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# Subcritical water hydrolysis of brewer's spent grains: A sustainable pretreatment for value-added products and bioenergy production in a biorefinery concept

# Hidrólise em água subcrítica de bagaço de malte: Um pré-tratamento sustentável para produção de produtos de valor agregado e bioenergia em um conceito de biorrefinaria

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Thesis presented to the School of Food Engineering of the University of Campinas in partial fulfillment of the requirements for the degree of Doctor in Food Engineering.

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Este exemplar corresponde à versão final da tese defendida pelo aluno William Gustavo Sganzerla, e orientado pela Prof. Dr. Tânia Forster Carneiro.

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# RESUMO

O tratamento da biomassa lignocelulósica e a produção de bioenergia e produtos de valor agregado é uma procura constante na indústria. O bagaço de malte é o principal resíduo lignocelulósico gerado pela indústria da cerveja, representando 20 kg por 100 L de cerveja produzida, sendo uma matéria-prima com potencial para aplicação em uma biorrefinaria. No entanto, a produção de biocombustíveis e produtos de valor agregado a partir de biomassa requer um pré-tratamento para quebrar a estrutura lignocelulósica. Uma revisão da literatura e análise bibliométrica elucidou que o pré-tratamento sustentável do bagaço de malte é uma das etapas mais limitantes na conversão da biomassa lignocelulósica em produtos de valor agregado e bioenergia. Desse modo, o objetivo desta tese foi avaliar a aplicação de hidrólise em água subcrítica do bagaço de malte como um processo tecnológico e sustentável para produzir produtos de valor-agregado e bioenergia em um conceito de biorrefinaria. O pré-tratamento do bagaço de malte foi otimizado em um processo semi-contínuo, operado com um único e dois reatores sequenciais para obter um hidrolisado contendo açúcares, xilo-oligossacarídeos e aminoácidos. A hidrólise em água subcrítica foi realizada a 15 MPa, 5 mL min$^{-1}$, e em diferentes temperaturas (80 - 180 °C). Experimentos integrando hidrólise em água subcrítica e digestão anaeróbia foram conduzidos para produzir bioenergia (biometano, electricidade e calor) e fertilizante. Em um conceito de biorrefinaria, foi avaliado os parâmetros econômicos dos bioprocessos, visando identificar a opção mais rentável para a implementação industrial. Os resultados obtidos demonstraram que a hidrólise em água subcrítica rompeu a estrutura do bagaço de malte, libertando monossacarídeos (47 mg g$^{-1}$ carboidratos), aminoácidos (42 mg g$^{-1}$ proteínas), e xilo-oligossacarídeos (204 mg g$^{-1}$ hemicelulose). A avaliação econômica da produção de açúcares revelou que a separação dos monossacáridos obtidos por hidrólise em água subcrítica é uma vantagem para a rentabilidade do processo industrial. No caso dos xilo-oligossacarídeos, o lucro bruto do processo de hidrólise com dois reatores sequenciais foi 30% superior quando comparado ao uso de um único reator subcrítico. Além disso, o pré-tratamento é rentável para a recuperação de xilo-oligossacarídeos. O hidrolisado obtido pela hidrólise em água subcrítica foi aplicado na digestão anaeróbia para verificar a produção de biogás rico em metano e bioenergia. Os resultados demonstraram que a integração entre pré-tratamento e a digestão anaeróbia aumentou o rendimento do metano (747 L $CH_4$ $kg^{-1}$ TVS) quando comparado com o processo de digestão anaeróbia sem pré-tratamento (53 L $CH_4$ $kg^{-1}$ TVS). Para o processo com pré-tratamento, a produção de electricidade (134 kWh $ton^{-1}$) e calor (604 MJ $ton^{-1}$) foram responsáveis pela mitigação de 44 kg de $CO_{2\text{-eq}}$ $ton^{-1}$. Em escala industrial, a digestão anaeróbia sem pré-tratamento é uma opção economicamente viável para produzir bioenergia e fertilizante, com tempo de retorno do investimento inferior a 5 anos, taxa interno de retorno de cerca de 20%, e valor presente líquido de até 1 milhão de USD. Portanto, a aplicação da hidrólise em água subcrítica do bagaço de malte pode ser considerado um pré-tratamento sustentável para produzir produtos de valor agregado e bioenergia, contribuindo para a transição a uma economia circular através da redução da pegada de carbono da indústria da cerveja.

**Palavras-chave:** *Lignocelulose; Biomassa; Biogás; Açúcares; Xilo-oligossacarídeos.*

# ABSTRACT

The management of lignocellulosic biomass and the production of bioenergy and biobased products is a constant demand in the industry. Brewer’s spent grains are the main lignocellulosic by-product generated by the beer industry, accounting for 20 kg per 100 L of beer produced, being a suitable feedstock for potential application in a biorefinery. However, the production of biofuels and biobased products from biomass requires a pretreatment to break down the lignocellulose structure. A comprehensive review and bibliometric analysis elucidated that the sustainable pretreatment of brewer’s spent grains is one of the most limiting steps in lignocellulosic biomass conversion into biobased products and bioenergy. Therefore, the objective of this PhD thesis was to evaluate the application of subcritical water pretreatment of brewer’s spent grains as a sustainable technological process to produce biobased products and bioenergy in a biorefinery concept. The pretreatment of brewer’s spent grains was optimized in a semi-continuous subcritical water hydrolysis process operated with a single and two sequential flow-through reactors to obtain a hydrolysate containing sugars, xylo-oligosaccharides, and amino acids. The subcritical water hydrolysis was carried out at 15 MPa, 5 mL water $\text{min}^{-1}$, and at different temperatures (80 – 180 °C). Experiments integrating subcritical water hydrolysis and anaerobic digestion were conducted to produce bioenergy (biomethane, electricity, and heat) and agricultural fertilizer. In a biorefinery concept, the techno-economic assessment of the designed bioprocesses was studied to identify the most profitable option for industrial implementation. The results obtained demonstrated that subcritical water hydrolysis attacked the brewer’s spent grains structure, releasing monosaccharides (47 mg $g^{-1}$ carbohydrate), amino acids (42 mg $g^{-1}$ proteins), and xylo-oligosaccharides (204 mg $g^{-1}$ hemicellulose). The economic analysis of sugar production revealed that the separation of monosaccharides obtained by subcritical water hydrolysis is an advantage for the profitability of the industrial-plant process. In the case of xylo-oligosaccharides, the gross profit of the process with two sequential reactors was 30% higher when compared to the single subcritical reactor. In addition, the pretreatment was demonstrated to be profitable for recovering xylo-oligosaccharides. The hydrolysate obtained by subcritical water hydrolysis was applied in anaerobic digestion to elucidate the production of methane-rich biogas and bioenergy. The results demonstrated that the integration of subcritical water hydrolysis and anaerobic digestion increased the methane yield (747 L $CH_4$ $kg^{-1}$ TVS) when compared to the anaerobic process without pretreatment (53 L $CH_4$ $kg^{-1}$ TVS). For the process with pretreatment, the generation of electricity (134 kWh $ton^{-1}$) and heat (604 MJ $ton^{-1}$) were responsible for the mitigation of 44 kg $CO_{2\text{-eq}}$ $ton^{-1}$. The industrial scale of anaerobic digestion without pretreatment was also found to be a feasible option to recover bioenergy and fertilizer, with payback lower than 5 years, internal return rate around 20%, and net present value up to 1 million USD. Finally, the application of subcritical water hydrolysis of brewer's spent grains is a sustainable pretreatment to produce value-added products and bioenergy, supporting the circular economy transition by reducing the carbon footprint of the beer industry.

***Keywords:*** *Lignocellulose; Biomass; Biogas; Sugars; Xylo-oligosaccharides.*

## TABLE OF CONTENTS

# CHAPTER I

## Introduction, hypothesis, objectives, and thesis structure

# CHAPTER I: Introduction, hypothesis, objectives, and thesis structure

## 1. Introduction

There is a worldwide demand for renewable energy obtained from eco-friendly processes, aiming to substitute petroleum-based fuels and decrease greenhouse gas emissions. In this case, there is a demand for cleaner technologies to produce materials and energy. In addition, there is a great interest in using renewable fuels derived from biomass (Toor et al., 2020). Through the production of ethanol, methane, and hydrogen, these biofuels can be used as a potential source of bioenergy with the capacity to diversify the country's energy matrix, which contributes to public policies to reduce greenhouse gas emissions (Cheah et al., 2020; Ricciardi et al., 2020).

The food industry is one of Brazil's most significant and promising sectors, representing 9.6% of the national gross domestic product in 2019 (ABIA, 2020). In addition, Brazil is the world's second-largest exporter of processed foods such as orange juice, beer, meat, dairy products, and coffee (ABIA, 2020). Indeed, world beer production reached 194 billion liters in 2018, and Brazil is one of the largest beer producers in the world, behind China and the United States. In Brazil, 400 thousand tons of barley were cultivated, and 644 thousand tons were imported from South America for beer production (IBGE, 2019; CONAB, 2020). Approximately 1 million tons of barley were destined to produce 14 billion liters of beer, only in Brazil (MAPA, 2020).

Due to the high food production, the food industry generates a large amount of solid organic waste (Ricciardi et al., 2020). However, most of these solid residues do not receive the correct treatment and destination, contributing to environmental problems (Margallo et al., 2019). The incorrect destination of organic waste causes greenhouse gas emissions, the proliferation of disease vectors, and the production of leachate, which can contaminate the soil and groundwater (Das et al., 2019). In the beer industry, the production of beer also generates a high amount of solid residues and wastewater. The main solid by-product obtained after malting is an insoluble and non-degraded fraction of the malted barley grain, denominated as brewer’s spent grains (Mussatto, 2014). Brewer’s spent grains represent 85% of the total by-products generated by the brewery, which account for 20 kg per 100 L of beer produced (Mussatto, 2014). Therefore, Brazilian brewer’s spent grains BSG production can be estimated at $2.8\times10^{6}$ ton year$^{-1}$ (wet weight) (Sganzerla et al., 2021a). Brewer’s spent grains is a lignocellulosic material suitable for *in loco* application to energy recovery.

Based on the mentioned above, for final and environmentally adequate disposal of solid waste from the food industry, reuse, recycling, composting, recovery, and energy use are some routes that could be implemented (Weber et al., 2020). The technologies for energy recovery in a biorefinery concept could reduce the organic load of industrial waste while producing bioenergy, biofuels, and value-added products (Dragone et al., 2020). Therefore, the food industry can adopt treating waste and producing new compounds, reversing production costs, increasing efficiency, and decreasing environmental risks.

Otherwise, there is a challenge in using lignocellulosic materials for biofuel production. Recently, several pretreatments (i.e., acid, alkaline, mechanical, and chemical hydrolysis) have been used to promote the rupture of the lignocellulosic material structures (Kumari & Singh, 2018). However, conventional pretreatments have high costs and generate contaminants after hydrolysis, requiring additional steps to post-treatment of the waste generated (Muharja et al., 2018). In many cases, conventional pretreatments are not economically viable due to the high demand for reagents, energy, water, and waste treatment costs (Cheah et al., 2020). Therefore, the use of subcritical water is considered a promising alternative for the hydrolysis of lignocellulosic biomass to obtain chemicals, biofuels, and energy products in a clean, sustainable, and renewable way (Prado et al., 2016; Wang et al., 2018). Water in the subcritical state is defined as that which maintains the liquid state under pressure at a temperature greater than its natural boiling point (100 °C) and lower than its critical temperature (374 °C) (Torres-Mayanga et al., 2019). During the subcritical process, the temperature, pressure, and residence time are important factors that control the fractionation of the lignocellulosic complex into simple sugars (Prado et al., 2016). Finally, subcritical water is a relevant strategy for developing the circular economy, as it is advantageous in valorizing lignocellulosic agro-industrial waste and, in many cases, this technology can be profitable for industrial implementation (Sganzerla et al., 2021b).

The integration between waste treatment and bioenergy production could be a means of reconciling energy production and environmental conservation within the Sustainable Development Goals established by the United Nations Framework Convention on Climate Change (UN, 2020). The International Energy Agency defines biorefining as the sustainable processing of biomass into a spectrum of marketable biobased products (food and feed ingredients, chemicals, materials) and bioenergy (fuels, power, heat) (IEA, 2019). The implementation of biorefinery acts as a strategic mechanism for a transition from the circular economy (Ubando et al., 2020). The circular economy is a conceptual model widely used to the agro-industrial waste management to perform sustainable development based on the bioeconomy (Keijer et al., 2019). The circular economy is a powerful concept that contributes to the decisions of environmental and economic policymakers, creating economic, social, and environmental value (Geng et al., 2019). Also, the circular economy is a system that adopts the practice of reducing, reusing, recycling, and recovering materials to achieve sustainable development (Weber et al., 2020).

## 2. Hypothesis

- Subcritical water hydrolysis is an eco-friendly process to produce sugars, xylo-oligosaccharides, and amino acids from brewer’s spent grains.
- The integration of subcritical water hydrolysis and anaerobic digestion can increase the methane yield and bioenergy recovery.
- Subcritical water hydrolysis is a profitable process for industrial implementation.
- The recovery of bioenergy from brewer’s spent grains decreases greenhouse gas emission and contribute to the circular economy transition of the beer industry.

## 3. Objective

### *3.1 General Objective*

This PhD thesis evaluated the application of subcritical water hydrolysis of brewer’s spent grains as a technological process to produce value-added biobased products and bioenergy in a biorefinery concept. For this, the pretreatment of brewer’s spent grains was conducted in a semi-continuous subcritical reactor to obtain a hydrolysate with a high amount of bioproducts (sugars, xylo-oligosaccharides, and amino acids). Experiments integrating subcritical water hydrolysis and anaerobic digestion were conducted to produce bioenergy (biomethane, electricity, and heat) and agricultural fertilizer. Finally, in a biorefinery concept, the techno-economic assessment of the bioprocesses was studied to identify the most profitable option for industrial implementation.

### *3.2 Specific Objectives*

- Determine the optimum experimental conditions for subcritical water hydrolysis of brewer’s spent grains in a single and two sequential reactors to increase the productivity of value-added products.
- Quantify the sugars, amino acids, xylo-oligosaccharides, organic acids, furfural, and 5-hydroxymethylfurfural.
- Determine the economic feasibility of sugars and xylo-oligosaccharides production by process intensification and scale-up.
- Investigate the integration between subcritical water hydrolysis and anaerobic digestion for biomethane, bioenergy, and fertilizer production.

## 4. Thesis structure

This thesis was assembled in IX chapters. The chapters correspond to scientific articles that were published in international journals. **Figure 1** shows an illustration of the main items covered in each chapter of the thesis.

**Chapter I (Introduction, justification, objectives, and thesis structure)** briefly presents the theme, novelty, justification, objectives, and steps involved for its realization.

**Chapter II (A bibliometric analysis on potential uses of brewer’s spent grains in a biorefinery for the circular economy transition of the beer industry)** is a bibliographic overview of the theme. In this chapter, the generation of brewers’ spent grains and the potential application to produce biobased products and bioenergy in a biorefinery concept was revised. The review paper presented in this chapter was published in *Biofuels, Bioproducts and Biorefining*, 15(6), 1965-1988, 2021.

**Chapter III (Recovery of sugars and amino acids from brewers' spent grains using subcritical water hydrolysis in a single and two sequential flow-through reactors)** address the process design and experimental results of the optimization of subcritical water hydrolysis of brewers' spent grains to produce a hydrolysate composed by sugars and amino acids. The results obtained in this chapter were published in the journal *Food Research International*, 157, 111470, 2022.

**Chapter IV (Techno-economic assessment of subcritical water hydrolysis process for sugars production from brewer's spent grains)** is composed of the techno-economic analysis of subcritical water hydrolysis of brewer's spent grains to produce sugars. This chapter focuses on the strategies to scale-up the production of purified sugars. The paper presented in this chapter was published in *Industrial Crops and Products*, 171, 113826, 2021.

**Chapter V (Techno-economic assessment of subcritical water hydrolysis of brewer's spent grains to recover xylo-oligosaccharides)** address the laboratory-scale experimental results for xylo-oligosaccharides production by subcritical water hydrolysis, followed by a techno-economic assessment. The results obtained in this chapter were published in *The Journal of Supercritical Fluids*, 196, 105895, 2023.

**Chapter VI (Subcritical water pretreatment enhanced methane-rich biogas production from the anaerobic digestion of brewer's spent grains)** evaluates the integration of subcritical water hydrolysis and anaerobic digestion to produce bioenergy and fertilizer. The paper presented in this chapter was published in *Environmental Technology*, 2022.

