharide in plants serves as the foundation to form the plant cell wall, along with hemicelluloses and lignin. Although it is highly affected by the pretreatment and the extraction process, cellulose is less affected during the roasting process and volatile compounds extraction than the hemicelluloses arabinogalactans and galactomannans. In fact, processes with higher severity conditions extract more hemicelluloses polysaccharides, consequently, glucan content increases[2].

Besides cellulose, hemicelluloses are the second most abundant polysaccharides in nature. In SCG's hemicelluloses, the main polysaccharides are mannans and arabinogalactans, containing β-D-glucose, β-D-xylose, β-D-mannose, α-D-galactose, and α-L-arabinose monomers. Due to their amorphous and less strength structure, hemicelluloses are less recalcitrant than cellulose, being easily hydrolyzed by acid or alkali treatments[6].

Galactomannans and galactoglucomannans are mannans branched polysaccharides, whose main chain backbone is formed of (1,4)-β-D-mannopyranose units and glucopyranose units, with galactoses as substituents. Like cellulose, mannan is water-insoluble and remains more recalcitrant than other polysaccharides. This property is influenced by mannose: galactose ratio (generally 23:1) and side-group substitutions (around 15%). When polysaccharides have low side-group substitution, they self-associate and form a crystalline structure, decreasing their solubility. While highly branched structure (>30%) potentiates solubility[1]. However, when submitted to pretreatments like microwave irradiation, dilute acid, steam explosion, and enzymatic hydrolysis, mannans are more susceptible than celluloses[2].

Differently, the arabinogalactans establish a polysaccharide network by physical interaction or covalent linkage with the cell wall and can be associated with pectin or proteins. This polymer can present two structural organizations: Type I - linear short chains (1,4)-β-galactopyranose backbone, with C3 substitutions by α-arabinofuranoside residues linked by (1,5) links; or Type II – (1,3)-β-galactopyranose backbone with C6 substitution with mono- and oligosaccharides of L-arabinosyl and D-galactosyl residues[2].

During roasting, arabinogalactan and galactomannan solubility increase due to cell-wall loosening and polysaccharide depolymerizing and, 30% of polysaccharides are extracted while the remaining stay bound to the SCG matrix[1]. However, arabinogalactan is more reactive and easily degraded during coffee roasting than galactomannans thus, their content in SCG is lower[2].

The last lignocellulosic component is lignin, a three-dimensional amorphous phenylpropane polymer with carbonyl, hydroxyl, and methoxyl substitutions, responsible to offer protection against mechanical and biological stresses. Although the green coffee beans are poor in lignin content (3.0 wt%), the concentration of total lignin in SCG increases to around 19.8-29.8% due to all processing applied to the beans, which contributes to the high calorific value of SCG. In order to obtain the fermentable sugars, lignin removal is necessary through hydrolysis processes during which some microbial inhibitors can be released[6].

2.2.2.2. Oleic fraction

The major compounds in SCG's oleic fraction are glycerides (80-90%), containing mainly linoleic, palmitic, stearic, or oleic acids; the remaining fraction is composed of terpenes (cafestol and kahweol), sterols (sitosterol, stigmasterol, and campesterol) and tocopherols[2]. The oleic fraction composition varies according to characteristics linked to coffee plant and coffee processing methods but also lipid extraction methods employed. In general, coffee oil is composed of approximately 75% triacylglycerols, 14% terpene esters, 5% partial acylglycerols, 1% free fatty acids, 1.5% free sterols, 1% sterol esters, and 1% polar lipids, and slightly differs from SCG oil[4,6].

2.2.2.3. Bioactive compounds

Caffeine, the most relevant bioactive compound in coffee, is mostly extracted during coffee brewing, therefore, its presence in SCG is almost insignificant. However, SCG are rich in phenols. The main phenolic compounds are esters formed by quinic acid and one or two moieties of caffeic acid, the chlorogenic acids (CGA)[1]. Apart from quinic acid, CGA also includes its analogs: caffeoylquinic acids, feruloylquinic acids, and p-coumaroylquinic acids[2]. CGA content reduces during roasting, yet antioxidant activities continue similar to

green beans antioxidant activity because other antioxidant compounds, such as Maillard reaction products, are formed during that process. One of the products formed during Millard reactions is melanoidins[6]. Beyond antioxidant activities, phenolic compounds also have radical scavenging, oxygen radical absorbance capacity, anti-inflammatory, anticarcinogenic, antimutagenic, and antimicrobial activities[1].

Due to their actions, SCG bioactive compounds act as microbial inhibitors. Besides inhibitors naturally present in SCG, such as phenolics and alkaloids, SCG can also contain acetic acid, furfural, and hydroxymethylfurfural, whose formation is promoted by the extremal conditions (temperatures above 150 ºC) applied during coffee processing and, during thermal degradation of carbohydrates. Inhibitors can constrain microbial growth (phenols, alkaloids, and melanoidins), affect cell maintenance functions (acetic acid), or interfere with the electron transport chain in the mitochondria (furfural and hydroxymethyl-furfural), affecting fermentation processes. Although microorganisms exhibit different inhibitor sensitivities, it is recommended their remotion. This can be achieved through pre- or post-treatment methods, by enzymatic degradation, application of adsorbent media, alkali treatment, or chemical conversion of phenolics. Inhibitors extraction previews hydrolysate application is a more advantageous option since the post-treatment detoxification can induce the reduction of sugar concentration and promote osmotic stress[6].

2.2.3. Valorization of SCG

As mentioned before, SCG are a rich substrate and can be valorized in a myriad way (Figure 3). The most common and older applications pass by the valorization without any fractionation such as additive for animal feed, composting additive, or to produce fuel, as pellets. However, these applications do not fully recover SCG potential and the high content of phenolic compounds can be problematic due to their toxicity, and burning SCG as fuel has environmental implications[6,23].

Recently, the fractionation, extraction, and application of specific SCG fractions or compounds to obtain high value from this organic waste have been studied. Coffee oil is associated with desirable characteristics for the cosmetic industry, such as antioxidant activity and the presence of ultraviolet (UV) radiation protectors. Therefore, its incorporation

in cosmetic formulations was studied[33]. On the other hand, SCG lipids are the potential substrate for other chemical and biotechnological processes such as the transesterification reaction of oil, allowing the conversion of SGC lipids in biodiesel[34–37]. Finally, the most studied biotechnological products obtained from coffee oil are polyhydroxyalkanoates (PHA), bacterial biodegradable and biocompatible polyesters accumulated by *Cupriavidus necator* and other strains[38].

The bioactive compounds isolated are known for their antioxidant, antibacterial, hepatoprotective, hypoglycemic, anticancer, and antiviral properties, having an economic interest in pharmaceutical, cosmetic, and food industries[6].

In the lignocellulosic fraction, the main goal passes to obtain a high amount of fermentable sugars through chemical and enzymatic hydrolysis or combining both. The used methodologies and their efficiency along with SCG characteristics and the application of pre- or post-treatment may have implications on the number of fermentable sugars and inhibitors present in the hydrolysates[22] and, represent more than 45% of the operational cost. In general, thermochemical methods are preferred over biological treatment, since these can be time-consuming, and enzymes are relatively expensive. However, thermochemical treatments enhance the formation of inhibitors, associated with intense energy consumption and the use of polluting chemicals[39].

SCG hydrolysates contain 0.192 mg/g SCG to 521.6 mg/g SCG fermentable sugars and are rich in hexoses (mannose, galactose, and glucose). This represents a great advantage over other lignocellulosic pentose-rich hydrolysates since the possibility of carbon catabolite repression occurs, i.e. the microorganism preferring one substrate instead of others is low[6,22]. The hydrolysates obtained have been recently used for biotechnological applications such as the production of SCOA[5,40–42], PHA[43–46], ethanol[47,48], and carotenoids[45].

Most applications referred to above are not commercially employed yet or focus on a single and low market value product/application. However, SCG characteristics and composition assign its prospective application as a biorefinery substrate[22].

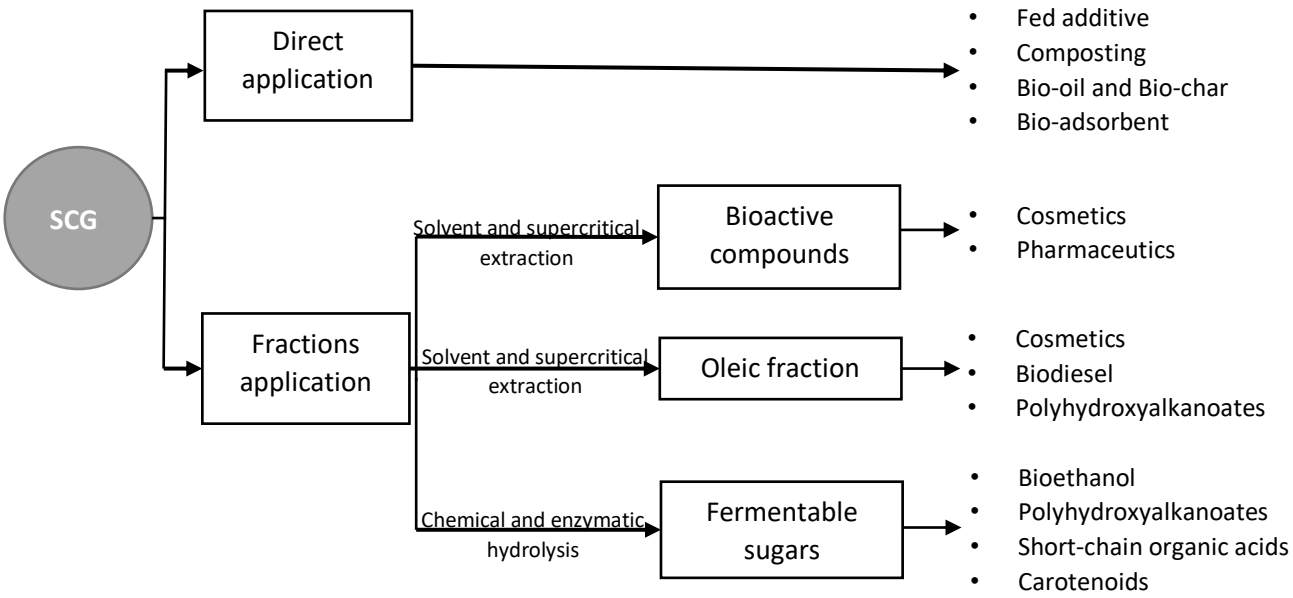

Figure 3. Spent coffee grounds applications.

2.3. Short-Chain Organic Acids

Short-chain organic acids (SCOA) (Table 1) are aliphatic monocarboxylic acids with two to six carbon atoms, such as acetic, propionic, butyric, isobutyric, valeric, and lactic acid. SCOA play an important role in microbial metabolism but also in plants and animals. These metabolites have a wide range of applications and can be applied to bioplastics and bioenergy production[49]. SCOA are usually produced by petrochemical processes, however, the environmental impact associated with it and the increasing oil's cost promoted the interest in biological production especially using organic waste as substrate[50].

2.3.1. Biological production of SCOA

Anaerobic digestion (AnD) is an anaerobic process used for organic waste management and also as a strategy for recovering high added valued products[51]. In this process, acidogenic bacteria and acetogens catalyze organic matter's conversion to methane and carbon dioxide. And, since SCOA are important intermediates in AnD, this process has been studied for their production[52].

Table 1. Representation of the most abundant short-chain organic acids characteristics. Based on[49,52,53]

SCOA	Molecular formula	Production methods	Metabolic pathway	Market size (kton/year)	Market price (€/ton)	Applications
Acetic acid	CH_3COOH	Chemical synthesis (carboxylation of methanol) Microbial fermentation (oxidative and anaerobic).	Acetate-ethanol type Butyrate-type Mixed-acid type	14000–17000	400–800	Vinyl acetate monomer (polymers, adhesives, dyes), food additive, solvent, vinegar, ester production.
Propionic acid	CH_3CH_2COOH	Chemical synthesis (oxidation of butyraldehyde) Extraction from butter Microbial fermentation.	Propionate-type Mixed-acid type	90–105	1500–1650	Food preservative, animal feed, herbicide, chemical intermediates of wastewater biological nutrient removal.
Butyric acid	$CH_3CH_2CH_2COOH$	Chemical Synthesis (ethylene hydroformylation, carboxylation of ethylene, direct oxidation of hydrocarbons) By-product of acetic acid manufacturing Microbial fermentation.	Butyrate-type Mixed-acid type	350–470	2000–2500	Human health care, pharmaceutical, cosmetics, chemical industry.

There are several metabolic pathways (Table 1) involved in AnD, but it relies on four sequential steps (Figure 4) hydrolysis, acidogenic fermentation (AF), acetogenesis, and methanogenesis performed by distinct and symbiotic microorganism groups[49]. Firstly, hydrolytic microorganisms hydrolyze the organic waste and enzymes convert complex polymers into simple monomers. Thus, allowing acidogenic bacteria to ferment the monomers into SCOA, H_2, and CO_2. On other hand, acetogenic bacteria will convert other SCOA into acetic acid, H_2, and CO_2. The process is concluded by methanogenesis, where methanogenic archaea generate methane from the previous metabolites[54]. To guarantee that SCOA are not consumed by methanogenic archaea, methanogenesis must be avoided.

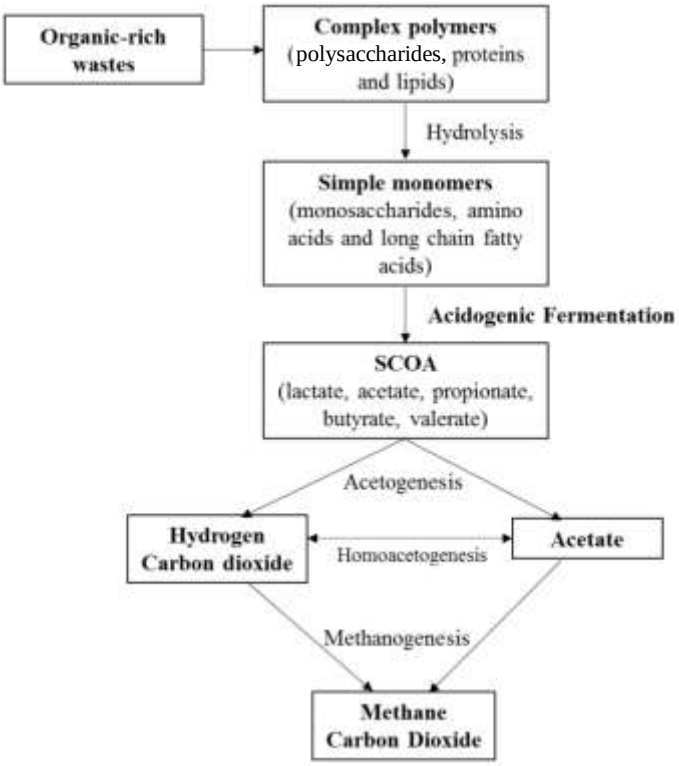

Figure 4. Anaerobic digestion representation[55].

2.3.2. Operational parameters

Like other bioprocesses, SCOA production has been performed with glucose and other pure sugars as the main carbon source which is associated not only with process cost increase but also ethical problems. On another hand, organic wastes provide not only a more rentable carbon source but also a profit waste management strategy[54]. In the last years, several studies explored SCOA production using organic waste such as food waste[56–58], lignocellulosic waste[59,60], waste activated sludge[61,62], among others (Table 2). When selecting substrates for AF it is important to take notice to substrate chemical characteristics. Additionally, it could be needed to feed the system with micronutrients and trace elements, such as nitrogen and metallic compounds, to keep an optimal growth. The supplementation need can be overcome with mixed wastes which could provide suitable nutrients and carbon-to-nitrogen (C/N) ratio for AF[52].

The microbial population present in the bioreactor plays a crucial role in AnD end products[49]. For AF inoculum can be used pure cultures or Mixed Microbial Cultures (MMC), microbial communities with the unknown composition and collected from wastewater treatment tanks (WWTT)[63]. On one hand, pure cultures can be used to achieve specific SCOA production with a high rate of purity. On another hand, MMC does not require sterile conditions, the operation control and equipment requirements are simpler, and they can successfully convert complex substrate therefore, the process cost is lower[49]. Nevertheless, it is necessary to select SCOA producer microorganisms by manipulating biological system parameters and creating a pressuring enviroment[64].

AnD is an anaerobic process, thus MMC used in AF are usually collected from anaerobic WWTT. Methanogens present on MMC use SCOA as substrate so they must be inhibited which can be achieved by heat inactivation of non-pore forming methanogens or by applying inhibitors that act exclusively on methanogens enzymes[52]. Since methanogens prefer pH ranges 7.8-8.2, their growth can be avoided by lower or higher pH. Another alternative to avoid methanogens is selecting anaerobic facultative microorganisms from an aerobic population and used as an inoculum[63]. Besides that, aerobic MMC are reported as more robust microorganisms, tolerating more changes in process parameters.

AF is usually performed at mesophilic temperatures (20-42 ºC) since it is the most efficient and environmentally favorable temperature range for SCOA accumulation[52]. Nonetheless, the increase in temperature has a positive effect on SCOA production since it increases substrate solubility and bacterial activity, thus boosting SCOA production and the SCOA yield[64]. Additionally, each microbial taxon has its optimal temperature, therefore it can be used to promote the growth of certain groups to detriment of others[49].

The pH plays a crucial role not only in AF but also in hydrolysis, thus it must be chosen an optimal pH for both processes. The optimal pH for AF is highly connected with the type of substrate used, but in general, pH ranges from 3.0 to 12, since acidogenic bacteria are negatively affected at extreme pH[52]. In fact, extremely acidic conditions promote the increase of undissociated SCOA which have a strong inhibition effect[65] leading to system perturbations and lactic acid bacteria predominance[63].

It has been reported that SCOA production is promoted by alkaline conditions when using waste sludge as substrate, by neutral conditions for food wastes, and by acidic conditions for wastewaters[64]. Nonetheless, pH 6.0 has been identified as suitable to enhance SCOA production from a variety of substrates[49]. For example, Castilla-Archilla et al.[65] after testing the effect of different pH (4.5-8.0) on SCOA producing from brewery spent grain conclude that pH 6.0 was the most propitious for AF, producing mainly acetic and butyric acids. On the contrary, Bermúdez-Penabad et al.[56] studied the effect of pH 5.0-10 on SCOA production from tuna cannery wastes. They observed that pH 8.0 was the most appropriate for hydrolysis and therefore for SCOA synthesis. However, pH strong alkaline conditions (pH 10) demonstrated to inhibited bacteria metabolism.

Besides that, pH is also an important factor in SCOA's profile. Generally, it is reported that acetic acid production increases with the pH increase from 4.0 to 7.0, while the butyric acid production decreases. Nonetheless, at neutral pH (6.5-7.0), the production of these SCOA became equally abundant. On another hand, pH ranging from 4.0 to 4.5 are the most propitious for propionic acid synthesis[52]. Queirós et. al[63] observed that although acetic, propionic, and butyric acids were the most prevalent SCOA on AF of hardwood sulfite spent liquor, pH lower than 4.6 promoted the production of propionic acid. On the opposite, butyric acid was stimulated at a pH higher than 4.6. Besides that, it was also observed the pH effect

on valeric and lactic since the first reached its maximum at pH 4.8 and decreased as the pH decreased while lactic acid was observed at more acid conditions, reaching its maximum at pH 4.3. Kumar et al.[66] observed that acetic acid was the most produced SCOA in AF of vegetable waste under neutral and alkaline pH, but acidic pH led to a shift of SCOA profile and more butyric acid was produced.

Other operating parameters such as hydraulic retention time (HRT), organic loading rate (ORL), and reactor conformation also affect SCOA's yield and chemical profile and must be taken into account. The HRT refers to the average period that substrate and biomass remain in the bioreactor[49]. The HTR regulation can be used to enhance SCOA production by promoting the contact between microorganisms and substrate for more time and thus increasing substrate solubilization. Additionally, HRT manipulation can be used to wash out slower growing microorganisms and consequently select a specific microbial population[52]. ORL indicates the amount of substrate fed into the bioreactor per day per unit of working volume[49]. By rising ORL, substrate availability increases and consequently SCOA production, too[52].

2.3.3. Strategies to enhance SCOA production

Although AF is a promising process it is necessary to increase its competitiveness counter to the petrochemical process. The strategies for enhancing SCOA production are generally classified into improving hydrolysis rate to produce more soluble substrates for further fermentation, promoting the acidogenic process by manipulating operating parameters, and removing inhibitors factors such as the presence of methanogens or undissociated acids[52].

Hydrolysis is regarded as the rate-limiting step in the AF due to lignocellulosic substrates characteristics. The improvement in the hydrolysis step increases readily available sugars disponibility and enhances SCOA production[52]. There are several physicals, chemical, and biological pretreatment that can be employed to enhance this process (Table 2). Physicochemical pretreatments are widely applied to improve substrate solubility and sugar availability, by removing lignin and hemicelluloses, reducing cellulose crystallinity, and increasing surface area for posterior enzymatic hydrolysis[67]. Girotto et al.[68] observed an improvement in productivity and stability of AnD of SCG after alkaline pretreatment. Later,

the same group verified that ensured a 20 % higher SCOA production than the control, in AF with brewers' spent grains pretreated with alkaline hydrolysis. Similarly, Azizi et al.[69] observed that source-separated organic wastes were effectively disintegrated after hydrothermal pretreatment, and SCOA production increased at 10-18%. More recently, Xu et al.[62] observed a 151.2% increase in maximum SCOA production as well as an increase in acetic, propionic, iso-valeric, iso-butyric, butyric, and valeric acids of 206%, 83%, 97%, 284%, 167%, and 238%, respectively, after AF of waste activated sludge pretreated with HCl trough isoelectric-point pretreatment.

Due to technical, physicochemical pretreatment's economic, and environmental drawbacks, it has been studied the application of biological strategies as an alternative. Fungal pretreatment has aroused interest due to low energy demands, cost-effectiveness process, and enzymatic systems[70]. Tsafrakidou et al.[70] registered similar SCOA production from AF with wheat straw pretreated with alkaline hydrolysis and wheat straw after delignification by the white-rot fungi *Phanerochaete chrysosporium*, demonstrating biological pretreatment efficiency. Besides fungal delignification, lignocellulosic wastes have been also treated with commercial enzymatic cocktails. Bahreini et al.[71] observed a positive effect on AF of wastewater treatment biosolids after adding a broad mixture of cellulase and xylanases. The enzymatic treatment promoted cellulose removal and consequently an increase in SCOA production, with a 86 % yield increase under optimal conditions. More recently its been studied combination of pretreatments to improve the process efficiency. Islam et al.[72] observed a 97 % and 143 % increase in acetic and butyric acids, respectively, after AF of sweet sorghum stalks pretreated with NaOH and cellulase.

Table 2. Review of some of the results achieved for SCOA production with different substrates submitted to different pretreatments. The SCOA profiles are presented in the order lactic, acetic, propionic, isobutyric, butyric, and valeric acids.

Pretreatment	Substrate	Composition	Pretreatment conditions	Operational conditions	[SCOA]	SCOA profile (%)	Reference
Acid Hydrolysis	Brewery spent grain	glucose (12.8 g/L) xylose (12.1 g/L) arabinose (11.8 g/L) *	1.5 % H_2SO_4 121 ºC 20 min	batch 1.75 L anaerobic sludge 37 ºC; 1500 rpm; pH 6	16.89 g COD/ L	0.00/41.7/0.10/0.00/57.8/0.40	[65]
	Corn stover	glucan (36.2%) xylan (19.0 %) araban (2.9%) acetyl group (4.4%) lignin (14 %)	0.6 % HNO3 150 ºC 2 min	batch 200 mL anaerobic sludge 150 rpm 37 ºC pH uncontrolled	2513.4 mg COD/L	0.00/54.1/22.8/0.00/14.1/9.00	[59]
	Waste activated sludge	n.d	4M HCl 25 ºC 5h pH 3.8	batch 250 mL anaerobic sludge 120 rpm 37 ºC pH 7	523.1 g COD/ g VS	0.00/33.0/26.9/8.56/4.74/3.67	[62]
	SCG	n.d	5% H_2SO_4 121 ºC 1 h	batch 100 mL aerobic sludge 28 ºC pH uncontrolled	2.52 gCOD/L	13.5/43.3/11.4/0/31.6/0.2	This work
Alkaline Hydrolysis	Waste activated sludge	soluble protein (20.6 mg/L) soluble carbohydrate (11.6 mg/L)	0.07 g/g TS CaO 15 h	Fed-batch 1L Anaerobic sludge 35 ºC 60 rpm pH uncontrolled	2611.6 mg COD/L	0.00/35.1/14.4/10.1/21.0/5.57	[61]

	Chemically enhanced primary sedimentation sludge	n.d	10 mM NaOH 25 ºC 30 min 200 rpm pH 12	Batch 500 mL 25 ºC pH uncontrolled	138.1 mg COD/ gVS	0.00/48.3/17.4/5.86/10.7/4.71	[73]
	SCG	cellulose (23.5%) hemicellulose (26.4%) lignin (14.3%)	8% NaOH 20 ºC 24h pH > 12	Batch 250 mL anaerobi sludge 35 ºC pH 9	30.0 g/L	0.00/76.7/8.33/0.00/13.3/1.67	[5]
	SCG	n.d	2.0% NaOH 121 ºC 1 h	batch 100 mL aerobic sludge 28 ºC pH uncontrolled	2.21 gCOD/L	5.4/46.1/42.2/2.4/3.2/0.7	This work
Supercritical extraction	Wild dog rose seed residue	crude protein (85.3 g/kg DM) crude fiber (536 g/kg DM) crude fat (7.25 g/kg DM)	2.5 kg/m2 CO_2	batch 40 mL ruminal fluid 39 ºC pH 6.5	72.7 mmol/L	0.00/65.7/16.7/0.92/13.8/1.47	[74]
	Grape pomace	n.d	10 kg/h CO_2 45 °C 3 h 28 MPa	batch 560 mL anaerobic sludge 37 ºC 150 rpm pH 7	22.2 g/L	00.0/77.5/0.50/0.00/21.5/0.50	[75]
	SCG	n.d	12 g/min CO_2 50 ºC 2 h 300 bar	batch 100 mL aerobic sludge 28 ºC pH uncontrolled	1.80 gCOD/L	2.6/55.5/18.9/3.0/18.1/1.9	This work
Enzymatic Hydrolysis	Oil palm fronds	hemicellulose (21%)	Enzymatic extracts from *G.*	Batch 100 mL	71.11 mmol	0.00/64.9/21.1/0.00/14.0/0.00	[76]

		cellulose (49%) lignin (19%)	*lucidum* 40 ºC 5 d $MnSO_4$ and H_2O_2 addition	Rumen fluid 39 ºC pH uncontrolled			
	Corn silage	crude protein (154.5 g/kg) ether extract (26.1 g/kg) neutral detergent fibre (397.5 g/kg) acid detergent fibre (238.5 g/kg) non-fibre carbohydrate (326.8 g/kg)	0.4 g/ kg DM Laccase (100 000 U/g)	Batch Rumen Fluid	126 mM	0.00/66.7/19.1/1.02/10.4/1.21	[77]
	SCG	n.d	Enzymatic extract from *T. versicolor* 40 ºC 7 d 100 rpm	batch 100 mL aerobic sludge 28 ºC pH uncontrolled	0.97 gCOD/L	0.00/84.8/14.7/0.40/0.00/0.00	This work
SSF	Wheat straw	n.d	SSF *Phanerochaete chrysosporium* 35 ºC 21 days 50-85 %MC	Batch 250 mL 37 ºC	6.99 g/L	10.7/55.8/16.3/0.00/17.2/0.00	[70]
	Solid digestate	cellulose (18.1%) hemicellulose (14.6% lignin (31.1%)	SSF *Pleurotus sajor caju* 25 ºC 70 % MC	Batch 30 ºC	240 mg COD/g VS	0.00/47.0/23.0/0.00/15.0/14.0	[78]
	Cow manure and rye residues	n.d	*T. versicolor* 25 ºC pH 4.2 75 % MC 10 days	Batch 1L 100 rpm 37 ºC anaerobic sludge	6500 mg/L	0.00/61.5/30.8/1.92/1.92/0.00	[79]

	Wheat straw	cellulose (30.5 %) hemicellulose (17.8%) lignin (9.4 %)	*T. versicolor* 25 ºC 70 % MC	Batch 400 mL 30 ºC	300 mg COD/g VS	0.00/45.0/35.0/0.00/15.0/5.00	[80]
	Polysaccharidic wastes	cellulose (31.65%) hemicellulose (26.53%) lignin (9.72%)	*Aspergillus fumigatus* pH 5 70 % MC 30 ºC 180 rpm 5 days	Batch 1 L pH 7 37 ºC 300 rpm	9983.49 mg/L	0.00/25.8/5.90/0.00/55.4/12.9	[58]
	SCG	n.d	*T. versicolor* 28 ºC 1:4 (w/V) MC 3 months	batch 100 mL aerobic sludge 28 ºC pH uncontrolled	1.51 gCOD/L	1.3/49.7/28.4/4.6/14.8/1.2	This work
Combined pretreatments	Wheat straw	n.d	1% NaOH 100 ºC 3h SmF *T. viride* 28 ºC 12 dias	Batch 250 mL 37 ºC	7.45 g/L	48.2/18.2/0.00/0.00/12.1/0.00	[70]
	Deoiled microalgae biomass	glucose (220 mg/g), galactose (72 mg/g)*	2 % HCL 121 ºC	Batch 250 mL 30 ºC Anaerobic sludge PH 8	944 mg/L	0.00/85.7/0.00/0.00/14.3/0.00	[81]
	SCG	n.d	12 g/min CO_2 50 ºC 2 h 300 bar 2.0% NaOH 121 ºC 1 h	batch 100 mL aerobic sludge 28 ºC pH uncontrolled	2.06 gCOD/L	8.00/48.2/37.3/2.00/3.80/0.80	This work

n.d – not defined: * composition recorded after pretreatment; The SCOA profiles lower than 100% included other organic acids than the referred above.