**Chapter VII (Techno-economic assessment of bioenergy and fertilizer production by anaerobic digestion of brewer's spent grains in a biorefinery concept)** presents the techno-economic assessment of bioenergy and fertilizer production from anaerobic digestion of brewer's spent grains. The paper presented in this chapter was published in the *Journal of Cleaner Production*, 297, 126600, 2021.

The discussion of the results obtained, and the conclusion of this thesis are presented in **Chapter VIII** and **Chapter IX**, respectively.

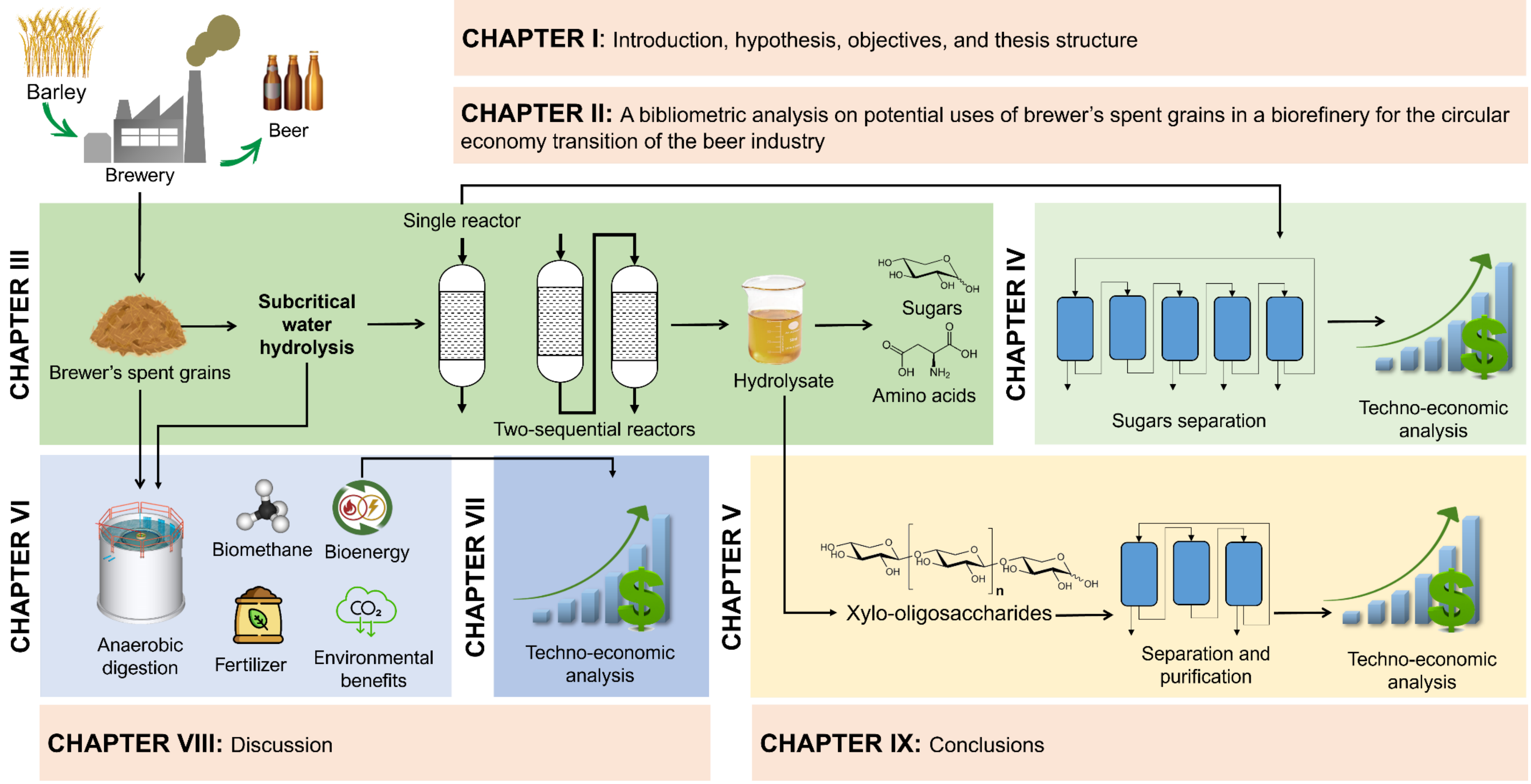

**Figure 1.** Representation of the subjects covered in the chapters of the thesis.

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# CHAPTER II

## A bibliometric analysis on potential uses of brewer’s spent grains in a biorefinery for the circular economy transition of the beer industry

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# CHAPTER II: A bibliometric analysis on potential uses of brewer's spent grains in a biorefinery for the circular economy transition of the beer industry

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## Abstract

This comprehensive review aimed to elucidate the state of art related to research trends on the use of brewer's spent grains (BSG), the main solid by-product generated in breweries. The status, future opportunities, and emerging trends were evaluated by bibliometric analysis, which contributed to clarifying the knowledge and perspectives in the field. In total, 510 documents were obtained from the Web of Science© database in a timespan from 1900 to 2020. VosViewer software and R-bibliometrix package were used to investigate and map the top-cited papers, international collaborative networks, keywords, top countries, and journals. The results showed that globally 65 countries have been engaged in research related to BSG, and Brazil has been the most productive country with up to 70 publications. Keyword's analysis revealed that the studies on BSG are divided into four main knowledge areas: *i)* pretreatment; *ii)* nutritional quality; *iii)* bioactive compounds; and *iv)* pyrolysis. It was also found that a biorefinery can be designed for the complete utilization of BSG to obtain several valuable compounds. In this sense, different technological routes can be applied to produce arabinoxylans, proteins, ferulic acid, xylitol, xylose, lactic acid, butanol, biogas, fertilizer, and ethanol. In conclusion, BSG is a cost-effective and feasible raw material for the sustainable production of bio-based products and bioenergy and its valorization in a biorefinery is a powerful strategy to promote the circular economy transition of the beer industry.

**Keywords:** *Bioeconomy; Biofuels; Bioenergy; Value-added products; Bioactive compounds.*

## 1. Introduction

The replacement of fossil-based carbon by renewable carbon from biomass leads to biorefinery facilities' development to support the sustainable management of biomass residues to produce bioenergy and value-added products.[1] According to the International Energy Agency [2] “biorefining is the sustainable processing of biomass into a spectrum of marketable bio-based products (food and feed ingredients, chemicals, materials) and bioenergy (fuels, power, heat)”. Therefore, biorefineries are the processes to produce fuels, biomaterials, and chemicals from biomass resources.[3] The biorefinery concept has been proposed as a technological route to optimize the overall technical, economic, and energetic efficiencies in producing bio-products.[1] In a biorefinery approach, technological processes can be combined and applied for biomass valorization to produce value-added products and bioenergy.[4,5]

According to Cherubini [6] a biorefinery can be classified as i) platforms, ii) products, iii) feedstocks, and iv) processes. Platforms are intermediates between feedstocks and products, which might be reached through different eco-friendly technological processes applied to the raw materials. Some examples of energy-driven biorefinery platforms are the technical route to produce biogas (a mixture of $CO_2$ and $CH_4$), syngas (a mixture of CO and $H_2$), hydrogen ($H_2$), C-6 sugars ($C_6H_{12}O_6$, i.e., glucose, fructose, and galactose), C-5 sugars ($C_5H_{10}O_5$, i.e., xylose and arabinose), electricity, and heat. Regarding the biorefinery products, biomass can be used to produce secondary energy carriers (energy-driven biorefinery) or to generate bio-based products (material-driven biorefinery). In both cases, value-added products aim to improve the biomass supply chain's economic, ecological, and technical performances. Some energy products generated include biodiesel, bioethanol, biomethane, electricity, and heat. In a material-driven biorefinery, food, fertilizer, glycerin, and polymers are some of the value-added products generated. Beyond, the third classification is the feedstock-based biorefinery, which can be provided in four sectors: agriculture (crops and crop residues); forestry (wood, short-rotation poplar, logging residues); industry and domestic activities (process and organic residues); and aquaculture (algae, seaweed). The feedstock-based biorefinery uses oil, sugar, starch, lignocellulose biomass, or agro-industrial residues (organic residues, oil-based residues, and lignocellulose-based biomass). Finally, the biorefinery classification as a process consists of routes to obtain the bioenergy and value-added products.

Mechanical, physical, biochemical, chemical, and thermochemical processes can be designed for biomass biorefinery. Additionally, some subgroups of biorefinery processes include anaerobic digestion, gasification, electrolysis, fermentation, and pyrolysis, which can also be applied to produce bio-based products.

A biorefinery can be proposed in a circular economy model to valorize solid residues and lignocellulosic biomass from the agriculture and food industries. The circular economy is a conceptual model widely used for the management of agro-industrial residues to support the development of a sustainable bio-based bioeconomy.[7] Some raw materials used mainly in the industry include sugarcane, soybean, rice, maize, cotton, orange, barley, wheat, cassava, tobacco, poultry, swine, and bovine.[8] From all of these raw materials, a high amount of solid residues is generated. In some cases, the residues generated from the industrial processing do not present appropriate management. Still, they represent a high amount of underutilized feedstock, which could be better explored in the context of a biorefinery. Moreover, the solid residues from industrial processing require appropriate management according to national and international laws established to promote a green economy.[9] In this sense, it is possible to adopt different technological routes to convert such lignocellulosic biomass into fermentable sugars, organic acids, renewable fuels, bioactive compounds, and biogas. These technological routes can be a promising alternative to support a biorefinery implementation, relevant for the circular economy transition of the agro-industrial sector.[10–12]

Bibliometric tools can be used to perform a complete analysis of scientific publications related to a subject area.[13,14] This systematic method can support the analysis of emerging trends, addressing gaps, and identifying all the collaboration networks of the research area.[13] Moreover, the bibliometric analysis identifies the possible knowledge gaps and supports the establishment of future studies to better understand the state of the art of a research field.[15] Furthermore, the dynamic evolution of scientific literature can also be a strategic tool available in the bibliometric study. Bibliometric analysis can highlight the key journals, trending articles, citation evolution, and the dynamics of publications. Therefore, bibliometric studies have been constantly applied to highlight the challenges and support future research studies. For instance, Goyal et al.[16] conducted a bibliometric study of research insights in the circular economy. Ubando et al.[17] identified the research clusters to achieve sustainable utilization of biowaste in a biorefinery, including the thematic areas of mitigation, sustainable utilization, and cleaner disposal. Andreo-Martínez et al.[18] performed a descriptive bibliometric study to observe the science production of pesticide residues in honey. In the energy area, Arriola et al.[19] analyzed the progress of the research on energy and sustainability that utilizes a fuzzy optimization approach. Jiménez-Castro et al.[20] elucidated the bioenergy production from orange agro-industrial residues, highlighting the use of anaerobic digestion technology to produce methane-rich biogas. Nazari et al.[21] evaluated the connection between biofuels and sustainable development goals to find an alternative to reduce the dependence on fossil fuels. In brief, a bibliometric study helps to elucidate the current knowledge and predict future directions of a research field, which can directly contribute to the development of the research field.[16]

Due to the constant generation of residues from the agro-industrial sector, sustainable technologies for waste management and valorization are urgently necessary. Therefore, this study aimed to address the current utilization of the solid residues generated by breweries (brewer's spent grains, BSG). Hence, after evaluating the most important research by bibliometric analysis, new technological routes and new policy-making decisions were proposed for BSG valorization, advocating the application of the biorefinery concept to support the circular economy transition of the beer industry.

## 2. General aspects of brewer's spent grain

In 2018, the worldwide beer production reached $194\times10^9$ L.[22] In Brazil, $400\times10^3$ tons of barley (*in natura*) were cultivated [23] and $644\times10^3$ tons were imported from South America,[24] which were the main feedstock used for the national productivity of $14\times10^9$ L of beer [25]. The industrial process of beer production consists of six key stages: malting, milling, mashing, brewing, cooling, and fermentation. The residual solid fraction obtained after the lautering is an insoluble and undegraded part of the malted barley grain, which is the main solid by-product of the beer production process, denominated as brewer's spent grain (BSG).[26] BSG represents 85 % of the total by-products generated by the brewery, which account for 20 kg of BSG per 100 L of beer produced. In this case, approximately $87\times10^6$ tons of BSG are generated from $194\times10^9$

L of beer produced per year worldwide. Then, $2.8\times10^6$ tons of BSG are generated in Brazil each year, taking into account the annual statistics presented.[27]

BSG consists of the husk of the original barley grain in a mixture with pericarp and seed coat layers obtained as residual solid material after the wort preparation.[28] In this context, BSG is a lignocellulosic material rich in sugars, proteins, and minerals. BSG contains (in a dry weight basis) 3.9 % ash, 19.2 % crude proteins, 6.1 % soluble lignin, 11.7 % insoluble lignin, 17.9 % cellulose, and 35.7 % hemicellulose.[29] The elemental composition of BSG presents 43.59 % carbon, 6.18 % hydrogen, 37.22 % oxygen, and 3.46 % nitrogen, with a higher heat value of 18.70 MJ $kg^{-1}$.[30] Regarding the mineral composition, BSG contains calcium (3515.0 mg $kg^{-1}$), sodium (309.3 mg $kg^{-1}$), potassium (258.1 mg $kg^{-1}$), magnesium (1958.0 mg $kg^{-1}$), aluminum (36.0 mg $kg^{-1}$), iron (193.4 mg $kg^{-1}$), barium (13.6 mg $kg^{-1}$), strontium (12.7 mg $kg^{-1}$), manganese (51.4 mg $kg^{-1}$), copper (18.0 mg $kg^{-1}$), zinc (178.0 mg $kg^{-1}$), phosphorus (5186.0 mg $kg^{-1}$), sulfur (1980.0 mg $kg^{-1}$), chromium (5.9 mg $kg^{-1}$), and silicon (10,740.0 mg $kg^{-1}$).[31] Furthermore, BSG is a source of bioactive compounds, such as syringic acid (hydroxybenzoic acid), catechin (flavan-3-ol), gallic acid (hydroxybenzoic acid), ferulic acid, kaempferol (flavonol), p-coumaric, and ferulic acid.[32,33] From the mentioned bioactive compounds, BSG presents a strong antioxidant capacity by ABTS (2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)), DPPH (2,2-diphenyl-1-picrylhydrazyl), FRAP (ferric reducing antioxidant power), and ORAC (oxygen radical absorbance capacity) assay.[34] Finally, the lipophilic phase of the BSG contains palmitic acid (252.78 g $kg^{-1}$), stearic acid (9.81 g $kg^{-1}$), oleic acid (103.71 g $kg^{-1}$), linoleic acid (566.74 g $kg^{-1}$), and linolenic acid (43.47 g $kg^{-1}$).[33]

Nonetheless, BSG contains around 85% of moisture after brewing.[35] Due to the high water content, a common practice is to discard this rich material in the landfill, animal feed, or incineration. Therefore, from an environmental perspective, it is necessary to improve the management of BSG by producing new high-value chemicals and materials to have a more sustainable destination.

## 3. Methodology for literature search and bibliometric analysis

The systematic literature search was carried out by selecting documents to demonstrate how the research has developed and changed over the years. Based on the number of publications, it was possible to hypothesize the influences, trends, and technical decisions on the field. The keywords associated with BSG were selected to explore the potential uses of this by-product worldwide generated by breweries. This sought to retrieve the publications that simultaneously cover technological routes for BSG valorization and production of valuable compounds and bioenergy. Hence, based on the documents selected in the literature search, new technological routes can be proposed in a biorefinery, supporting the circular economy transition of the beer industry.

For the bibliometric analysis of BSG, the data collection was based on scientific publications published and indexed in the Science Citation Index Expanded (SCI-E) – Clarivate Analytics' ISI – Web of Science© (WoS) database. **Fig. 1** displays a global illustration of the methodology established for the bibliometric review.

The research in WoS core collection was conducted in the section “advanced search”, to obtain reliable and accurate data in the specific field of residues from brewery. The WoS search was performed applying the following logic operation: TS=(“brewer’s spent grain” OR “brewer’s spent grains” OR “brewers’ spent grain” OR “brewers’ spent grains” OR “malt bagasse”) NOT KP=(“brewer’s spent grain” OR “brewer’s spent grains” OR “brewers’ spent grain” OR “brewers’ spent grains” OR “malt bagasse”). Through this search query, it was possible to filter the publications based in selected words included in the title, abstract, author’s keywords, and not in the keywords-plus of each document, avoiding mismatches with words that only appeared on references and are not the topic of the studies. The research was done between 1900 and 2020, which is the longest timespan configured in the database, providing results that correspond to all publications available on WoS. Furthermore, a filter based on the document type was used, where “article” and “review” were selected, resulting in a total of 510 documents. The data from the 510 documents were exported and analyzed on bibliometric software’s, namely the VosViewer [36] and the “Bibliometrix” package software for the bibliometric analysis.[37] Maps based on the main keywords, authors, and the connections among them were performed in the VosViewer software. The Bibliometrix was applied to the three-field plot construction and illustrated the most productive authors over the last years. Lastly, the overlay visualization was chosen to identify the documents that have received more attention from the scientific community and visualize the most important articles published. After the data collection and bibliometric analysis regarding the studies conducted with BSG, the biorefinery concept was applied to assess the possibilities for BSG valorization.

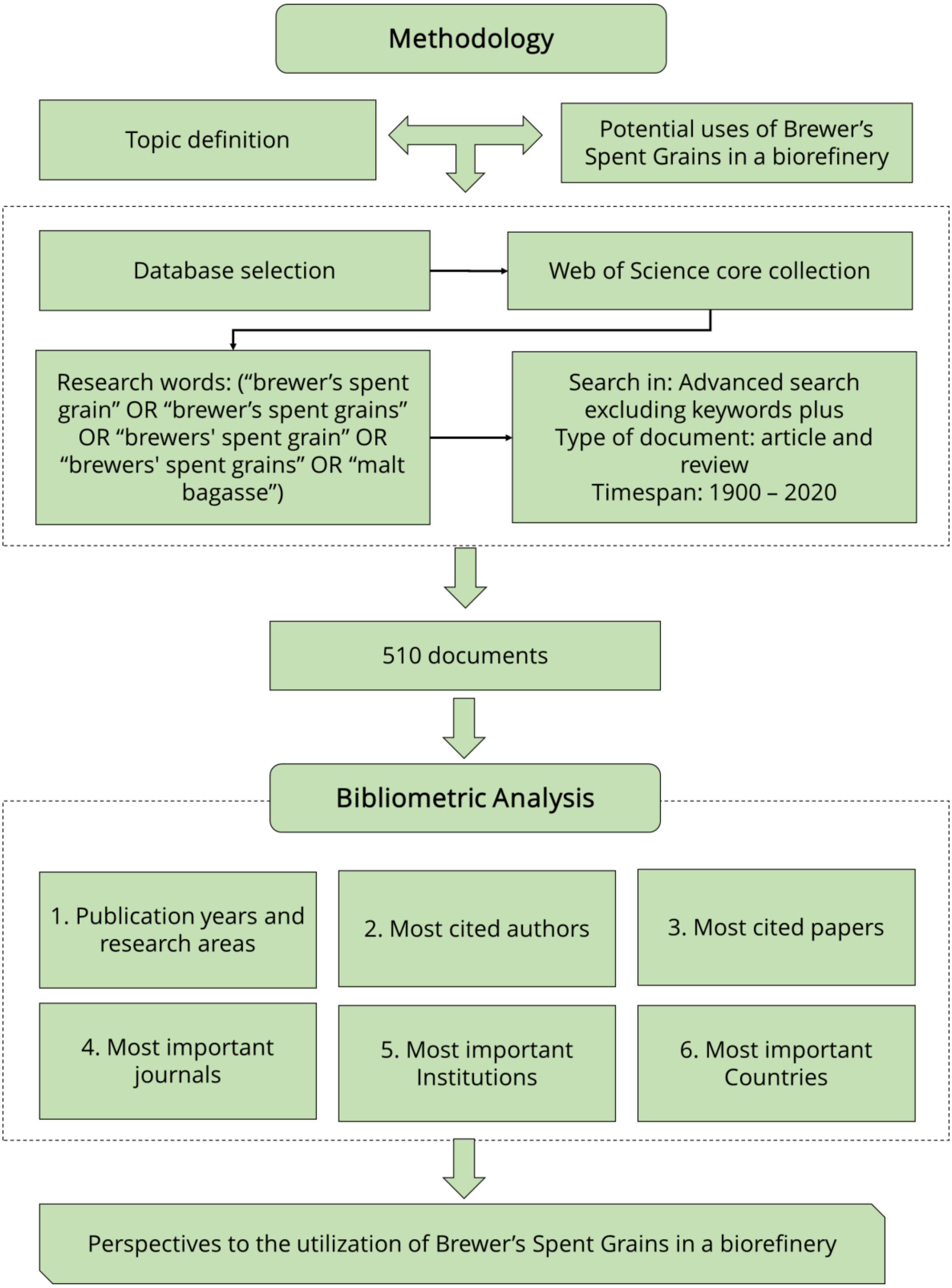

**Figure 1.** Global illustration established for the current bibliometric overview.

## 4. Bibliometric results and research trends of brewer’s spent grains

### *4.1 Publications and research areas*

The research on WoS covered the years between 1900 and 2020, however, the first article was published in 1976. In total, 490 articles and 20 reviews were obtained for the search. **Fig. 2** shows the total number of publications using the BSG and the research areas associated with the research. In general, it is possible to observe an exponential increase in the number of publications, which means that research on the use of BSG is still of great interest and non-completely explored. The increasing number of publications regarding the residues from beer production can be directly associated with the increase in worldwide beer consumption.[38] Therefore, considering the high productivity demand for the industry, the number of publications with BSG should also be considered since BSG is the main solid residue generated in breweries.

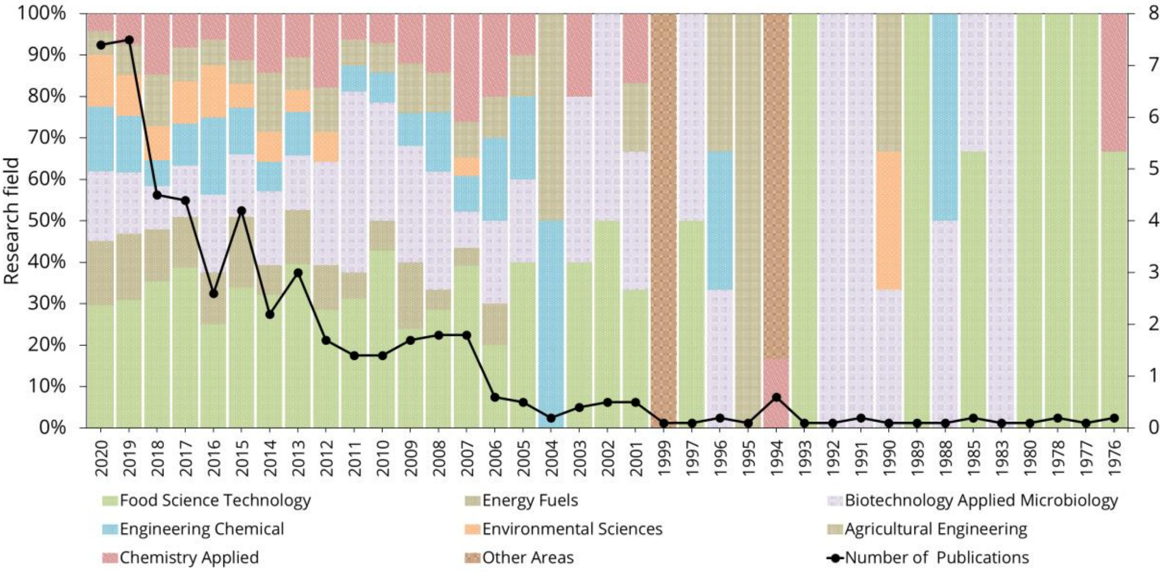

**Figure 2.** Evolution of the publications on the use of BSG during the period between 1976 and 2020 in the different research fields.

A significant increase in the total number of publications is observed starting in 2007, which can be associated with the international concern related to the correct management of solid residues from all the industrial sectors. Additionally, after 2009, the constant increase in publications and citations can also be correlated with the high price of petroleum and the global economic crisis that affects the world.[39] Therefore, the research worldwide focuses on developing methods to produce BSG derived biofuels, aiming to gradually replace petroleum as the center of the economy.[39] Thereby, since 2009, the bioenergy trend related to solid residues' valorization (especially BSG) is continually growing. This fact can be explained by the interest in obtaining technical, economic, and sustainable methods for waste management and valorization to produce biofuels and value-added products.

The most expressive research fields during all the periods of analysis were Food Science Technology (195 publications), Biotechnology Applied Microbiology (107 publications), Agriculture (105 publications), and Energy Fuels (68 publications). In the initial years of publications, Food Science Technology was almost exclusively studied, followed by Biotechnology Applied Microbiology. The Energy Fuel classification shares more than 35 documents with Biotechnology Applied Microbiology and has been growing since 2006 due to the global energy demands. Other important research fields covered in the studies with BSG are Chemistry Applied, Engineering Chemical, Agricultural Engineering, and Environmental Sciences. The evolution of these research fields according to the WoS categories along the 44 years of publication was also evaluated (**Fig. 2**). The WoS categories refine the documents from different subjects into specific groups for a systematic analysis. More than thirty (30) WoS categories were displayed in this study, and the most expressive research fields were selected, classified, and analyzed.

From 1976 to 1993 the research field of the documents published with BSG was classified in the Food Science Technology and Biotechnology Applied Microbiology. During these 17 years, 16 publications were recorded with 317 citations. From the most important documents published in this period, some studies aimed to incorporate BSG in food formulation to increase the fibers content [40–42] since BSG is a rich source of polysaccharides. Otherwise, from 1991 to 2001 some articles were published in research fields different from Food Science Technology. Therefore, the research on Biotechnology Applied Microbiology stands out from 1991 to 2001, mainly with studies associated with nutrition and dietetics, which focused on the protein content available in the BSG and its effects of feed supplementation.[43–45]

After 2004, additional research fields started to appear, and a standard profile can be observed until 2020. The Chemistry Applied, Chemical Engineering, Environmental Sciences, and Energy Fuels were the differential knowledge areas, especially after 2010, totalizing almost 400 publications and 10 thousand citations. The last decade was responsible for producing up to 75 % of all the knowledge related to the use, characterization, and application of BSG. This behavior in the publications trend can be associated with the interest to control climate change through achieving zero emissions, which alleviates the demand for petroleum products.[21] For this, sustainable technologies of food industry waste management started to be explored.[39] Then, the research fields of Environmental Sciences and Energy Fuels highlighted after 2005, as evidenced in **Fig. 2**.

Moreover, studies applied BSG as a low-cost feedstock for many biotechnological applications, using different pretreatments to hydrolyze the lignocellulosic biomass [46,47] and support the recovery of proteins,[48] activated carbon,[49] ethanol, [50,51] among other value-added products. Therefore, waste management technology can be considered the future trend to bioenergy production from agro-industrial residues.[20] Currently, in 2020, the most important areas after Food Science Technology (28.37 %) are Biotechnology Applied Microbiology (16.21 %), Energy Fuels (14.85 %), and Environmental Sciences (12.16 %). Therefore, the articles published in 2020 shared different knowledge lines with a range of objectives due to the worldwide concern in the valorization of agro-industrial residues.

### *4.2 Main keywords*

**Fig. 3** shows the keywords that were frequently used by the authors in publications related to the systematic search presented in this study. From the current search, over the last 44 years of publications, 510 studies have been found, and 2587 keywords were related to BSG. In the bibliometric coupling, the keywords were grouped into four (04) clusters based on the similarities in the research field. For this study, consideration was given to keywords with a minimum of seven (07) occurrences. It was found in **Fig. 3** that “brewer’s spent grain” was the keyword with the highest total link strength and occurrence. Indeed, there are different words-combination to express the BSG, such as “brewer’s spent grain”, “brewer’s spent grains”, “brewers’ spent grain”, “brewers’ spent grains”, and “malt bagasse”, as previously elucidated in the WoS search, and therefore, most of the studies employed these keywords to express the residues generated by the beer industry. The most frequent keywords used in the selected documents after BSG were fermentation, hydrolysis, dietary fiber, biomass, extraction, optimization, and antioxidant activity. This result suggests relevant concern about techniques to valorize BSG and showed the possibility of applying the technological routes to produce new industrial products.

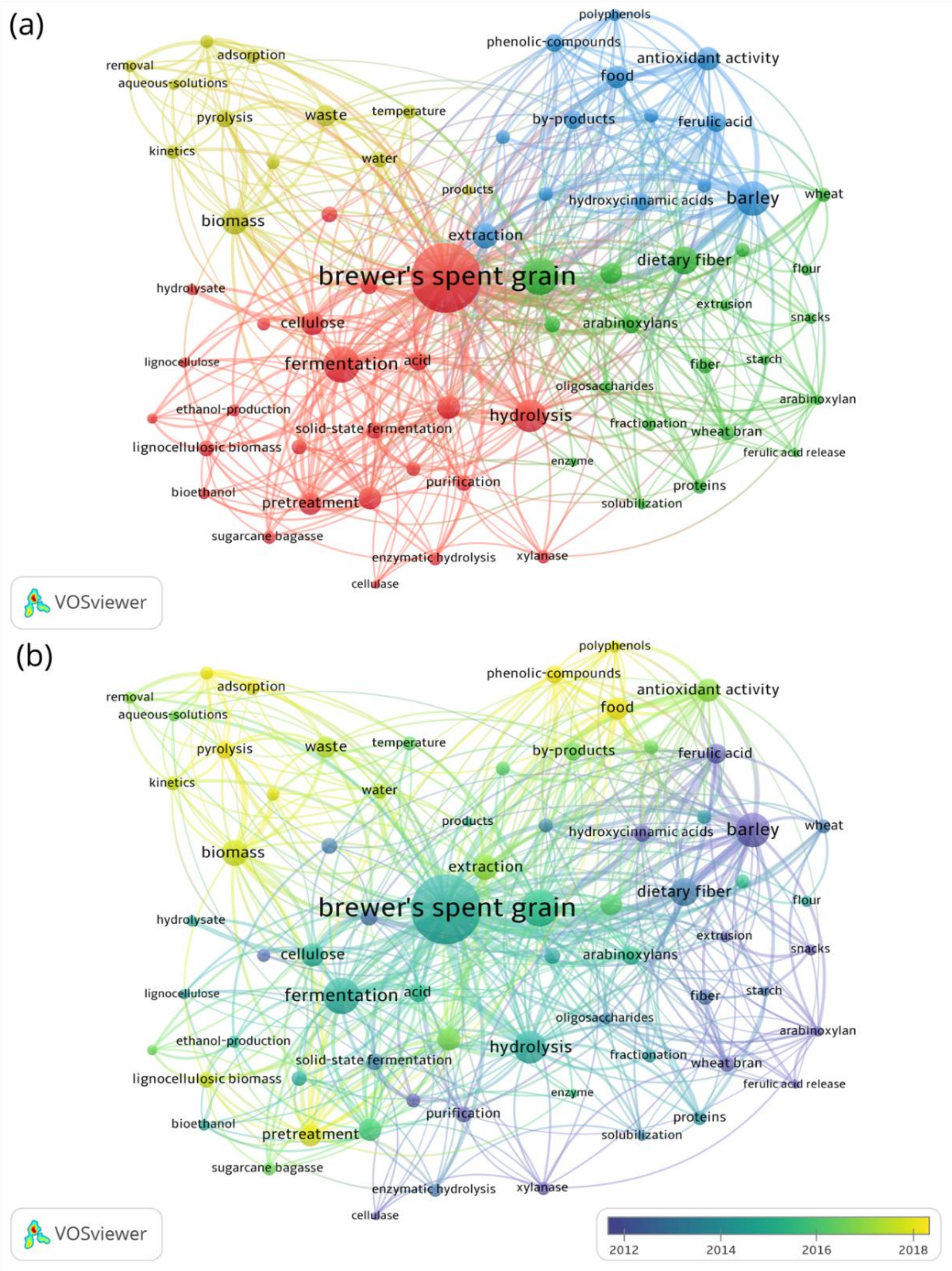

**Figure 3.** Illustration of the 70 most employed keywords used in the 510 publications. (a) Term map based on different clusters; (b) Term average year map.