2.4. Enzymes of industrial interest

Enzymes are highly specific biocatalysts that accelerate chemical reactions without suffering any chemical change and are used by humans for centuries. However, the purified enzyme's application just became to be used in recent decades. Due to their high substrate and product specificity, moderate operational conditions, low by-products formation, and high yields, they are selected over chemical catalysts for applications in various industries such as food, paper, pharmaceutical, detergent, fine chemical industries, and more recently in biorefineries[82,83]. There are over 3,000 different enzymes described and isolated from a diverse range of organisms[84], and three main industrial categories: carbohydrases, representing around 50% of the industrial market; proteases, with 25 to 30% of the market; and lipases having around 10% market share[85]. With the crescent interest in using lignocellulosic materials as raw materials for bio-based processes, the lignocellulosic enzymes (Table 3) market is expected to increase. The industrial enzyme market is expected to grow from $5.0 billion in 2016 to $6.3 billion in 2021, representing a 4.7% increase at a compounded annual growth rate[86], with over 400 companies producing these proteins[84].

2.4.1. Lignin-degrading enzymes

Lignin degradation involves two enzymatic groups: lignin-modifying enzymes (LME) and lignin-degrading auxiliary (LDA) enzymes. LME are produced by different microorganisms and are directly involved in lignin degradation, comprising phenol oxidase, laccase (Lac), and heme-containing peroxidases, lignin, manganese, and multifunctional peroxidases. On another hand, LDA cannot degrade lignin by themselves but play an indispensable role in the degradation process through the sequential action of multiple proteins. Auxiliary enzymes include several enzymes frequently secreted by white-rot fungi, glyoxal oxidase, aryl alcohol oxidase, pyranose 2-oxidase, cellobiose dehydrogenase, and glucose oxidase. Recently it was found that heme-thiolate haloperoxidases have a role in lignin degradation. Although these enzymes have not yet been classified as LME or LDA, there is no experimental demonstrating that they can perform degradation alone[87].

Lac is one of the most important ligninolytic enzymes and can oxidize multiple phenolic compounds, reducing oxygen to water[88]. This metalloprotein contains two to four copper atoms per molecule in the catalytic site[8,87] and removes one electron from the phenolic nucleus, generating phenoxy free radical products that can be converted to quinone[83,87]. Its oxidative potential can be increased, with the addition of mediators, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid (ABTS), hidroxibenzotriazole, 2,5-xylidine, veratryl alcohol, and ethanol. These mediators are oxidized and release free radicals that can react with diverse substrates, even non-phenolic compounds[8,87]. Lac can be produced by bacteria and fungi, with white-rot fungi being the most efficient lignin-degrading microorganism and secreting several isozymes. Lac production is stimulated by monosaccharides' low concentration, polymeric substrates, and lignocellulose presence. In fact, several lignocellulosic substrates have been reported as a suitable source for Lac production[86].

Peroxidases are heme proteins with a protoporphyrin IX as a prosthetic group[87] which oxidize substrate one-electron in three steps, requiring a peroxide as the electron acceptor. Firstly, H_2O_2 oxidizes peroxidase (state Fe^{3+}), removing one electron from the porphyrin ring forming Fe^{4+} and the first intermediate compound (FIC). This unstable FIC will oxidize the substrate, releasing a free radical and originating a second intermediate compound (SIC). The SIC is reduced by a second substrate molecule and releases a substrate radical, allowing the enzyme to return to the native state[8,87]. Lignin peroxidase (LiP) is a non-specific enzyme involved in the cleavage of phenolic and non-phenolic lignin compounds through the endogenous synthesis of H_2O_2 and releasing aryl and alkyl radicals. Manganese peroxidase (MnP) is responsible for phenolic compounds oxidation in the presence of Mn^{2+}, additionally, products of peroxidation of unsaturated fatty acids can also degrade non-phenolic substrates[87,88].

2.4.2. Tannases

Tannase transforms galotannins, also known as complex or hydrolyzable tannins, into glucose and gallic acid by hydrolyzing ester bonds (galloyl ester of alcohol moiety) and depside bonds (galloyl ester of gallic acid)[89]. This versatile catalyst is responsible for industrial bioconversion of tannic acid to gallic acid, during this process is also released

glucose and various galloyl ester glucose[90]. Tannase can be produced by microorganisms, plants, and animals, but like other enzymes, industrial production is performed by microorganisms. *Aspergillus* and *Penicillium* genus represents the major and more efficient tannase producers, tolerate even 20% tannin[91]. Tannase production depends on the source and culture conditions[90] but the most important factor is the carbon source since it is induced by phenolic compounds as a self-defense mechanism for microorganisms[92]. Tannases can be applied in food, beverage, pharmaceutical, cosmetic industries, and bioremediation[89,90].

Table 3. Industrially produced lignocellulosic enzymes.

Substrate	Enzyme	E.C number	Synonyms	Action	Reference
Cellulose	endoglucanase	3.2.1.4	endo-(1,4)-β-D-glucanase endocellulase carboxymethyl cellulase	β-1,4-glycosidic bonds cleavage	[86]
	exoglucanase	3.2.1.176	exo-(1,4)-β-D-glucanase exocellulase microcrystalline cellulase cellobiohydrolase	cellobiose removal	
	β-glucosidase	3.2.1.21	cellobiase	release glucose from cellobiose and short-chain cellooligosaccharides	
Mannan	β-mannanase	3.2.1.78	1,4-β-D mannohydrolases	glycosidic bonds cleavage	[93]
	β-mannosidase	3.2.1.25	1,4-β-D mannopyranoside hydrolases	mannobiose degradation	
	β-glucosidase	3.2.1.21	1,4-β-D glucoside glucohydrolases	β-1,4-glucopyranose units excising	
Xylan	endoxylanase	3.2.1.8	endo-1,4-β-xylanase	xylose hydrolysis	[94]
	β-xylosidase	3.2.1.37	exo-1,4-β-xylosidase	xylooligomers hydrolysis	
Lignin	laccase	1.10.3.2	oxygen oxidoreductase	phenolic compounds oxidation	[87]
	lignin peroxidase	1.11.1.14	ligninase diarylpropane oxygenase		
	Manganese peroxidase	1.11.1.13	Mn^{2+}: hydrogen peroxide oxidoreductase		

2.5. Microbial enzyme production

All organisms produce enzymes, nonetheless, more than 90% of the industrial enzymes are produced by microorganisms[84]. These are the preferred biocatalyst's producers since their life cycle is brief and fast, with regular and high enzyme production in a short time. Another great advantage is the fact that their extracellular enzymes are secreted into cultivation media (favoring enzyme extraction) and enzymes can be stored without losing activity under ideal conditions. Besides that, microorganisms are easy to handle and industrial scale-up is well established[83]. However, it is necessary to guarantee that the selected microorganism is safe, non-toxic, and non-pathogenic, to be commercially exploited. The majority of bacterial and fungal strain industrially applied are widely studied and have been used in many commercial applications, but wild-type strains can also be a commercial opportunity[82].

Although it is a growing market, enzyme production is still capital-intensive with the major cost being equipment and installation (48%) as well as the raw material used (28%), reflecting in highly-priced products[82]. To increase biological treatment competitiveness is necessary to lower enzyme costs which can be achieved by using cheap substrates and integrating enzyme production in a biorefinery plant. This way, the costs associated with transportation, purification, and stabilization will decrease and the enzymes obtained will be more suitable for substrate hydrolysis[7].

Like other biobased compounds, enzymes are majorly produced by Submerged Fermentation (SmF). In this process, the microorganism is cultivated in a liquid medium inside a reactor providing a sterile and homogenous environment, where physical and chemical parameters (pH, temperature, nutrients, and oxygen distributions) can be controlled. On the other hand, the homogenous environment can induce oxidative stress and affect the process, when harmful compounds are present. Beyond this, the large and complex equipment increase substantially the process price[82]. And in order to use lignocellulosic residues as substrate, biomass needs to be submitted to pretreatments that involve high energy and water demand and can result in environmental damage[95].

More recently, another fermentation strategy has aroused interest in biotechnology applications, especially in regions with abundant agro-industrial wastes. Solid-state

fermentation (SSF) is an ancient process where microorganisms are cultivated in a resembling natural environment. For this, moist solid substrates are used and none or very low free water is present. This strategy is propitious to aerobic fungal species, especially filamentous fungi, and does not imply necessarily fermentation metabolic pathways. Compared to SmF (Table 4), SSF is the more eco-friendly strategy with low effluent production, and it is more rentable, once microorganisms are less susceptible to metabolic repression, ensuring high volumetric productivity with high product concentration[82,96,97]. In fact, reports can be found in the literature for enzyme production through SSF being more favorable[95,98]. However, the difficulty of guarantee process and standard physicochemical parameters has been appointing as a problem to allow the process scale-up which have been shown as backdowns to SSF large-scale application[95].

Table 4. Comparison between submerged fermentation and solid-state fermentation for enzyme production.

SmF	SSF
• Easy control of operational parameters	• High productivity and stability of products
• Sterile and homogenous environment	• Simple and cheap
• Well implemented process	• Environmentally friendly
• Large and complex equipment	• Possibility of detoxification of organic waste
• Higher cost and environmental impact.	• Difficult control of operational parameters

When applying SSF the microbial strain used, substrate characteristics and fermentation physicochemical parameters should be taken into account[99]. From these, microorganisms selection is the most important criterion and it is necessary to ensure its suitability for the substrate and capacity of synthesizing the target product. To improve the success the substrate must be inoculated with high inoculum concentration to speed up substrate colonization and avoid possible contaminations with other microorganisms[100].

To select the substrate, particle size, water absorption capacity, and chemical composition should be considered[96]. The most favorable particle size depends on other substrate factors and in the microorganism used but particles need to be small enough to provide a high surface area permitting microorganisms to attach, but large enough to avoid clustering and allow appropriated gas diffusion. This can be achieved by selecting substrates

with heterogeneous particle sizes or particles with intermediate size[100]. The substrate physical characteristic can be directly affected by the substrate water retention capacity, i.e. substrate capacity to absorb water; and the critical humidity point, the amount of water bound to the support, and not available to the microorganism. The ideal substrate should have high water absorption capacity but low critical humidity point in order to ensure a constant and adequate moisture content (MC) during the fermentation process but without compromise gas exchange, nutrient diffusion, and consequently microorganism biological activities. If the desirable solid waste substrate does not have the previous characteristic it can be balanced with the continuous water addition during fermentation or by using solid substrates mixtures with water absorption capacities[101]. Besides physical characteristics, substrate chemical composition is crucial to obtain the desired product during fermentation. It is necessary to guarantee the adequate C/N ratio to allow microbial growth and the presence of expression inductors will stimulate metabolites production[102]. After selecting the most suitable substrate, it can be submitted or not to pretreatment to reduce particle size and increasing nutrients availability. However, it is necessary to take into account that pretreatments can be expensive and, consequently, increase product price[99].

During fermentation, metabolic processes will lead to pH variations and heat release. When accumulated, the heat generated by microorganisms increases temperature and provokes moisture loss, impacting microorganism's growth, enzyme production, and consequently its denaturation. This is especially problematic in large-scale systems, but it can be overcome by blown air into the system, which forces the heat to exit or installing a cooling system. Contrary to temperature, pH in SSF is difficult to measure and control since the available electrodes are not fitted for pH measuring in a solid medium, thus it is necessary to select microorganisms with wide-range pH[100].

The main drawback of SFF, regarding the industrial application, is the fact that the scale-up process can be difficult. The key issues rely on heat removal, substrate compaction, and limited oxygen transfer. Currently, there are two main reactor configurations, the static reactors, tray or packed bed reactors, where the aeration is forced; or mixing reactors such as rotatory drum or horizontal paddle[95]. Static reactors are a simpler and economical option and have been successfully used for enzyme production. However, it can be difficult to control

substrate temperature, leading to protein denaturation. On the other hand, the mixed reactors can overcome the heat removal problem and improve substrate homogeneity, but it implies major costs and agitation can damage filamentous fungi cells[100]. The type of bioreactor along with the substrate selected will also impact the downstream process: extraction and conservation[95]. Enzyme recovery can be performed by multiple fraction technics such as precipitation, centrifugation, and ultrafiltration. When isolating the enzyme is important to find a balance between quality and quantity, ensuring a suspension with high activity and low contaminants. For that, the process must be performed under the enzyme's optimal pH, this can be achieved by employing antimicrobial buffers, which will avoid pH variations and decrease possible contaminations. Besides that, detergents can be added to facilitate the release of enzymes from the membrane, reducing agents to avoid the oxidation of thiol groups when the enzyme is released for the exterior, and proteolytic inhibitors to prevent protease action on the protein of interest[82]. Although SSF studies focus on enzyme production, this strategy can also be applied to obtain antibiotics, organic acids, biopesticides, aromas, biofuels, bioplastics, biosurfactants, single-cell protein, and food[95,97,99].

Due to their characteristics, SCG have been applied as a substrate for enzyme production under SSF (Table 5). Murthy et al.[103] studied the production of α-amylase by *Neurospora crassa* under SSF using coffee waste as a substrate. From the various substrates, SCG gave the lowest enzyme activity (2844 U/g). The same group studied xylanase production from *Penicillium sp*. using coffee residues and once again, SCG had the lowest performance (1542 U/g)[104]. The explanation for these results is not explored, but substrate particle size is appointed as an important factor for the process. In fact, Machado et al.[105], who studied the removal of phenolic compounds from coffee residues, using fungal strains observed that fungal biomass growth was superior on coffee silverskin than in SCG. They suggested that SCG smaller particles promoted compaction and consequently less aeration, preventing fungal growth in between the particles. More recently, Mansor et al. [106], evaluated the production of tannase using different substrates. Once again, SCG registered lower enzyme activity and fungal growth when compared with the other substrates. It was proposed that the high tannin content might inhibit fungal growth and consequently tannin metabolism. However, when used as a supplement in rice bran, SCG enhanced tannase activity by 1.8-

fold and 3-fold, when compared with single rice bran and rice bran supplemented with tannic acid.

Besides enzyme production, studies have been performed to remove SCG bioactive compounds and producing edible mushrooms (Table 5.)[107,108]. All these works demonstrated that SSF is a feasible strategy to perform SCG detoxification and producing microbial metabolites. However, the microorganism strains and conditions applied could not be the most adequated resulting in low productivity when compared with other substrates. The authors conclude that SSF products by themselves did not represent a profitable option but, it can be integrated as important detoxification and hydrolyzing step in an SCG biorefinery[7].

Table 5. Studies performed using spent coffee grounds as substrate under solid-state fermentation.

Strain	Product/ application	Reference
N. crassa	α-amylase	103
Penicillium sp.	xylanase	104
P. porpurogenum *N. crassa* *N. Mucor*	phenolic compounds removal	105
Aspergillus niger	tannase	106
Flammulina velutipes	edible mushroom caffeine and tannin degradation	107
Bacillus clausii	increase bioactive compounds content antioxidant and antimicrobial activity evaluation	108

2.5.1. Filamentous fungi as enzymes producers

Being decomposers, filamentous fungi play an important ecological role by recycling organic matter, degrading complex polymers such as lignocellulosic materials into small and simpler molecules. To obtain the necessary nutrients, fungi secrete a wide variety of metabolites including enzymes, organic acids, and antimicrobial compounds, which have

industrial and commercial value. Filamentous fungi, such as *Trichoderma reesei*, are the main enzyme producer for biomass conversion since they can use complex materials as substrates with high productivity of enzymes with high activity and thermal stability[39]. Therefore, fungal enzymes have been exploited for industrial production for decades and applied in multiple industries. To improve metabolites production, mutagenesis along with suitable fermentation conditions have resulted in more efficient strains[109].

2.5.1.1. Trametes versicolor

Trametes genus is widely identified as a rich source of bioactive compounds for medicinal, biotechnological, and environmental applications. *T. versicolor* was formerly classified as *Agaricus versicolor, Boletus versicolor, Coriolus versicolor*, *Polyporus versicolor, Polystictus versicolor, and Poria versicolor*[110]. Its characteristics can be influenced by genetic and harvesting conditions allowing its utilization for multiple finalities such as food and pharmaceutical applications[8]. In fact, in China is used in folk medicine due to its antioxidant, anti-inflammatory, immune-enhancing, anticancer, antiviral, antimicrobial, and prebiotic activity[88,111]. As a with-rot fungus, *T. versicolor* plays an important ecological function as a wood primary decomposer by the production of ligninolytic enzymes, especially LiP, MnP, cellobiose dehydrogenase, and Lac isoenzymes[97]. Due to its widens degrading spectrum and intense oxidative action, these enzymes degrade other complex products other than lignin such as xenobiotic compounds, pesticides, aromatic hydrocarbons, chlorinated organic compounds, among others[8,88].

From all enzymes produced by *T. versicolor*, Lac production is the most studied, with its application on lignin degradation and degradation of toxic compounds. Lac production is often associated with secondary metabolism[112] and after an active cellular growth. Therefore, in the early stages, the fungus must have the appropriate carbon supply. Sugars availability can be increased when the substrate is submitted to pre-treatments[113] or by adding sugar from external sources. From several carbon sources, glucose, fructose, sucrose, maltose, and molasses (1.0% w/w), Iqbal et al.[114] found that for Lac production, glucose was the most efficient option with 98.05 U/mL. However, excessive concentrations can be inhibitory[112]. Besides that, nitrogen source and content directly affect Lac production[115]. Aydinoğlu et al.[116]

observed that yeast extract was the most favorable nitrogen source from inorganic and organic supplements, but concentrations above 1.0% (w/w) affected negatively enzyme activity. Almeida et al.[117] reported that non-protein sources such as urea as a good inducer for Lac. More recently, Pinheiro et al.[118] reported an increase of 30 % in Lac production by adding 0.1 % peptone into the fermentation medium.

Additionally, some xenobiotics such as inorganic metallic salts and phenolic compounds can be used as inducers[112]. These will reduce the exponential phase, promoting an early stationary phase and lignin degradation[119]. Xin et al.[120] tested copper sulfate, veratryl alcohol, guaiacol, pyrogallol, syringic acid, caffeic acid, and ethanol, and enzyme activity increased in 2.8, 1.9, and 1.5-fold when using veratryl alcohol, copper sulfate, and ethanol, respectively, while the others compounds did not have significative effects. Mishra et al.[119] studies, showed that cooper sulfate (4.9-fold), gallic acid (3.5-fold), and synringic acid (5.6-fold) were the supplements that most improved Lac activity. This can be due to synringic acid and gallic acid structural similarities with lignin. On another hand, surfactants can promote water permeation on the substrate and increase superficial areas. On Lac production, Tween 80 is the surfactant used since it transforms cellular membrane structure and promotes enzyme excretion. Iqbal et al.[114] tested multiple concentrations of this compound and found that 0.3 mL of 1 mM Tween 80 was the most adequate.

As a white-rot fungus, ligninolytic enzyme production by *T. versicolor* is more propitious in a range of 25 to 30 ºC[114]. When applying higher or lower temperatures, Lac activity will decrease since it may occur because of enzyme denaturation or metabolic deceleration[120]. Lac optimal pH ranges from 4.5 to 6.0[112], therefore its production may be performed under these conditions. Iqbal et al.[114] performed SSF at pH from 3.0 to 5.0 and obtained maximum activity at pH 4.0. However, Adekunle et al. [113] verified that there are no significant differences between pH 4.0 or 5.0.

On lignocellulosic valorization, Wyman et al.[121] treated corn stover with ligninolytic enzymes from *Pleurotus eryngii*, *Pleurotus ostreatus*, and *T. versicolor*, for posterior biogas production. When treated, the biomass showed a 19% increase in biogas production, proving that enzyme production by filamentous fungi and biogas production can be carried as a sequential strategy. In addition to Lac ligninolytic activity, SSF studies have been performed

to degrade pollutants as a potential strategy to treat water effluents. Merino et.[122], used corncob as dye absorbent and posteriorly treated it with *T. versicolor* and *Pleuorus ostreatus* co-culture obtaining maximum activity of 19.8 and 5.4 U/g of Lac and manganese peroxidase, respectively. Lac expression was stimulated by the presence of nitrogen from organic nature, and enzyme production was favored by C/N ratios lower than 30, because of synthesis activation. Since Lac contains coppers, several studies reported that it induces Lac production. However, in this case, the increase in copper concentration affected negatively the fungus growth, especially in the inoculum initial phase, and MnP.

Besides ligninolytic enzymes, *T. versicolor* has also a high cellulolytic activity which can be improved when supplementing with $CuSO_4$, gallic, and syringic acid. Mishra et al.[119] evaluated sorghum bagasse lignin degradation and enzymatic saccharification, obtaining a wide range of enzymes which activity was improved when supplemented with these compounds, observing Lac 4.9-fold, polyphenol oxidase 1.9-fold, MnP 2.5-fold, LiP 13-fold, and arylalcohol oxidase 2.8-fold. In Cardoso et al.[123] works, *T. versicolor* was revealed to have the highest cellulase activity (144.88 U/mL) among *Pycnosporus sanguineus*, *Pleurotus ostreatus*, *Pleurotus eryngii*, and *Phanerochaete chrysosporium*.

2.5.1.2. Paecilomyces variotii

The genus *Paecilomyces* is based on the species *Paecilomyces variotii*, an asexual fungus that can be found worldwide in soils, animals, indoor environments, and food products and even cause human infections[124,125]. Although the optimum growth temperatures for most genus *Paecilomyces* members range from 30 to 37 ºC[125], *P. variotii* can tolerate brief exposure to high temperatures (80-100 ºC) for up to 15 min[124]. Besides its thermotolerance is also capable of growing in low oxygen levels[10] and maintaining a high-level substrate degradation, even when the conditions do not allow other microorganisms to grow. The ability to grow in common agro-industrial derivatives and degrade their toxic contaminates, aliphatic and aromatic hydrocarbons as well as phenolic derivates, is possible due to *P. variotii* secretome. The fungus presents a promising source of numerous enzymes such as amylases, chitinases, pectinases, phytases, tannases, and xylanases[126].

This ascomycete represents an interesting option for biotechnological applications, particularly in food and animal feed. The earliest application was the fermentation of Maotai-flavored liquor, an alcoholic Chinese drink, by SSF. Later, *P. variotii* became to be applied in the production of Single Cell Protein (SCP) by the Pekilo process, where agro-industrial waste was used as a low-cost substrate allowing not only substrate detoxification but also fungi biomass increase. The Pekilo biomass, rich in fungal protein (50%), can be used as protein origin in animal feed or for human consumption[126]. Besides this, enzymes from *P. variotii* also represent a valuable product. From an industrial point of view, *P. variotii* enzymes, tannase, glucoamylase, β-glucosidase, and alcohol oxidase, have favorable properties, being stable at high temperatures and over a wide pH range[10].

Enzyme production by *P. variotii* has been focusing on tannase production. Although tannases can be produced by different fermentation strategies, Raaman et al.[127] demonstrated that SSF is the most efficient fermentation strategy allowing maximal extracellular tannase production to reach 167 U/mL, over 123 U/mL in SmF, and 102 U/mL in liquid-surface fermentation. Besides higher yields, the enzyme produced under SSF did not decrease as much as in the other fermentation strategies, demonstrating that SSF proteins are more stable. Tannase was successfully produced using organic waste as a substrate and its production is carbon source-dependent. It is directly proportional to microorganism growth and can be enhanced by an additional carbon source. From arabinose, fructose, glucose, lactose, maltose, mannitol, raffinose, rhamnose, sucrose, sorbose, starch, and xylose, the addition of 1.0 % (w/v) glucose empowered enzyme production, however, values greater than 3.0 % (w/v) were inhibitory. In another way, the substrate may not have the necessary amount of nitrogen which can also be added by an external source such as ammonium nitrate, ammonium sulfate, ammonium phosphate, sodium nitrate, sodium nitrite, potassium nitrate, thiourea, urea, and ascorbic acid. Raaman et al.[127] observed that 0.2 % (w/v) of sodium nitrate was the best option, however, Madeira et al.[128] observed an increase in tannase activity when adding 5.0 % (w/v) of ammonium sulfate. Besides carbon and nitrogen source, tannase production can also be maximized by the addition of tannic acid, a tannase inductor. Madeira et al.[129] improved the process by applying 6.0 % (w/w) tannic acid, later, the same group succeed using only 3.0 % (w/w), and by reducing the amount of inductor necessary, it also reduced the costs associated with the process[130].

It is also necessary to have into account other factors on tannase production. Tannase can be active in a wide range of temperatures (20 to 60 ºC) and it remains active in an acidic environment (pH 4.0 to 6.5). Regarding substrate physical characteristics, Madeira et al.[128] observed that the most appropriate particle size was 0.7 mm, which ensures a suitable contact area between the organism and the substrate while the increase in particle size is associated with a decrease in the contact area and consequently in the production of enzymes. Another important factor in SSF is relative humidity applied, it is important to ensure the water disponibility, however, MC above 90 % can limiting enzyme production[129].

This enzyme can also be simultaneously produced with phytase using agro-industrial residues. Madeira et al.[129], performed simultaneous production of tannase (2600 U/g) and phytase (260 U/g), while detoxification and reducing antinutritional factors in castor bean residues. Later, the same group obtained successfully tannase (5000 U/g) and phytase (350 U/g) production using orange pomace as substrate, showing that phytase can be produced under conditions for tannase production. The production of the two enzymes was stimulated by the supplementation of tannic acid and contrary to other studies, concentrations above 7.0 % (w/w) led to a reduction in enzymatic activity[130]. Besides detoxification, tannase produced by SSF was also employed to produce phenolic compounds and subsequently increased antioxidant activity from citrus residues[131].

Besides tannase, studies also explored other enzyme production. In Amutha et al.[132] work, wheat bran was used as the substrate for xylanase production under SSF and after 7 days it was obtained 0.0206 U/g. Even though this value would imply that *P. variotii* is not an optimal xylanase producer, more recently, Pathak et al.[133] efficiently obtained carboxymethyl-cellulase (383.95 U/g), filter paper activity (27.37 U/g), and xylanase (4842.93 U/g), using *Jatropha curcas* deoiled cake, using an inoculum dose of 10 %.

2.6. Objectives

As previously referred, coffee is one of the most consumed beverages and its processing generates annually tons of organic wastes such as SCG. Due to their composition rich in polysaccharides, this residue is an interesting substrate for SCOA production. Nonetheless, SCG recalcitrant composition requires the application of pretreatments. From various pretreatments used, enzymatic hydrolysis is highly efficient and environmentally friendly, however, enzyme application is associated with high costs. This drawback can be overpassed by using organic wastes as substrate and integrating enzyme production on a biorefinery plant. As decomposers, the filamentous fungi *Trametes versicolor* and *Paecilomyces variotii* secrete a wide variety of hydrolytic and oxidative enzymes capable of degrading SCG 's complex polymers.

Therefore, this work aims to study the enzymatic production by filamentous fungi *Trametes versicolor* and *Paecilomyces variotii* and select the best conditions for some enzymes production. Moreover, this work also pretends to evaluate biological and physicochemical pretreatments efficiency for short-chain organic acids production by acidogenic fermentation.

Bearing in mind the previously described, this study can be viewed from a CE perspective since it allows a more environmental-friendly and profitable waste management strategy, following United Nations sustainable development goals.

3. Materials and Methods

3.1. Experimental Design

Figure 6 represents a schematic overview of the several set-up and methodologies used in this thesis.

3.2. Enzymatic Fermentation

3.2.1. Microorganisms and maintenance conditions

The filamentous fungi used in this study were *Paecilomyces variotii*, NRRL-115 supplied by Agricultural Research Service Culture Collection at National Center for Agricultural Utilization Research, USDA; and *Trametes versicolor* CBS 109428 supplied by the Westerdjik Fungal Biodiversity Institute. Cultures were maintained in Petri plates with malt extract agar (30 g malt extract, 5 g peptone, and 15 g agar per liter of distilled water) kept at 4 ºC. Every month, replication was made using a sterile loop: an agar square was transferred from a fully grow plate to a new medium plate and was kept at 28 ºC for 7 days to get a fully grown plate (Figure 5).