The fundamental use of keywords in scientific publications is to evaluate the research trends and identify the research areas and gaps.[39] Therefore, each cluster obtained in **Fig. 3** was directly associated with a specific research field on the use of BSG. In Cluster 1 (red color in **Fig. 3a**), the keywords were related to biotechnological processes and pathways to support the production of value-added products. For this, enzymatic hydrolysis of lignocellulosic biomass (hydrolysis of BSG) was the main process observed.[31,47,52–58] Following, the fermentation to produce ethanol/bioethanol was a necessary technological process covered in Cluster 1.[51,53,59–61] Hence, the studies observed in this cluster were related to the biorefinery concept and the possibility to produce second-generation ethanol.

The second cluster (green color in **Fig. 3a**) was grouped by 18 keywords. The focuses of this cluster were related to documents that concerned the utilization of BSG to food formulation and functionalization. For this, dietary fibers, starch, and proteins were studied in some technological processes, such as extrusion to the production of snacks with high nutritional quality and considerable good sensorial acceptance.[62–68] In Cluster 3 (blue color in **Fig. 3a**), the research was focused on the antioxidant potential of BSG, and therefore, the extraction and identification of bioactive compounds were studied. Specifically, the presence of ferulic acid was constantly studied over the years of publication since this is the main phenolic compound extracted from BSG.[69–76] Finally, in the last cluster (yellow color in **Fig. 3a**), the pyrolysis process was used to upgrade the biomass, and then, some operational parameters, i.e., temperature and kinetics, were optimized for the pyrolysis process of BSG.[77–83]

Additionally, it is possible to plot the main research keywords over publication years (**Fig. 3b**). For this, the color ranging from blue to yellow was selected to better observe the history of BSG over the years of publication, considering the average terms used. The research fields related to the antioxidant capacity of BSG were most expressive around 2012. In this period, linking with the antioxidant potential, the food-processing capacity of BSG was also studied. In the next years (between 2014 and 2016), the studies regarding hydrolysis and fermentation to ethanol production were highlighted. After this, the pyrolysis research field received attention, and even some studies of antioxidant capacity and protein extraction were highlighted between 2016 and 2017. After 2018, the studies started focusing on biorefinery, and the keywords “pretreatment”, “lignocellulosic biomass”, “phenolic compounds”, and “food” were the most expressive. Hence, the studies of biorefinery of BSG are still non-explored since few recent studies reported in the research field.

### *4.3 Main articles in the research field*

**Table 1** shows the top 10 most cited documents related to the research on the use of BSG. The most cited paper was published in the *Journal of Cereal Science* and accounts for 421 citations. The mentioned review evaluated the generation, characteristics, and potential applications of BSG, with a focus on alternative uses as a raw material in foods, energy production and, biotechnological processes.[26] The high attention given to this study shows the great interest in establishing new routes to BSG valorization and sustains the global environmental care policies.

The second most cited paper (203 citations) was published at *Enzyme and Microbial Technology* and evaluated two pretreatments of BSG. Pretreatment by diluted acid (cellulignin) and diluted alkali (cellulose pulp) was conducted to forthcoming enzymatic hydrolysis. The best performance of cellulose enzymatic hydrolysis into glucose was achieved according to the lower hemicellulose content and lignin in BSG.[84] Additionally, it was not necessary to promote a complete removal of hemicellulose and lignin to achieve a high cellulose conversion ratio during the enzymatic hydrolysis of BSG, which can be an advantage to the industrial application of this process.

Following a chronological sequence, the article “Hydroxycinnamic acids and ferulic acid dehydrodimers in barley and processed barley” published in the *Journal of Agricultural and Food Chemistry* were the oldest from ten most cited papers ranking in the third position with 148 citations. In this study, Hernanz et al. [85] determined the content of hydroxycinnamic acid and ferulic acid after alkaline hydrolysis in barley grain and processed barley. From the main results, ferulic acid (359–624 μg $g^{-1}$), p-coumaric acid (79–260 μg $g^{-1}$), and caffeic acid (<19 μg $g^{-1}$) were the compounds quantified. Otherwise, significant variations among the barley varieties were observed for all the chemical compounds analyzed. Finally, BSG presented 5-fold higher content of ferulic acid, p-coumaric acid, and ferulic acid than barley grains.

**Table 1.** Top 10 most cited papers about BSG.

| Ranking* | Title | Journal | Publication year | Citations | Citation per year | Reference |
|---|---|---|---|---|---|---|
| 1st | Brewer's spent grain: generation, characteristics and potential applications | Journal of Cereal Science | 2006 | 421 | 26.31 | [26] |
| 2nd | Effect of hemicellulose and lignin on enzymatic hydrolysis of cellulose from brewer's spent grain | Enzyme and Microbial Technology | 2008 | 203 | 14.50 | [84] |
| 3rd | Hydroxycinnamic acids and ferulic acid dehydrodimers in barley and processed barley | Journal of Agricultural and Food Chemistry | 2001 | 148 | 7.05 | [85] |
| 4th | Brewer's spent grain: a valuable feedstock for industrial applications | Journal of the Science of Food and Agriculture | 2014 | 146 | 18.25 | [28] |
| 5th | Influence of extraction solvents on the recovery of antioxidant phenolic compounds from brewer's spent grains | Separation and Purification Technology | 2013 | 144 | 16 | [71] |
| 6th | Variability of brewer's spent grain within a brewery | Food Chemistry | 2003 | 141 | 7.42 | [86] |
| 7th | Brewer's spent grain: A review of its potentials and applications | African Journal of Biotechnology | 2011 | 125 | 11.36 | [87] |
| 8th | The effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made from food by-products | Food Chemistry | 2009 | 124 | 9.54 | [66] |
| 9th | Ferulic and p-coumaric acids extraction by alkaline hydrolysis of brewer's spent grain | Industrial Crops and Products | 2007 | 124 | 8.27 | [88] |
| 10th | Acid hydrolysis and fermentation of brewer's spent grain to produce xylitol | Journal of the Science of Food and Agriculture | 2005 | 106 | 6.24 | [31] |

*Data recorded at WoS database in January 2021.

In 2014, another critical review published received attention from the scientific community, and 146 citations were recorded. The article “brewer's spent grain: a valuable feedstock for industrial applications” published in the *Journal of the Science of Food and Agriculture* summarizes the main aspects of the generation of agricultural residues, focusing on the potential of BSG.[28] The review also covered and discussed the most recent technologies used to valorize BSG through application in food, energy, and biotechnological processes to produce value-added products and recover compounds of interest from promoting beneficial effects on health.

Focusing on the presence of bioactive compounds with antioxidant capacity, Meneses et al. [71] evaluated the extraction of antioxidant phenolic compounds from BSG by different solvents (methanol, ethanol, acetone, hexane, ethyl acetate, water, methanol/water, ethanol/water, and acetone/water mixtures). The study published in *Separation and Purification Technology* is the 5th most cited paper (144 citations). The main results demonstrated that acetone and water mixtures at 60% (v/v) were the most efficient solvents to extract bioactive compounds from BSG. Moreover, the study is among the first attempts for recovering antioxidant phenolic compounds from BSG by solid-liquid extraction, which directly contribute to future applications of BSG in the food, cosmetic and pharmaceutical industries as an alternative to substitute the use of synthetic antioxidants.

Next, the 6th most cited paper determined the BSG composition (moisture, protein, fat, ash, and phenolic). Santos et al. [86] also conducted an experiment related to the preservation of BSG by oven drying, freeze-drying, and freezing. The paper “Variability of brewer's spent grain within a brewery” was published in *Food Chemistry* in 2003 and accounts for 141 citations. In summary, the authors concluded that oven drying was similar in comparison to freeze-drying, considering the BSG composition, and then, due to the lowest economic cost, oven drying should be applied as the most suitable method for the preservation of BSG.

The 7th most cited study (125 times) was attributed to Aliyu et al. [87], which conducted a review on the potentials and applications of BSG for different purposes. As evidenced in previous review studies of BSG, Aliyu et al. [87] highlighted that BSG is a promising feedstock for producing several products such as animal nutrition products, construction bricks, metal adsorption, growth medium for microorganisms bioethanol production, but especially for sustainable reuse through biotechnological processes.

The 8th most cited paper, “the effect of extrusion cooking using different water feed rates on the quality of ready-to-eat snacks made from food by-products”, was published in *Food Chemistry* and accounted for 124 citations. Stojceska et al. [66] investigated the effect of different feed moisture levels during extrusion cooking on the nutritional and textural quality of expanded products. For this, BSG was used as dietary fiber and incorporated in wheat flour and corn starch before the extrusion. From the main results, the extrusion cooking increased the level of phenolic compounds and antioxidant capacity, demonstrating that BSG could be applied as feedstock with rich antioxidant capacity.

The subsequent publications' research fields evidenced the alkaline hydrolysis of BSG to produce ferulic and p-coumaric acids. This paper was published by Mussatto et al. [88] in *Industrial Crops and Products* and accounts for 124 citations. This paper can be classified as a sequence of the study published by Hernanz et al. [85], since the study evaluated hydroxycinnamic acid and ferulic acid content. Finally, the 10th most cited paper (106 citations) was published in the *Journal of the Science of Food and Agriculture,* and it is entitled “Acid hydrolysis and fermentation of brewer's spent grain to produce xylitol”. In this study, Mussatto et al. [31] concluded that it is possible to use BSG hydrolysate to produce xylitol as an alternative for the valorization of this agro-industrial by-product.

### *4.4 Main authors*

After evaluating the most cited papers of the research field, it is possible to identify the most productive researchers. **Fig. 4** shows an illustration generated on Bibliometrix of the top-authors’ productivity over time, based on the number of publications and total citations per year. It is important to note the authors shown in **Fig. 4** are not necessarily the first authors of each publication. This study includes the 20 most important authors. From the perspective of **Fig. 4**, the size of the markers represents the number of articles published, i.e., the bigger the circles, the bigger the number of articles. Additionally, it is possible to observe the number of citations through the makers’ colors, i.e., the more transparent the marker, the lower is the number of total citations.

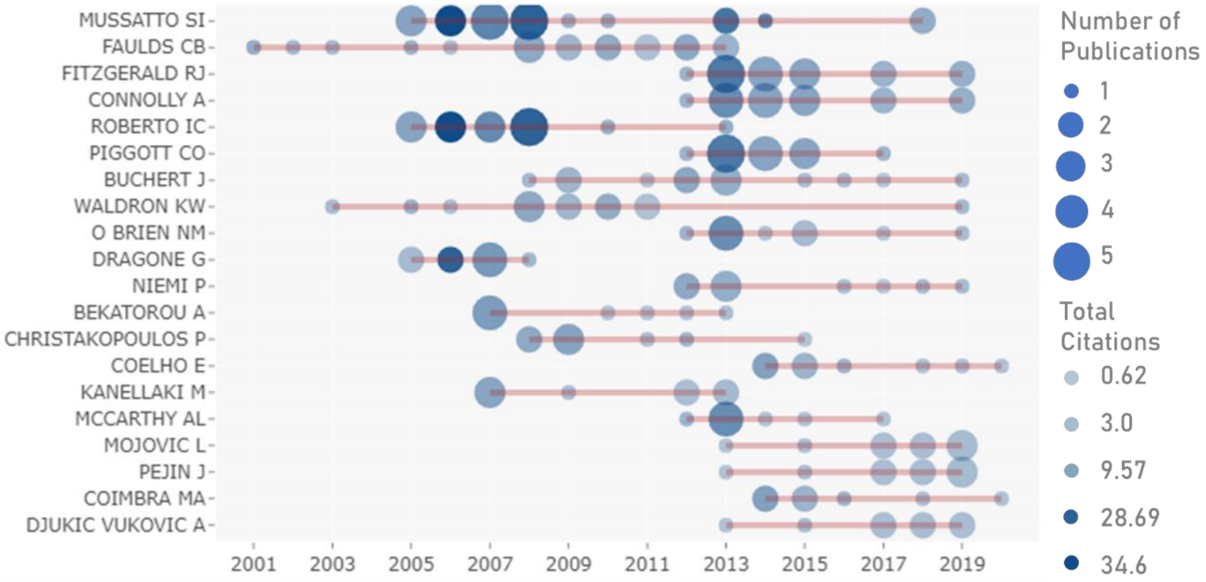

**Figure 4.** Illustration of the most important authors publishing on BSG over the last years. Label: the size of circles indicates the number of publications, and the color of the sizes indicates the number of citations.

Mussatto S.I. was the most productive researcher in the field, with 23 documents. In 2006 the number of citations per year registered by this author reached its maximum, with 34.6 citations. In collaboration with her, Roberto I.C. and Dragone G. are the co-authors of the articles published from 2005 to 2013. Faulds C.B. was the second most productive researcher (totalizing 18 documents) since 2000', and his last publication was recorded in 2013. The highest number of publications per year for this author was in 2012, with 8.33 citations. Following, Fitzgerald R.J. (17 publications), Connolly A. (16 publications), and Piggott C.O. (14 publications) presented a collaboration network among them. After 2013, Mojovic L., with eight (08) publications, has published some studies in collaboration with Pejin J. and Djukic-Vukovic A.

### *4.5 Journals, institutions, and countries*

The most critical journals in the research field were *Bioresource Technology*, *Journal of Agricultural and Food Chemistry*, and *Food Chemistry*, accounting for 59 publications (**Table 2**), which means that 10.22 % of all documents were published in these journals. The scope of these journals presents a similarity with the keywords previously discussed. From the most cited papers, *Food Chemistry* and the *Journal of* *the Science of Food and Agriculture* were responsible for publishing 40 % of the ten (10) most cited articles. Therefore, it can be considered the most important journal in the field.

**Table 2.** The 10 most prestigious journals publishing studies on BSG.

| Ranking* | Journal | Publications |
|---|---|---|
| 1st | Bioresource Technology | 26 |
| 2nd | Journal of Agricultural and Food Chemistry | 19 |
| 3rd | Food Chemistry | 14 |
| 4th | Journal of Cereal Science | 14 |
| 5th | Journal of the Institute of Brewing | 14 |
| 6th | Industrial Crops and Products | 13 |
| 7th | LWT Food Science and Technology | 11 |
| 8th | Journal of the Science of Food and Agriculture | 10 |
| 9th | Applied Biochemistry and Biotechnology | 9 |
| 10th | Carbohydrate Polymers | 9 |

*Data recorded at WoS database in January 2021.

**Fig. 5** presents a three-field plot relating the authors, author's keywords, and journals. In this plot, it is possible to visualize the main items of three fields and how they are related through a Sankey diagram.[37] This visualization is used to depict a flow from one set of values to another, and the heavier the links, the stronger is the connection between the items. In **Fig. 5** it is possible to notice Mussatto S.I. had the heaviest link to the keyword "brewer's spent grain", followed by Roberto I.C., and Buchert J. Among these three authors, the same second heavier link is shared, which occurs to the keyword "lignin". Following the flow from the field of the keyword to the journal's one, it is noticeable the heaviest link from "brewer's spent grain" is related to the *Journal of Agricultural and Food Chemistry* and the *Bioresource Technology*. Additionally, "lignin" has the heaviest link with the *Journal of Agricultural and Food Chemistry*, suggesting that the journal covered most publications with this keyword.