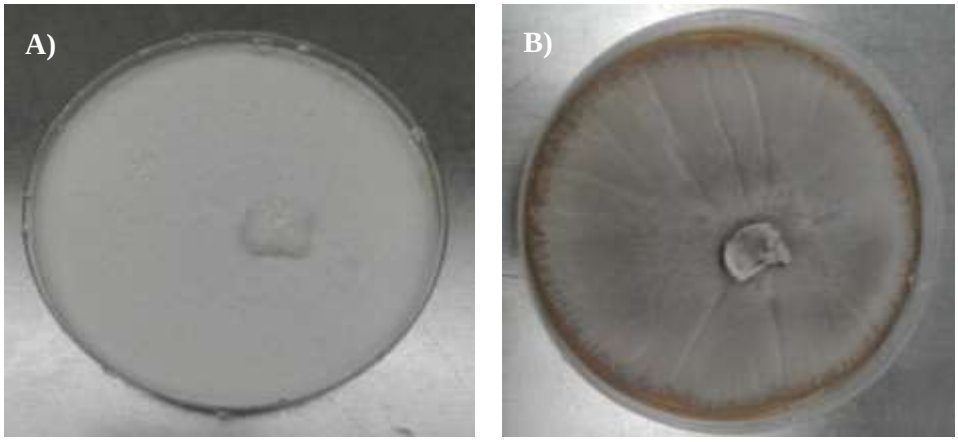

Figure 5. Filamentous fungi after 7 days of growing: A) *Trametes versicolor*, B) *Paecilomyces variotii*

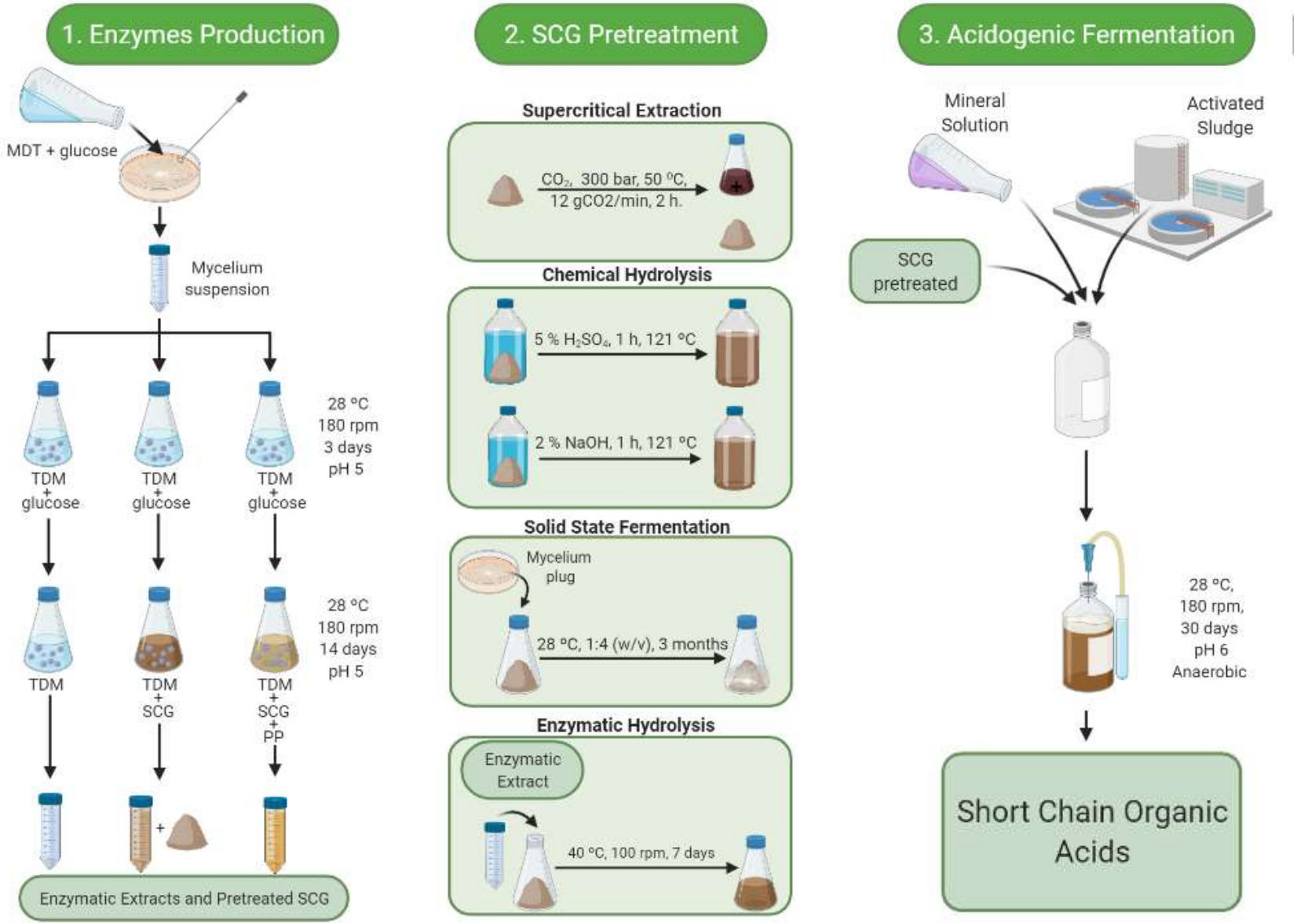

Figure 6. Experimental work overview (created with BioRender.com)

3.2.2. Enzymatic production medium

Enzyme production was evaluated using the *Trametes* Defined Medium (TDM)[134] with 1.0 mL/L of vestigial elements solution to stimulate enzyme excretion (Table 6), and the pH was adjusted to 5.0. All the media, solutions, and materials were autoclaved at 120 ºC for 20 min. To avoid the Maillard reaction, the glucose solution was autoclaved separately.

Table 6. *Trametes* defined medium and vestigial elements solution composition.

TDM		**Vestigial elements solution**	
Nutrient	Concentration (mM)	**Nutrient**	Concentration (µM)
Glucose	83	**$FeSO_4.7H_2O$**	20
Glutamine	5.0	**$CuSO_4.5H_2O$**	2.0
NaCl	5.0	**$ZnCl_2$**	5.0
KH_2PO_4	5.0	**$CoCl_2.6H_2O$**	6.0
$MgSO_4.7H_2O$	1.0	**$NiCl_2.6H_2O$**	0.1
$CaCl_2$	0.1	**$MnSO_4.H_2O$**	20
2,2-dimethyl succinate	10	**$(NH_4)_6MO_7O_{24}.4H_2O$**	0.5

3.2.3. Inoculum preparation

For inoculum preparation, 10.0 mL of TDM was added to a fully grown plate. The mycelium was removed and suspended in the medium with a sterile loop and transferred to a previously sterilized Erlenmeyer to obtain a concentrated mycelium suspension. 1 mL of mycelium suspension was used to inoculate 250 mL TDM with glucose, in 500 mL Erlenmeyer flasks and incubated at 28 ºC under agitation at 180 rpm.

To estimate biomass concentration in the inoculum, 5.00 mL mycelium suspension was filtered with a paper filter (0.45 µm) under sterile conditions and transferred into a crucible, which was previously weighted along with the filter paper. The sample was dried at 105 ºC for 24 h and cooled at a desiccator. When cool, the sample was weighed, and the dry weight was calculated. This procedure was replicated three times per sample.

3.2.4. Enzyme production

Three days after inoculum preparation (Figure 7. A) Trametes versicolor mycelium growth on Trametes defined medium with glucose. B) Submerged fermentation setup for enzyme production using spent coffee grounds and pineapple peel as inducers., the culture was filtered through a filter paper (0.45 µm) under sterile conditions. The biomass retained on the filter was washed with TDM without glucose and transferred to 500 mL Erlenmeyer flasks with 250 mL TDM without glucose with a cotton plug. The flasks were incubated at 28 ºC, under the agitation of 180 rpm, for 14 days.

3.2.4.1. Study of food waste inducer effect

On the third day after inoculation, SCG, and pineapple peel (PP) were added to study the inducer effect of food waste (Figure 7B). The four conditions tested were the sole effect of SCG using two distinct concentrations (0.4 and 1.0 % w/v) and the combined effect of SCG and PP using two concentrations (0.4 % w/v of SCG + 0.2 % w/v of PP and 1.0 % w/v of SCG and 0.4 % w/v of PP).

Every day, a 2.0 mL sample of the fermented medium was collected, the sample was centrifuged at 13,000 rpm for 10 min (Centrifuge MiniSpin, Eppendorf). The pellet was discharged, and the supernatant was used to assess the enzymatic activity.

At the end of the fermentation assay, the remaining fermentation medium was centrifugated at 5,000 rpm for 1 h (Centrifuge Thermo, Thermofisher). The pellet was discharged except for the assays where SCG was used as an inducer. For those, the SCG was sterilized under UV radiation for 30 min, dried at 105 ºC for 24 h, and stored to be further used in acidification assays. The supernatant was filtered with a paper filter (0.45 µm) under sterile conditions and stored at -16 ºC for further enzymatic treatment.

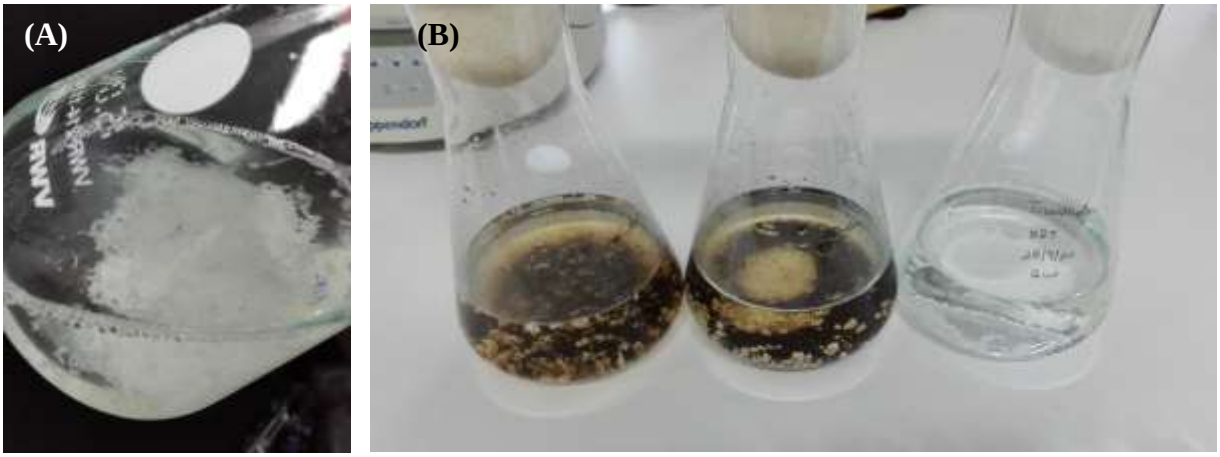

Figure 7. A) *Trametes versicolor* mycelium growth on *Trametes* defined medium with glucose. B) Submerged fermentation setup for enzyme production using spent coffee grounds and pineapple peel as inducers.

3.3. SCG pre-treatment

SCG were submitted to different physicochemical and biological pretreatment, and the pretreated SCG were used as the substrate for acidification assays. The pretreatment conditions employed are summarized in

Table *7*.

3.3.1. Supercritical extraction

SGC were submitted to supercritical CO_2 extraction at 300 bar, 50 ºC, 12 gCO_2/min for 2 h. After pretreatment, the extracted oil portion was separated and chemical oxygen demand (COD) from the extracted SCG were used in the acidification assays was evaluated.

3.3.2. Chemical hydrolysis

Two types of chemical hydrolysis were performed with 1.5 g of SCG suspended in 40.0 mL of deionized water. Acid hydrolysis was performed with 5% H_2SO_4 at 121 ºC for 1 hour in the autoclave. Similarly, alkali hydrolysis was performed with 2% NaOH. After pretreatment, COD from the suspension obtained was evaluated.

3.3.3. Enzymatic hydrolysis

The pretreatment was performed using 1.5 g of SCG in 40.0 mL of enzymatic extracts obtained in section 3.2.4 Enzyme production, at 40 ºC with an agitation of 100 rpm, for 7 days and, COD of the suspension obtained was evaluated.

3.3.4. Solid-State Fermentation

Solid-state fermentation was performed in cotton plugged 250 mL Erlenmeyer using 5 g of SCG as substrate (Figure 8). The moisture content was assured by adding water on a 1:4 (w/v) ratio. The substrate was inoculated with a 3 cm diameter agar plug from a fully grow plate and the cultures were incubated at 28 ºC for 3 months.

Figure 8. Solid-state fermentation of spent coffee grounds by filamentous fungi *Trametes versicolor and Paecilomyces variotii.*

Table 7. Pretreatment and respective conditions used to treat spent coffee grounds.

Pretreatment		Conditions
Acid Hydrolysis	AH	5% H_2SO_4 at 121 ºC for 1 h in autoclave
Alkaline Hydrolysis	BH	2% NaOH at 121 ºC for 1 h in autoclave
Supercritical extraction	SE	CO_2 extraction at 300 bar, 50 ºC, 12 g CO_2/min for 2 h
Supercritical extraction + Acid Hydrolysis	SE+AH	CO_2 extraction at 300 bar, 50 ºC, 12 g CO_2/min for 2 h followed by 5% H_2SO_4 at 121 ºC for 1 h in the autoclave
Supercritical extraction + Alkaline Hydrolysis	SE+BH	CO_2 extraction at 300 bar, 50 ºC, 12 g CO_2/min for 2 h followed by 2% NaOH at 121 ºC for 1 h in the autoclave
Solid State Fermentation	TvSSF	28 ºC for 3 months 1:4 (w/v) MC with *T. versicolor*
	PvSSF	28 ºC for 3 months 1:4 (w/v) MC with *P. variotii*
Submerged Fermentation	TvSmF	28 ºC, 180 rpm for 15 days with *T. versicolor*
	PvSmF	28 ºC, 180 rpm for 15 days *P. variotii*
Enzymatic Hydrolysis	TvS	Enzymatic extract obtained from *T. versicolor* with SCG as an inducer (Laccase 73 U/L) at 40 ºC, 100 rpm for 7 days
	TvP	Enzymatic extract obtained from *T. versicolor* with SCG and PP as inducers (Laccase 49 U/L) at 40 ºC, 100 rpm for 7 days
	PvM	Enzymatic extract obtained from *P. variotii* (enzymatic activity not determined) with no inducer at 40 ºC, 100 rpm for 7 days
	PvS	Enzymatic extract obtained from *P. variotii* (enzymatic activity not determined) with SCG as inducer at 40 ºC, 100 rpm for 7 days
	PvP	Enzymatic extract obtained from *P. variotii* (enzymatic activity not determined) with SCG and PP as inducers at 40 ºC, 100 rpm for 7 days

AH- SCG pretreated with acid hydrolysis; BH – SCG pretreated with alkaline hydrolysis; SE – SCG pretreated with supercritical extraction; SE+AH - SCG pretreated with supercritical extraction and acid hydrolysis; SE+BH - SCG pretreated with supercritical extraction and alkaline hydrolysis; TvSSF – SCG pretreated with solid-state fermentation by *T. versicolor*; PvSSF - SCG pretreated with solid-state fermentation by *P. variotii*; TvSmF - SCG pretreated with submerged fermentation by *T. versicolor;* PvSmF - SCG pretreated with submerged fermentation by *P. variotii;* TvS – SCG pretreated with the enzymatic extract obtained from *T. versicolor* with SCG as an inducer; TvP - SCG pretreated with the enzymatic extract obtained from *T. versicolor* with SCG and PP as inducer; PvM – SCG pretreated with the enzymatic extract obtained from *P. variotii* without inducers; PvS - SCG pretreated with the enzymatic extract obtained from *P. variotii* with SCG as inducer; PvP - SCG pretreated with the enzymatic extract obtained from *P. variotii* with SCG and PP as inducers.

3.4. Acidogenic fermentation assays

3.4.1. Inoculum

An anaerobic mixed microbial culture, MMC, collected from an aerobic tank of wastewater treatment plant (WWTP) Aveiro Sul, SIMRia maintained at 4 ºC was used as inoculum.

3.4.2. Substrate

SCG were provided by the coffee shop at the Chemistry Department of the University of Aveiro. The substrate was dried in an oven at 105ºC, at the list for 24 h until a constant weight was observed.

3.4.3. Mineral Solution Supplementation

Acidification assay medium was supplemented with a mineral solution of 160 mg/L of NH_4Cl, 160 mg/L of KH_2PO_4, 80 mg/L of $CaCl_2$, 160 mg/L of $MgSO_4$, 800 mg/L of $NaHCO_3$, 200 mg/L of $CoCl_2$, 30 mg/L of $MnCl_2$, 10 mg/L of $CuCl_2$, 100 mg/L of $ZnSO_4$, 300 mg/L of H_3BO_3, 30 mg/L $(NH_4)_6Mo_7O_2$ and 20 mg/L of $NiCl_2$.

3.4.4. Batch tests

To study the pretreatment effect on SCG, batch tests were conducted on flasks with 100 mL of working volume (Figure 9). Each flask was inoculated with the 0.15 g COD of aerobic sludge, supplemented with the mineral solution, and 1 g COD of SCG submitted to different pretreatments (

Table *7*). The flasks were encapsulated and purged with N_2, to ensure anaerobic conditions, and incubated at 28 ºC with constant stirring for 26 days on control assay and 29 to 30 days for pretreatment assays.

Every day, a 2.0 mL sample was collected under anaerobic conditions and centrifuged at 13,000 rpm for 10 min (Centrifuge MiniSpin, Eppendorf). The pellet was discharged, and the supernatant pH was assessed. The supernatant was stored at -16 ºC for further determination of SCOA, glucose, and xylose concentrations.

Figure 9. Batch experiments setup for short-chain organic acid production.

3.5. Analytic methods

3.5.1. Enzymatic activity determination

3.5.1.1. Laccase

The Lac activity was measured according to Ander and Messner[135] procedure, using 2,2-Azino-bis(3-ethyl- benzothiazoline-6-sulfonic acid) (ABTS) as substrate and assuming that one Lac unit corresponds to enzyme quantity that oxide 1 µmol of ABTS per minute. 500 µL ABTS (0.4 mM) was added to 1,400 µL citrate phosphate buffer (0.05 M/0.1 M, pH 4.5) and incubated at 40 ºC for 30 min. Then, the solution was transferred into a plastic cuvette and the spectrophotometer was calibrated at 420 nm. A 100 µL properly diluted enzyme sample was added, and the Lac activity was determined by ABTS cationic radical formation, which has a 36,000 M^{-1} cm^{-1} molar extinction coefficient (ε) at 420 nm (UV Visible Spectrophotometer UVmini-1240, Shimadzu). The value obtained in Δabs/s was converted in U/L using the following equation (Equation 1):

$$\frac{U}{L} = \frac{\Delta Abs \times 60 \times df \times 10^6}{\varepsilon} \quad \text{(Equation 1)}$$

Where,

Δ Abs = measured absorbance

60 = conversion of second to minute

df = dilution factor

10^6 = conversion of µL to l

ε = molar extinction coefficient (M^{-1} cm^{-1})

3.5.1.2. Lignin Peroxidase

The LiP activity was determined through the variation in optical density resulting from veratraldehyde formation according to Tien and Kirk[136]. A solution containing 500 µL veratryl alcohol (0.8 mM), and 1.2 mL tartaric acid (20 mM, pH 2.5) was incubated at 40 ºC for 30 min. The solution was transferred into a quartz cuvette and the spectrophotometer calibrated at 310 nm. A 200 µL properly diluted enzyme sample and 100 µL hydrogen peroxide (0.8 mM) were added, and the LiP activity was measured. The value obtained in abs/s was converted in U/L using Equation 1 and molar extinction coefficient 9,300 M^{-1} cm^{-1}.

3.5.1.3. Manganese Peroxidase

The MnP activity was evaluated according to Roy and Archibald[134] by the oxidation of phenol-red. A solution containing 500 µL $MnSO_4$ (0.2 mM), 100 µL phenol red (0.067 mM), and 1.1 mL sodium malonate buffer (50 mM, pH 4.5) was incubated at 40 ºC for 30 min. The solution was transferred into a plastic cuvette and the spectrophotometer calibrated at 431 nm. A 200 µL properly diluted enzyme sample and 100 µL hydrogen peroxide (1.0 mM), were added and the MnP activity was measured. The value obtained in Δabs/s was converted in U/L using Equation 1 and molar extinction coefficient 22,751 M^{-1} cm^{-1}.

3.5.1.4. Tannase

Tannase activity was assessed according to Sharma et al.[137] by measuring the variation of a chromogen formed between gallic acid (released by the action of tannase on methyl gallate) and rhodanine (2-thio-4-ketothiazo-lidine). Two tubes containing 0.25 mL methyl gallate solution (0.01 M) prepared in citrate buffer (0.05 M, pH 5) were used as blank, and test. The control tube was prepared by adding 0.25 mL buffer. To each tube, 0.25 mL of the enzyme sample and incubating at 30 ºC. After 5 min, 0.3 mL ethanolic rhodamine (0.667% w/v) was added to all the tubes. The solutions were maintained at 30 ºC for more 5 min and 0.2 mL potassium hydroxide (0.2 N) was added. Then, each solution was diluted with 4.0 mL distilled water and incubated for 10 min. The absorbance was recorded against water at 520 nm and the change in absorbance was assessed with the following equation (Equation 2):

$$\Delta A_{520} = (A_{test} - A_{blank}) - (A_{control} - A_{blank}) \quad \text{(Equation 2)}$$

A calibration curve (Figure 23 on section Appendix A: calibration curves) for gallic acid estimation was prepared as previously described in the blank tube, replacing methyl gallate solution with a gallic acid solution (0.5 mM) prepared in citrate buffer, with a concentration between 5-100 nmol. And used to convert the ΔA_{520} into enzymatic activity, where one unit of the enzyme was defined as one micromole of gallic acid formed per minute.

3.5.2. Chemical Oxygen Demand

The COD was measured accordingly to Standard Methods[138], using a digestive aqueous solution with $K_2Cr_2O_7$, $HgSO_4$, and H_2SO_4, and an acid solution with H_2SO_4 and $AgSO_4$. Tests tubes containing 2.0 mL properly diluted sample, 1.2 mL digestive aqueous solution, and 2.8 mL acid solution were agitated and incubated at 150 ºC for 2 h. After cooling in a dark room, sample absorbance was measured at 600 nm with a colorimeter (Spectroquant Picco COD/CSB, Merck Millipore).

A standard curve (Figure 24 on section Appendix A: calibration curves) was prepared as previously described using glucose solution, with a concentration between 0-1 g/L.

3.5.3. Volatile Suspended Solids

Biomass concentration was assessed by volatile suspended solids (VSS) determination according to Standard Methods[138]. A 5.0 mL sample was filtered through microfiber filters with 1.0 µm pore size (VWR 692) previously calcined for 30 minutes at 550 ºC and weighted. The filters were dried in the oven for 24 h at 105 ºC and after cooled at a desiccator, each sample was weighed. Once again, each sample went to the muffle furnace for 30 min at 550 ºC and after cooled at a desiccator, each sample was weighed.

3.5.4. Determination of Sugars and SCOA

Glucose, xylose, and SCOA concentration were quantified by high-performance liquid chromatography (HPLC). 650 µL of the sample were filtered with cellulose acetate membrane filters with 0.2 µm pore size (CoStar Spin-x) by centrifugation for 20 minutes at 8,000 rpm. Then, 500 µL of the sample was injected (Autosampler Hitachi L-2200) in an anion exchange column (RezexTM ROA – Organic Acid H+ (8%), Phenomenex, 300 x 7.8 mm) connected to a refraction index detector (Hitachi RI L- 2490) and a pump (Gilson). The column was at 65 ºC in a Gecko 2,000 external oven and the eluent used was 0.005 N H_2SO_4, prepared with Milli-Q water, at a flow rate of 0.5 mL/min (Hitachi L-2130).

A calibration curve was obtained by preparing solutions with 0.0 to 5.0 g/L of glucose, xylose, and, acetic, butyric, iso-butyric, propionic, lactic, and valeric acids.

3.6. Calculations

3.6.1. Conversion of Units

The values of SCOA, xylose, glucose, and biomass in g/L were converted in gCOD/L using conversion factors that represent the mass (g) of oxygen required to oxidize 1 g of the compound based on the oxidation reactions for each compound. The overall oxidation equation is represented in $a\ compound{+}bO_2 \rightarrow cCO_2 + dH_2O + eNH_3$ (Equation *3*.

$$a\ compound + bO_2 \rightarrow cCO_2 + dH_2O + eNH_3 \quad \text{(Equation 3)}$$

In which, *a*, *b*, *c*, *d*, and *e* represent the stoichiometric coefficients of the equation. Therefore, the conversion factor (cf) was calculated according to Equation 4.

$$cf(gO_2/g) = \frac{b \times M(O_2)}{a \times M(compound)}$$ (Equation 4)

The conversion factors were 1.07 g O_2/g for glucose, xylose, lactic and acetic acids, 1.51 g O_2/g for propionic acid, 1.82 g O_2/g for butyric acid, and 2.04 g O_2/g for valeric acid.

3.6.2. Yield

The yield on SCOA was calculated relatively to the COD of the feed using Equation 5.

$$Y_{SCOA}(gCOD/gCOD) = \frac{[SCOA]_{produced}}{COD_{in}}$$ (Equation 5)

3.6.3. Acidification degree

The acidification degree (AD) represents the amount of substrate consumed to produce SCOA taking into account the organic matter feed into the batch assays and was calculated using equation 6.

$$AD(gCOD/gCOD) = \frac{[SCOA]_{produced}}{COD_{in}} \times 100$$ (Equation 6)

4. Results and discussion

4.1. Enzymes production

Ligninolytic enzyme production in wastes Solid State Fermentation (SSF) or Submerged Fermentation (SmF) culture media containing lignocellulosic wastes as carbon source, and enzymes inducer, has been reported in the literature for a long time. However, this is still a research topic since residues pretreatment must be improved (Table 8). In this work, the use of Spent Coffee Grounds (SCG) and Pineapple Peel (PP) as enzyme inducers for ligninolytic or hydrolytic enzymes production by *T. versicolor* and *P. variotii* respectively under submerged fermentation was evaluated. Enzymatic activity was only observed on *T. versicolor* samples.

To assure *T. versicolor* initial mycelium development, the fermentation medium was supplemented with glucose as the sole carbon source for the first three days of cultivation. During this period, it was expected that the disponibility of a simple carbon source stimulated primary metabolism and consequently fungal growth[139]. The metabolic pathways related to cellular growth must have been active during this first period of incubation since mycelium growth was observed (Figure 7A).

Ligninolytic enzymes are related to the secondary metabolism of white-rot fungi[140,141] and their induction is dependent on environmental conditions[142]. Thus Lac, LiP, and MnP significant production and/or activation were only expected after glucose depletion[143] and inducers addition[144]. Being wastes of lignocellulosic origin, SCG and PP are rich in phenolic compounds[145,146], which usually are responsible to trigger ligninolytic enzymes production[147]. Consequently, enzymatic activities were only assessed after the mycelium transference for a new flask with the cultivation medium without glucose and supplemented with the lignocellulosic wastes. From all the enzymes tested, LiP did not show any enzymatic activity.

Figure 10 and Figure 11 show the effect of different SCG concentrations on Lac production. The results indicate that the presence of the lignocellulosic waste promoted Lac production and a significant activity has begun on day 5, varying between 70 to 270 U/L. Indeed, in the first days, fungus might be consuming any residual glucose or were still adapting to the new conditions by starting to express the genetic potential for different carbon

sources. The best result, 350 U/L, was obtained on day 6 when using only SCG as an inducer (0.4 % w/v) improving Lac production by 10.6 fold compared with the control experiment performed in TDM medium without any inducer. After reaching the peak, a decrease in Lac activity was observed. This fact could indicate that cellulose and hemicelluloses needed for fungi growth became available and ligninolytic enzymes synthesis requirements were lower[148]. Under these conditions, significant MnP activity (Figure 11) was not detected. Nonetheless, with the higher SCG concentration (1.0 % w/v) the maximum enzymatic activity was only reported on day 16, achieving 85 and 70 U/L for Lac and MnP, respectively. For this condition, enzymatic activity increase was observed along the time, which can suggest that the enzymatic peak was not reached. Therefore, it would be interesting to extend the assay period.

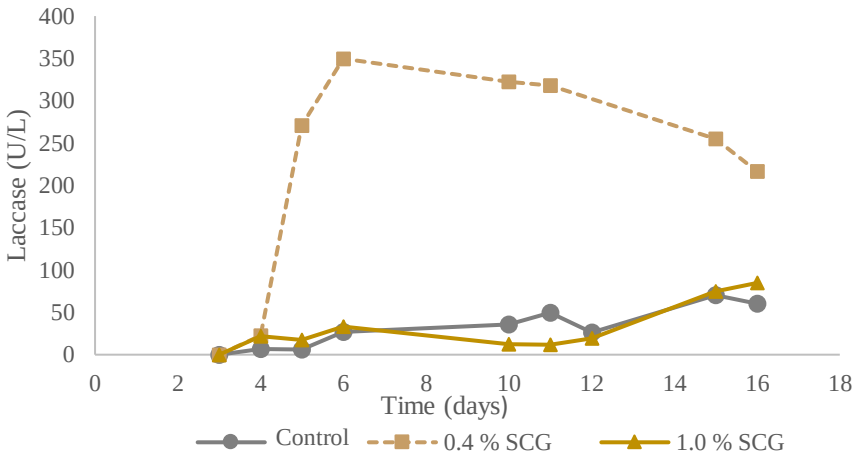

Figure 10. Laccase production by *Trametes versicolor* in submerged fermentation using different concentrations of spent coffee grounds as inducer.

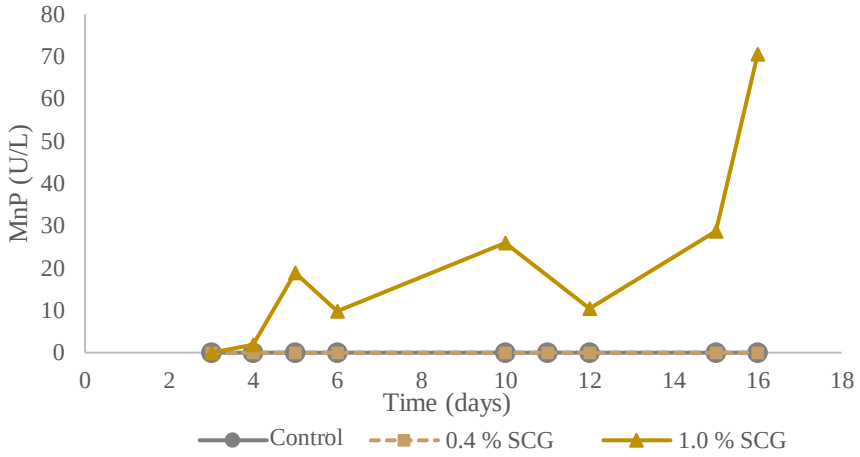

Figure 11. Manganese Peroxidase production by *Trametes versicolor* in submerged fermentation using different concentrations of spent coffee grounds as inducer.

As previously referred, SCG are rich in phenolic and polyphenolic compounds, such as caffeic, ferulic, gallic, p-coumaric, syringic, and vanillic acids[146]. The presence of these compounds was reported to act as a trigger of fungi secondary metabolism as a biological response to oxidized these compounds and lower the toxic effects[115]. Thus, explaining the enzymatic activity obtained in this work with this residue. In the literature (Table 8), coffee industry residues and by-products have been successfully used as substrates for fungi growth and enzyme production[149,150]. Gonzalez et al.[151] registered lower Lac activity (177 U/L) when studying Lac production by *Trametes pubescens* using coffee husk as substrate SmF with copper supplementation. Even under SSF without copper supplementation, the maximum Lac activity was only 204 U/L. The results from the literature suggest that the operational conditions and substrate used in the present work were more adequated for Lac production even without cooper supplementation. Contrary, Almeida et. al[117] achieved Lac production 118 times higher by using a different species, *Lentinus crinitus* with coffee husk under similar conditions.

Besides the stimulation effect, exogenous aromatic compounds when present in high concentrations can negatively affect fungi activity[152]. Bautista et al.[144] tested the supplementation effect of coffee pulp to enhance Lac production and consequently improve sugarcane bagasse delignification by *Pycnoporus sanguineus*. They observed a 33 % increase

in Lac production when using 10 % coffee pulp but, the addition of 25 % lead to a decrease in Lac activity. Even so, SCG are highly recalcitrant and when in high concentration can have antifungal activity[153]. Therefore, a concentration of 1.0 % w/v probably inhibited *T. versicolor* metabolism[152] and, consequently, lower Lac activity was observed. Nevertheless, it important to notice that 1.0 % w/v SCG also promoted higher MnP activity.

Lignocellulosic wastes are known for their complex composition, but usually do not always meet the nutritional requirements for fungi growth and enzyme production. This can be overcome by combining different wastes as substrates. In literature, the positive effect of mixed substrate for enzyme production has been reported[118,154,155]. Therefore, the combined effect of SCG and PP for Lac production under similar concentrations was tested. Contrary to the results previously observed, where 0.4 % w/v of SCG led to the best results, in the presence of PP (Figure 12 and Figure 13), maximum enzymatic activities were obtained with higher concentrations of 1.0 % w/v SCG and 0.4 % w/v PP on day 11 for Lac (223 U/L) and on day 12 for MnP (41 U/L). While at lower concentrations 0.4 % w/v of SCG and 0.2 % w/v of PP, Lac activity reached 133 U/L on day 11, but MnP activity was not detected.

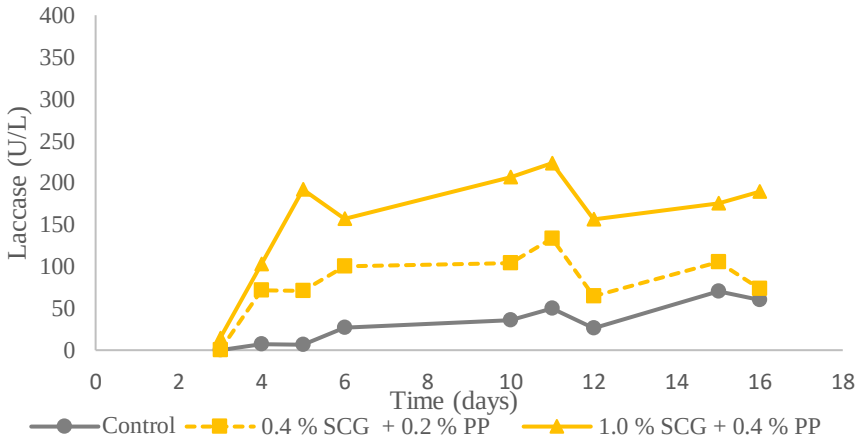

Figure 12. Laccase production by *Trametes versicolor* in submerged fermentation using different concentrations of spent coffee grounds and pineapple peel as inducers.

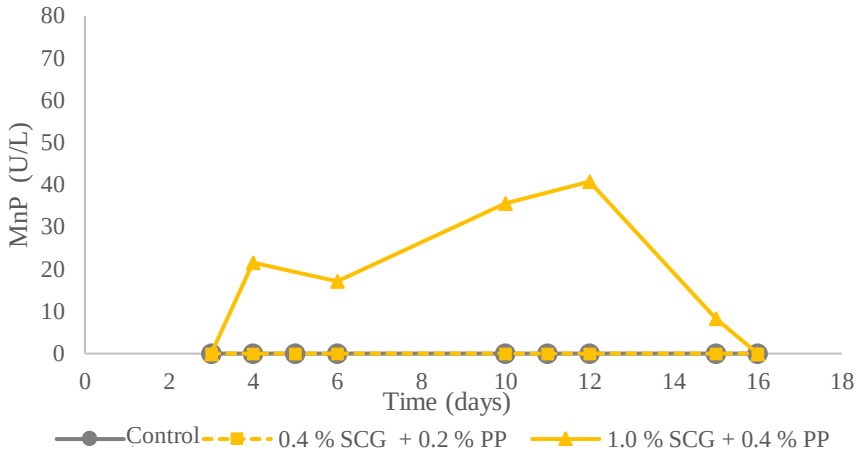

Figure 13. Manganese Peroxidase production by *Trametes versicolor* in submerged fermentation using different concentrations of spent coffee grounds and pineapple peel as inducers.

Although the inhibitory effect of 1.0 % w/v of SCG was observed, the use of PP as a co-substrate revealed a positive effect. Montoya et al.[154] also reported an increase in Lac and MnP activity and process efficiency when combining two different substrates, oak-sawdust and corn bran under SSF of *Grifola frondosa*. Even so, when supplementing oak-sawdust with SCG, they observed significantly lower biomass growth but Lac and MnP production did not seem to be significantly affected. Zeng et al.[155] also observed that a mixture of lignocellulosic substrates was beneficial for Lac production by *T. versicolor* with wheat bran and corn straw promoting the best results (32.09 U/g). The reported results were significantly lower than the present work, but both these studies were performed under SFF. On another hand, Pinheiro et al.[118] described a positive influence on Lac production by *T. versicolor* when using cotton gin wastes as support for fungus growth. Due to its larger particles, PP could have served as support for fungus growth, increasing the stability and growth rate[156] and thus explaining the better results when compared with assays with 1.0 % SCG.

Bazanella et al.[157] reported PP as a good substrate for Lac (1400 U/L) and MnP (2200 U/L) production by *Pleurotus pulmonarius* in SSF. Similarly, Durán-Aranguren and Cruz[158] observed that pineapple waste was the most promising substrate for Lac production among several tropical fruit wastes on the semi-submerged culture of *Pleurotus ostreatus*. Nonetheless, pineapple wastes are rich in cellulose and have low lignin content[159] allowing fungi easy access to fermentable sugars, which promote primary metabolism and cellular growth[160]. It is also possible that by promoting mycelial growth in the early stage, fungi

became more robust and capable to tolerate SCG toxicity. Indeed, this may also explain why Lac production was lower and appear later when PP was present in the fermentation medium.

When compared with studies of a similar scale using other lignocellulosic wastes, the results of this work reveal lower Lac production. Tišma et al.[161] studied the use of paper industry waste as a substrate achieving Lac activity almost seven times higher than in this study. But it is important to notice that $CaCO_3$ microparticles present on the substrate had an important role in Lac production by promoting the formation of dispersed mycelium. Wang et al.[115] verified that corn steep liquor can be successfully applied as a nitrogen source and Lac inducer on SmF, improving the Lac production up to 91.8% and producing 633 U/L. It was reported that the substrate chemical characteristics are important factors for Lac production[151]. Besides phenolic compounds, Lac gene expression is highly regulated by the presence of nitrogen[162]. The best nitrogen source and optimal C/N ratio for ligninolytic enzyme synthesis differ according to fungi species[163]. The C/N ratio of SCG has been reported to be around 23-31[164–167] and some studies stated similar ranges as the most propitious for Lac production[148,168,169]. Merino-Restrepo et al.[170] observed that the C/N ratio of 20 was the most propitious for ligninolytic enzymes and consequently on the decolorization of synthetic dyes, while a ratio of 40 had a negative effect, leading to a 30% decrease in dye degradation. Nonetheless, it has been also described that C/N higher than 40 improves microorganism growth[171] and consequently stimulates higher Lac production[172]. Medina et al.[173] achieved 2000 U/L during the biodegradation of wheat straw, with a C/N ratio of ~130, by *T. versicolor*. Therefore, it would be interesting to test the effect of different nitrogen sources and to access the best C/N ratio to improve inductive Lac production.

Fungi grow in static conditions in nature and SSF has been reported as a good strategy for enzyme production since it resembles fungi's natural environment[119,121,174–177]. Asgher et al.[169] studied the use of corncobs as a substrate for Lac production from *T. versicolor* in SFF. Under the optimal C/N ratio (25) and with Tween-80 and $CuSO_4$ supplementation, it was achieved 1012 U/mL on day 5. Even in submerged fermentation assays, agitation was revealed to be an important factor for white-rot fungi growth and enzyme production[178–181]. This parameter is necessary to assure good oxygenation and nutrients distribution of the medium[180], but higher agitations could lead to biomass collision and cellular damage as well

as enzyme deactivation or proteolysis[181]. Pinheiro et al.[118] observed a reduction in Lac production by 44 % when applying agitation at 25 rpm while agitations of 50 and 100 rpm lead to a drop of more than 75 % on enzyme production. Nonetheless, Zahra et al.[156] did not observe a significant effect when applying 50, 100, or 150 rpm on black liquor decolorization by *T. versicolor* implying that the enzymatic production was not affected by this parameter. Similarly, Tavares et al.[182] did not observe a significant effect on Lac production by *T. versicolor* by applying 100 or 180 rpm (agitation used in this work). But as Zhao et al.[181] pointed out, this could be due to the range of tested agitation that could be near to the optimal agitation value. Thus, further studies should assess the effect of agitation and SSF strategy for Lac production using SCG and PP as the substrate for *T. versicolor*.

Regarding the pH (Figure 14), an increase in all the assays with inducers was observed, ranging from pH ~5.0 at the beginning up to pH ~5.8-7.2 at the end of the assay. This increase could indicate transitions from primary to secondary metabolism[139] and might result from the secondary metabolites accumulation[141]. In fact, Tavares et al.[139] reported a direct correlation between pH decrease and Lac production. The same results were observed by other authors[118,160,183].

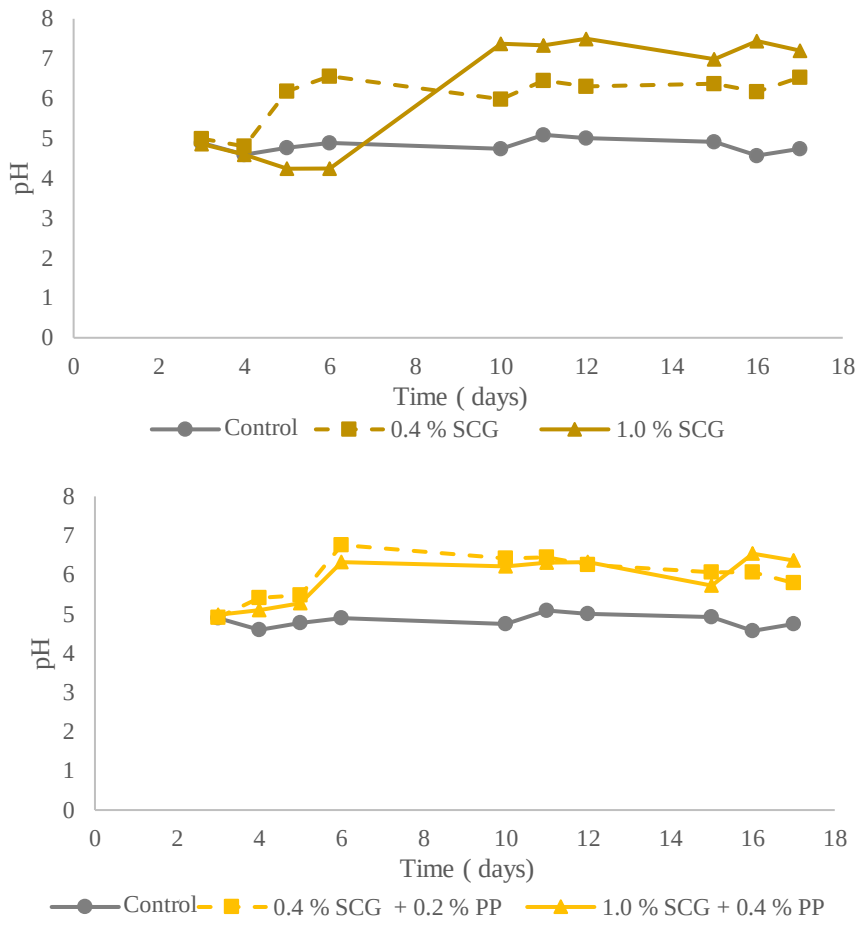

Figure 14. pH variation during enzyme production by *Trametes versicolor* using: A) spent coffee grounds and; B) spent coffee grounds and pineapple peels as inducers.

The assay with 0.4 % SCG and 0.2 % PP showed the lowest increase in pH variation. On one hand, 1.0 % SCG registered the highest pH variation, but on another hand, it also showed a pH decrease at the beginning of the experiment with the lowest pH, 4.2 on day 5. This initial decrease could be associated with the consumption of residual sugar and SCOA production[139,173,182]. In this assay, the lowest pH coincided with the maximum Lac activities on days 5 and 6 followed by a decrease in Lac activity. It could be a connection between these factors since it was reported that a pH around 4.5 was the most appropriate for fungal growth[143] and Lac production by *T. versicolor*[141]. However, pH by itself can not be used to make conclusions.

Table 8. Lignocellulosic materials used as a substrate for laccase production by fungi and different enrichment techniques used to enhance laccase activity.

Fermentation Conditions							Laccase activity	Ref.
Substrate	Scale	T (ºC)	Time (days)	Agitation (rpm)	Culture complementation	C/N ratio		
T. versicolor								
SCG	500 mL Erlemeyer flask	28	6	180	-	n.d	350 U/L	This work
Wheat bran	3.0 L airlift reactor	35	11	150	-	n.d	223 U/mL	[141]
Corn steep liquor	250 mL flasks	26	5	150	-	n.d	633 U/L	[147]
Sugarcane bagass	500 mL Erlenmeyer flask	30	3	-	urea (n.d)	10	~35 U/g*	[148]
Wheat brand and corn straw	250 mL Erlenmeyer flaks	30	7	-	yeast extract (1.0 g/L) urea (0.3 g/L)	n.d	32.09 U/g*	[155]
Vinasse and cotton gin wastes	Bioreactor 3.3 L	30	12	-	peptone (0.1 %)	~15	5006 U/L	[118]

Paper industry waste	500 mL Erlenmeyer flask	27	17	140	peptone (0.6 g/L) yeast extract (0.8 g/L)	n.d	2378 U/L	[161]
Corn cobs	500 mL Erlenmeyer flask	30	5	-	yeast extract (0.2%) glucose (1.0 %) Tween 80 (1.0 %) $CuSO_4$ (1.0 mM)	25	1012 U/mL*	[169]
Corncob	50 mL flask	25	6	-	binary dye mixture (10.39 mg/L)	30	16 U/g*	[170]
Wheat straw	1.5 kg bags	28	126	-	Fe_2O_3 (2.0 %)	~130	2000 U/L	[173]
Rice husk	n.d	~20	5	130	-	n.d	1081 U/mL	[184]
				Grifola frondosa				
Oak sawdust and SCG	1 kg bag	20-25	30	-	-	n.d	15 U/g*	[154]
				Trametes pubescens				
Coffee husk	1000 mL Erlenmeyer flask	30	23 19*	-	$CuSO_4$ (5.0 µM)	n.d	177 U/L 204 U/L*	[151]

Lentinus crinitus								
Coffee husk	250 mL Erlenmeyer flask	28	11	-	urea (0.7 g/L)	12.5	41246 U/L	[117]
Pycnoporus sanguineus								
Sugarcane bagasse	500 mL container	30	n.d	-	coffee pulp (10 %)	n.d	2 μmol/g /h	[144]
Pleurotus pulmonarius								
Coffee pulp	100 g bag	27-28	12	-	-	n.d	1 U/g*	[149]
Coffee pulp	100 g bag	28	12	-	confrontation with *Trichoderma viride*	n.d	180 U/g*	[150]
PP	250 L Erlenmeyer flask	28	10	-	-	n.d	2450 U/L	[157]
Pleurotus ostreatus								
PP	1.5 L container	27	6	150	-	n.d	~1500.0 U/L	[158]

n. d – not defined; * - under SSF.

4.2. Acidogenic fermentation of SCG

4.2.1. Pretreatment study

Several studies report different strategies to enhance SCG saccharification including physical-chemical and biological pretreatments or a combination of them, such as acid hydrolysis, enzymatic hydrolysis, alkali hydrolysis, hydrothermal pretreatment, among others[185]. A good pretreatment should allow high sugar recovery, reduced time and cost associated, and limiting the presence of inhibitor compounds[186]. Extreme pretreatment conditions can result in inhibitors such as furfural and hydroxymethylfurfural[187], resulting in a need for a posterior detoxification step, increasing process cost[185]. Nonetheless, its been reported that mild conditions, i.e low chemical reagent concentration and low reaction time, allow carbohydrates efficient solubilization while avoiding inhibitor extraction[65], high environmental impact, and higher costs[188]. Bearing this in mind, several pretreatments with mild conditions were applied to SCG. The conditions applied, and sugars released are described in Table 9.

As can be observed, diluted acid hydrolysis (AH) resulted in better sugar release (2.23 g/L) when compared with other pretreatments. Among different pretreatments, acid hydrolysis has been reported as a relevant pretreatment with high hemicellulose recoveries[185]. However, the sugar yield (5.95%) obtained is low when compared with the results reported in the literature. Juarez et al.[187] obtained a maximum sugar yield of 29.0% when submitting SCG to dilute acid hydrolysis with 5% v/v sulfuric acid at 95 ºC during 3 h. Similarly, Go et al.[189] achieved 34% sugar yield when applying 4% v/v sulfuric acid at 95 ºC during 3-4 h. In both cases, researchers achieved a sugar yield 5 to 6 times superior to the obtained in this work. This could indicate that the conditions selected for AH were not the most appropriated. Parameters such as solvent-to-solid ratio, acid concentration, hydrolysis time, and temperatures employed have a significant impact on SCG hydrolysis[189]. Since in both studies the acid concentration is similar or even equal to used in this work, it is possible that temperature and reaction time applied played a crucial role to increase the process efficiency. On the other hand, López-Linares[185] obtained a more similar result (5.8 g/L) with the concentration obtained in this work when pretreating SCG with dilute acid hydrolysis.

Although this result was obtained with only 0.29% sulfuric acid concentration it is important to notice that SCG were previously submitted to microwave pretreatment.

Acid hydrolysis was also applied on SCG previously submitted to supercritical extraction (SE+AH). Although the literature reports that non-delipidated SCG when pretreated with acid hydrolysis, resulted in similar amounts of sugar with the delipidated samples[187]. By removing the lipidic fraction, the SCG structure changed with increasing accessibility for acid hydrolysis[186]. Consequently, by combining these processes the sugar yield obtained was expected to be higher than with AH. However, the sugar yield (4.03 %) achieved was almost 1.5 lower when compared with AH. Contrary to the results obtained in this study, Kovalcik et al.[44] reported a 20.8% increase of total sugar when submitting deffated SCG to 4.0 % sulfuric acid at 100 ºC for 120 min on the autoclave when compared with SCG pretreated at the same conditions. Nonetheless, the oil extraction was performed with a mixture of hexane and isopropyl using a Soxhlet extractor for 4h. Therefore, the method used for oil extraction could have been a crucial parameter and the lower sugar yields in SE+AH can be linked-to partial elimination of hemicelluloses during supercritical extraction.

Acid hydrolysis is reported as an adequate pretreatment to enhance sugars disponibility being hydrolysates pH usually between 1.0 and 1.5[185]. At this pH, it is required an additional step of neutralization since microorganisms can not handle pH so low. This step will impact the process cost. Nonetheless, it is also important to notice that by avoiding microbial growth, acidic hydrolysates can be stored without any risk of contamination[187].

The remaining physicochemical pretreatments resulted in sugar concentrations neer to zero. This was expected since supercritical extraction (SE) is used to extract oil and diterpenes from SCG[190] while alkaline hydrolysis (BH) when combined with thermal pretreatment promotes fibers swelling, macromolecules losing, and consequently damage on lignin fibers[191]. Passadis et al.[188] performed alkaline pretreatment using 0.7 M NaOH for 6 h at 50 ºC achieving a 79.2 % lignin degradation. Besides delignification, they achieved 0.488 g/L, 16 times higher than the sugar detected in this work. They used a NaOH solution 1.4 times more concentrated and longer reaction times than used in this work. Thus, it can explain why lower concentration sugars were obtained. Nonetheless, alkaline reagents were reported

as more efficient than acid or oxidative reagents for SCG fiber solubilization[5]. Therefore, it would be interesting to adjust BH parameters in further studies.

More recently, enzymatic hydrolysis was reported as an advantageous strategy to enhance monosaccharides production[186]. SCG is a mannan-rich substrate and its enzymatic hydrolysis is usually performed by mannanase and/or cellulase cocktails[186,188,192,193]. The enzymatic extracts produced previously contained oxidative enzymes. As mentioned before, Lac, MnP, and LiP are responsible for the oxidation of lignin and phenolic compounds present in SCG[87]. Therefore, an increase in sugars release was not expected. Nonetheless, it is important to point that enzymatic extracts were stored at -16 ºC, which could negatively affect enzymatic activity. Consequently, Lac activity was lower than reported in section 4.1 Enzymes production.

It is also important to consider that only glucose and xylose were detected after the pretreatments were performed. In the future, it is important to evaluate the effect of pretreatment on cellulose, hemicellulose, and lignin fractions as well as the concentration of inhibitors of microbial activity that could result from the pretreatment and influence negatively the AF.

Table 9. Pretreatment conditions tested and obtained sugar concentration and extraction yields from spent coffee grounds.

Pretreatment		Conditions	[Sugars] (g/L)	%Yield (gSugar/gSCG)
Acid Hydrolysis	AH	5% H_2SO_4 at 121 ºC for 1 h in autoclave	2.23	5.95
Alkaline Hydrolysis	BH	2% NaOH at 121 ºC for 1 h in autoclave	0.03	0.08
Supercritical extraction	SE	CO_2 extraction at 300 bar, 50 ºC, 12 g CO_2/min for 2 h	0.02	0.05
Supercritical extraction + Acid Hydrolysis	SE+AH	CO_2 extraction at 300 bar, 50 ºC, 12 g CO_2/min for 2 h followed by 5% H_2SO_4 at 121 ºC for 1 h in the autoclave	1.51	4.03
Supercritical extraction + Alkaline Hydrolysis	SE+BH	CO_2 extraction at 300 bar, 50 ºC, 12 g CO_2/min for 2 h followed by 2% NaOH at 121 ºC for 1 h in the autoclave	0.03	0.08
Solid State Fementation	TvSSF	28 ºC for 3 months 1:4 (w/v) MC with *T. versicolor*	n.d	
	PvSSF	28 ºC for 3 months 1:4 (w/v) MC with *P. variotii*	n.d	
Submerged Fermentation	TvSmF	28 ºC, 180 rpm for 15 days with *T. versicolor*	n.d	
	PvSmF	28 ºC, 180 rpm for 15 days *P. variotii*	n.d	
Enzymatic Hydrolysis	TvS	Enzymatic extract obtained from *T. versicolor* with SCG as an inducer (Laccase 73 U/L) at 40 ºC, 100 rpm for 7 days	n.d	
	TvP	Enzymatic extract obtained from *T. versicolor* with SCG and PP as inducers (Laccase 49 U/L) at 40 ºC, 100 rpm for 7 days	n.d	
	PvM	Enzymatic extract obtained from *P. variotii* (enzymatic activity not determined) with no inducer at 40 ºC, 100 rpm for 7 days	n.d	
	PvS	Enzymatic extract obtained from *P. variotii* (enzymatic activity not determined) with SCG as inducer at 40 ºC, 100 rpm for 7 days	n.d	
	PvP	Enzymatic extract obtained from *P. variotii* (enzymatic activity not determined) with SCG and PP as inducers at 40 ºC, 100 rpm for 7 days	n.d	

n.d – not detected.

4.2.2. Acidogenic Fermentation of SCG with different pretreatments

Substrate hydrolysis is known as the limiting step of AF, which can be overcome with pretreatments that enhance sugars disponibility[191]. To assess, the effect of different physicochemical and biological pretreatments on the AF of SCG, a set of batch experiments was performed. Usually, AF is performed with anaerobic sludge where two groups of bacteria have fundamental roles: acidogens and methanogens. Acidogens contribute to the formation of SCOA, but methanogens convert SCOA to methane negatively affecting SCOA yield[58]. Since methanogens were found to be mainly strict anaerobes and acidogens facultative anaerobes, to avoid a decrease in SCOA yield, activated sludge from an aerobic reactor of a WWTP was chosen as inoculum for these assays[63]. Therefore, the assays were performed with aerobic activated sludge and lasted 26 days on control assay and 29 to 30 days for pretreatment assays in the absence of aeration. Conditions such as the fermentation medium, COD concentration of SCG, temperature, and pH used were chosen accordingly to the parameters reported in the literature[194].

4.2.2.1. Overall results

The maximum SCOA produced, the yield of SCOA, and acidification degree (AD) are resumed in Table 10. The best result for maximum SCOA concentration was obtained with AH (2.52 g COD/L). The remaining physicochemical pretreatments lead to a higher maximum SCOA concentration than the control (1.31 g COD/L) assay except for SE+AH (1.20 g COD/L). On another hand, the only biological pretreatment more efficient than the control were submerged fermentation with *T. versicolor* (TvSmF) and solid-state fermentation with *T. versicolor* (TvSSF). With maximum SCOA production of 2.44 and 1.51 g COD/L, respectively. Although AH resulted in a slightly higher SCOA concentration, TvSmF led to the highest yield and AD demonstrating that this could be the most adequate pretreatment applied.

The average AD for all experiments was 18.5 %, ranging from 4.51 to 48.0 % for pretreatment with the enzymatic extract obtained by *P. variotii* with SCG and PP as inducers (PvP) and TvSmF, respectively. These values indicate that a significant part of the substrate was not degraded. The best result obtained was slightly higher than Hemalathe and al.[81] results (35-40%) during AF of pretreating deoiled *Azolla pinnata* biomass with acid hydrolysis, but lower than the majority of the batch assays. These results could suggest that the pretreatment used in this work

did not allow the full conversion of hemicelluloses into fermentable sugars following the results obtained in section 4.2.1Pretreatment study. Nonetheless, they achieved slightly lower total SCOA concentrations, than in this work. Once again, Castilla-Archilla[65] reported similar results, 44.0% AD using brewery spent grain pretreated with thermal acid diluted hydrolysis at uncontrolled pH, but it increased to 93.0% with controlled pH 6. This could indicate that pH 6.0 is the most promising for the process. Assays with pH around ~5-6 demonstrated to be the best conditions for SCOA production, translating in higher acid concentration and AD. Nonetheless, pH control plays an important factor in AF, and future studies with controlled pH should be assessed.

It is also important to notice that, although higher AD implies higher substrate consumption, it did not necessarily result in higher SCOA production. As can be observed in Table 10, pretreatment with the enzymatic extract obtained by *T. versicolor* with SCG as an inducer (TvS), pretreatment with the enzymatic extract obtained by *T. versicolor* with SCG and PP as inducers (TvP), and pretreatment with the enzymatic extract obtained by *P. variotii* with SCG as inducer (PvS), had higher AD than control, but lower SCOA production, indicating that the substrate consumption was directed to other metabolisms.

As can be seen in Figure 15, the main SCOA produced during batch assays were generally acetic acid followed by propionic acid, and this relation was only reverted on PvSmF and PvM. The prevalence of acetic acid is in accordance with the literature (Table 2 in section 2.3.3 Strategies to enhance SCOA production). Other SCOA appears in lower concentration and lactic acid is present on AH, BH, SE, SE+AH, SE+BH, and TvSSF, which could be due to the dominance of lactic acid bacteria on some stages and indicate system perturbations[63].

Table 10. Summary of the results from the batch tests with spent coffee grounds submitted to different pretreatments

Pretreatment	day	pH	[SCOA] (g COD/L)	Yscoa (g COD/ g COD)	AD (%)
Control	25	4.82	1.31	0.13	13.3
AH	28	4.71	2.52	0.23	22.6
BH	20	5.70	2.21	0.22	21.7
SE	22	4.65	1.80	0.31	30.8
SE+AH	25	4.86	1.20	0.12	12.3
SE+BH	18	5.75	2.06	0.14	13.8
TvSSF	25	5.00	1.51	0.15	14.6
PvSSF	19	6.03	0.45	0.05	4.64
TvSmF	24	4.90	2.44	0.48	48.0
PvSmF	16	5.85	0.89	0.12	11.8
TvS	18	6.61	0.97	0.19	19.1
TvP	24	6.94	0.71	0.16	16.4
PvM	18	5.52	1.23	0.26	25.9
PvS	14	5.68	0.81	0.18	17.8
PvP	11	6.76	0.16	0.05	4.51

AH- SCG pretreated with acid hydrolysis; BH – SCG pretreated with alkaline hydrolysis; SE – SCG pretreated with supercritical extraction; SE+AH - SCG pretreated with supercritical extraction and acid hydrolysis; SE+BH - SCG pretreated with supercritical extraction and alkaline hydrolysis; TvSSF – SCG pretreated with solid-state fermentation by *T. versicolor*; PvSSF - SCG pretreated with solid-state fermentation by *P. variotii*; TvSmF - SCG pretreated with submerged fermentation by *T. versicolor;* PvSmF - SCG pretreated with submerged fermentation by *P. variotii;* TvS – SCG pretreated with the enzymatic extract obtained from *T. versicolor* with SCG as inducer; TvP - SCG pretreated with the enzymatic extract obtained from *T. versicolor* with SCG and PP as an inducer; PvM – SCG pretreated with the enzymatic extract obtained from *P. variotii* without inducers; PvS - SCG pretreated with the enzymatic extract obtained from *P. variotii* with SCG as an inducer; PvP - SCG pretreated with the enzymatic extract obtained from *P. variotii* with SCG and PP as inducers.

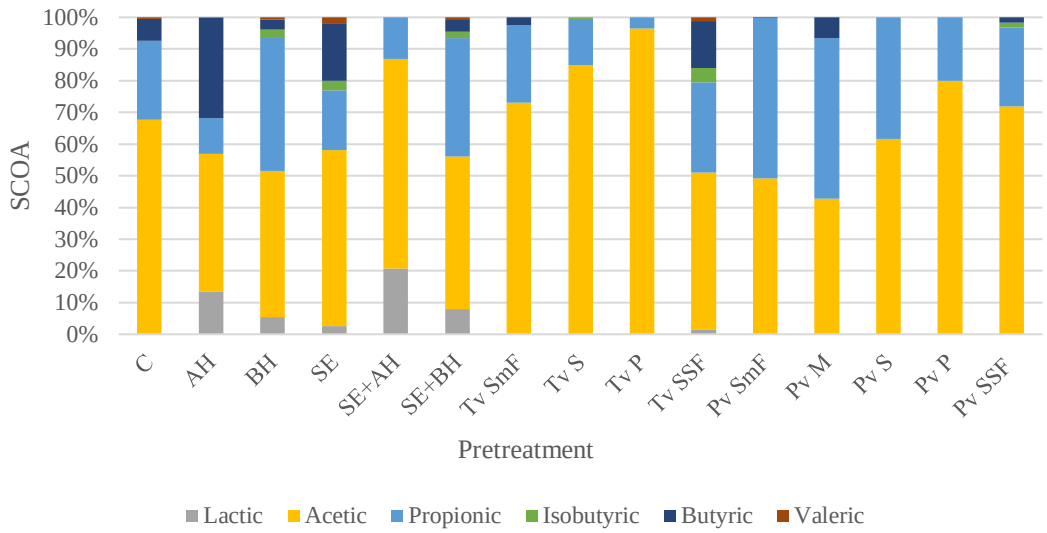

Figure 15. Average short-chain organic acids profile from acidogenic fermentation with spent coffee grounds submitted to different pretreatments.