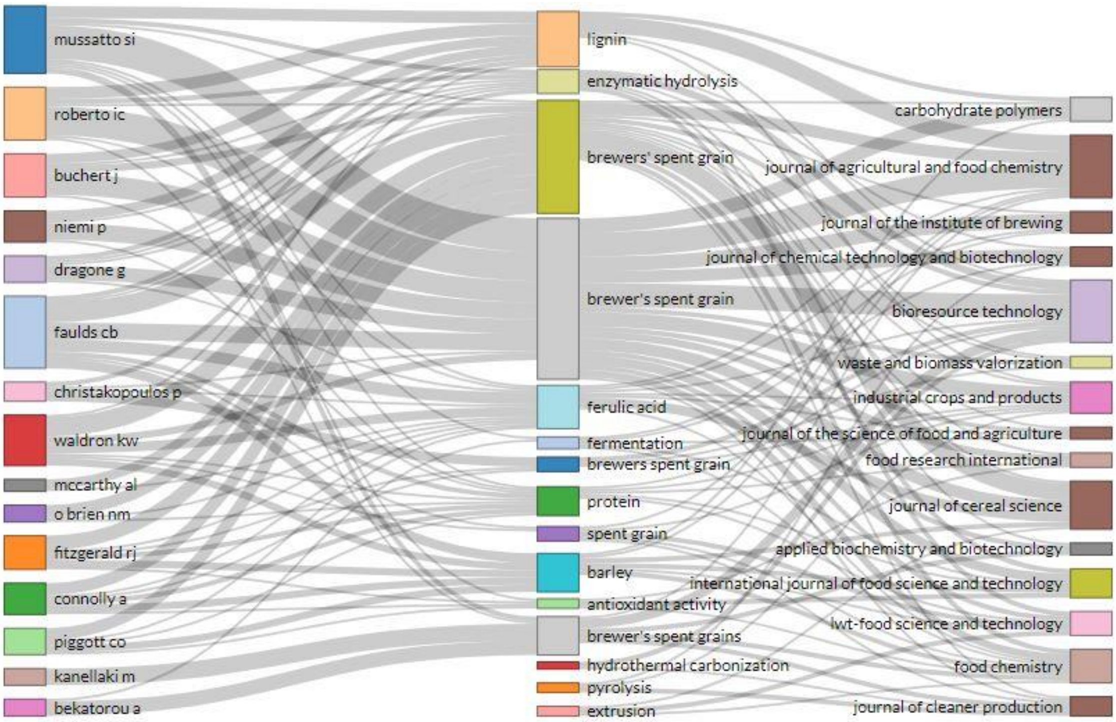

**Figure 5.** The three-field plot of BSG research relating the main authors, keywords, and journals.

The number of publications related to BSG was used to rank countries and institutions involved in the bibliometric analysis. Globally, a total of 65 countries are engaged in research related to the use of BSG. **Fig. 6** shows the number of publications per country on the research on BSG. Brazil is the most productive country in this research area, accounting for 73 publications (14.3 %), followed by Spain (47 publications, 9.2 %), Portugal (37 publications, 7.2 %), Italy (36 publications, 7.1 %), England (34 publications, 6.6 %), and Ireland (34 publications, 6.6 %). It is noticeable that countries from Europe are more interested in study bio-waste to bioenergy as a strategic tool to be involved in the reduction of climate change. Then, these countries are introducing research on renewable and alternative energy sources.[39] Beyond, regarding the most prestigious institutions (**Table 3**), the University of São Paulo (Brazil, 20 publications) stand out from the research involving BSG, followed by the University of Minho (Portugal, 18 publications), Biotechnology and Biological Sciences Research Council (England, 17 publications), University of Limerick (Ireland, 17 publications), Quadram Institute (England, 16 publications), and the VTT Technical Research Center (Finland, 16 publications).

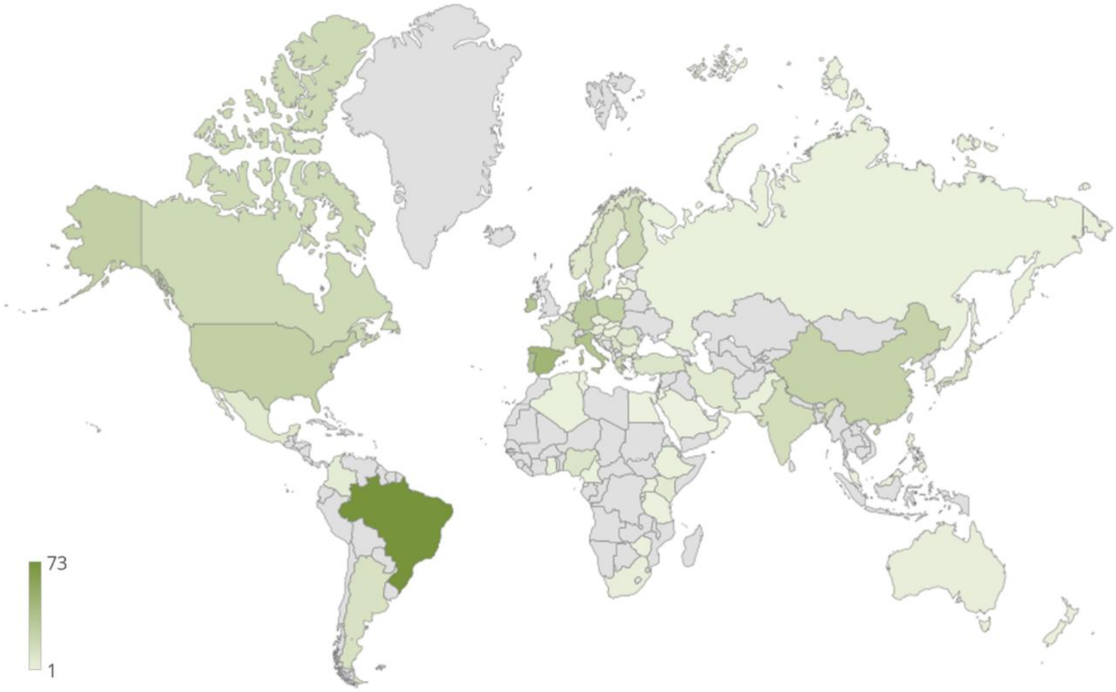

**Figure 6.** The number of scientific publications on BSG per country.

Beyond, the collaboration network between the countries was also studied (**Fig. 7**). In general, the clusters are assembled into eight clusters, and the shorter the distance between the circles, the more similar are the research fields.[39] The first cluster was composed of Argentina, Australia, Brazil, Colombia, Hungary, Ireland, Portugal, and Spain. Following, Algeria, Austria Czech Republic, France, Italy, Japan, and Sweden were responsible for the second cluster. There are four clusters composed of five countries (Cluster 3: England, Finland, Norway, Scotland, and Turkey; Cluster 4: Canada, Greece, India, Mexico, and North Ireland; Cluster 5: Belgium, Cameroon, Germany, Kenya, and the Netherlands; Cluster 6: Nigeria, Pakistan, China, Singapore, and the United States of America. Finally, Cluster 7 was composed by Denmark, Serbia, and Uganda; and Cluster 8 was joined by Iran and Poland.

**Table 3.** List of the most prestigious institutions by the number of publications on BSG.

| Ranking* | Affiliation | Country | Number of Publication |
|---|---|---|---|
| 1st | University of São Paulo | Brazil | 20 |
| 2nd | University of Minho | Portugal | 18 |
| 3rd | Biotechnology and Biological Sciences Research Council | England | 17 |
| 4th | University of Limerick | Ireland | 17 |
| 5th | Quadram Institute | England | 16 |
| 6th | VTT Technical Research Center | Finland | 16 |
| 7th | University of East Anglia | England | 13 |
| 8th | National Scientific and Technical Research Council | Argentina | 12 |
| 9th | University College Cork | Ireland | 12 |
| 10th | Spanish National Research Council | Spain | 12 |

*Data recorded at WoS database in January 2021.

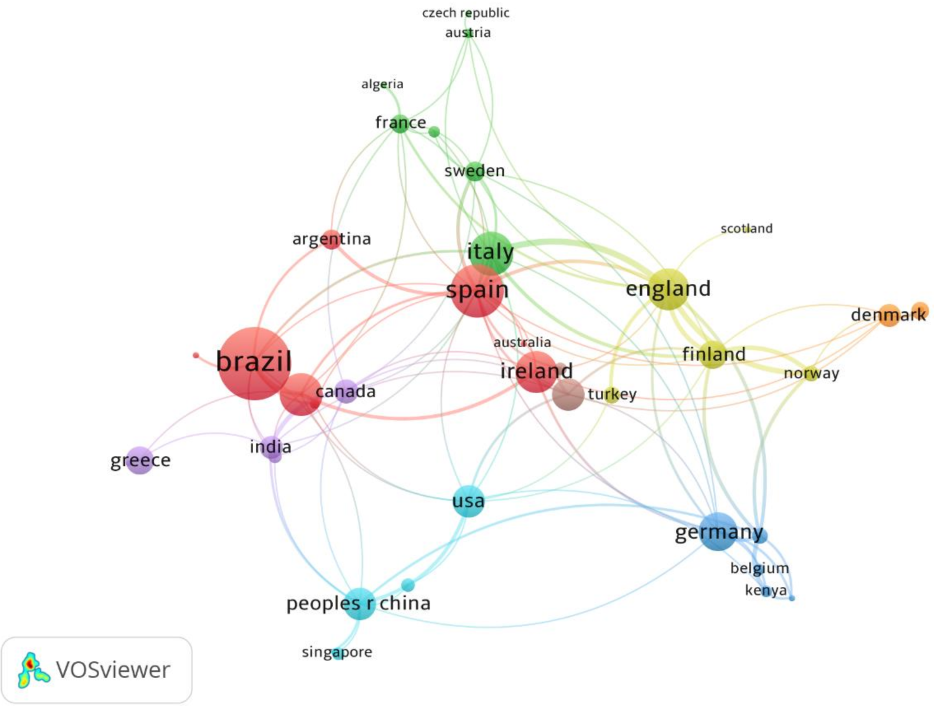

**Figure 7.** Collaboration network strength between the countries according to co-authorship.

## 5. Biorefinery to the valorization of BSG

Under-developing countries do not have good management policies to prevent the burning of residues, open-air, and landfill disposal, which is unsuitable for decreasing environmental impacts, such as greenhouse gas emissions. The development of a new management system, annexed in a biorefinery, could be an essential strategy to minimize environmental, social, and economic problems.[9] Therefore, based on the bibliometric search conducted, the studies related to the production of value-added products from BSG were selected. A general scheme to propose a biorefinery to the valorization of BSG was performed (**Fig. 8**). This scheme was used to detail the most promising technological routes to produce bioenergy, biofuels, and value-added products. **Table 4** detailed the technological routes usually used to the production of value-added products from BSG. **Fig. 9** demonstrates a scheme related to the scientific topic of BSG generation and valorization through a biorefinery implementation.

The valorization of different components available in biomass supports the industrial implementation of biomass-based biorefinery,[1] and in the current study, a waste biorefinery can be designed. However, one of the most limiting steps to the lignocellulosic biomass conversion is fractionation. As evidenced in **Fig. 8**, different pretreatments were applied in the BSG, such as acid,[31] alkali,[89] acid-alkali,[90] chemical,[91] supercritical,[70] pulsed electric,[92] hydrothermal,[29] and enzymatic hydrolysis.[93] Most of them can be a promising approach to upgrade the sugar yield.

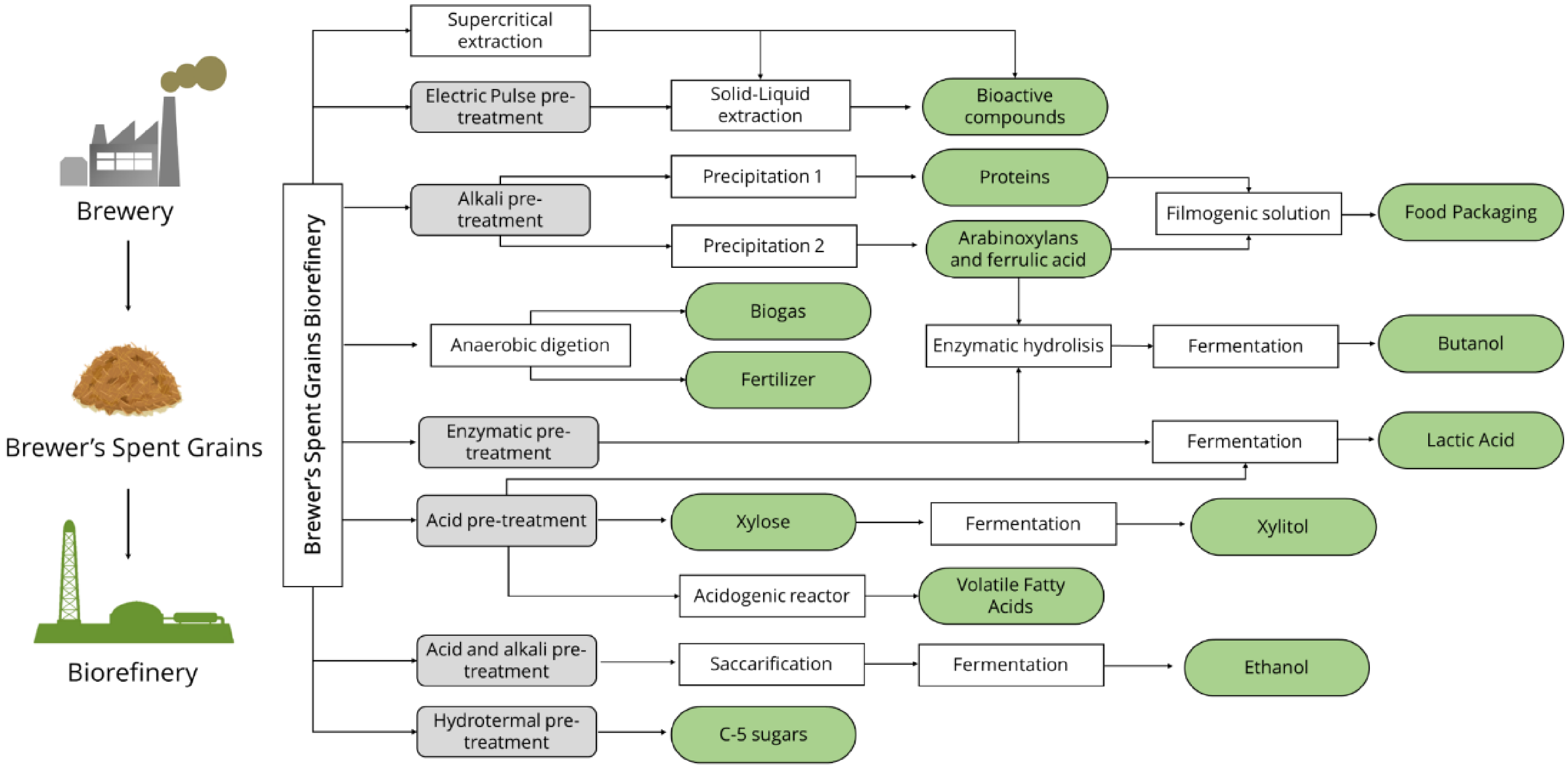

**Figure 8.** The overall scheme of the technological routes for a biorefinery on the valorization of BSG and possible value-added products generated.

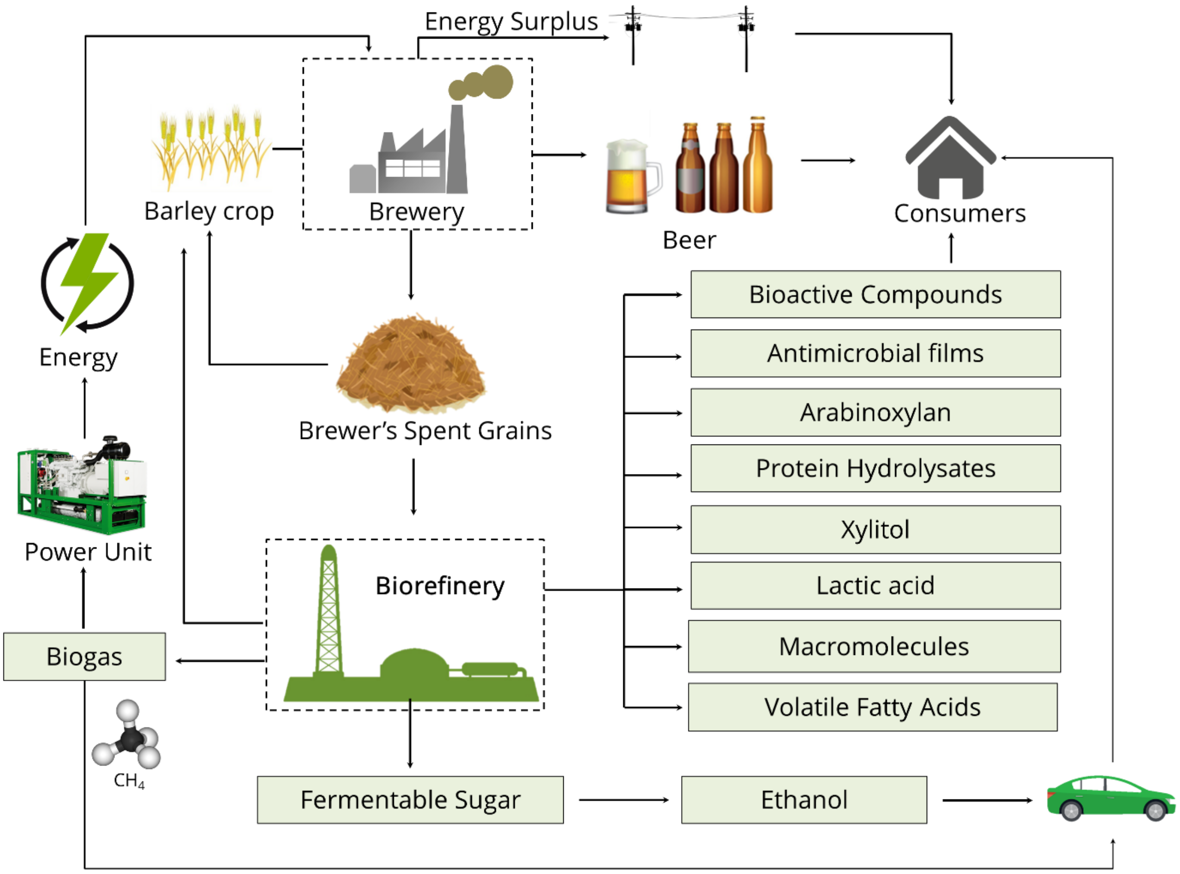

**Figure 9.** General scheme related to the scientific topic of BSG generation and valorization through a biorefinery implementation.

**Table 4.** Possible technological routes applied in a biorefinery for the valorization of BSG and production of value-added products.

| Value-added products | Technological route | Products yield | Additional information | References |
|---|---|---|---|---|
| Bioactive compounds | Solid-liquid extraction with hydroethanolic solution (60 %, v v$^{-1}$) | 4-Hydroxybenzoic acid (104.6 ± 0.2 µg g$^{-1}$ BSG), vanillin (109.2 ± 0.5 µg g$^{-1}$ BSG), catechin (223.6 ± 1.9 µg g$^{-1}$ BSG), and vanillic acid (122.0 ± 4.4 µg g$^{-1}$ BSG) | The extracts presented antioxidant, antihypertensive, and antibacterial activity | 73 |
| | Pulsed electric field extraction pre-treatment (2.5 kV cm$^{-1}$, 50 Hz, 14.5 s) | Tricin (49.76 ± 1.20 µg g$^{-1}$ BSG), sinapoyl hexose (23.00 ± 0.73 µg g$^{-1}$ BSG), hydroferuloyl glucose (8.60 ± 0.12 µg g$^{-1}$ BSG), catechin (3.96 ± 0.34 µg g$^{-1}$ BSG), and *p*-*h*ydroxybenzaldehyde (3.80 ± 0.05 µg g$^{-1}$ BSG) | Twelve (12) free phenolic compounds were quantified, totalizing 101 ± 2 µg g$^{-1}$ BSG | 92 |
| | Supercritical carbon dioxide extraction (35 MPa, 40 °C) and ethanol (60 %, v/v) | Total phenolic compounds (0.35 ± 0.01 mg g$^{-1}$ BSG) and total flavonoids (0.22 ± 0.01 mg g$^{-1}$ BSG) | The extract presented antioxidant potential by the DPPH assay (2.09 ± 0.04 g g$^{-1}$ BSG) | 70 |
| Arabinoxylan | Microwave assisted alkaline pretreatment (172 °C, 0.38 mol L$^{-1}$ NaOH) | Arabinoxylan (133 kg t$^{-1}$ BSG) | The pretreatment recovery 52.6 % of the arabinoxylan contained in the untreated BSG | 93 |
| | The extraction was carried out by alkaline process (0.5 mol L$^{-1}$ NaOH) | Arabinoxylan yield was approximately 5 %, and pure arabinoxylan represented 71.64 ± 1.18 % of the BSG arabinoxylan | BSG- arabinoxylan was found to contain glucose (17.94 %), arabinose (33.74 %), and xylose (37.90 %). Ferulic acid is mainly | 94 |

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| | | | responsible of the arabinoxylan antioxidant capacity. | |
| Arabinoxylan and Proteins | Sequential extraction of arabinoxylan and proteins | Yield of 66–73 % of total arabinoxylan.<br>Yield of 82–85 % of total proteins | The arabinoxylan were recovered by ethanol precipitation; and citric acid was applied to obtain the protein-rich fractions. | 95 |
| Food packaging | Arabinoxylan was obtained by alkaline process, and then, casting technique was used to obtain arabinoxylan film packaging | Films containing arabinoxylan enhanced thermal (up to 230 °C) and mechanical properties (up to 7.5 GPa). Active agents (ferulic acid) incorporated in the films improved the UV-barrier, antioxidant, and antimicrobial properties. | The films produced have potential for active food packaging. | 89 |
| | BSG-protein concentrate films were prepared by water solubilization with polyethylene glycol as plasticizer | Films prepared at pH 2 were homogeneous in appearance and could be manipulated. The films presented good mechanical, water-barrier properties, and the antioxidant capacity. | BSG proteins could be a cheap alternative for the preparation of biodegradable films, which are capable of being used as active food packaging | 98 |
| Butanol | Enzymatically hydrolysis of arabinoxylan fraction with cellulases and fermented by *Clostridium beijerinckii* | 37 kg acetone-butanol-ethanol $t^{-1}$ BSG and 28 kg butanol $t^{-1}$ BSG | Potential for the efficient use of BSG in an integrated biorefinery | 93 |
| | Pretreatment of BSG with laccase from *P. ostreatus*, following by enzymatic hydrolysis, Saccharification and | 12.6 g $L^{-1}$ acetone-butanol-ethanol and 7.83 g $L^{-1}$ butanol | High detoxification and delignification yields achieved by laccase pretreatment resulted in great Saccharification, with 79 % of sugar conversion | 99 |

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| | fermentation with *Clostridium acetobutylicum* | | | |
| | Peroxide alkaline pretreatment in BSG (5 % $H_2O_2$ w w$^{-1}$, pH 11.5, 50 °C, 5–15 % w w$^{-1}$ BSG, 60–180 min) following by enzymatic hydrolysis and acetone-butanol-ethanol fermentation using *C. beijerinckii* | 13.7 ± 0.2 g acetone-butanol-ethanol L$^{-1}$ and 11.0 ± 0.2 g butanol L$^{-1}$. 45.1 kg butanol ton$^{-1}$ BSG and 56.1 g acetone-butanol-ethanol kg$^{-1}$ BSG | Authors evaluated that ozonolysis was not an effective treatment | 91 |
| Xylitol | BSG was hydrolyzed with diluted sulfuric acid concentration to produce a liquor with a large amount of xylose and good fermentability to produce xylitol by *Candida guilliermondii* yeast | Xylose consumption ranged from 67 to 96.9 % and good fermentation results were demonstrated ($Y_{P/S}$= 0.70 g g$^{-1}$ and $Q_P$= 0.45 g dm$^{-3}$ h$^{-1}$). | High extraction efficiency of hemicellulose sugars (92.7 %) was obtained with the pretreatment. The results were obtained without nutrient supplementation in the fermentation. | 55 |
| Lactic acid | BSG hydrolysates fermentation by *Lactobacillus rhamnosus* | Lactic acid yield (91.29 %) and lactic acid volumetric productivity (1.69 g L$^{-1}$ h$^{-1}$) | Initial reducing sugar content of 54 g L$^{-1}$. | 111 |
| | BSG hydrolysates fermentation by *Lactobacillus delbrueckii* | Lactic acid yield (73 %) and lactic acid productivity (5.4 g L$^{-1}$) | Initial sugar content of 50 g glucose L$^{-1}$. | 102 |

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| Sugars | Hydrothermal fractionation of BSG in a semi-continuous subcritical reactor | Arabinose (22 g kg$^{-1}$), galactose (1.5 g kg$^{-1}$), glucose (2.5 g kg$^{-1}$), xylose (11 g kg$^{-1}$), and fructose (3 g kg$^{-1}$) | The flow rate of 20 mL min$^{-1}$ with an S F$^{-1}$ 64 at 210 °C maximized the production of sugars. | 29 |
| | Hydrolysis using supercritical CO$_2$ technology to assist the enzymatic hydrolysis of BSG | 219.39 g sugar kg$^{-1}$ BSG; 26.5 % glucose, 2.2 % cellobiose, 1.7 % xylose, and 0.7 % arabinose | The use of near-critical CO$_2$ increased up to 3.6 times the sugars yield. The optimum conditions were enzyme-substrate mixture and CO$_2$ at 40 °C, 175 bar, and 80 % moisture for 240 min. | 112 |
| Ethanol | Acid-alkali pretreatment of BSG, following by Saccharification with cellulase and glucosidase; and fermentation with *S. cerevisiae*. | 12-12.79 g L$^{-1}$ | 97 % efficiency of cellulose conversion into glucose. | 90 |
| | Sake fermentation system with *A. oryzae* and *S. cerevisiae* NCYC479 | 37 g L$^{-1}$ of ethanol. 1 t of BSG (dry weight) yield 94 kg of ethanol. The volumetric productivity was 3.7 g L$^{-1}$ day$^{-1}$ | The final residue contained higher than 22 % of crude protein. | 61 |
| | High solid loadings and use of whole slurry from the pretreatment were evaluated | Ethanol concentration (42.27 g L$^{-1}$) and ethanol yield (94.0 %) | High concentration of gluco-oligosaccharides (51.6 g L$^{-1}$) was obtained from BSG autohydrolysis. | 59 |
| | Sulfuric acid pretreatment, following by enzymatic hydrolysis and fermentation | 22.9 L bioethanol 100 kg$^{-1}$ of dry biomass. | 94 % of sugars in raw BSG were recovered at optimized pretreatment conditions. | 60 |
| | Phosphoric acid pretreatment, following by enzymatic hydrolysis and fermentation | Ethanol yield of 17.9 g 100 g$^{-1}$ BSG | The use of pretreatment and enzymatic hydrolysis recovered 92 % of total sugars in BSG | 53 |

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| | | 69 % of the total sugars in the BSG to be converted to ethanol. 227 L of ethanol ton$^{-1}$ of dry BSG | | |
| | Solid-state fermentation with mesophilic fungus *N. crassa* and submerged bioreactor | 74 g of ethanol kg$^{-1}$ dry BSG (5-6 g L$^{-1}$) | The optimum conditions were aeration 0.1 vvm, pre-treatment with 1 g NaOH 10 g$^{-1}$ dry BSG. | 46 |
| Volatile fatty acids (VFA) | Fed batch stirred tank reactor was maintained at acidogenic conditions | The maximum volumetric VFA productivity of 91.3 ± 9.1 mg COD L$^{-1}$ h$^{-1}$ and VFA concentration of 24.9 ± 2.6 g L$^{-1}$ were obtained for 16 days of HRT and 16 g $TS_{in}$ L$^{-1}$d$^{-1}$ of OLR | Propionic, acetic, and butyric acids were the main VFA produced. | 106 |
| | Thermal diluted acid hydrolysis following by acidogenic batch fermentation in an anaerobic granular sludge | The highest VFAs concentration was obtained at pH 6.0 and reached 16.89 g COD L$^{-1}$, composed of mainly (99.5-99.8 %) acetate and butyrate. | A mixed liquor rich in carbohydrates with 0.44 g of carbohydrates per gram of total solid of BSG was obtained using a single step of a thermal diluted sulfuric acid hydrolysis (1.5 % $H_2SO_4$ v v$^{-1}$). | 105 |
| Biogas | Biotransformation of BSG by thermophilic and mesophilic anaerobic methanogenic communities | Thermophilic conditions (64 % methane)<br>methane yield of up to 58.7 L kg$^{-1}$<br>biodegradation of 63.5 % | The optimal BSG concentration in biogas production was 50 and 100 g L$^{-1}$. | 108 |
| | Two-stage process composed of solid-state anaerobic | Biogas production of 414 ± 32 L kg$^{-1}$, methane production of 224 ± 34 L kg$^{-1}$, | The excellent adaptability of granular biomass was confirmed by | 107 |

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| | digestion and granular biomass reactors | and solids degradation between 75.9 and 83 % | 68.2 % shift in bacterial and a 31.8 % shift in archaeal community | |
| | Thermal pretreatment of BSG before anaerobic digestion | daily biogas yield of 430 Nm$^3$ kg$^{-1}$ VS<br>methane yield of 409.8 Nm$^3$ kg$^{-1}$ VS methane (58 %) | Positive effect on biogas production was observed with the thermal pretreatment | 109 |

For the production of bioactive compounds in a biorefinery, solid-liquid extraction is the most employed method. The extraction solvent is an important factor in recovering these compounds and then should be continuously optimized to each feedstock.[71] Methanol, ethanol, acetone, hexane, ethyl acetate, water, and its mixtures were continuously studied for extracting antioxidant phenolic compounds from BSG. From the majoritarian compounds obtained from solid-liquid extraction, 4-hydroxybenzoic acid (104.6 ± 0.2 μg $g^{-1}$ BSG), vanillin (109.2 ± 0.5 μg $g^{-1}$ BSG), catechin (223.6 ± 1.9 μg $g^{-1}$ BSG), and vanillic acid (122.0 ± 4.4 μg $g^{-1}$ BSG) stand out.[73] Otherwise, the pretreatment of BSG by pulsed electric field can be applied to upgrade the yield, and then, the profile of free bioactive-antioxidant compounds changes to tricin (49.76 ± 1.20 μg $g^{-1}$ BSG), sinapoyl hexose (23.00 ± 0.73 μg $g^{-1}$ BSG), hydroferuloyl glucose (8.60 ± 0.12 μg $g^{-1}$ BSG), catechin (3.96 ± 0.34 μg $g^{-1}$ BSG), and p-hydroxybenzaldehyde (3.80 ± 0.05 μg $g^{-1}$ BSG) as majoritarian compounds.[92] Beyond, the supercritical technology with carbon dioxide can also be employed to recover bioactive compounds from BSG. This technique can also be coupled with solid-liquid extraction, which improves the extraction and yield of antioxidant compounds.[70] The BSG extracts also presented antioxidant, antihypertensive, and antibacterial activity, which can be a great source to application in food, medicines, and packaging.[71,73]

The BSG macromolecules composition is lignocellulose, and then, different technological routes to upgrade this material can be designed in a biorefinery. One of the most explored products from BSG is the arabinoxylans, which can be recovered from alkaline pretreatment. Since BSG is an excellent source of bioactive compounds, the conventional extraction method of arabinoxylan also extracts a great amount of ferulic acid, a phenolic compound with biological importance.[94] Based on the literature, 1 ton of BSG can produce 133 kg of arabinoxylan, which means a yield of up to 50 % of the untreated BSG.[93] Otherwise, it is still possible to integrate the extraction of arabinoxylan and proteins since BSG presents up to 20 % of crude proteins in its composition. For this technological route, after the alkaline pretreatment, the arabinoxylans are recovered by precipitation with ethanol, and then protein-rich fractions can be obtained employing citric acid.[95] Adopting this process 66–73 % of arabinoxylans and 82–85 % of total proteins can be recovered from BSG.[95] Beyond, Qin et al.[96] evaluated different pretreatments strategies for protein extraction from brewer’s spent grains. The study concluded that hydrothermal pretreatment at 60 °C was found as an interesting option for protein extraction from BSG, being also advantageous in terms of costs and more environmentally friendly, requiring low temperature and no addition of chemicals.[96] From an industrial perspective, it is possible to produce protein-rich fractions from BSG using an enzyme-assisted fractionation process.[97] After one (01) h of hydrolysis with the enzyme Alcalase (5 μL $g^{-1}$), 46 % of protein can be obtained with a separation efficiency of 80 %. The scaled-up process for protein-rich fraction production presents a total capital investment of 11.2 million USD for an annual processing capacity of 590 ton BSG $day^{-1}$.[97] Beyond, the process' economic performance revealed a minimum selling price of protein of 1044 USD $ton^{-1}$, which is an amenable price for ensuring its competitiveness in the market.

Another technological route to be applied in a biorefinery is producing food packaging from arabinoxylan and proteins.[89] An additional benefit of packaging production is that the arabinoxylans extract presents high antioxidant compounds. Then, the packaging formulated can be a promising alternative to substitute the conventional and synthetic food additives.[98]

Otherwise, from a biotechnological perspective, butanol can be produced from BSG hydrolysates. For this, the arabinoxylan fraction should be hydrolyzed with cellulases for the fermentation with *Clostridium*. Beyond, BSG can be pre-treated with laccase from *Pleurotus ostreatus*, promoting high detoxification and delignification yields, resulting in a sugar conversion of around 80 %.[99] The yield of butanol produced from BSG range from 7 to 11 g butanol $L^{-1}$, which means that 45 kg butanol $ton^{-1}$ BSG can be produced.[91,93,99]

In the experiment with an acid pretreatment, xylose is the majoritarian sugar obtained from BSG, with an extraction efficiency of up to 90 %.[31] Xylose can also be converted into xylitol by *Candida guilliermondii* yeast, a technological route to upgrade the economic indicators of a biorefinery.[100] According to Swart et al.[101], small-scale biorefinery can be implemented annexed to a brewery, for the conversion of BSG into xylitol and xylo-oligosaccharides. In this process, the xylitol and xylo-oligosaccharides presented a minimum required selling price of 4153 and 4500 USD $ton^{-1}$, respectively.[101] These results provide a good platform for developing the cost-effective commercial production of xylitol and xylo-oligosaccharides from BSG.