4.2.2.2. SCOA distribution

The control assay (Figure 16) demonstrated that MMC were capable of using SCG as substrate producing a combination of acetic, propionic, butyric, and valeric acids on an average molar proportion of 67.6/25.0/6.80/0.60%. Along with the assays, an increase of SCOA concentration was observed until it reached a maximum of 0.99 g/L, followed by a decrease. The SCOA production corresponded to the reduction in pH on the first days of the assay, resulting from the accumulation of acids on the fermentation medium. After that, the pH remained near to 5.

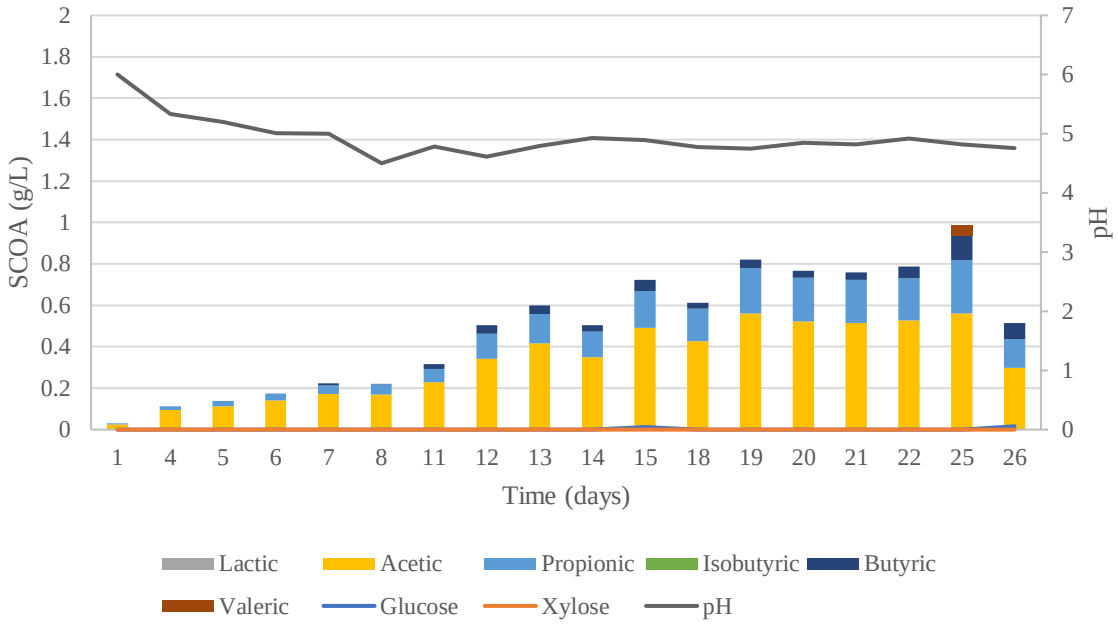

Figure 16. Acidogenic fermentation of spent coffee grounds without pretreatment.

4.2.2.2.1. AF of SCG with physicochemical pretreatment

Acidic pretreatment is an efficient process that relies on the application of acids that break down hemicellulose bonds, exposing cellulose for microbial degradation[67]. The assay of AF with SCG submitted to AH is represented in Figure 17. The production of lactic, acetic, propionic, butyric, and valeric acids corresponded to an average molar proportion of 13.5/43.3/11.4/0/31.6/0.2%. In the first days, lactic acid was the prevalent SCOA produced. As previously referred, pH plays an important influence on SCOA production by promoting certain metabolisms over others. As an acidic substrate, SCG pretreated with AH may have promoted lactic acid bacterias prevalence over the other microorganism and thus promoting lactic acid production[63]. Similarly, Castilla-Archilla et al.[65] observed that at pH 6 lactic acid was produced simultaneously with other SCOA while lower pH resulted in longer adaptation periods. During this time, lactic acid bacterias will consume sugars and accumulate pyruvate. This will promote a decrease in intracellular pH due to NADH accumulation, resulting in the activation of lactate dehydrogenase and the conversion of pyruvate on lactic acid. In fact, when AF was performed with AH without correctly adjusted pH at the beginning of the assay, it was observed that the MMC produced almost exclusively lactic acid (Figure 25Figure 26, on section 8.2 Appendix B:

AF batch experiments) After day 9, a shift in SCOA production was observed, and it was not observed lactic acid production besides day 22, where its production was almost residual. This could demonstrate that the MMC was adapted to the new conditions and other microbial groups prevailed over lactic acid bacterias and were able to consume lactic acid.

Some fluctuations in SCOA production were observed, which could indicate SCOA consumption or MMC instability. This instability could be explained by the age of the inoculum and the fact that it was stored for a long time at 4 ºC. Nonetheless, the inoculum used was the same in all experiments, and in some of them, this instability was not observed. Finally, on day 28 the maximum SCOA production was achieved with 1.78 g/L, almost 2 times higher than the observed in control. By increasing sugars disponibility, SCG treated with AH allowed not only higher concentrations of SCOA in general but also higher concentrations of butyric acid. Zhang et al.[59] obtained similar SCOA concentrations when pretreating corn stover with diluted nitric acid. Acetic and propionic acids were the dominant SCOA produced during the firsts 48 h but then, butyric acid became more prevalent than propionic acid. Although pretreatment applied was equally efficient it was employed lower HNO_3 concentration (0.6%) and shorter reaction time (2 min). This could indicate that HNO_3 is a more efficient acid but certainly, the lower lignin content of corn stover requires lower acid concentration.

Differently, Castilla-Archilla et al.[65] using brewery spent grain pretreated with thermal diluted hydrolysis 1.5% H_2SO_4, obtained a maximum SCOA concentration almost 4 and 7 times superior in assays without pH control and at constant pH 6.0, respectively. In both assays, butyric acid was the most prevalent SCOA. These authors observed that acidic conditions were the most favourable for butyric acid production while acetic acid increased with the increase of pH. However, they started the assay with 14 g/L of sugars which may be the reason why they obtained higher concentrations than the obtained in this work. Kumar et al.[195] also reported similar results when combining autoclave with 1 % H_2SO_4 to pretreat vegetal waste.

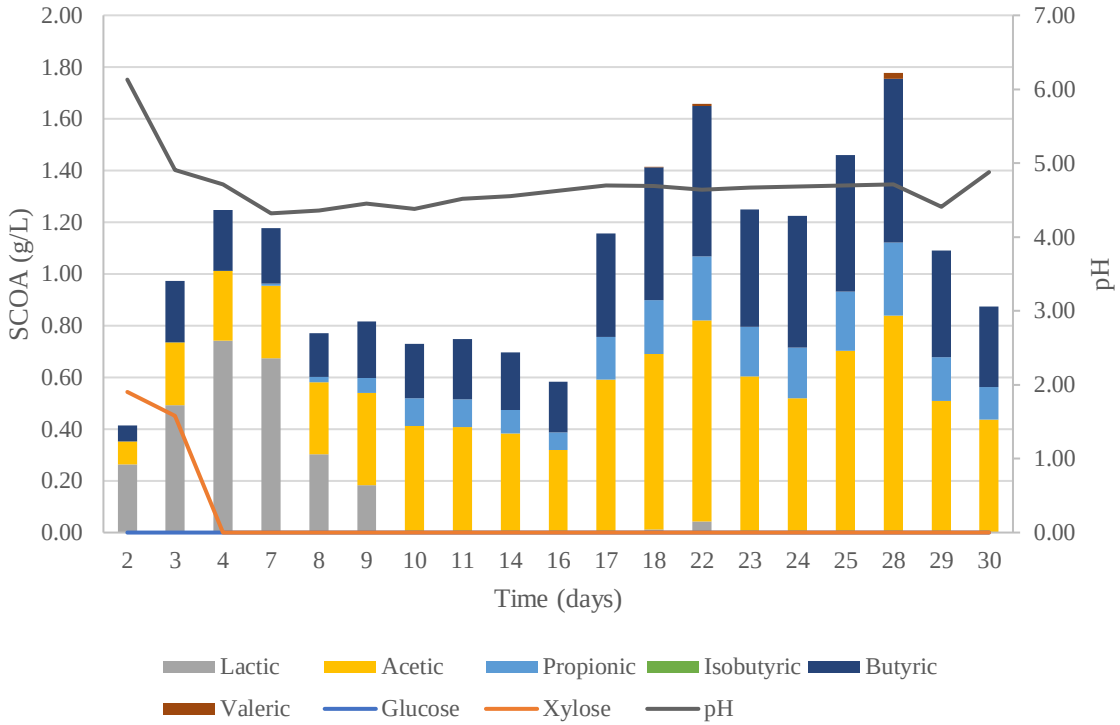

Figure 17. Acidogenic fermentation of spent coffee grounds with acidic hydrolysis pretreatment.

A similar profile was obtained on SE+AH pretreatment (Figure 27, on section 8.2 Appendix B: AF batch experiments) but no butyric acid was produced and the maximum SCOA concentration (1.04 g/L) was almost similar to the obtained in the control assay. This could indicate that the lipidic fraction of SCG can play an important role in butyric acid production. However, on the assay with SCG pretreated only with SE (Figure 18), the production of butyric acid alongside others SCOA contradicts the explanation theorized above. As suggested in section 4.2.1 Pretreatment study, SE could have promoted an increase of structural availability for AH[186] leading to the elimination of hemicellulose portions which resulted in lower sugar release and consequentially in lower SCOA concentrations[196]. It was also possible that the degraded portion of SCG was responsible for butyric acid production, explaining why this acid was not produced under these conditions. Hude et al.[196] had previously reported that by increasing sulfuric acid above 0.6% when combining supercritical extraction and diluted acid hydrolysis, SCOA yield and biogas production was negatively affected and decreased. They pointed out that acidic medium neutralization led to sodium concentration increase which created a toxic medium and could have inhibited acetogenic microorganisms. More recently, Hemalatha et al.[81] achieved slightly lower

total SCOA concentrations, than in this work, when pretreating deoiled *Azolla pinnata* biomass with 2% HCl. Acetic acid was also the main SCOA produced, however, contrary to this study, they obtained butyric acid and none propionic acid. These differences in the results could be explained by the different pretreatment conditions as well as the substrate chemical characteristics.

Even so, in assays where MMC was supplied with SCG pretreated with SE (Figure 18), it was observed a combination of lactic, acetic, propionic, isobutyric, butyric, and valeric acid in an average proportion of 2.6/55.5/18.9/3.0/18.1/1.9%. The SCOA production increase along the assay reaching a peak on day 22 with a maximum of 1.36 g/L, 1.4 times higher than on the control test. Besides that, it is also observed more variety of SCOA production when compared with control, AH, and AH+SE where isobutyric acid is not observed and valeric acid is less frequent. And once again, after a slight decrease on the firsts days, pH maintained relatively stable over the rest of the time. Szumacher-Strabel et al.[74] also registered slightly better SCOA production using wild dog rose seed pretreated with supercritical extraction when compared with the control assays, with the production of acetic, propionic, isobutyric, butyric, and valeric acids. Similarly, Hernandéz et al.[197] reported greater SCOA and consequently methane production after lipids extraction from microalgae through supercritical extraction with CO_2. They suggested the pretreatment was capable of degrading proteins and carbohydrates into aminoacids and monosaccharides, thus increasing microalgae biomass digestibility.

Contrary, Martines et al.[75] did not observe significant improvements on AF after polyphenol and oil extraction from grape pomace with supercritical CO_2. They hypothesized that even untreated grape pomace had readily biodegradable compounds in large disponibility and the high SCOA concentrations obtained could have inhibited AF, preventing SCOA productions above 22.2 g/L. Besides higher SCOA concentrations the researchers also obtained different

SCOA profiles with acetic acid representing almost 70 %, followed by butyric acid, 19 %, which can be explained by grape pomace high sugar content.

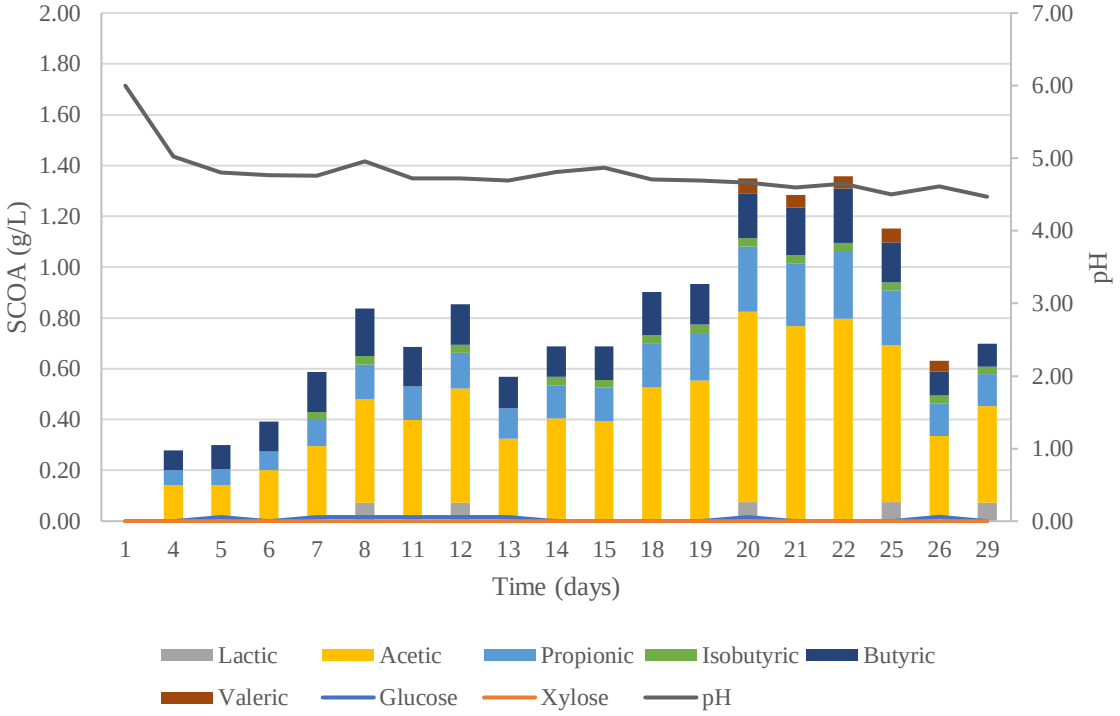

Figure 18. Acidogenic fermentation of spent coffee grounds with supercritical extraction pretreatment.

Alkaline pretreatment was reported as the most efficient among chemical treatments in lignin depolymerization and superior increase of the surface area[67]. From BH pretreated SCG (Figure 19) MMC were also capable of producing a combination of lactic, acetic, propionic, isobutyric, butyric, and valeric acid in a proportion of 5.4/46.1/42.2/2.4/3.2/0.7% reaching a maximum of 1.62 g/L on day 19, decreasing in SCOA production after that. Similar production was observed with SCG pretreated with SE+ BH (Figure 28, on section 8.2 Appendix B: AF batch experiments). Xin et al.[61] achieved a similar SCOA concentration as the obtained in this study when using waste-activated sludge pretreated with calcium oxide. But, they obtained higher productions of isobutyric, butyric, and valeric acids. Previously, Lin et al.[73] also reported higher productions for isobutyric, butyric, and valeric acids than the obtained in this study, when using waste sludges pretreated with 10 mM NaOH. A different microbial group present on each MMC could promote these differences. Nonetheless, the substrate used could be an important factor since acetic and propionic acids can be converted from soluble carbohydrates and low molecules, while

valeric acid and other complexes SCOA requires the bioconversion of macromolecular proteins and waste-activated-sludge have higher protein content.

Girotto et al.[5] also observed predominantly acetic acid, and lower concentrations of propionic, butyric, and valeric acids when performing AF with SCG pretreated with 8 % w/w NaOH. Nonetheless, the maximum SCOA production was 18.5 times higher than the obtained in this work, suggesting that the NaOH concentration was a determinant factor in AF efficiency. Previously, the same group has tested the effect of different NaOH concentrations (2, 4, 6, and 8% w/w) to pretreat SCG for 24 h at ambient temperature. They observed a direct relationship between the increase of NaOH concentration and SCG lignin degradation and a consequent improvement on anaerobic digestion without seeing any decrease in process efficiency[68]. Therefore, NaOH concentration must increase to improve the pretreatment process and AF process.

Nonetheless, Tsafrakidou et al.[70] performed AF of wheat straw pretreated with 1% w/w NaOH at 100 ºC during 3h. Even using lower alkali concentration, after 48h they obtained 3.08 g/L of SCOA, almost 2 times more than the results from this study but lactic and acetic acids were the only SCOA produced. After that, lactic acid was no longer produced and SCOA concentration became more similar to the obtained in this work. This can suggest that wheat straw is less recalcitrant due to less lignin content than SCG and milder pretreatment conditions are required to be efficiently treated. In this study, researchers also concluded that by immobilizing cells on γ-alumina, SCOA production increase up to 6.99 g/L. Therefore, it would be interesting to explore this strategy in future assays. Besides that, it is also important to notice that MMC microbial composition is unknown and operational parameters such as pH, temperature, and substrate and inoculum ration applied were different from the above-described studies, which could also impact SCOA production.

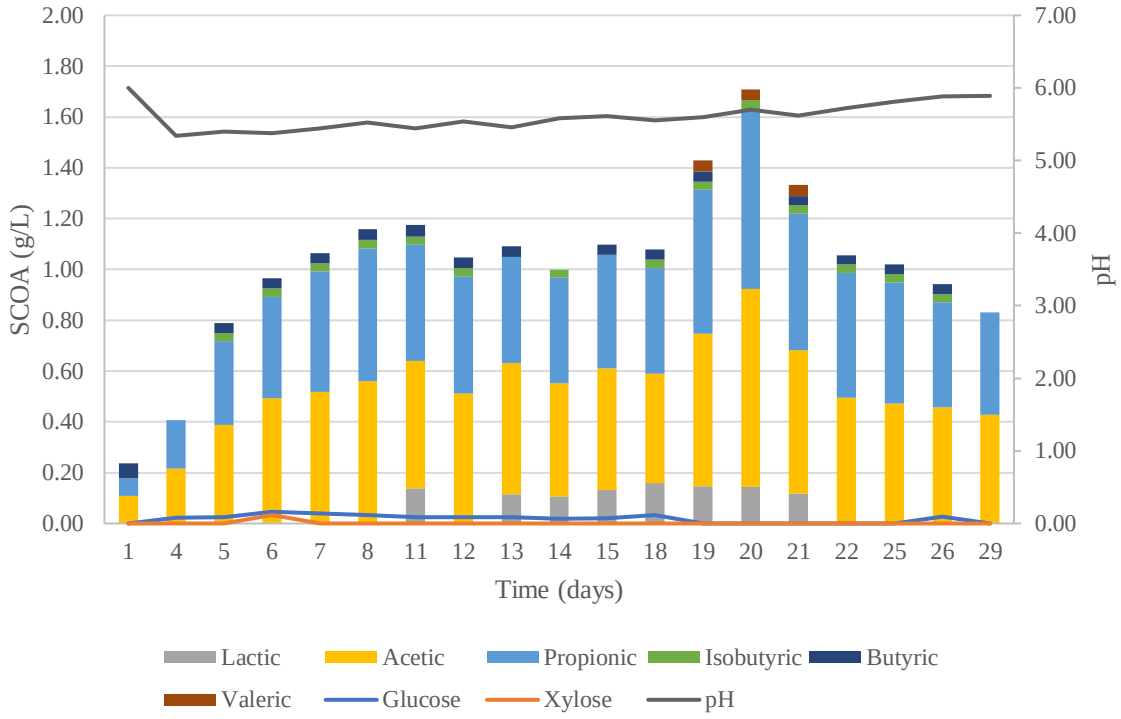

Figure 19. Acidogenic fermentation of spent coffee grounds pretreated with basic hydrolysis.

In both SE (Figure 18), BH (Figure 19), and SE+BH (Figure 28, on section 8.2 Appendix B: AF batch experiments) it is observed a wider variety of SCOA produced which can suggest that by promoting a change in SCG structure they allow easier access for MMC and thus promoting the production of isobutyric which is not observed on AH and SE+AH nonetheless it is important to notice that samples were analyzed in different runs and that can explain this variability on SCOA production.

4.2.2.2.2. AF of SCG with biological pretreatment

Physico-chemical pretreatments are the most frequently employed to treat lignocellulosic biomass (Table 2 in section 2.3.3 Strategies to enhance SCOA production**Erro! A origem da referência não foi encontrada.**). However, these can have multiple technical, economic, and environmental drawbacks. In the last years, biological strategies were applied. Fungal pretreatment has aroused interest due to low energy demands, cost-effectiveness process, and enzymatic

systems[70]. These microorganisms secrete lignocellulosic enzymes capable of loose lignocellulose structure and posteriorly degrading it[198].

Enzymatic hydrolysis of substrates for AF is usually performed with commercial cellulases and xylanases to convert cellulose and xylans to sugars[71,72]. Nonetheless, for lignin degradation, oxidative enzymes are employed[67]. The enzymatic extracts produced in section 4.1Enzymes production were applied for SCG pretreatment. All the assays (Figure 30 to Figure 34, on section 8.2 Appendix B: AF batch experiments) resulted in similar profiles to the obtained after SmF pretreatment (Figure 20 and Figure 21). However, it was achieved a lower SCOA concentration, even in the control assays.

Contrary to these findings, Rusli et al.[76] observed a positive effect on SCOA production when pretreating oil palm founds with enzymatic extracts in-house produced from the white-rot fungus, *Ganoderma lucidum*. After pretreatment, hemicellulose and lignin content decreased while cellulose content was maintained unaltered. They suggested that Lac, LiP, and MnP present in the extracts promoted *in vitro* rumen digestibility, and consequently significantly increased SCOA production (4.75 g/L). Acetic acid was the main acid produced, followed by butyric and propionic acids in the proportion of 58.3/23.4/18.3%. Yue et al.[77] also demonstrated the positive effect of Lac on ruminal SCOA production. In their study, the addition of Lac from *Aspergillus orzaye* promoted corn silage degradability and a shift in fermentation from acetic acid to more propionic acid formation. Similarly, Zhang et al.[199] observed a shift in human gut bacteria that increased butyrate-producing bacteria after pretreating corn bran arabinoxylan with Lac. On another hand, Wang et al.[200] reported that by increasing Lac activity on ruminal fluid, acetic acid production increase while propionic acid decreased. Nonetheless, it was observed a positive effect on SCOA production, with acetic, propionic, isobutyric, and valeric acids productions.

The lower efficiency of this pretreatment here obtained could be linked with the fact that enzymatic extracts produced in section 4.1Enzymes production, had lower Lac activity than the reported in the previous works. It is also possible that the strategy used to remove fungi cells was not the most efficient. In fact, in some of these assays, mycelium growth was observed on the surface of the AF medium. Therefore, the easily accessible SCOA could have been used for fungi metabolism. In the future, it is necessary to try a better strategy to recover active enzymes, avoiding fungal growth and SCOA consumption.

Although the poor results were obtained with enzymatic extracts, enzymatic activity from *T. versicolor* was demonstrated a positive effect in SCOA production after TvSmF pretreatment (Figure 20). MMC were able to produce acetic, propionic, and butyric acids in the proportion of 73.0/24.4/2.6 %. While acetic acid production occurs through the assay, a significant propionic acid production starts on day 10 and butyric acid is only obtained from day 18. Like on the other assays, SCOA production increase until reaching a peak on day 24, decreasing acid concentration after that. The maximum SCOA concentration achieved was 1.99 g/L, 2 times higher than the control revealing that *T. versicolor* can be successfully used to pretreat SCG for AF. Regarding pH, it decreases until reaching pH 5.

On contrary, Tsafrakidou et al.[70] allied alkali hydrolysis with hydrolytic pretreatment of wheat straw with *Trichoderma viride* under SmF. Although they observed an increase in glucose and cellobiose release, SCOA production increase when compared with non-treated wheat straw was not relevant. And they conclude that this pretreatment was too time-consuming and not efficient enough. The difference in results could be linked with the fact that they used a different microorganism and the substrate was previously pretreated with alkali hydrolysis which affected substrate structure and lignin concentration.

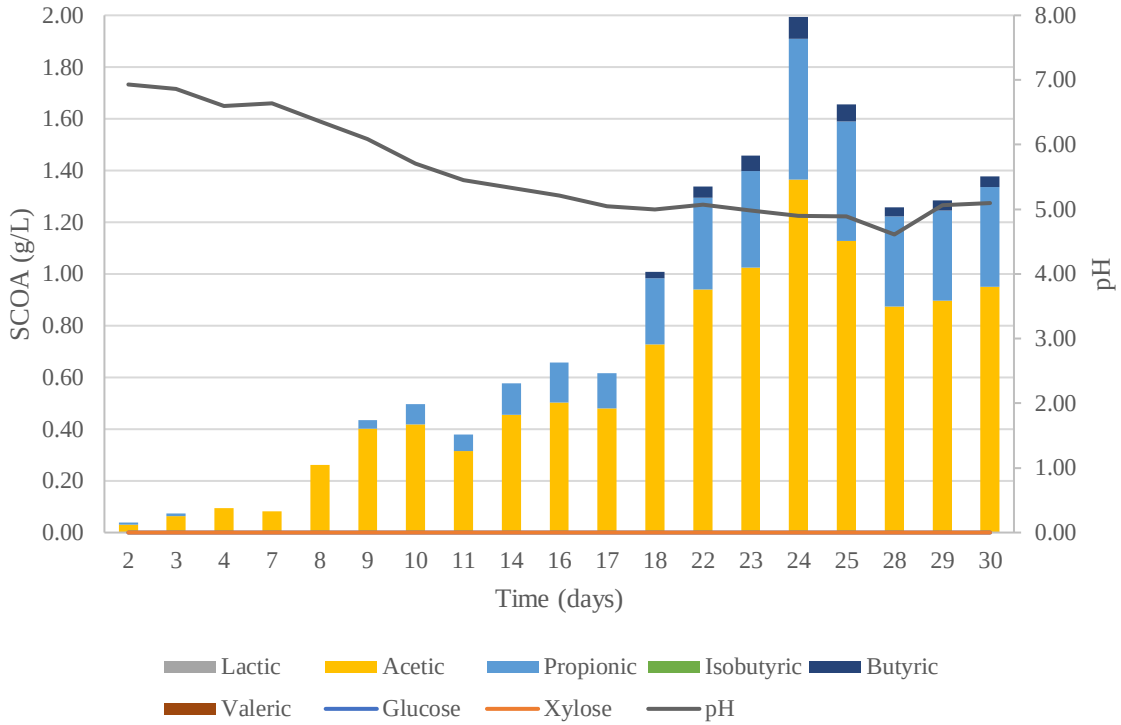

Figure 20. Acidogenic fermentation of spent coffee grounds pretreated with submerged fermentation by *Trametes versicolor*.

On the other hand, when treating SCG with PvSmF (Figure 21), the production of acetic and propionic acid was observed in the proportion of 49.1/50.8%. Acetic acid was the most prevalent acid on other assays, being produced on all days, but in PvSmF, acetic acid production starts to decrease after the 16th day which is accompanied by an increase in pH. In fact, after day 22, samples only contained propionic acid, these results suggested that a change in the MMC population occurred, and metabolism from propionic acid became more prevalent. Nonetheless, it is also possible that the UV sterilization performed on SCG before AF was not effective to destroy *P. variotii* cells. Therefore, the fungus was capable of growth in the fermentation medium and started to consume acetic acid which can be linked to an increase in pH. Although this pretreatment leads to slightly lower SCOA production, compared with the control assay, it could be an interesting strategy to produce only propionic acid by manipulating HRT and thus the microbial community.

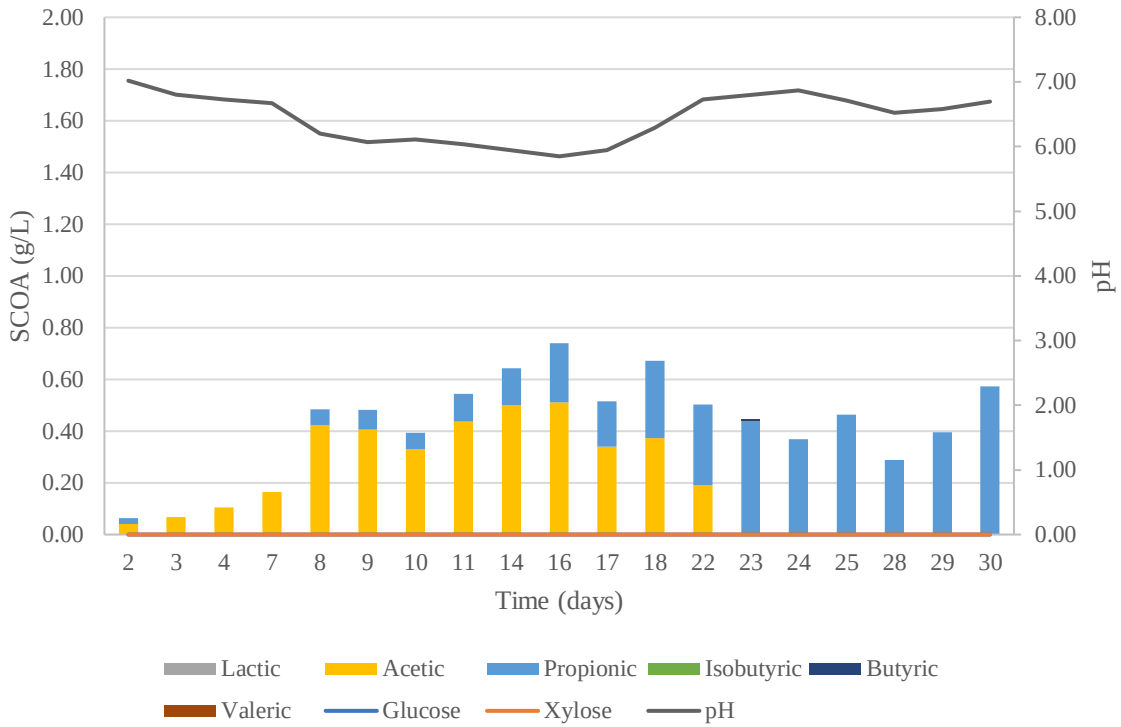

Figure 21. Acidogenic fermentation of spent coffee grounds pretreated with submerged fermentation by *Paecilomyces variotii.*

By mimicking natural conditions of fungal growth, SSF as pretreatment can lead to higher enzymatic production, when compared whit SmF, and thus a more efficient pretreatment[86]. In fact, by promoting fungal delignification it is avoided the occurrence of lignin degradation products and there is no need for lignin recovery[70]. From the SCG pretreated with SSF, the only with significant SCOA production was TvSSF (Figure 22) while the PvSSF (Figure 29 on section 8.2 Appendix B: AF batch experiments) resulted in SCOA production near to zero. After the pretreatment, it was observed the production of lactic, acetic, propionic, isobutyric, butyric, and valeric acids on the proportions of 1.3/49.7/28.4/4.6/14.8/1.2%. Like the other pretreatments, it is observed an increase in SCOA production until it reaches 1.13 g/L on day 25, and after that, SCOA production started to decrease. Although the highest concentration obtained is near to the obtained on control assay, it is observed more variability on SCOA produced.