Beyond, the BSG hydrolysates can be upgraded into lactic acid by fermentation with *Lactobacillus*, a technological process with a yield of up to 70 %.[102] In a biorefinery concept, xylitol, lactic acid, activated carbon, and phenolic acids can be produced in the facility.[100] The production capacity for xylitol, lactic acid, and activated carbon represents values from 197 to 249 ton day$^{-1}$. Due to the low cost of BSG and high productivity, the industrial process simulated by Mussatto et al.[100] is profitable. It increases the value when the integration level increases with combined energy and mass integration.

Another pretreatment that is still rarely explored is the utilization of hydrothermal technology with subcritical water. Employing a flow rate of 20 mL min$^{-1}$ in a semi-continuous reactor with a solvent/feed ratio of 64 and 210 °C of reactor's temperature, arabinose (22 g kg$^{-1}$), galactose (1.5 g kg$^{-1}$), glucose (2.5 g kg$^{-1}$), xylose (11 g kg$^{-1}$), and fructose (3 g kg$^{-1}$) can be produced from the subcritical fractionation of BSG.[29] In an economic perspective, the implementation of subcritical water pretreatment of BSG can be a promising alternative to produce different concentrated sugars in an industrial-plant.[103] For this, BSG should be hydrolyzed in an extractor vessel of 500 L, and the hydrolysate obtained should be separated in a five-zone simulated moving bed process. The cost of manufacturing for the subcritical water process without purification was 2.92 USD kg$^{-1}$, and with the adoption of a separation system, the cost of manufacturing increase to 6.63 USD kg$^{-1}$, which was associated with the high implementation costs. However, the selling price of purified sugars (59 USD kg$^{-1}$ for arabinose, i.e., the highest concentrated sugar obtained from BSG) is higher than the market price of non-concentrated sugars (3 USD kg$^{-1}$),[104] which allow obtaining positive profitability indicators in the process with a purification system.[103] Additionally, in a biorefinery concept, the hydrolysates obtained via subcritical water can be a promising approach to further fermentation to obtain butanol, xylitol, and lactic acid. However, these studies were not explored and should be investigated to propose innovative routes for a biorefinery with the subcritical technology, indicating a gap in experimental approaches that would possibly amplify the opportunities of BSG usage.

Regarding biofuels, BSG is also a promising feedstock for bioethanol production. Beyond, one of the most studied technological processes for BSG valorization was ethanol production, as evidenced in the bibliometric study. In general, acid-alkali pretreatment followed by saccharification and fermentation is the main steps applied to ethanol production. The literature describes that acid pretreatment is the most favorable to recover fermentable sugars from BSG, with a yield up to 90 % (w/w).[53,90] For this, a pretreatment with dilute phosphoric acid (2 %, w/v) at 155 °C is necessary.[60] The global yield obtained for the mentioned study was 17.9 g ethanol 100 $g^{-1}$ BSG, which can be considered a promising yield for industrial application.

Additionally, strategies to increase the efficiency of ethanol production were proposed.[59] In general, mild pretreatment increased the solubilization of glucose from BSG in the saccharification step. Then, the use of different *Saccharomyces cerevisiae* strains was responsible for producing ethanol with high concentration (42.27 g $L^{-1}$) and yield (94.0 %). Another technique used for ethanol production from BSG consisted of a fungal process.[61] For this, BSG needs to be inoculated with yeast and filamentous fungi and incubated in a semi-solid static bioreactor to the fungal growth. The best combination of the fungus *Aspergillus oryzae* and the yeast *S. cerevisiae* resulted in 94 kg ethanol $ton^{-1}$ BSG.

From the anaerobic fermentation process, recent studies demonstrated the potential of BSG to produce volatile fatty acids. The substrate is applied in an anaerobic stirred tank reactor maintained at acidogenic conditions.[105] Propionic, acetic, and butyric acids were the main volatile fatty acids produced from the process.[106] The process to obtain volatile fatty acids are still a few explored, and some additional steps should be optimized for further implementation in a biorefinery. Otherwise, under methanogenic conditions of anaerobic digestion, biogas rich in methane can be produced. Some studies demonstrate that the degradation of the solid can reach up to 80 %, with the methane production of 224 L $CH_4$ $kg^{-1}$.[107] Beyond, under thermophilic conditions, 64 % of methane with a methane yield up to 58.7 L $kg^{-1}$ can be obtained from the digestion of BSG.[108] Moreover, by applying a thermal pretreatment in the BSG, the daily methane production can increase to 409.8 $m^3$ $kg^{-1}$ volatile solids, demonstrating a positive effect of thermal pretreatment on biogas production.[109] In an industrial and economic perspective, the waste management system with AD implementation for BSG treatment presents profitability indicators, with payback lower than five (05) years, internal rate of return around 20 %, and net present value up to 1 million USD, and therefore, it is possible to produce bioenergy and fertilizer, as a powerful strategy for implementation in breweries.[27]

As evidenced in **Fig. 8**, numerous routes for BSG valorization are available. Moreover, these diverse routes can be chosen according to the industry's interest and machinery, making the process more economically favorable as activities are coupled. The challenging key point for a biorefinery implementation is to study industries individually, evaluating which route fits better the processes already developed in the industrial plant.[110] For instance, the lignocellulosic by-products valorization through subcritical water hydrolysis followed by anaerobic digestion can be a strategy to be evaluated for BSG, stimulating the transition from linear to the circular economy. At the same time, residues are treated as new feedstocks. Concerns around environmental issues are increasing. Consequently, experimental research, implementation of biorefineries, and even new technological routes will be discovered for the correct management of agro-industrial wastes.

## 6. Circular economy transition of the beer industry

Nowadays, the circular economy plays an essential role in materials reinsertion in a productive chain, and it is a supportive way to achieve economic growth and change the linear economy based on fossil resources. Besides, a circular economy also aims at the creation of economic, social, and environmental value. Therefore, the reuse of by-products from the conventional industrial process as feedstock in innovative technological routes leads to a novel approach for biorefinery development in the circular economy.

From the perspective of integrated biorefinery, BSG could be exploited to produce value-added products and bioenergy. An integrated close to zero waste system exploiting a sequential process with internal recycling can be proposed as a model system for the circular economy transition of the beer industry. Therefore, integration between technological routes can be an alternative for breweries. For instance, the implementation of anaerobic digestion technology in breweries can be a strategy for the management of BSG and recovery of biogas and digestate (**Fig. 10**). The biogas can be upgraded into biomethane, electricity, and thermal energy. The use of biogas can be an alternative to the decentralized production of electric energy, which supports the diversification of energy resources, improves supply capability, reliability, and efficiency. Also, thermal energy can be used by breweries and avoid the use of biomass or natural gas based on fossil fuel, which in an environmental perspective is a positive strategy to decrease greenhouse gases emissions. Moreover, the digestate obtained from the anaerobic digestion of BSG can be used as an agricultural fertilizer and replace nitrogen, phosphorus, and potassium, avoiding greenhouse gases emissions.

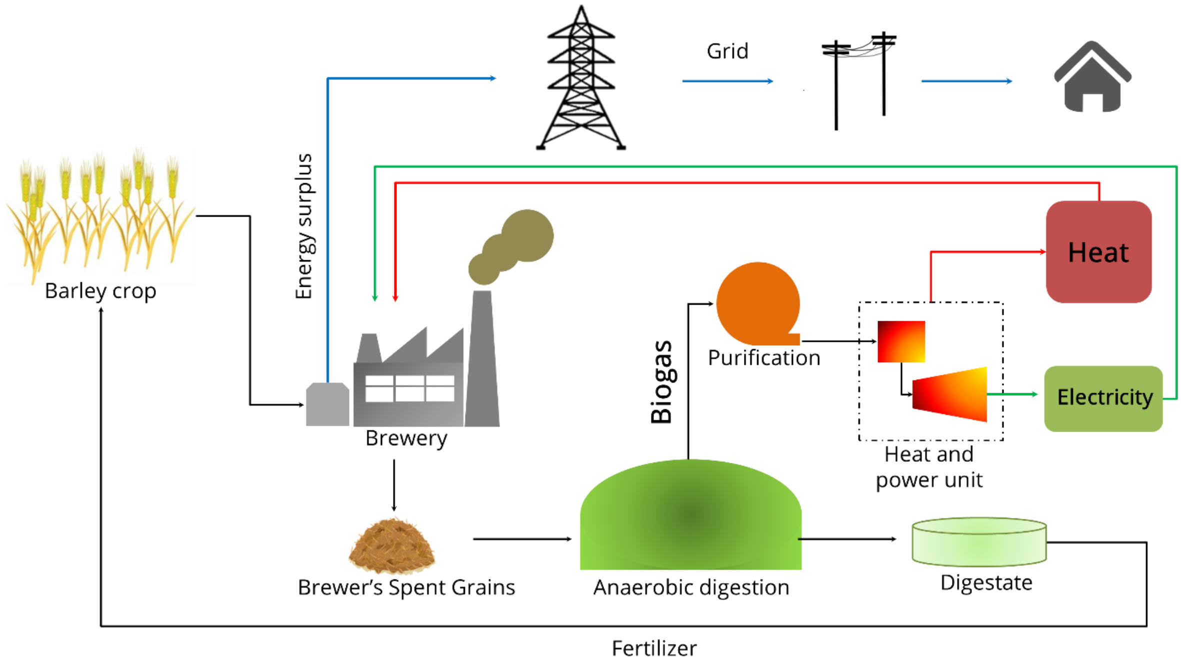

**Figure 10.** Waste management of BSG through anaerobic digestion as a possible integrated technological route for implementation in a biorefinery.

Notwithstanding, there are some challenges for more practical applications of BSG processing in integrated technological routes. For instance, there are few studies in the literature related to the practical adoption of eco-friendly pretreatments of BSG for biofuels production. Beyond, future studies should avoid using acid, alkali, acid-alkali, other chemicals, and enzymatic hydrolysis since these pretreatments are expensive, generate high amount residues, and decrease the process sustainability. Therefore, the biorefinery should adopt the most profitable and environmental route, contributing to local, sustainable development and strengthening the economy, and providing job opportunities at the local, national, and international levels. The BSG biorefinery is a sustainable model and contributes to the development of the agriculture and food sector by decreasing the wastes generated and reducing the greenhouse gases emissions by producing renewable resources and marketable products, reaching the goals of the circular economy.

## 7. Concluding remarks and perspectives

The bibliometric analysis showed that, although there is a great interest in the valorization of BSG, research in this area is still little directed towards a possible industrial application. This opens opportunities for researchers to conduct new and highly impacting studies, positively affecting this research field and leading to solutions for BSG valorization able to be scaled. There is a relevant concern about techniques for the valorization of BSG, especially in terms of enzymatic hydrolysis, food-formulation, extraction of bioactive compounds, and pyrolysis. An evaluation of the most cited papers highlighted that the application of biotechnological processes for hydrolysis of BSG stands out, especially to obtain xylitol, bioactive compounds, and fermentable sugars. The most prestigious journals publishing studies in these areas are *Bioresource Technology* and the *Journal of Agricultural and Food Chemistry*. However, considering the most cited papers, *Food Chemistry* and the *Journal of the Science of Food and Agriculture* is responsible for publishing 40% of the ten (10) most cited papers. Therefore, it can be considered the most important journal in the field. Globally, 65 countries are engaged in research related to the use of BSG, and Brazil was the most productive country with up to 70 publications.

One of the most important achievements of this bibliometric study was to demonstrate the possibility of developing a biorefinery using BSG as raw material. However, pretreatment is one of the most limiting steps to the lignocellulosic biomass conversion. Different types of pretreatments (acid, alkali, and hydrothermal) can be applied to upgrade the product’s yield. After applying the most suitable pretreatment and enzymatic hydrolysis, the hydrolysates can be used to produce value-added products, biofuels, and other economically viable molecules, such as protein and lignin, which can also be incorporated in a biorefinery. However, the environmental impacts of chemical pretreatments should be investigated before industrial implementation. The industrial plants should be individually studied before a biorefinery implementation, and therefore, the best route for BSG conversion can be chosen.

Implementation of a biorefinery can be an interesting strategy to support the circular economy transition of the beer industry. In a biorefinery, BSG valorization can be maximized, contributing to environmental, economic, and social benefits since emissions of greenhouse gases and costs are avoided, and jobs are generated. Beyond, in the circular economy transition, the beer industry should adopt reducing, reusing, recycling, and recovering materials, with the perspective of achieving sustainable development with the generation of value-added products. Finally, it was proved that BSG can be a cost-effective and feasible raw material to produce a range of bio-based products and bioenergy. From an industrial perspective, BSG is still considered a low-value by-product; however, this should change in the future due to environmental worries, giving BSG a more appropriate destination to produce value-added bio-based products.

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# CHAPTER III

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## Recovery of sugars and amino acids from brewers' spent grains using subcritical water hydrolysis in a single and two sequential semi-continuous flow-through reactors

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# CHAPTER III: Recovery of sugars and amino acids from brewers' spent grains using subcritical water hydrolysis in a single and two sequential semi-continuous flow-through reactors

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## Abstract

This study evaluated the subcritical water hydrolysis (SWH) of brewer's spent grains (BSG) to obtain sugars and amino acids. The experimental conditions investigated the hydrolysis of BSG in a single flow-through reactor and in two sequential reactors operated in semi-continuous mode. The hydrolysis experiments were carried out for 120 min at 15 MPa, 5 mL water $min^{-1}$, at different temperatures (80 – 180 °C) and using an S/F of 20 and 10 g solvent $g^{-1}$ BSG, for the single and two sequential reactors, respectively. The highest monosaccharide yields were obtained at 180 °C in a single reactor (47.76 mg $g^{-1}$ carbohydrates). With these operational conditions, the hydrolysate presented xylose (0.477 mg $mL^{-1}$) and arabinose (1.039 mg $mL^{-1}$) as main sugars, while low contents of furfural (310.7 µg $mL^{-1}$), 5-hydroxymethylfurfural (<1 mg $L^{-1}$), and organic acids (0.343 mg $mL^{-1}$) were obtained. The yield of proteins at 180 °C in a process with a single reactor was 43.62 mg amino acids $g^{-1}$ proteins, where tryptophan (215.55 µg $mL^{-1}$), aspartic acid (123.35 µg $mL^{-1}$), valine (64.35 µg $mL^{-1}$), lysine (16.55 µg $mL^{-1}$), and glycine (16.1 µg $mL^{-1}$) were the main amino acids recovered in the hydrolysate. In conclusion, SWH pretreatment is a promising technology to recover bio-based compounds from BSG; however, further studies are still needed to increase the yield of bioproducts from lignocellulosic biomass to explore two sequential reactors.

***Keywords:*** *Brewery residue; Green chemistry; Biorefinery; Biomass; Bio-based products; Circular economy; Waste valorization.*

## 1. Introduction

Nowadays, the circular economy is receiving significant interest in replacing the linear economy based on fossil resources (D'Amato & Korhonen, 2021). This interest is associated with materials reinsertion in a productive chain, and it is a supportive way to achieve economic growth (Fogarassy & Finger, 2020). Therefore, reusing by-products from the conventional industrial process as feedstock in innovative technological routes can lead to novel approaches for biorefinery development in a circular economy (Sheldon, 2020).

The increased beer production worldwide has led to environmental concerns regarding appropriately managing of the wastes generated. Therefore, strategies for management must be developed to valorize agro-industrial by-products (Lorente et al., 2019). The valorization of the by-products addresses opportunities for breweries to incorporate innovative processes within the biorefinery concept (Alonso-Riaño et al., 2021). Brewers' spent grain (BSG) is the main solid by-product obtained during beer production, accounting for approximately 20 kg per 100 L of beer produced (Mussatto et al., 2006). In Brazil, approximately $2.8\times10^6$ tons (wet weight) of BSG are generated annually (Sganzerla, Buller, et al., 2021). BSG contains (on a dry weight basis) ash (2–5%), crude proteins (19–30%), fibers (20–70%), lignin (10–28%), cellulose (12–25%), and hemicellulose (20–35%) (Amoriello & Ciccoritti, 2021; Moreira et al., 2013). Furthermore, BSG is a source of bioactive compounds (syringic acid, catechin, gallic acid, ferulic acid, kaempferol, p-coumaric, and ferulic acid) with high antioxidant and antimicrobial properties (Stefanello et al., 2018).