Tsafrakidou et al. [70] performed wheat straw lignin degradation through SFF with *P. chrysosporium.* After AF, MMC produced lactic, acetic, and butyric acids on the proportion 26.8/47.2/26.0% with a maximum SCOA concentration of 3.96 and 5.85 g/L with free and

immobilized cells, respectively. SCOA production was 3.5 and 5.2 times higher than the obtained in this work. Similar results were obtained by Tišma et al.[201]. After corn silage pretreatment with *T. versicolor*, they registered the production of acetic, isobutyric, propionic, and valeric acids on the proportion of 67.4/27.6/2.6/2.4% totalization 5.43 g/L of SCOA. Once again, the results obtained from the researchers were almost 5 times higher than in this work. This discrepancy of results could be related to SFF incubation time. In Tsafrakidou and Tišma works, fungi were only incubated for 21 and 7 days, respectively, while in this work, SFF was performed for 3 months. During this time, besides lignin oxidation *T. versicolor* could have consumed hemicellulose fraction of SCG, lowering the sugars available for AF.

Opposing this, Fang et al. [78] observed that solid digestate pretreated with *T.versicolor* yielded 9 % lower SCOA than the control test. Although *T. versicolor* exhibited relatively high Lac activity (284.9 U/ g VS) it also obtained a low cellulose/lignin ratio of the pretreated substrate. This led researchers to conclude that lignin degradation was accompanied by high cellulose degradation by *T. versicolor*, explaining the poor results obtained. More recently, the same group studied the pretreatment effect on cellulose-rich compost by *T. versicolor*. Although it was observed an increase in SCOA production in the first weeks, the SCOA yield was 39 % lower than the control group. No substantial changes in acids profile were observed, with acetic and propionic acids as the main SCOA produced[80]. Once again, Akyol et al.[79] registered more significant cellulose degradation when pretreating a mixture of cow manure and cereal crops with *T. versicolor*. Nonetheless, untreated substrate AF produced higher SCOA, mainly propionic acid. Pretreatment with *T. versicolor* lead to a shift in SCOA composition and acetic acid became the most prevalent SCOA, indicating that *T. versicolor* positively affected acetic acid metabolism.

More recently Li et al.[198] studied the use of a mixture of microorganisms including *T. versicolor, Phanerochaete chrysosporium, Trichoderma viride, Aspergillus niger, Gloeophyllum trabeum, Bacillus circulans, Pseudomonas aeruginosa*, and *Streptomyces badius* to pretreat corn straws. They observed that by promoting interactions between different fungi and bacteria, they obtained a more efficient lignin degradation and consequently, could shorten the AF fermentation period and increase biogas production. Therefore, it would be interesting to test SCG pretreatment including other microorganisms on SFF.

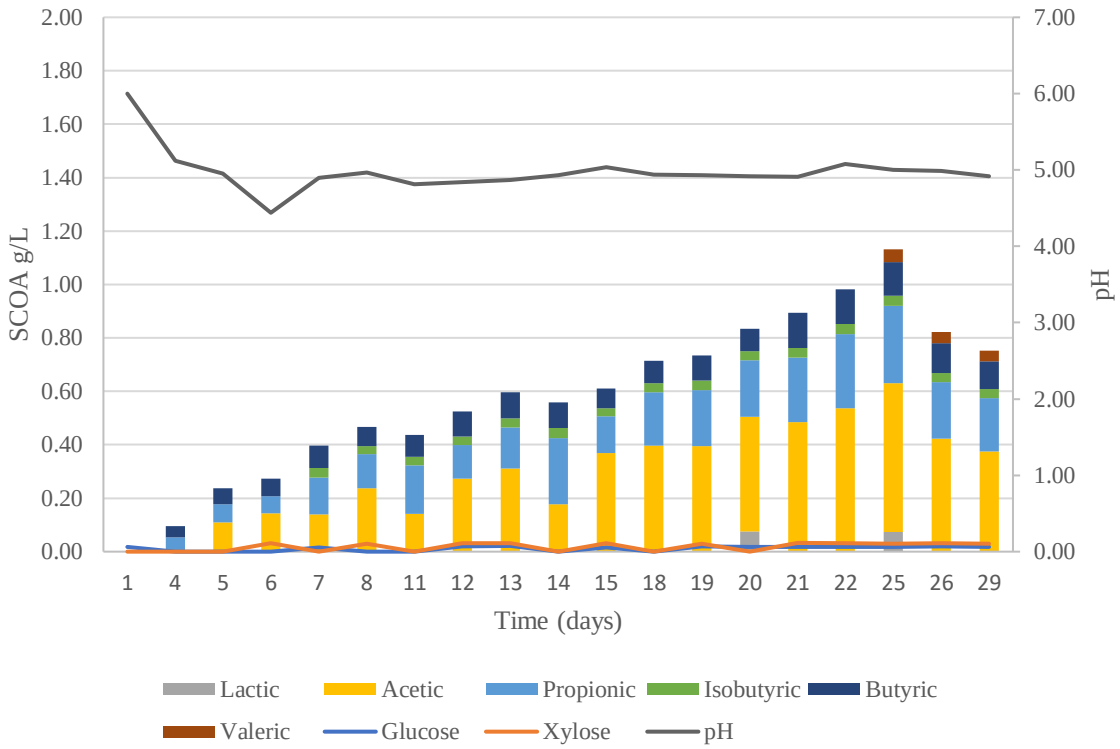

Figure 22. Acidogenic fermentation of spent coffee grounds pretreated with solid-state fermentation by *Trametes versicolor*.

5. Conclusions

SCG are an interesting substrate to employ in biotechnological processes, such as SCOA production. Nonetheless, their recalcitrant structure implies the application of pretreatments to increase sugars disponibility and lower lignin presence. Physicochemical pretreatments are widely studied but can have multiple technical, economic, and environmental drawbacks. Enzymatic hydrolysis is a more environmentally friendly alternative, however, enzymes are expensive proteins and increase process costs.

During the present study, the inducer effect of SCG was studied, by itself and mixed with PP, on enzyme secretion by *T. versicolor* and *P. variotti*. The enzymatic extracts obtained were used to pretreat SCG alongside other pretreatments such as acid hydrolysis, alkaline hydrolysis, supercritical extraction, and SSF of *T. versicolor* and *P. variotti*. Finally, pretreated SCG were submitted to AF to assess the effect of different pretreatments on SCOA production.

With this work, it was possible to conclude that *T. versicolor* was able to produce Lac in significant concentrations when using SCG and a combination of SCG with PP as inducers. The maximum Lac activity achieved was 350 and 223 U/L for 0.4% w/v SCG and 1.0 and 0.4% w/v SCG+PP, respectively. It was also observed that lignocellulosic wastes concentration played an important factor in enzyme production since higher SCG concentrations resulted in lower Lac activity but led to MnP production.

From the pretreatment applied acid hydrolysis resulted in higher sugar released however the yield obtained, 5.95%, was lower than the reported on literature thus indicating that the conditions used in this study were too mild and consequently, acid concentration, temperature, and/or time of hydrolysis should be increased. It was also observed a negative effect when submitting SCG to acid hydrolysis after being treated with supercritical extraction.

In general, for AF batch assays, the main SCOA produced were acetic and propionic acids, reflecting a dominance on acetic and propionic bacteria. It was also registered the production of isobutyric, butyric, valeric, and lactic acids at lower concentrations. Lactic acid production was associated with AF disturbance specially promoted by lower pH.

From all pretreatments, acid hydrolysis led to higher SCOA concentration (2.52 g COD/L) but submerged fermentation by *T. versicolor* led to similar SCOA concentration (2.44 g COD/L) and

higher acidification degree (48.0%) showing a great potential of *T. versicolor* enzymes for SCG pretreatment. However, results obtained were lower than the reported in the literature indicating that a great part of SCG was not consumed and pretreatment must be optimized. Another finding was that pretreatments with *P. variotii* produced the lowest SCOA but demonstrated the exclusive production of propionic acid after 23 days of incubation, suggesting that it can be applied to produce this SCOA exclusively.

Overall, it is possible to conclude that SCG has great potential as a substrate for AF and Lac production by *T. versicolor*. Nonetheless, future optimizations of the processes must be performed to increase their efficiency and competitiveness.

6. Future prospects

Despite the conclusions reached in this work, due to time constraints, further work goes through enzyme production and SCG pretreatment optimization.

Enzymatic production is closely linked to physical and chemical parameters. Besides phenolic compounds, Lac gene expression is highly regulated by the presence of nitrogen[162]. and cupric ions[142,152]. Therefore, it would be interesting to test the effect of different C/N ratios and copper in combination with SCG to access the best concentration for Lac production. Moreover, further studies should assess the impact of agitation and SSF strategy for Lac production using SCG and PP as the substrate for *T. versicolor*.

As previously pointed, SCG pretreatment was not as efficient as reported in the literature, reflecting low sugars release, low SCOA productions, and low acidification degree. Therefore, it would be interesting to adjust acid and alkaline hydrolysis parameters in further, studies, namely chemical concentration, reaction time, and temperature but always keeping them under mild conditions. In the future, it is important to evaluate the effect of pretreatment on cellulose, hemicellulose, and lignin fractions as well as the concentration of inhibitors of microbial activity that could result from the pretreatment and influenced the AF negatively.

Regarding biological pretreatments, when using enzymatic hydrolysis with the extracts produced, lower SCOA were obtained and it was also visible the growth of fungi in the batch assays. Therefore it is necessary to try a better strategy to purify enzymes to avoid fungal growth and SCOA consumption. The SSF pretreatment also reveals some constraints related to incubation time thus should be explored the pretreatment with a lower incubation time. Additionally, it would be interesting to test SCG pretreatment including other microorganisms on SFF.

7. Bibliography

1. Franca, A. S., & Oliveira, L. S. Coffee and its by-products as sources of bioactive compounds. in *Coffee: Production, Consumption and Health Benefits* (ed. Massey, J. L.) 1–28 (Nova Science Publishers, Inc, 2016).

2. Brienzo, M., García-Aparicio, M., & Görgens, J. Spent coffee ground properties and application in bioenergy and bioproducts. in *Coffee: Production, Consumption and Health Benefits* (ed. Massey, J. L.) 67–96 (Nova Science Publishers, Inc, 2016).

3. Esmaeilian, B., Wang, B., Lewis, K., Duarte, F., Ratti, C., & Behdad, S. The future of waste management in smart and sustainable cities: A review and concept paper. *Waste Manag.* **81**, 177–195 (2018).

4. Karmee, S. K. A spent coffee grounds based biorefinery for the production of biofuels, biopolymers, antioxidants and biocomposites. *Waste Manag.* **72**, 240–254 (2018).

5. Girotto, F., Kusch, S., & Lavagnolo, M. C. Biological metabolites recovery from beverage production solid residues through acidogenic fermentation. *Detritus* **5**, 19–28 (2019).

6. Kovalcik, A., Obruca, S., & Marova, I. Valorization of spent coffee grounds: a review. *Food Bioprod. Process.* **110**, 104–119 (2018).

7. Sperandio, G. B., & Filho, E. X. F. Fungal co-cultures in the lignocellulosic biorefinery context: A review. *Int. Biodeterior. Biodegrad.* **142**, 109–123 (2019).

8. Tavares, A. P. M., Pereira, S. R., & Xavier, A. M. R. B. Biotechnological applications of Trametes versicolor and their enzymes. *Curr. Biotechnol.* **6**, 78–88 (2017).

9. Saloheimo, M., Pakula, T., Aro, N., & Joensuu, J. J. Protein Production—Quality Control and Secretion Stress Responses in Trichoderma reesei. in *Biotechnology and Biology of Trichoderma* (eds. Gupta, V. K., Schmoll, M., Herrera-Estrella, A., Upadhyay, R. ., Druzhinina, I. & Tuohy, M. G.) 81–88 (Elsevier, 2014). doi:10.1016/B978-0-444-59576-8.00006-0.

10. Urquhart, A. S., Mondo, S. J., Mäkelä, M. R., Hane, J. K., Wiebenga, A., He, G., Mihaltcheva, S., Pangilinan, J., Lipzen, A., Barry, K., de Vries, R. P., Grigoriev, I. V., & Idnurm, A. Genomic and Genetic Insights Into a Cosmopolitan Fungus, Paecilomyces variotii (Eurotiales). *Front. Microbiol.* **9**, 1–21 (2018).

11. Sharma, B., Sarkar, A., Singh, P., & Singh, R. P. Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. *Waste Manag.* **64**, 117–132 (2017).

12. Kaza, S., Yao, L., Bhada-Tata, P., & Woerden, F. Van. *What a waste 2.0: A global snapshot of solid waste management to 2050*. (World Bank, 2018). doi:10.1596/978-1-4648.

13. Bagheri, M., Esfilar, R., Sina Golchi, M., & Kennedy, C. A. Towards a circular economy: A comprehensive study of higher heat values and emission potential of various municipal solid wastes. *Waste Manag.* **101**, 210–221 (2020).

14. Abila, B., & Kantola, J. Waste management: relevance to environmental sustainability. *Int. J. Environ. Waste Manag.* **23**, 337–351 (2019).

15. Sharma, S., Basu, S., Shetti, N. P., & Aminabhavi, T. M. Waste-to-energy nexus for circular economy and environmental protection: Recent trends in hydrogen energy. *Sci. Total Environ.* **713**, 136633 (2020).

16. The United Nations. About the Sustainable Development Goals - United Nations Sustainable Development. *Sustainable Development Goals* 3–6 https://www.un.org/sustainabledevelopment/sustainable-development-goals/ (2015).

17. Vitorino de Souza Melaré, A., Montenegro González, S., Faceli, K., & Casadei, V. Technologies and decision support systems to aid solid-waste management: a systematic review. *Waste Manag.* **59**, 567–584 (2017).

18. Kirchherr, J., Reike, D., & Hekkert, M. Conceptualizing the circular economy: An analysis of 114 definitions. *Resour. Conserv. Recycl.* **127**, 221–232 (2017).

19. Millar, N., McLaughlin, E., & Börger, T. The Circular Economy: Swings and Roundabouts? *Ecol. Econ.* **158**, 11–19 (2019).

20. Collivignarelli, M. C., Canato, M., Abbà, A., & Carnevale Miino, M. Biosolids: What are the different types of reuse? *J. Clean. Prod.* **238**, 117844 (2019).

21. Zabaniotou, A., & Kamaterou, P. Food waste valorization advocating Circular Bioeconomy - A critical review of potentialities and perspectives of spent coffee grounds biorefinery. *J. Clean. Prod.* **211**, 1553–1566 (2019).

22. Mata, T. M., Martins, A. A., & Caetano, N. S. Bio-refinery approach for spent coffee grounds valorization. *Bioresour. Technol.* **247**, 1077–1084 (2018).

23. Murthy, P. S., & Madhava Naidu, M. Sustainable management of coffee industry by-products and value addition - A review. *Resour. Conserv. Recycl.* **66**, 45–58 (2012).

24. International Coffee Organization. *Coffee market report*. (2019).

25. Tsai, W. T., Liu, S. C., & Hsieh, C. H. Preparation and fuel properties of biochars from the pyrolysis of exhausted coffee residue. *J. Anal. Appl. Pyrolysis* **93**, 63–67 (2012).

26. McNutt, J., & He, Q. (Sophia). Spent coffee grounds: A review on current utilization. *J. Ind. Eng. Chem.* **71**, 78–88 (2019).

27. Mata, T. M., Martins, A. A., & Caetano, N. S. Bio-refinery approach for spent coffee grounds valorization. *Bioresour. Technol.* **247**, 1077–1084 (2018).

28. Pauli, E. D., Barbieri, F., Garcia, P. S., Madeira, T. B., Acquaro, V. R., Scarminio, I. S., da Camara, C. A. P., & Nixdorf, S. L. Detection of ground roasted coffee adulteration with roasted soybean and wheat. *Food Res. Int.* **61**, 112–119 (2014).

29. Franca, A. S., & Oliveira, L. S. Coffee processing solid wastes: Current uses and future perspectives. in *Agricultural Wastes* (eds. Ashworth, G. S. & Azevedo, P.) 155–190 (Nova Science Publishers, Inc, 2009).

30. Mussatto, S. I., Carneiro, L. M., Silva, J. P. A., Roberto, I. C., & Teixeira, J. A. A study on chemical constituents and sugars extraction from spent coffee grounds. *Carbohydr. Polym.* **83**, 368–374 (2011).

31. Simões, J., Nunes, F. M., Domingues, M. R., & Coimbra, M. A. Extractability and structure of spent coffee ground polysaccharides by roasting pre-treatments. *Carbohydr. Polym.* **97**, 81–89 (2013).

32. Ballesteros, L. F., Teixeira, J. A., & Mussatto, S. I. Chemical, Functional, and Structural Properties of Spent Coffee Grounds and Coffee Silverskin. *Food Bioprocess Technol.* **7**, 3493–3503 (2014).

33. Ribeiro, H., Marto, J., Raposo, S., Agapito, M., Isaac, V., Chiari, B. G., Lisboa, P. F., Paiva, A., Barreiros, S., & Simões, P. From coffee industry waste materials to skin-friendly products with improved skin fat levels. *Eur. J. Lipid Sci. Technol.* **115**, 330–336 (2013).

34. Caetano, N. S., Silva, V. F. M., Melo, A. C., Martins, A. A., & Mata, T. M. Spent coffee grounds for biodiesel production and other applications. *Clean Technol. Environ. Policy* **16**, 1423–1430 (2014).

35. Oliveira, L. S., Franca, A. S., Camargos, R. R. S., & Ferraz, V. P. Coffee oil as a potential feedstock for biodiesel production. *Bioresour. Technol.* **99**, 3244–3250 (2008).

36. Al-Hamamre, Z., Foerster, S., Hartmann, F., Kröger, M., & Kaltschmitt, M. Oil extracted from spent coffee grounds as a renewable source for fatty acid methyl ester manufacturing. *Fuel* **96**, 70–76 (2012).

37. Park, J., Kim, B., & Lee, J. W. In-situ transesterification of wet spent coffee grounds for sustainable biodiesel production. *Bioresour. Technol.* **221**, 55–60 (2016).

38. Obruca, S., Benesova, P., Kucera, D., Petrik, S., & Marova, I. Biotechnological conversion of spent coffee grounds into polyhydroxyalkanoates and carotenoids. *N. Biotechnol.* **32**, 569–574 (2015).

39. Troiano, D., Orsat, V., & Dumont, M. J. Status of filamentous fungi in integrated biorefineries. *Renew.*

Sustain. Energy Rev. **117**, 1–18 (2020).

40. Hudeckova, H., Neureiter, M., Obruca, S., Frühauf, S., & Marova, I. Biotechnological conversion of spent coffee grounds into lactic acid. *Lett. Appl. Microbiol.* **66**, 306–312 (2018).

41. Kopp, D., Willows, R. D., & Sunna, A. Cell-free enzymatic conversion of spent coffee grounds into the platform chemical lactic acid. *Front. Bioeng. Biotechnol.* **7**, 1–13 (2019).

42. Kim, J., Jang, J. H., Yeo, H. J., Seol, J., Kim, S. R., & Jung, Y. H. Lactic acid production from a whole slurry of acid-pretreated spent coffee grounds by engineered Saccharomyces cerevisiae. *Appl. Biochem. Biotechnol.* **189**, 206–216 (2019).

43. Kucera, D., Pernicová, I., Kovalcik, A., Koller, M., Mullerova, L., Sedlacek, P., Mravec, F., Nebesarova, J., Kalina, M., Marova, I., Krzyzanek, V., & Obruca, S. Characterization of the promising poly(3-hydroxybutyrate) producing halophilic bacterium Halomonas halophila. *Bioresour. Technol.* **256**, 552–556 (2018).

44. Kovalcik, A., Kucera, D., Matouskova, P., Pernicova, I., Obruca, S., Kalina, M., Enev, V., & Marova, I. Influence of removal of microbial inhibitors on PHA production from spent coffee grounds employing Halomonas halophila. *J. Environ. Chem. Eng.* **6**, 3495–3501 (2018).

45. Obruca, S., Benesova, P., Kucera, D., Petrik, S., & Marova, I. Biotechnological conversion of spent coffee grounds into polyhydroxyalkanoates and carotenoids. *New Biotechnology* (2015) doi:10.1016/j.nbt.2015.02.008.

46. Obruca, S., Benesova, P., Petrik, S., Oborna, J., Prikryl, R., & Marova, I. Production of polyhydroxyalkanoates using hydrolysate of spent coffee grounds. *Process Biochem.* **49**, 1409–1414 (2014).

47. Kwon, E. E., Yi, H., & Jeon, Y. J. Sequential co-production of biodiesel and bioethanol with spent coffee grounds. *Bioresour. Technol.* **136**, 475–480 (2013).

48. Mussatto, S. I., Machado, E. M. S., Carneiro, L. M., & Teixeira, J. A. Sugars metabolism and ethanol production by different yeast strains from coffee industry wastes hydrolysates. *Appl. Energy* **92**, 763–768 (2012).

49. Wainaina, S., Lukitawesa, Kumar Awasthi, M., & Taherzadeh, M. J. Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: A critical review. *Bioengineered* **10**, 437–458 (2019).

50. Sun, L., Gong, M., Lv, X., Huang, Z., Gu, Y., Li, J., Du, G., & Liu, L. Current advance in biological production of short-chain organic acid. *Appl. Microbiol. Biotechnol.* **104**, 9109–9124 (2020).

51. Romero-Güiza, M. S., Vila, J., Mata-Alvarez, J., Chimenos, J. M., & Astals, S. The role of additives on anaerobic digestion: A review. *Renew. Sustain. Energy Rev.* **58**, 1486–1499 (2016).

52. Zhou, M., Yan, B., Wong, J. W. C., & Zhang, Y. Enhanced volatile fatty acids production from anaerobic fermentation of food waste: A mini-review focusing on acidogenic metabolic pathways. *Bioresour. Technol.* **248**, 68–78 (2018).

53. Atasoy, M., Owusu-Agyeman, I., Plaza, E., & Cetecioglu, Z. Bio-based volatile fatty acid production and recovery from waste streams: Current status and future challenges. *Bioresour. Technol.* **268**, 773–786 (2018).

54. Liang, T., Elmaadawy, K., Liu, B., Hu, J., Hou, H., & Yang, J. Anaerobic fermentation of waste activated sludge for volatile fatty acid production: Recent updates of pretreatment methods and the potential effect of humic and nutrients substances. *Process Saf. Environ. Prot.* **145**, 321–339 (2021).

55. Sousa, R. D. M. Industrial byproducts acidification to produce microbial substrates. (Universidade de Aveiro, 2016).

56. Bermúdez-Penabad, N., Kennes, C., & Veiga, M. C. Anaerobic digestion of tuna waste for the production of volatile fatty acids. *Waste Manag.* **68**, 96–102 (2017).

57. Cheah, Y. K., Dosta, J., & Mata-Álvarez, J. Enhancement of volatile fatty acids production from food waste by mature compost addition. *Molecules* **24**, 1–17 (2019).

58. Basak, B., Saha, S., Chatterjee, P. K., Ganguly, A., Woong Chang, S., & Jeon, B.-H. Pretreatment of polysaccharidic wastes with cellulolytic Aspergillus fumigatus for enhanced production of biohythane in a dual-stage process. *Bioresour. Technol.* **299**, 1–10 (2020).

59. Zhang, R., Liu, F., Liu, H., & Zhang, D. Pretreatment of Corn Stover with Diluted Nitric Acid for the Enhancement of Acidogenic Fermentation. *Energy and Fuels* **32**, 425–430 (2018).

60. Sawatdeenarunat, C., Sung, S., & Khanal, S. K. Enhanced volatile fatty acids production during anaerobic digestion of lignocellulosic biomass via micro-oxygenation. *Bioresour. Technol.* **237**, 139–145 (2017).

61. Xin, X., She, Y., & Hong, J. Insights into microbial interaction profiles contributing to volatile fatty acids production via acidogenic fermentation of waste activated sludge assisted by calcium oxide pretreatment. *Bioresour. Technol.* **320**, 1–9 (2021).

62. Xu, Y., Zheng, L., Geng, H., Liu, R., & Dai, X. Enhancing acidogenic fermentation of waste activated sludge via isoelectric-point pretreatment: Insights from physical structure and interfacial thermodynamics. *Water Res.* **185**, 1–11 (2020).

63. Queirós, D., Sousa, R., Pereira, S., & Serafim, L. S. Valorization of a Pulp Industry By-Product through the Production of Short-Chain Organic Acids. *Fermentation* **3**, 1–11 (2017).

64. Lee, W. S., Chua, A. S. M., Yeoh, H. K., & Ngoh, G. C. A review of the production and applications of waste-derived volatile fatty acids. *Chem. Eng. J.* **235**, 83–99 (2014).

65. Castilla-Archilla, J., Papirio, S., & Lens, P. N. L. Two step process for volatile fatty acid production from brewery spent grain: Hydrolysis and direct acidogenic fermentation using anaerobic granular sludge. *Process Biochem.* **100**, 272–283 (2021).

66. Naresh Kumar, A., & Venkata Mohan, S. Acidogenesis of waste activated sludge - Biohydrogen production with simultaneous short chain carboxylic acids. *J. Environ. Chem. Eng.* **6**, 2983–2991 (2018).

67. Hashemi, B., Sarker, S., Lamb, J. J., & Lien, K. M. Yield improvements in anaerobic digestion of lignocellulosic feedstocks. *J. Clean. Prod.* **288**, 1–27 (2021).

68. Girotto, F., Lavagnolo, M. C., & Pivato, A. Spent coffee grounds alkaline pre-treatment as biorefinery option to enhance their anaerobic digestion yield. *Waste and Biomass Valorization* **9**, 2565–2570 (2018).

69. Azizi, A., Hosseini Koupaie, E., Hafez, H., & Elbeshbishy, E. Improving single- and two-stage anaerobic digestion of source separated organics by hydrothermal pretreatment. *Biochem. Eng. J.* **148**, 77–86 (2019).

70. Tsafrakidou, P., Bekatorou, A., Koutinas, A. A., Kordulis, C., Banat, I. M., Petsi, T., & Sotiriou, M. Acidogenic Fermentation of Wheat Straw After Chemical and Microbial Pretreatment for Biofuel Applications. *Energy Convers. Manag.* **160**, 509–517 (2018).

71. Bahreini, G., Nazari, L., Ho, D., Flannery, C. C., Elbeshbishy, E., Santoro, D., & Nakhla, G. Enzymatic pre-treatment for enhancement of primary sludge fermentation. *Bioresour. Technol.* **305**, 1–10 (2020).

72. Islam, M. S., Zhang, Z., Qu, S. Ben, Liu, C. L., Guo, C., & Liu, C. Z. Coproduction of hydrogen and volatile fatty acids via integrated two-step fermentation of sweet sorghum stalks by alkaline and enzymatic treatment. *Biomass and Bioenergy* **145**, 1–8 (2021).

73. Lin, L., Li, R. hong, & Li, X. yan. Recovery of organic resources from sewage sludge of Al-enhanced primary sedimentation by alkali pretreatment and acidogenic fermentation. *J. Clean. Prod.* **172**, 3334–3341 (2018).

74. Szumacher-Strabel, M., Zmora, P., Roj, E., Stochmal, A., Oleszek, W., & Lechniak, D. The potential of the wild dog rose (Rosa canina) to mitigate in vitro rumen methane production. *J. Anim. Feed Sci.* **20**, 285–299 (2011).

75. Martinez, G. A., Rebecchi, S., Decorti, D., Domingos, J. M. B., Natolino, A., Del Rio, D., Bertin, L., Da Porto, C., & Fava, F. Towards multi-purpose biorefinery platforms for the valorisation of red grape pomace: production of polyphenols, volatile fatty acids, polyhydroxyalkanoates and biogas. *Green Chem.* **18**, 261–270 (2016).

76. Rusli, N. D., Azmi, M. A., Mat, K., Hasnita, C. H., Wan-Zahari, M., Azhar, K., Zamri-Saad, M., & Hassim, H. A. The effect of physical and biological pre-treatments of oil palm fronds on in vitro ruminal degradability. *Pertanika J. Trop. Agric. Sci.* **42**, 791–805 (2019).

77. Yue, Z. Q., Xu, Y. Z., Wang, C., Liu, Q., Guo, G., Huo, W. J., Zhang, J., Chen, L., Pei, C. X., Zhang, Y. L., & Zhang, S. L. Effects of dietary laccase supplementation on growth performance, nutrient digestion, rumen fermentation and microbiota in dairy bulls. *Anim. Feed Sci. Technol.* **269**, 1–9 (2020).

78. Fang, W., Zhang, P., Zhang, X., Zhu, X., van Lier, J. B., & Spanjers, H. White rot fungi pretreatment to advance volatile fatty acid production from solid-state fermentation of solid digestate: Efficiency and mechanisms. *Energy* **162**, 534–541 (2018).

79. Akyol, Ç., Ince, O., Bozan, M., Ozbayram, E. G., & Ince, B. Biological pretreatment with Trametes versicolor to enhance methane production from lignocellulosic biomass: A metagenomic approach. *Ind. Crops Prod.* **140**, 1–10 (2019).

80. Fang, W., Zhang, X., Zhang, P., Carol Morera, X., van Lier, J. B., & Spanjers, H. Evaluation of white rot fungi pretreatment of mushroom residues for volatile fatty acid production by anaerobic fermentation: Feedstock applicability and fungal function. *Bioresour. Technol.* **297**, 1–7 (2020).

81. Hemalatha, M., Sarkar, O., & Venkata Mohan, S. Self-sustainable azolla-biorefinery platform for valorization of biobased products with circular-cascading design. *Chem. Eng. J.* **373**, 1042–1053 (2019).

82. Ravindran, R., & Jaiswal, A. K. Microbial enzyme production using lignocellulosic food industry wastes as feedstock: A review. *Bioengineering* **3**, 1–22 (2016).

83. Subramanian, S. B., Yan, S., Tyagi, R. D., & Surampalli, R. Y. Enzymes: Production and Extraction. in *Sustainable Sludge Management* (eds. Tyagi, R. D., Surampalli, R. Y., Yan, S., Zhang, T. C., Kao, C. M. & Lohani, B. N.) 231–261 (American Society of Civil Engineers, 2009). doi:10.1061/9780784410516.ch10.

84. Hirose, Y. Production and Isolation of Enzymes. in *Enzyme Catalysis in Organic Synthesis* (eds. Drauz, K., Groger, H. & May, O.) 191–215 (Wiley-VCH Verlag GmbH & Co, 2012).

85. Guerrand, D. Lipases industrial applications: Focus on food and agroindustries. *OCL - Oilseeds fats, Crop. Lipids* **24**, 1–7 (2017).

86. Ravindran, R., Hassan, S. S., Williams, G. A., & Jaiswal, A. K. A review on bioconversion of agro-industrial wastes to industrially important enzymes. *Bioengineering* **5**, 1–20 (2018).

87. Janusz, G., Pawlik, A., Sulej, J., Świderska-Burek, U., Jarosz-Wilkolazka, A., & Paszczyński, A. Lignin degradation: Microorganisms, enzymes involved, genomes analysis and evolution. *FEMS Microbiol. Rev.* **41**, 941–962 (2017).

88. Córdoba M, K. A., & Ríos H, A. Biotechnological applications and potential uses of the mushroom Tramestes versicolor. *Vitae, Rev. La Fac. Química Farm.* **19**, 70–76 (2012).

89. Kumar, S. S., Sreekumar, R., & Sabu, A. *Green Bio-processes*. *Green Bio-processes* (Springer Singapore, 2019). doi:10.1007/978-981-13-3263-0.

90. Chandrasekaran, M., & Beena, P. S. *Tannase: Source, biocatalytic characteristics, and bioprocesses for production*. *Marine Enzymes for Biocatalysis: Sources, Biocatalytic Characteristics and Bioprocesses of Marine Enzymes* (Woodhead Publishing Limited, 2013). doi:10.1533/9781908818355.3.259.

91. Mäkelä, M. R., Marinović, M., Nousiainen, P., Liwanag, A. J. M., Benoit, I., Sipilä, J., Hatakka, A., de Vries, R. P., & Hildén, K. S. Aromatic metabolism of filamentous fungi in relation to the presence of aromatic compounds in plant biomass. *Adv. Appl. Microbiol.* **91**, 63–137 (2015).

92. Yao, J., Guo, G. S., Ren, G. H., & Liu, Y. H. Production, characterization and applications of tannase. *J. Mol. Catal. B Enzym.* **101**, 137–147 (2014).

93. Chauhan, P. S., Puri, N., Sharma, P., & Gupta, N. Mannanases: Microbial sources, production, properties and potential biotechnological applications. *Appl. Microbiol. Biotechnol.* **93**, 1817–1830 (2012).

94. Kunamneni, A., Plou, F. J., Alcalde, M., & Ballesteros, A. Trichoderma Enzymes for Food Industries. in *Biotechnology and Biology of Trichoderma* (eds. Gupta, V. K., Schmoll, M., Herrera-Estrella, A., Upadhyay, R. S., Druzhinina, I. & Tuohy, M. G.) 339–344 (Elsevier, 2014). doi:10.1016/B978-0-444-59576-8.00024-2.

95. Cerda, A., Artola, A., Barrena, R., Font, X., Gea, T., & Sánchez, A. Innovative Production of Bioproducts From Organic Waste Through Solid-State Fermentation. *Front. Sustain. Food Syst.* **3**, 1–6 (2019).

96. Rodríguez Couto, S., & Sanromán, M. A. Application of solid-state fermentation to ligninolytic enzyme production. *Biochem. Eng. J.* **22**, 211–219 (2005).

97. Jiménez, S., Velásquez, C., Mejía, F., Arias, M., & Hormaza, A. Comparative studies of pure cultures and a consortium of white-rot fungi to degrade a binary mixture of dyes by solid-state fermentation and performance at different scales. *Int. Biodeterior. Biodegrad.* **145**, 104772 (2019).

98. Farinas, C. S. Developments in solid-state fermentation for the production of biomass-degrading enzymes for the bioenergy sector. *Renew. Sustain. Energy Rev.* **52**, 179–188 (2015).

99. Sadh, P. K., Duhan, S., & Duhan, J. S. Agro-industrial wastes and their utilization using solid state fermentation: a review. *Bioresour. Bioprocess.* **5**, 1–15 (2018).

100. Manan, M. A., & Webb, C. Design Aspects of Solid State Fermentation as Applied to Microbial Bioprocessing. *J. Appl. Biotechnol. Bioeng.* **4**, 511–532 (2017).

101. de Castro, R. J. S., & Sato, H. H. Enzyme Production by Solid State Fermentation: General Aspects and an Analysis of the Physicochemical Characteristics of Substrates for Agro-industrial Wastes Valorization. *Waste and Biomass Valorization* **6**, 1085–1093 (2015).

102. Oliveira, F., Souza, C. E., Peclat, V. R. O. L., Salgado, J. M., Ribeiro, B. D., Coelho, M. A. Z., Venâncio, A., & Belo, I. Optimization of lipase production by Aspergillus ibericus from oil cakes and its application in esterification reactions. *Food Bioprod. Process.* **102**, 268–277 (2017).

103. Murthy, P. S., Naidu, M. M., & Srinivas, P. Production of α-amylase under solid-state fermentation utilizing coffee waste. *J. Chem. Technol. Biotechnol.* **84**, 1246–1249 (2009).

104. Murthy, P. S., & Naidu, M. M. Production and Application of Xylanase from Penicillium sp. Utilizing Coffee By-products. *Food Bioprocess Technol.* **5**, 657–664 (2012).

105. Machado, E. M. S., Rodriguez-Jasso, R. M., Teixeira, J. A., & Mussatto, S. I. Growth of fungal strains on coffee industry residues with removal of polyphenolic compounds. *Biochem. Eng. J.* **60**, 87–90 (2012).

106. Mansor, A., Ramli, M. S., Abdul Rashid, N. Y., Samat, N., Lani, M. N., Sharifudin, S. A., & Raseetha, S. Evaluation of selected agri-industrial residues as potential substrates for enhanced tannase production via solid-state fermentation. *Biocatal. Agric. Biotechnol.* **20**, 101216 (2019).

107. Leifa, F., Pandey, A., & Soccol, C. R. Production of flammulina velutipes on coffee husk and coffee spent-ground. *Brazilian Arch. Biol. Technol.* **44**, 205–212 (2001).

108. Rochín-Medina, J. J., Ramírez, K., Rangel-Peraza, J. G., & Bustos-Terrones, Y. A. Increase of content and bioactivity of total phenolic compounds from spent coffee grounds through solid state fermentation by Bacillus clausii. *J. Food Sci. Technol.* **55**, 915–923 (2018).

109. Wösten, H. A. B. Filamentous fungi for the production of enzymes, chemicals and materials. *Curr. Opin. Biotechnol.* **59**, 65–70 (2019).

110. Dou, H., Chang, Y., & Zhang, L. Coriolus versicolor polysaccharopeptide as an immunotherapeutic in China.

in *Progress in Molecular Biology and Translational Science* (ed. Zhang, L.) vol. 163 361–381 (Elsevier Inc., 2019).

111. Pop, R. M., Puia, I. C., Puia, A., Chedea, V. S., Leopold, N., Bocsan, I. C., & Buzoianu, A. D. Characterization of Trametes versicolor: Medicinal mushroom with important health benefits. *Not. Bot. Horti Agrobot. Cluj-Napoca* **46**, 343–349 (2018).

112. Brijwani, K., Rigdon, A., & Vadlani, P. V. Fungal laccases: Production, function, and applications in food processing. *Enzyme Res.* **2010**, 1–10 (2010).

113. Adekunle, A. E., Zhang, C., Guo, C., & Liu, C. Z. Laccase production from Trametes versicolor in solid-state fermentation of steam-exploded pretreated cornstalk. *Waste and Biomass Valorization* **8**, 153–159 (2017).

114. Iqbal, H. M. N., Asgher, M., & Bhatti, H. N. Optimization of physical and nutritional factors for synthesis of lignin degrading enzymes by a novel strain of Trametes versicolor. *BioResources* **6**, 1273–1287 (2011).

115. Wang, F., Hu, J. H., Guo, C., & Liu, C. Z. Enhanced laccase production by Trametes versicolor using corn steep liquor as both nitrogen source and inducer. *Bioresour. Technol.* **166**, 602–605 (2014).

116. Aydinoğlu, T., & Sargin, S. Production of laccase from Trametes versicolor by solid-state fermentation using olive leaves as a phenolic substrate. *Bioprocess Biosyst. Eng.* **36**, 215–222 (2013).

117. Almeida, P. H., de Oliveira, A. C. C., de Souza, G. P. N., Friedrich, J. C., Linde, G. A., Colauto, N. B., & do Valle, J. S. Decolorization of remazol brilliant blue R with laccase from lentinus crinitus grown in agro-industrial by-products. *An. Acad. Bras. Cienc.* **90**, 3463–3473 (2018).

118. Pinheiro, V. E., Michelin, M., Vici, A. C., de Almeida, P. Z., & Teixeira de Moraes Polizeli, M. de L. Trametes versicolor laccase production using agricultural wastes: a comparative study in Erlenmeyer flasks, bioreactor and tray. *Bioprocess Biosyst. Eng.* **43**, 507–514 (2020).

119. Mishra, V., Jana, A. K., Jana, M. M., & Gupta, A. Fungal pretreatment of sweet sorghum bagasse with supplements: improvement in lignin degradation, selectivity and enzymatic saccharification. *3 Biotech* **7**, 1–13 (2017).

120. Xin, F., & Geng, A. Utilization of horticultural waste for laccase production by Trametes versicolor under solid-state fermentation. *Appl. Biochem. Biotechnol.* **163**, 235–246 (2011).

121. Wyman, V., Henríquez, J., Palma, C., & Carvajal, A. Lignocellulosic waste valorisation strategy through enzyme and biogas production. *Bioresour. Technol.* **247**, 402–411 (2018).

122. Merino, A., Eibes, G., & Hormaza, A. Effect of copper and different carbon and nitrogen sources on the decolorization of an industrial dye mixture under solid-state fermentation. *J. Clean. Prod.* **237**, 117713 (2019).

123. Cardoso, W. S., Soares, F. E. de F., Queiroz, P. V., Tavares, G. P., Santos, F. A., Sufiate, B. L., Kasuya, M. C. M., & de Queiroz, J. H. Minimum cocktail of cellulolytic multi-enzyme complexes obtained from white rot fungi via solid-state fermentation. *3 Biotech* **8**, 1–8 (2018).

124. Mioso, R., Toledo Marante, F. J., & Herrera Bravo de Laguna, I. The Chemical Diversity of the Ascomycete Fungus Paecilomyces variotii. *Appl. Biochem. Biotechnol.* **177**, 781–791 (2015).

125. Nguyen, T. T. T., Paul, N. C., & Lee, H. B. Characterization of Paecilomyces variotii and Talaromyces amestolkiae in Korea based on the morphological characteristics and multigene phylogenetic analyses. *Mycobiology* **44**, 248–259 (2016).

126. Herrera Bravo de Laguna, I., Toledo Marante, F. J., & Mioso, R. Enzymes and bioproducts produced by the ascomycete fungus Paecilomyces variotii. *J. Appl. Microbiol.* **119**, 1455–1466 (2015).

127. Raaman, N., Mahendran, B., Jaganathan, C., Sukumar, S., & Chandrasekaran, V. Optimisation of extracellular tannase production from Paecilomyces variotii. *World J. Microbiol. Biotechnol.* **26**, 1033–1039 (2010).

128. Valdo Madeira, J., Rosas Ferreira, L., Alves Macedo, J., & Alves Macedo, G. Efficient tannase production

using Brazilian citrus residues and potential application for orange juice valorization. *Biocatal. Agric. Biotechnol.* **4**, 91–97 (2015).

129. Madeira, J. V., Macedo, J. A., & Macedo, G. A. Detoxification of castor bean residues and the simultaneous production of tannase and phytase by solid-state fermentation using Paecilomyces variotii. *Bioresour. Technol.* **102**, 7343–7348 (2011).

130. Madeira, J. V., Macedo, J. A., & Macedo, G. A. A new process for simultaneous production of tannase and phytase by Paecilomyces variotii in solid-state fermentation of orange pomace. *Bioprocess Biosyst. Eng.* **35**, 477–482 (2012).

131. Madeira, J. V., Nakajima, V. M., Macedo, J. A., & Macedo, G. A. Rich bioactive phenolic extract production by microbial biotransformation of Brazilian Citrus residues. *Chem. Eng. Res. Des.* **92**, 1802–1810 (2014).

132. Amutha, K., Haritha, Y., Gowdhaman, D., & Ponnusami, V. Production and optimization of xylanase from newly isolated. *Int. J. ChemTech Res.* **7**, 2064–2070 (2015).

133. Pathak, R., Sharma, A., Adak, A., Sharma, S., & Prasad, R. Role of Jatropha curcas deoiled cake as substrate for the production of cellulases and xylanase and additive in vermicomposting of kitchen waste. *J. Pure Appl. Microbiol.* **10**, 3163–3172 (2016).

134. Roy, B. P., & Archibald, F. Effects of kraft pulp and lignin on Trametes versicolor carbon metabolism. *Appl. Environ. Microbiol.* **59**, 1855–1863 (1993).

135. Ander, P., & Messner, K. Oxidation of 1-hydroxybenzotriazole by laccase and lignin peroxidase. *Biotechnol. Tech.* **12**, 191–195 (1998).

136. Tien, M., & Kirk, T. K. Lignin peroxidase of Phanerochaete chrysosporium. in *Methods in Enzymology* vol. 161 238–249 (Academic Press, 1988).

137. Sharma, S., Bhat, T. K., & Dawra, R. K. A spectrophotometric method for assay of tannase using rhodanine. *Anal. Biochem.* **279**, 85–89 (2000).

138. Clesceri, L., Greenberg, A., & Easton, A. *Standard Methods for the Examination of Water and Wastewater*. (American Public Health Association, 1998).

139. Tavares, A. P. M., Coelho, M. A. Z., Coutinho, J. A. P., & Xavier, A. M. R. B. Laccase improvement in submerged cultivation: Induced production and kinetic modelling. *J. Chem. Technol. Biotechnol.* **80**, 669–676 (2005).

140. Wu, J. M., & Zhang, Y. Z. Gene expression in secondary metabolism and metabolic switching phase of phanerochaete chrysosporium. *Appl. Biochem. Biotechnol.* **162**, 1961–1977 (2010).

141. Atilano-Camino, M. M., Álvarez-Valencia, L. H., García-González, A., & García-Reyes, R. B. Improving laccase production from Trametes versicolor using lignocellulosic residues as cosubstrates and evaluation of enzymes for blue wastewater biodegradation. *J. Environ. Manage.* **275**, 1–10 (2020).

142. Vrsanska, M., Voberkova, S., Langer, V., Palovcikova, D., Moulick, A., Adam, V., & Kopel, P. Induction of Laccase, Lignin Peroxidase and Manganese Peroxidase Activities in White-Rot Fungi Using Copper Complexes. *Molecules* **21**, 1–15 (2016).

143. Amaral, P. F. F., Fernandes, D. L. A., Tavares, A. P. M., Xavier, A. B. M. R., Cammarota, M. C., Coutinho, J. A. P., & Coelho, M. A. Z. Decolorization of dyes from textile wastewater by Trametes versicolor. *Environ. Technol.* **25**, 1313–1320 (2004).

144. González Bautista, E., Gutierrez, E., Dupuy, N., Gaime-Perraud, I., Ziarelli, F., & Farnet da Silva, A. M. Pre-treatment of a sugarcane bagasse-based substrate prior to saccharification: Effect of coffee pulp and urea on laccase and cellulase activities of Pycnoporus sanguineus. *J. Environ. Manage.* **239**, 178–186 (2019).

145. Azizan, A., Lee, A. X., Abdul Hamid, N. A., Maulidiani, M., Mediani, A., Abdul Ghafar, S. Z., Zolkeflee, N. K. Z., & Abas, F. Potentially Bioactive Metabolites from Pineapple Waste Extracts and Their Antioxidant and

α-Glucosidase Inhibitory Activities by 1H NMR. *Foods* **9**, 1–19 (2020).

146. Angeloni, S., Nzekoue, F. K., Navarini, L., Sagratini, G., Torregiani, E., Vittori, S., & Caprioli, G. An analytical method for the simultaneous quantification of 30 bioactive compounds in spent coffee ground by HPLC-MS/MS. *J. Mass Spectrom.* **55**, 1–13 (2020).

147. Wang, F., Hu, J. H., Guo, C., & Liu, C. Z. Enhanced laccase production by Trametes versicolor using corn steep liquor as both nitrogen source and inducer. *Bioresour. Technol.* **166**, 602–605 (2014).

148. Sakdaronnarong, C. K., Onsrithong, N., Suwankrua, R., & Jonglertjunya, W. Improving enzymatic saccharification of sugarcane bagasse by biological/physico-chemical pretreatment using trametes versicolor and bacillus sp. *BioResources* **7**, 3935–3947 (2012).

149. Velázquez-Cedeño, M. A., Mata, G., & Savoie, J. M. Waste-reducing cultivation of Pleurotus ostreatus and Pleurotus pulmonarius on coffee pulp: Changes in the production of some lignocellulolytic enzymes. *World J. Microbiol. Biotechnol.* **18**, 201–207 (2002).

150. Mata, G., Murrieta Hernández, D. M., & Iglesias Andreu, L. G. Changes in lignocellulolytic enzyme activites in six Pleurotus spp. strains cultivated on coffee pulp in confrontation with Trichoderma spp. *World J. Microbiol. Biotechnol.* **21**, 143–150 (2005).

151. Gonzalez, J. C., Medina, S. C., Rodriguez, A., Osma, J. F., Alméciga-Díaz, C. J., & Sánchez, O. F. Production of Trametes pubescens laccase under submerged and semi-solid culture conditions on agro-industrial wastes. *PLoS One* **8**, 1–14 (2013).

152. Piscitelli, A., Giardina, P., Lettera, V., Pezzella, C., Sannia, G., & Faraco, V. Induction and transcriptional regulation of laccases in fungi. *Curr. Genomics* **12**, 104–112 (2011).

153. Nonthakaew, A., Matan, N., Aewsiri, T., & Matan, N. Antifungal activity of crude extracts of coffee and spent coffee ground on Areca palm leaf sheath (Areca catechu) based food packaging. *Packag. Technol. Sci.* **28**, 633–645 (2015).

154. Montoya, S., Orrego, C. E., & Levin, L. Growth, fruiting and lignocellulolytic enzyme production by the edible mushroom Grifola frondosa (maitake). *World J. Microbiol. Biotechnol.* **28**, 1533–1541 (2012).

155. Zeng, S., Zhao, J., & Xia, L. Simultaneous production of laccase and degradation of bisphenol A with Trametes versicolor cultivated on agricultural wastes. *Bioprocess Biosyst. Eng.* **40**, 1237–1245 (2017).

156. Zahmatkesh, M., Spanjers, H., & van Lier, J. B. A novel approach for application of white rot fungi in wastewater treatment under non-sterile conditions: immobilization of fungi on sorghum. *Environ. Technol. (United Kingdom)* **39**, 2030–2040 (2018).

157. dos Santos Bazanella, G. C., de Souza, D. F., Castoldi, R., Oliveira, R. F., Bracht, A., & Peralta, R. M. Production of laccase and manganese peroxidase by Pleurotus pulmonarius in solid-state cultures and application in dye decolorization. *Folia Microbiol. (Praha).* **58**, 641–647 (2013).

158. Durán-Aranguren, D., Cruz-Reina, L., González, L., & Sierra, R. Use of tropical fruit waste for the production of lignocellulolytic enzymes and fungal biomass. in *European Biomass Conference and Exhibition Proceedings* 318–323 (2019). doi:10.5071/27thEUBCE2019-1DV.3.18.

159. Siti Roha, A. M., Zainal, S., Noriham, A., & Nadzirah, K. Z. Determination of sugar content in pineapple waste variety N36. *Int. Food Res. J.* **20**, 1941–1943 (2013).

160. Ire, F. S., Ahuekwe, E. F., & Harcourt, P. Production of fungal laccase using orange peelings as substrate by submerged static fermentation. **15**, 1–19 (2016).

161. Tišma, M., Žnidaršič-Plazl, P., Vasić-Rački, D., & Zelić, B. Optimization of laccase production by Trametes versicolor cultivated on industrial waste. *Appl. Biochem. Biotechnol.* **166**, 36–46 (2012).

162. Collins, P. J., & Dobson, A. D. W. Regulation of laccase gene transcription in Trametes versicolor. *Appl. Environ. Microbiol.* **63**, 3444–3450 (1997).

163. Kurt, S., & Buyukalaca, S. Yield performances and changes in enzyme activities of Pleurotus spp. (P. ostreatus and P. sajor-caju) cultivated on different agricultural wastes. *Bioresour. Technol.* **101**, 3164–3169 (2010).

164. Kim, J., Kim, H., Baek, G., & Lee, C. Anaerobic co-digestion of spent coffee grounds with different waste feedstocks for biogas production. *Waste Manag.* **60**, 322–328 (2017).

165. Orfanoudaki, A., Makridakis, G., Maragkaki, A., Fountoulakis, M. S., Kallithrakas-Kontos, N. G., & Manios, T. Anaerobic Co-digestion of Pig Manure and Spent Coffee Grounds for Enhanced Biogas Production. *Waste and Biomass Valorization* **11**, 4613–4620 (2020).

166. Chrysargyris, A., Antoniou, O., Xylia, P., Petropoulos, S., & Tzortzakis, N. The use of spent coffee grounds in growing media for the production of Brassica seedlings in nurseries. *Environ. Sci. Pollut. Res.* (2020) doi:10.1007/s11356-020-07944-9.

167. Cervera-Mata, A., Lara, L., Fernández-Arteaga, A., Ángel Rufián-Henares, J., & Delgado, G. Washed hydrochar from spent coffee grounds: A second generation of coffee residues. Evaluation as organic amendment. *Waste Manag.* **120**, 322–329 (2021).

168. D'Agostini, É. C., Mantovani, T. R. D., Valle, J. S. do, Paccola-Meirelles, L. D., Colauto, N. B., & Linde, G. A. Low carbon/nitrogen ratio increases laccase production from basidiomycetes in solid substrate cultivation. *Sci. Agric.* **68**, 295–300 (2011).

169. Asgher, M., Nasir Iqbal, H. M., & Asad, M. J. Kinetic characterization of purified laccase produced from Trametes versicolor IBL-04 in solid state bio-processing of corncobs. *BioResources* **7**, 1171–1188 (2012).

170. Merino-Restrepo, A., Mejía-Otálvaro, F., Velásquez-Quintero, C., & Hormaza-Anaguano, A. Evaluation of several white-rot fungi for the decolorization of a binary mixture of anionic dyes and characterization of the residual biomass as potential organic soil amendment. *J. Environ. Manage.* **254**, 1–11 (2020).

171. Sánchez, Ó. J., & Montoya, S. Assessment of polysaccharide and biomass production from three white-rot fungi by solid-state fermentation using wood and agro-industrial residues: A kinetic approach. *Forests* **11**, 1–23 (2020).

172. Robinson, T., Chandran, B., & Nigam, P. Studies on the production of enzymes by white-rot fungi for the decolourisation of textile dyes. *Enzyme Microb. Technol.* **29**, 575–579 (2001).

173. Medina, J., Monreal, C. M., Orellana, L., Calabi-Floody, M., González, M. E., Meier, S., Borie, F., & Cornejo, P. Influence of saprophytic fungi and inorganic additives on enzyme activities and chemical properties of the biodegradation process of wheat straw for the production of organo-mineral amendments. *J. Environ. Manage.* **255**, 1–10 (2020).

174. Howard, R. L., Abotsi, E., Van Rensburg, E. L. J., & Howard, S. Lignocellulose biotechnology: Issues of bioconversion and enzyme production. *African J. Biotechnol.* **2**, 702–733 (2003).

175. Chaali, M., Lecka, J., Suresh, G., Salem, M., Brar, S. K., Hernandez-Galan, L., Sévigny, J., & Avalos-Ramirez, A. Supplement comprising of laccase and citric acid as an alternative for antibiotics: In vitro triggers of melanin production. *Eng. Life Sci.* **18**, 359–367 (2018).

176. Lourenço, L. A., Alberton Magina, M. D., Tavares, L. B. B., Guelli Ulson de Souza, S. M. A., García Román, M., & Altmajer Vaz, D. Biosurfactant production by Trametes versicolor grown on two-phase olive mill waste in solid-state fermentation. *Environ. Technol. (United Kingdom)* **39**, 3066–3076 (2018).

177. Perdani, M. S., Margaretha, G., Sahlan, M., & Hermansyah, H. Solid state fermentation method for production of laccase enzyme with bagasse, cornstalk and rice husk as substrates for adrenaline biosensor. *Energy Reports* 22–25 (2019) doi:10.1016/j.egyr.2019.08.065.

178. Birhanli, E., & Yesilada, O. Enhanced production of laccase in repeated-batch cultures of Funalia trogii and Trametes versicolor. *Biochem. Eng. J.* **52**, 33–37 (2010).

179. Babici, J., & Pavko, A. Enhanced enzyme production with the pelleted form of D . squalens in laboratory bioreactors using added natural lignin inducer. *J Ind Microbiol Biotechnol* **39**, 449–457 (2012).

180. Tinoco-Valencia, R., Gómez-cruz, C., Galindo, E., & Serrano-carreón, L. Toward an understanding of the effects of agitation and aeration on growth and laccases production by Pleurotus ostreatus. *J. Biotechnol.* **177**, 67–73 (2014).

181. Zhao, L. ., Chen, W., Wang, L. ., Sun, H. ., & Zhu, Z. Improvement of laccase production by Pleurotus ostreatus by means of agroindustrial waste and fermentation kinetics. *Mycosphere* **8**, 147–161 (2017).

182. Tavares, A. P. M., Coelho, M. A. Z., Agapito, M. S. M., Coutinho, J. A. P., & Xavier, A. M. R. B. Optimization and modeling of laccase production by Trametes versicolor in a bioreactor using statistical experimental design. *Appl. Biochem. Biotechnol.* **134**, 233–248 (2006).

183. Hess, J., Leitner, C., Galhaup, C., Kulbe, K. D., Hinterstoisser, B., Steinwender, M., & Haltrich, D. Enhanced formation of extracellular laccase activity by the white-rot fungus Trametes multicolor. in *Biotechnology for Fuels and Chemicals: The Twenty--Third Symposium* (eds. Finkelstein, M., McMillan, J. D. & Davison, B. H.) 229–241 (Humana Press, 2002). doi:10.1007/978-1-4612-0119-9_18.

184. Nazilah, K. R., Koentjoro, M. P., Isdiantoni, Ekawati, I., Muslihatin, W., & Prasetyo, E. N. Effect of laccase oxidation pre-treatment on coffee (Coffea arabica) bean processing waste for composting substrate. in *AIP Conference Proceedings* vol. 2215 1–8 (2020).

185. López-Linares, J. C., García-Cubero, M. T., Coca, M., & Lucas, S. Efficient biobutanol production by acetone-butanol-ethanol fermentation from spent coffee grounds with microwave assisted dilute sulfuric acid pretreatment. *Bioresour. Technol.* **320**, 1–9 (2021).

186. Nguyen, Q. A., Cho, E. J., Lee, D. S., & Bae, H. J. Development of an advanced integrative process to create valuable biosugars including manno-oligosaccharides and mannose from spent coffee grounds. *Bioresour. Technol.* **272**, 209–216 (2019).

187. Juarez, G. F. Y., Pabiloña, K. B. C., Manlangit, K. B. L., & Go, A. W. Direct dilute acid hydrolysis of spent coffee grounds: a new approach in sugar and lipid recovery. *Waste and Biomass Valorization* **9**, 235–246 (2018).

188. Passadis, K., Fragoulis, V., Stoumpou, V., Novakovic, J., Barampouti, E. M., Mai, S., Moustakas, K., Malamis, D., & Loizidou, M. Study of Valorisation Routes of Spent Coffee Grounds. *Waste and Biomass Valorization* **11**, 5295–5306 (2020).

189. Go, A. W., Conag, A. T., & Bertumen, M. M. N. Taguchi method to improve the production of sugar-rich hydrolysate from non-delipidated spent coffee grounds, and subsequent recovery of lipids and bioactive compounds. *Biofuels* **10**, 193–205 (2019).

190. Acevedo, F., Rubilar, M., Scheuermann, E., Cancino, B., Uquiche, E., Garcés, M., Inostroza, K., & Shene, C. Spent coffee grounds as a renewable source of bioactive compounds. *J. Biobased Mater. Bioenergy* **7**, 420–428 (2013).

191. Kim, D., Kim, J., & Lee, C. Effect of mild-temperature thermo-alkaline pretreatment on the solubilization and anaerobic digestion of spent coffee grounds. *Energies* **11**, 1–14 (2018).

192. Chiyanzy, I., Brienzo, M., García-Aparicio, M., Agudelo, R., & Görgens, J. Spent coffee ground mass solubilisation by steam explosion and enzymatic hydrolysis. *J. Chem. Technol. Biotechnol.* **90**, 449–458 (2015).

193. Naydenova, G., & Yankov, D. Butyric acid production by fermentation of waste hydrolysates. *Bulg. Chem. Commun.* **51**, 111–114 (2019).

194. Pereira, J., Queirós, D., Lemos, P. C., Rossetti, S., & Sera, L. S. Enrichment of a mixed microbial culture of PHA-storing microorganisms by using fermented hardwood spent sul fi te liquor. *N. Biotechnol.* **56**, 79–86 (2020).

195. Kumar, A. N., & Mohan, S. V. Acidogenic valorization of vegetable waste for short chain carboxylic acids and biohydrogen production: Influence of pretreatment and pH. *J. Clean. Prod.* **203**, 1055–1066 (2018).

196. Hude, M. P., & Yadav, G. D. Process intensification in methane generation during anaerobic digestion of Napier grass using supercritical carbon dioxide combined with acid hydrolysis pre-treatment. *Can. J. Chem. Eng.* **92**, 2176–2184 (2014).

197. Hernández, D., Solana, M., Riaño, B., García-González, M. C., & Bertucco, A. Biofuels from microalgae: Lipid extraction and methane production from the residual biomass in a biorefinery approach. *Bioresour. Technol.* **170**, 370–378 (2014).

198. Li, P., He, C., Li, G., Ding, P., Lan, M., Gao, Z., & Jiao, Y. Biological pretreatment of corn straw for enhancing degradation efficiency and biogas production. *Bioengineered* **11**, 251–260 (2020).

199. Zhang, X., Chen, T., Lim, J., Xie, J., Zhang, B., Yao, T., & Hamaker, B. R. Fabrication of a soluble crosslinked corn bran arabinoxylan matrix supports a shift to butyrogenic gut bacteria. *Food Funct.* **10**, 4497–4504 (2019).

200. Wang, C., Liu, C., Zhang, G. W., Du, H. S., Wu, Z. Z., Liu, Q., Guo, G., Huo, W. J., Zhang, J., Pei, C. X., Chen, L., & Zhang, S. L. Effects of rumen-protected folic acid and betaine supplementation on growth performance, nutrient digestion, rumen fermentation and blood metabolites in Angus bulls. *Br. J. Nutr.* **123**, 1109–1116 (2020).

201. Tišma, M., Planinić, M., Bucić-Kojić, A., Panjičko, M., Zupančič, G. D., & Zelić, B. Corn silage fungal-based solid-state pretreatment for enhanced biogas production in anaerobic co-digestion with cow manure. *Bioresour. Technol.* **253**, 220–226 (2018).

8. Appendices

8.1. Appendix A: calibration curves

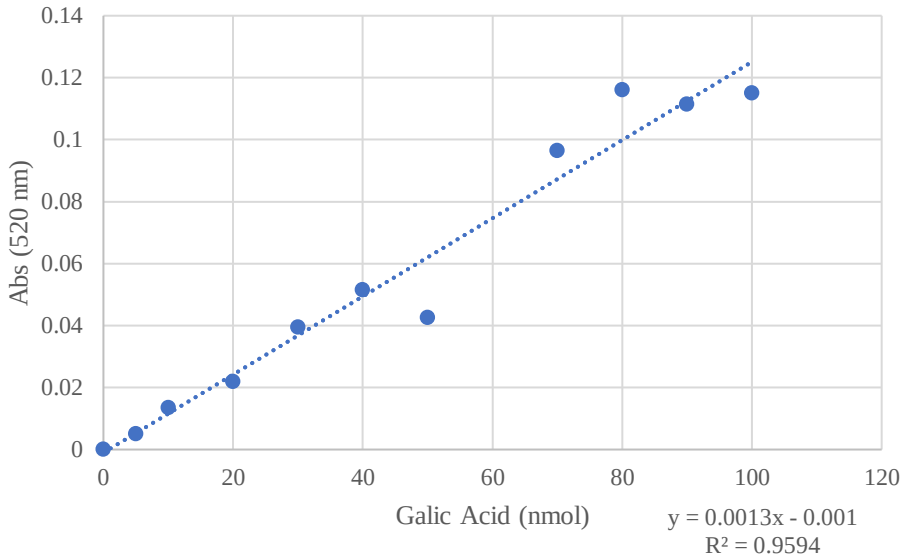

Figure 23. Galic acid calibration curve.

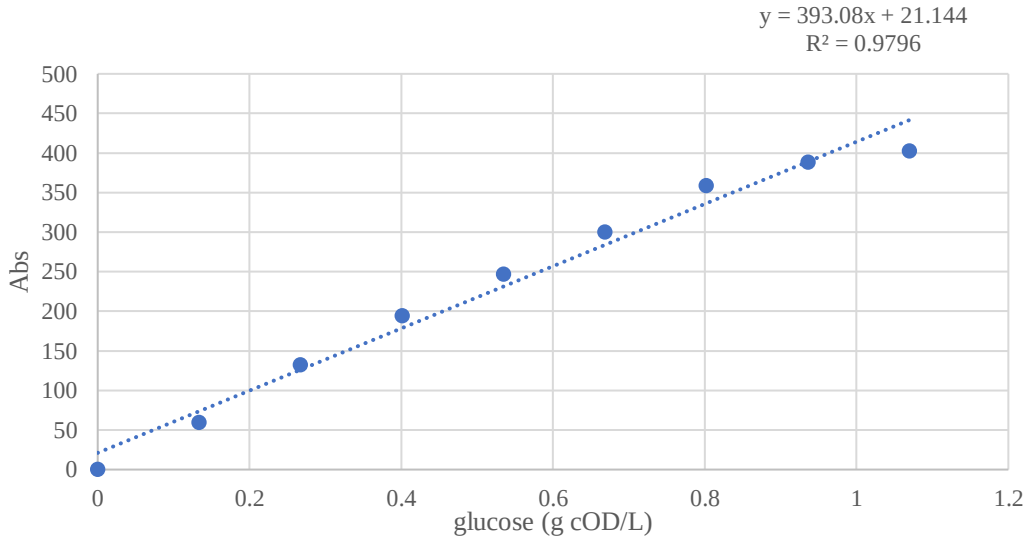

Figure 24. Glucose calibration curve.

8.2. Appendix B: AF batch experiments

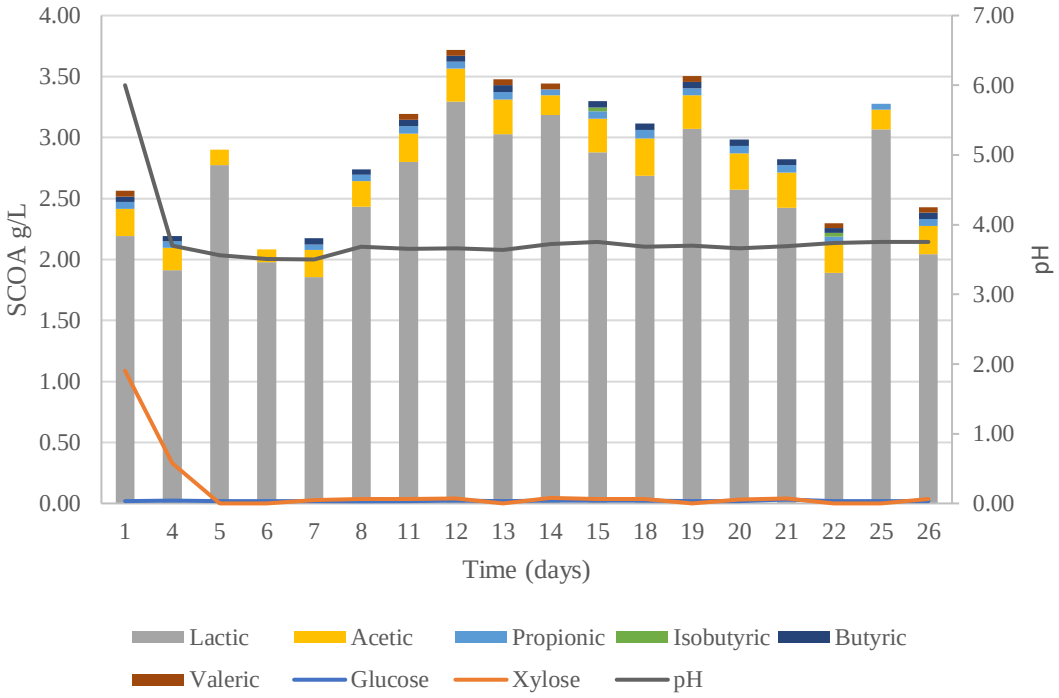

Figure 25. Acidogenic fermentation of spent coffee grounds pretreated with acid hydrolysis without a correct pH adjustment.

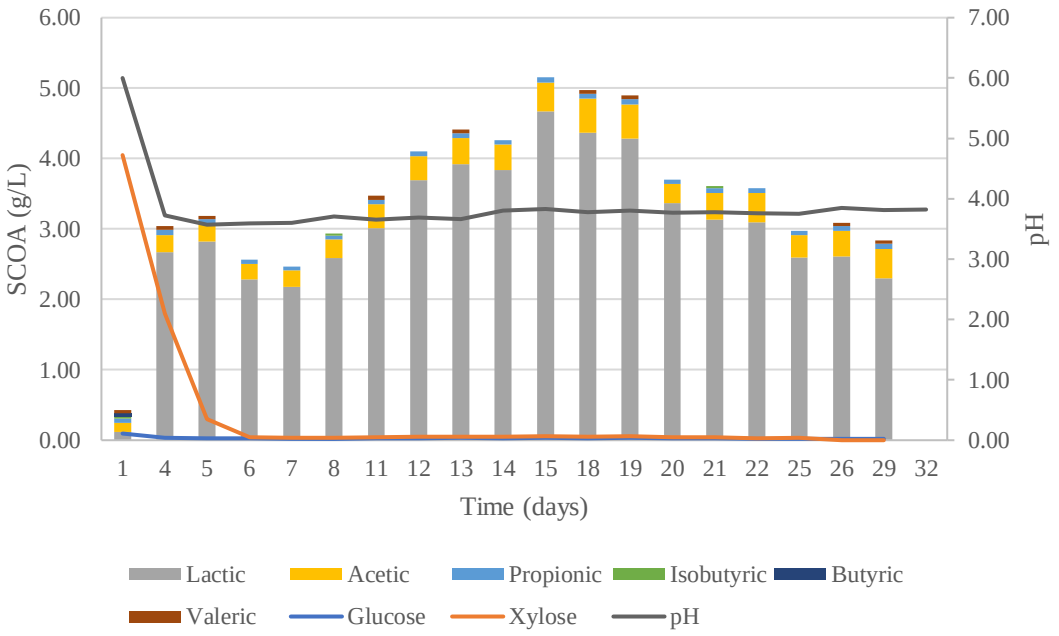

Figure 26. Acidogenic fermentation of spent coffee grounds pretreated with supercritical extraction and acid hydrolysis without a correct pH adjustment.

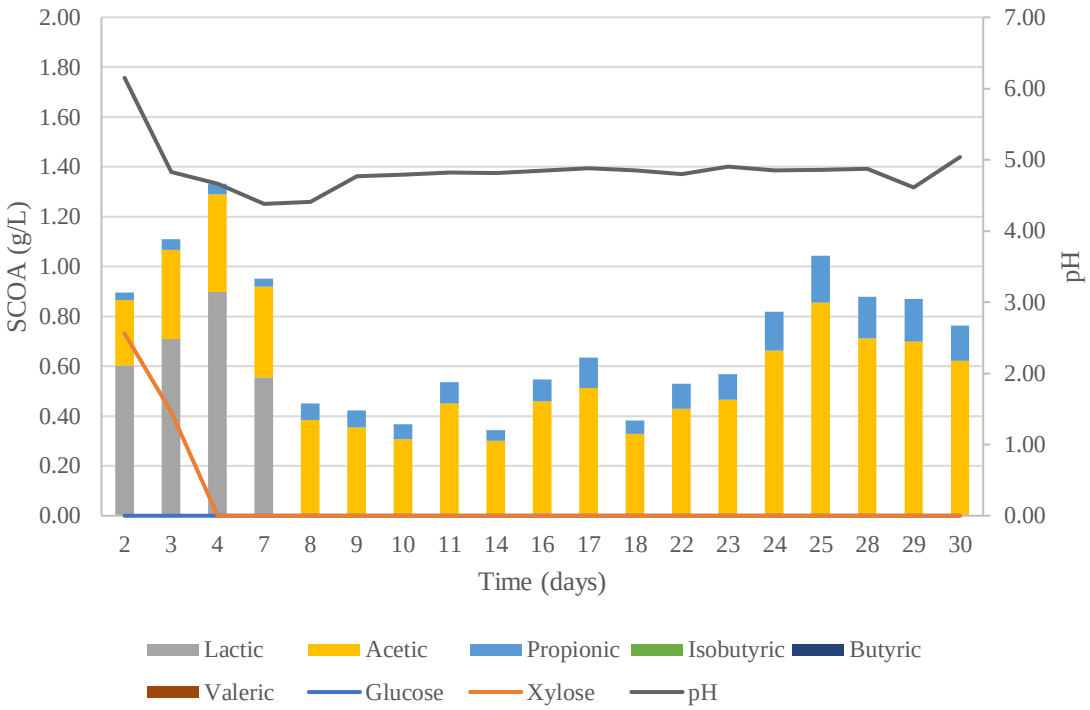

Figure 27. Acidogenic fermentation of spent coffee grounds pretreated with supercritical extraction and acid hydrolysis.

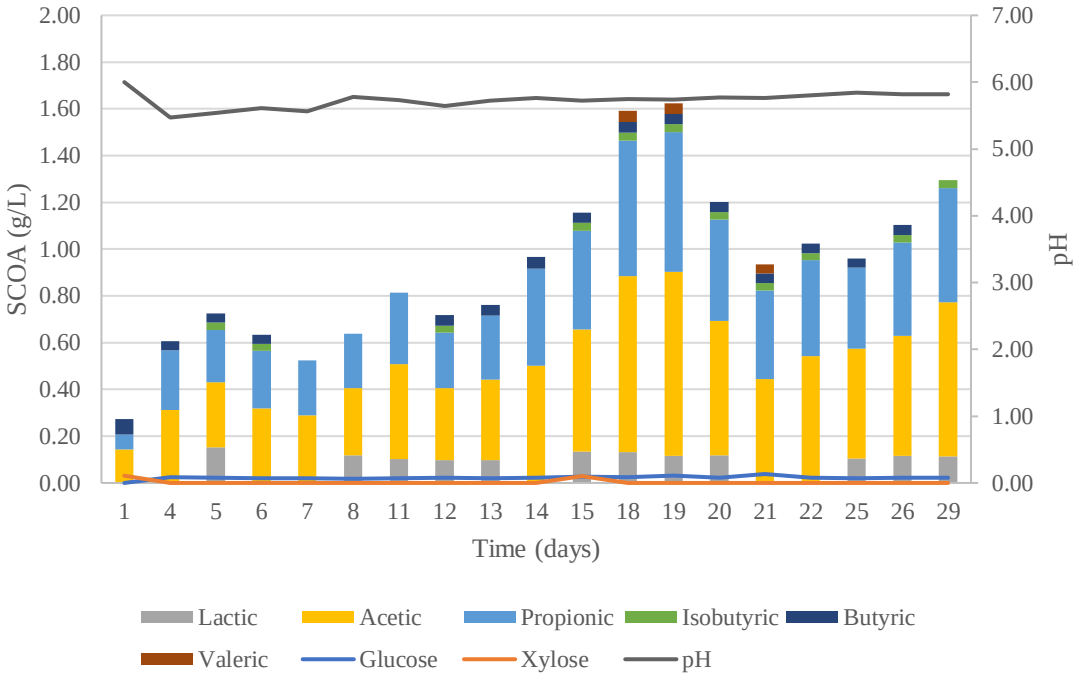

Figure 28. Acidogenic fermentation of spent coffee grounds pretreated with supercritical extraction and alkaline hydrolysis.

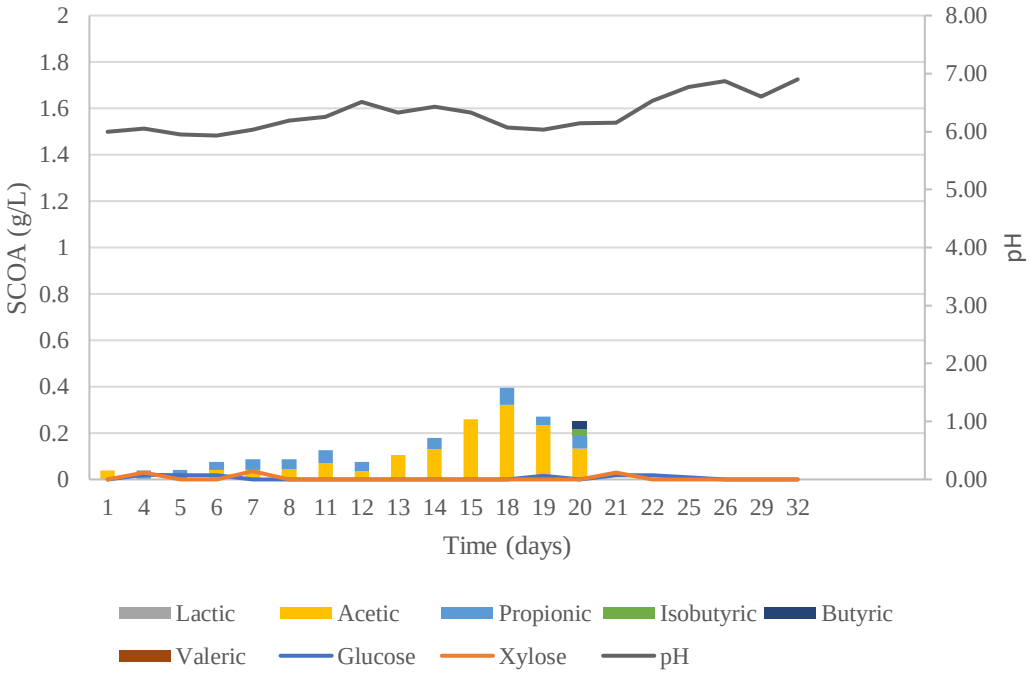

Figure 29 Acidogenic fermentation of spent coffee grounds pretreated with solid-state fermentation by *Paecylomyces variotii*.

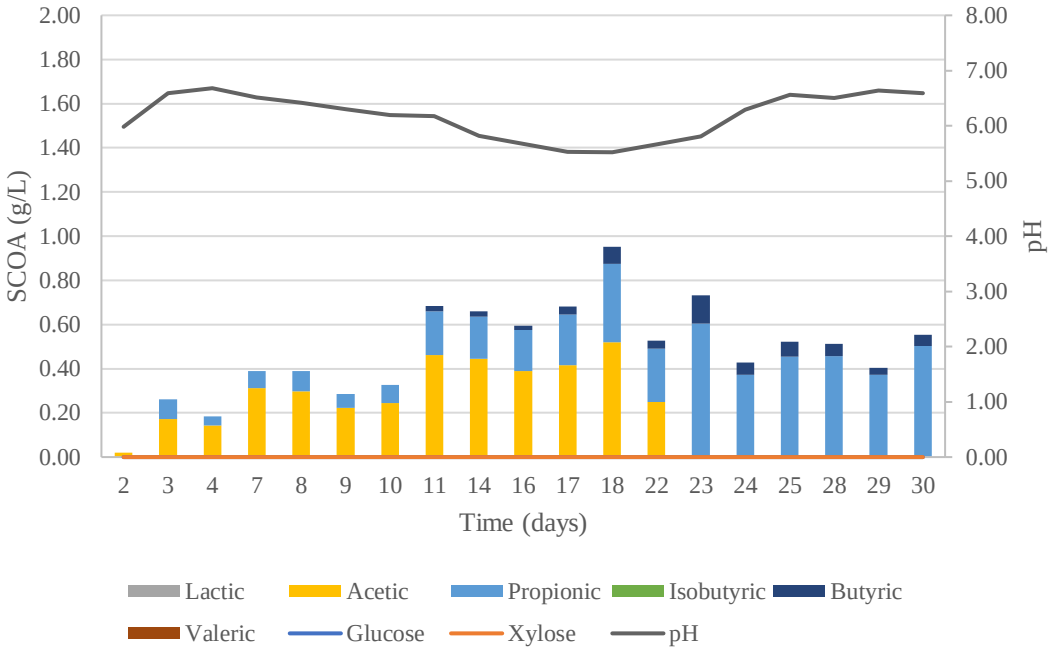

Figure 30. Acidogenic fermentation of spent coffee grounds pretreated with the enzymatic extract obtained from *Paecylomyces variotii* without inducers.

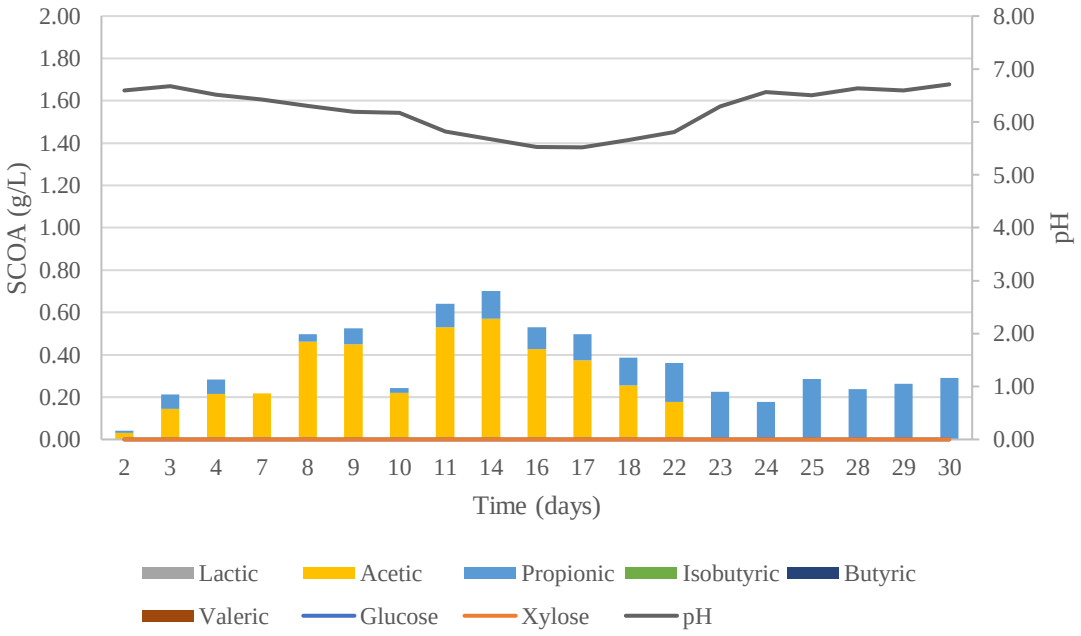

Figure 31. Acidogenic fermentation of spent coffee grounds pretreated with the enzymatic extract obtained from *Paecylomyces variotii* with spent coffee grounds as inducer.

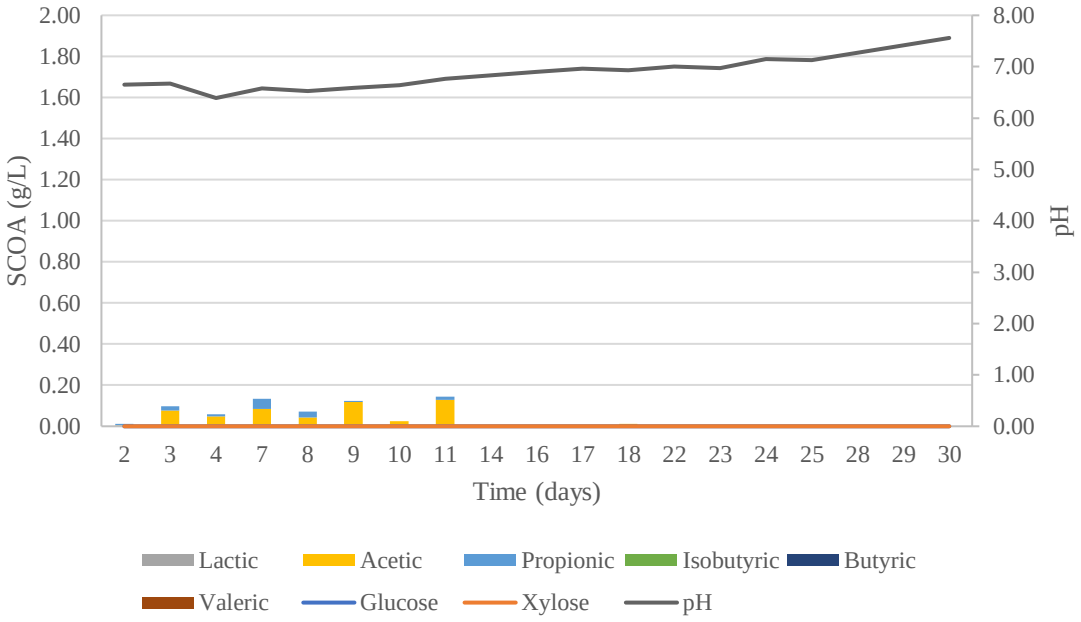

Figure 32. Acidogenic fermentation of spent coffee grounds pretreated with the enzymatic extract obtained from *Paecylomyces variotii* with spent coffee grounds and pineapple peel as inducers.

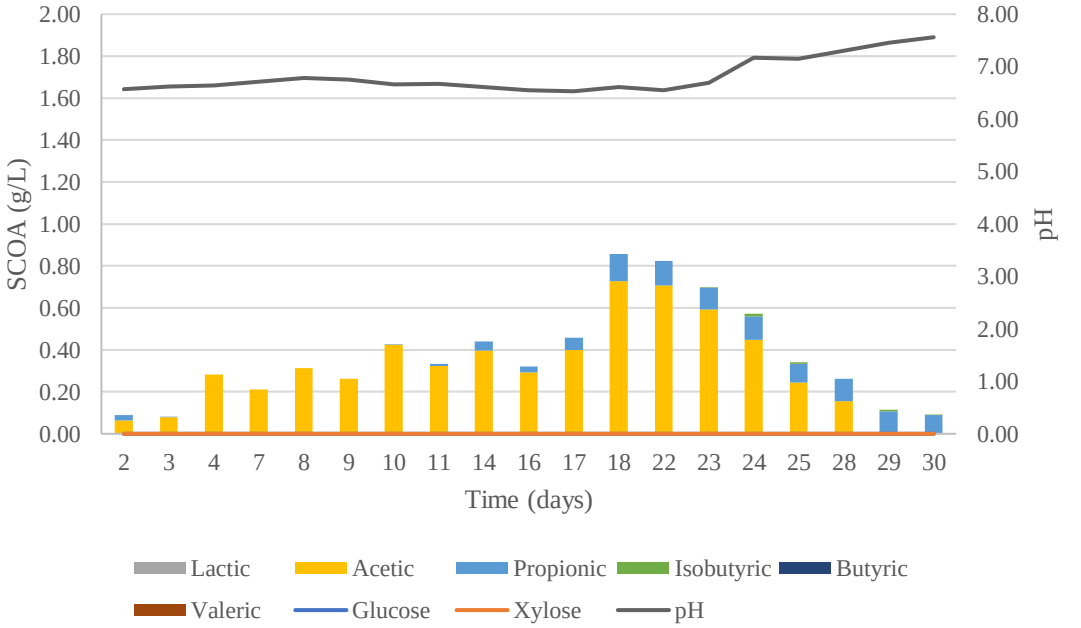

Figure 33. Acidogenic fermentation of spent coffee grounds pretreated with the enzymatic extract obtained from *Trametes versicolor* with spent coffee grounds as inducer.

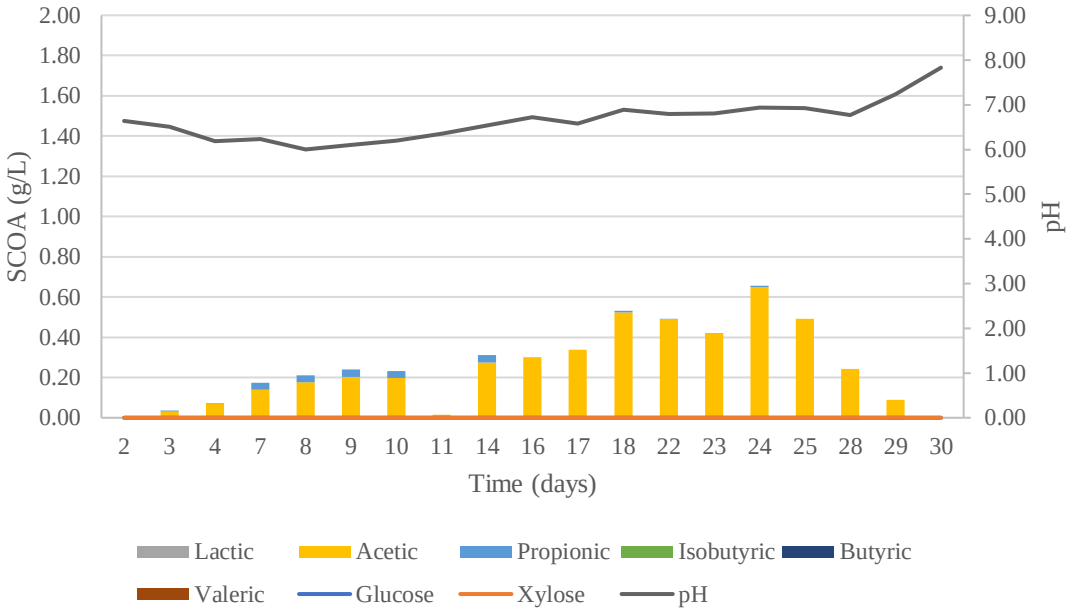

Figure 34. Acidogenic fermentation of spent coffee grounds pretreated with the enzymatic extract obtained from *Trametes versicolor* with spent coffee grounds and pineapple peel as inducers.