Considering the BSG generated by the beer industry, a variety of value-added products (i.e., sugars and amino acids) and bioenergy can be obtained in a biorefinery (Sganzerla, Buller, et al., 2021; Sganzerla, Zabot, et al., 2021). From the perspective of an integrated biorefinery, BSG could be exploited in an integrated close to zero waste system to recover bio-based products for the circular economy transition of the beer industry (Sganzerla, Ampese, et al., 2021). However, there are some challenges to more practical applications of BSG processing in integrated technological routes. For instance, there are few studies in the literature regarding the practical adoption of eco-friendly pretreatments of BSG to produce fermentable sugars and amino acids.

Nonetheless, the lignocellulosic composition of BSG demands a depolymerization process to release the sugars from the cell wall polysaccharides. The use of subcritical water hydrolysis (SWH) has been proposed as a promising hydrothermal pretreatment for depolymerizing the different components from the lignocellulosic biomass (Cocero et al., 2018a). Generally, subcritical water is pressurized in its liquid state, and temperature and residence time are critical parameters for biomass hydrolysis (Alonso-Riaño et al., 2021).

To reach the subcritical state, the temperature and pressure conditions of the water should be higher than its typical boiling point (100 °C, 1 MPa) and lower than its critical point (374 °C, 22 MPa) (Essien et al., 2020). Subcritical water behaves as a non-polar solvent that hydrolyzes complex matrices and releases organic molecules, including bio-based compounds (Gbashi et al., 2017). Simultaneously, the ionic product of subcritical water is much greater than water at room temperature and pressure, resulting in the promotion of both acid and base-catalyzed reactions (Motavaf & Savage, 2021). In subcritical conditions, the protons ($H^+$) content increases, thus increasing the acid content in water and converting hemicelluloses into monomeric xylose (Abaide, Ugalde, et al., 2019).

Several studies demonstrate the application of SWH as a faster process with low solvent consumption and high selectivity, resulting in hydrolysates with diverse compositions and high biological activity (Zhang et al., 2020). There are several applications of SWH in a single reactor to produce value-added chemicals from biomass, including bamboo (Mohan et al., 2015), coffee residues (Mayanga-Torres et al., 2017), brewer's spent grains (Steiner et al., 2018; Torres-Mayanga et al., 2019), rice husks (Draszewski et al., 2021), blue mussel (Jeong et al., 2021), pecan wastes ( dos Santos et al., 2020), soybean straw, and hull (Vedovatto et al., 2021). However, there is a lack in the literature regarding applying sequential subcritical reactors to produce value-added products. Despite the benefits of SWH, technological advance is still necessary to increase the yield of bio-products for an industrial application of this process. The adoption of two sequential reactors can be an alternative to increasing the yield of the products.

Therefore, this study aimed to verify the SWH of BSG for sugars and amino acid recovery. The novelty of this study lies in the technical point of view related to the use of a single and two sequential flow-through subcritical reactors to increase the productivity of value-added products.

## 2. Materials and methods

### *2.1. Brewery residues*

BSG was supplied by Ambev brewery (Jaguariúna, SP, Brazil). The raw material was dried in an air convection oven (45 °C, 24 h) until reaching moisture of 9% (w/w). The size reduction was carried out in a multiprocessor until it reached an average particle size of 0.41 mm, following the method reported by Levenspiel (Levenspiel, 1984). The BSG was physiochemically characterized regarding to the contents of moisture, ashes, protein, pH, lignin (soluble and insoluble), cellulose, and hemicellulose, using the methodologies recommended by the National Renewable Energy Laboratory (NREL) (Hames et al., 2008; Sluiter, Hames, Ruiz, Scarlata, et al., 2008; Sluiter, Hames, Ruiz, Sluiter, et al., 2008).

### *2.2. Subcritical water hydrolysis*

The process for SWH of BSG was conducted in a single (**Fig. 1a**) and two sequential reactors (**Fig. 1b**) (patent pending BR1020150314019). The system was composed of a high-pressure liquid pump (double piston pump, Model 36 preparation pump, Apple Valley, MN, USA) to pressurize and feed water to the subcritical reactor. The hydrolysis vessels consist of 316-stainless steel tubes with an internal volume of 110 mL. A thermal jacket rated to deliver 1500W was used to heat the reactor, insulated by a ceramic fiber jacket (RSA Equipment and Instrumentation, Campinas, SP, Brazil). The temperature was controlled using two thermocouples (type K) located in the entrance and outlet of the reactor. The product exiting the hydrolysis reactor was cooled in a serpentine coupled to a thermostatic bath (Marconi Equipment, model MA184, Piracicaba, SP, Brazil). The system's pressure is controlled by a micrometer valve (Parker Autoclave Engineers, model 10VRMM2812, Erie, PA, USA) located after the liquid exchanger. The pressure in the system was measured by pressure gauges (0 – 50 MPa), with an accuracy of up to 0.1% (WIKA company, Klingenberg am Main, Bavaria, Germany).

The initial and tested operational conditions used in the flow-through reactors were selected based on previous studies (Lachos-Perez et al., 2017; Torres-Mayanga et al., 2019). In each experiment, 10 g of BSG were loaded into each reactor, operated in semi-continuous mode. The reactor was filled with water from the pump to reach the pressure of 15 MPa, which was held constant for all experiments. The hydrolysis was performed with a water flow of 5 mL min$^{-1}$ for 120 min. The influence of the hydrolysis temperature in the subcritical process with a single and two sequential reactors were studied. The hypothesis tested was to evaluate the effect of different temperatures (80, 130, and 180 °C) in a process with a single reactor, and then, for the process with two sequential reactors, the temperature of the first reactor was kept stable at 80 °C and changed in the second reactor (80, 130, or 180 °C). The operational condition proposed was chosen to allow the extraction of bioproducts at low temperature in the first reactor, followed by an additional extraction/hydrolysis in the second reactor. For this, six experiments were randomly performed in duplicate to estimate the experimental uncertainty and establish reproducibility (**Table 1**).

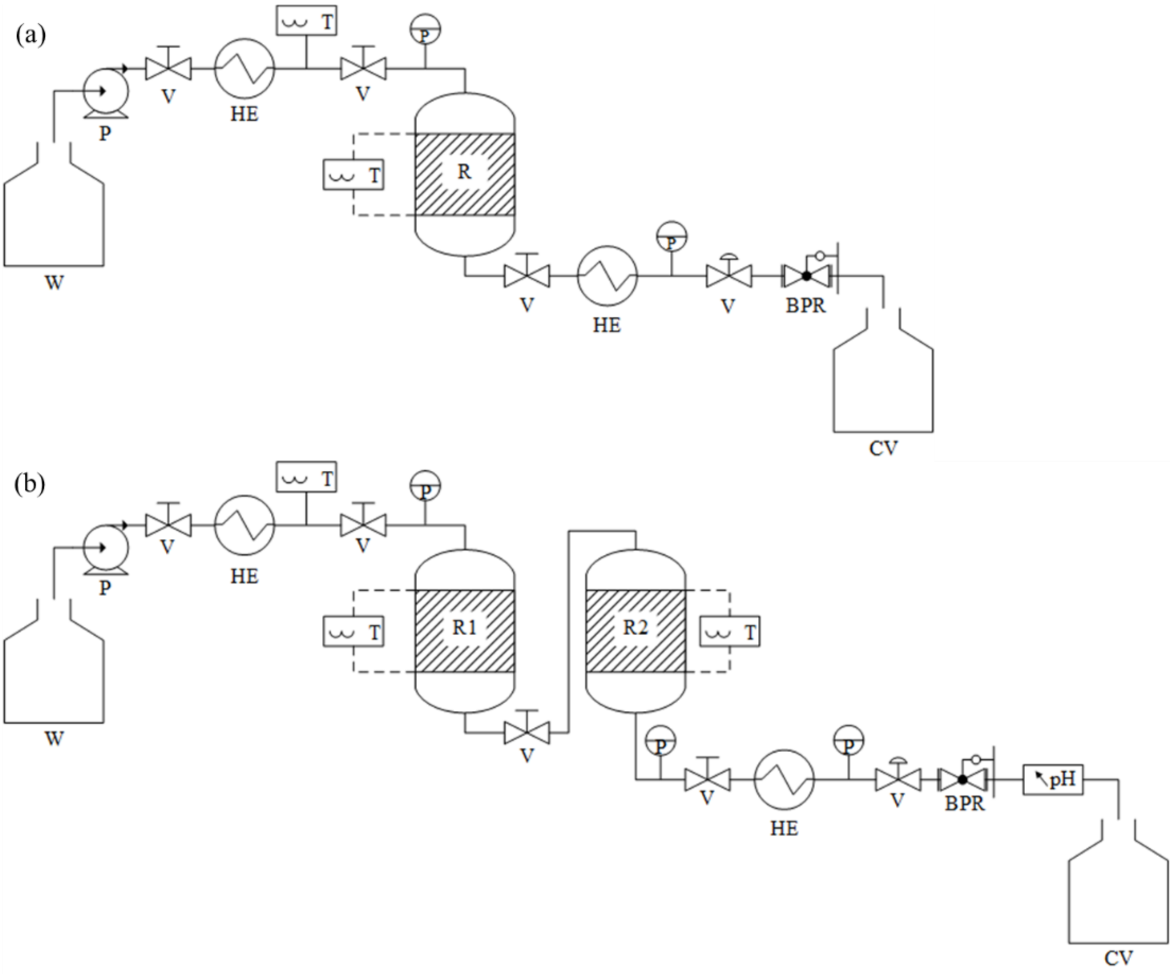

**Figure 1.** Laboratory-scale diagram of the subcritical water unit: (a) process with a single reactor, and (b) process with sequential reactors. Label: W, water tank; P, High-pressure pump; V, block valves; P, manometer; T, thermocouples; R, subcritical reactor; HE, heat exchanger; BPR, back pressure valve; and CV, collecting vessel.

**Table 1.** Temperature conditions for SWH of BSG in single and sequential reactors.

| Parameters | | Reactor 1 | Reactor 2 | Code |
|---|---|---|---|---|
| Single reactor | SWH 1 | 80 °C | - | R1 80°C |
| | SWH 2 | 130 °C | - | R1 130°C |
| | SWH 3 | 180 °C | - | R1 180°C |
| Sequential reactors | SWH 4 | 80 °C | 80 °C | R1 80°C \| R2 80 °C |
| | SWH 5 | 80 °C | 130 °C | R1 80°C \| R2 130 °C |
| | SWH 6 | 80 °C | 180 °C | R1 80°C \| R2 180 °C |

### *2.3. Characterization of hydrolysates*

#### *2.3.1. pH*

The pH was determined for each sample during the SWH using a digital pHmeter (IONLAB, model THS-3E). The measurements were done at 25 °C, and the pHmeter was previously calibrated with buffer solutions before the readings.

#### *2.3.2. Reducing sugars and total reducing sugars*

The content of reducing sugars and total sugars was determined using the colorimetric Somogyi-Nelson method (Nelson, 1944). To determine reducing sugars, aliquots of the hydrolysates were submitted to the reaction. When necessary, the samples were diluted for analysis. The content of sugars in the hydrolysates was analyzed at different intervals for 120 min. In the analysis of total sugars, the hydrolysates were subjected to acid hydrolysis conditions (HCl, 2 mol $L^{-1}$) for 1 h at 95 °C to decompose sugar oligomers into monomers. The absorbance of the reaction was measured at 540 nm in a spectrophotometer (Hach, model DR 4000U, SP, Brazil). The concentration of reducing sugars and total reducing sugars was calculated using an external calibration curve based on standard solutions of glucose ranging from 0.005 to 0.1 mg $mL^{-1}$. The analysis was conducted in triplicate, and the results were expressed as mg glucose $mL^{-1}$ hydrolysate.

#### *2.3.3. Monosaccharides and organic acids*

Sugar monomers were analyzed by high-performance liquid chromatography with a refractive index detector (HPLC-RID). Separation was performed using a Shodex$^{TM}$ column (model SH1011, 7 µm, 8 mm x 300 mm), adopting an isocratic flow of 1.0 mL min$^{-1}$ of $H_2SO_4$ 0.01 mol L$^{-1}$ at 30 °C. The RID was maintained at 30 °C. For the analysis, the hydrolysates were centrifuged (10,000 × *g*) and filtered (nylon 0.22 µm). Then, 10 µL of hydrolysates were injected, and the sample run was established as 20 min. The concentration of cellobiose, glucose, xylose, arabinose, formic acid, and acetic acid was calculated from each standard's calibration curves ranging from 0.01 to 1 mg mL$^{-1}$. When necessary, the samples were diluted for analysis. The analysis was conducted in triplicate, and the results were expressed as mg mL$^{-1}$ hydrolysate.

### *2.3.4. Soluble protein*

The soluble protein content in the hydrolysates was determined according to the method of Bradford (Bradford, 1976) with modifications. Aliquots of 100 µL of hydrolysate were reacted with 1000 µL of Bradford reagent (100 mg of Coomassie Brilliant Blue G-250; 50 mL of ethanol 95%; 100 mL of phosphoric acid 85%; and adjusted to a final volume of 1 L) and 900 µL of deionized water. After 5 min, the absorbance was measured at 595 nm in a spectrophotometer (Hach, model DR 4000U, SP, Brazil). The soluble protein concentration was calculated using an external calibration curve based on serum albumin bovine standard solutions ranging from 0.005 to 0.1 mg mL$^{-1}$. When necessary, the samples were diluted for analysis. The analysis was conducted in triplicate, and the results were expressed as mg albumin mL$^{-1}$ hydrolysate.

### *2.3.5. Free amino acids*

The free amino acid composition in the hydrolysates was determined using a Dionex UltiMate 3000 HPLC. The system was operated under the following chromatographic conditions: temperature of 37 °C; mobile phase of (A) 40 mM $Na_2HPO_4$ with 0.02 % $NaN_3$; and (B) 45% acetonitrile, 45% methanol, and 10% $H_2O$; flow rate of 1.0 mL min$^{-1}$; and injection volume of 56.5 µL. The free amino acids were separated with a Gemini C18 column (3 µm particle size, 4.6 × 150 mm) from Phenomenex (PN 00F-4439-E0, USA), Guard column (SecurityGuard Gemini C18, Phenomenex PN: AJO-7597), and UV+fluorescence detector (Thermo Scientific, USA).

### *2.3.6. Furfural and 5-hydroxymethylfurfural*

Furfural and 5-hydroxymethylfurfural (5-HMF) were quantified by Ultra-High-Performance Liquid Chromatography coupled with Photodiode Array Detector (UPLC-PAD) (Waters, Acquity H-Class, Milford, MA, USA). The chromatographic separation was performed using a C18 column (Kinetex, 100 × 4.6 mm i.d.; 2.6 µm; Phenomenex, Torrance, CA, USA) at 47 ºC. The mobile phase was ultra-pure water (A) and acetonitrile (B), both acidified with 0.1% acetic acid. The flow rate was 1.0 mL min$^{-1}$. The separation gradient was as follows: 0 min: 97% A; 3 min: 90% A; 4 min: 75% A; 7-7.5 min: 50% A; 8.5 min: 97% A. The column cleaning and equilibration times were 2 min. The PAD detector collected data between 210 and 500 nm, and the chromatograms were processed at 280 nm. Samples were properly diluted with ultra-pure water, and 5 µL were automatically injected. Quantitation using external calibration was performed by preparing a calibration curve ranging from 1 to 200 mg L$^{-1}$ of furfural and 5-HMF. Samples and standards were injected in duplicate, and the results were expressed in µg mL$^{-1}$ hydrolysate.

### *2.4. Calculations*

The yield of monosaccharides (Eq. 1) was calculated considering the amount of monosaccharides (sum of cellobiose, glucose, xylose, and arabinose) produced in the hydrolysate (mg mL$^{-1}$), the total carbohydrates content in BSG (71.74 g 100 g$^{-1}$, dry matter), and the solvent-to-feed ratio (S/F, g water g$^{-1}$ BSG) employed in the SWH, which is equal to 20 for the process with a single reactor and 10 for the process with two sequential reactors.

$$\text{Yield}\left(\frac{\text{mg}_{\text{monosaccharides}}}{\text{g}_{\text{carbohydrates}}}\right)=\frac{\text{Monosaccharides}_{\text{hydrolysate}}}{\text{Carbohydrates}_{\text{BSG}}}\times\frac{\text{S}}{\text{F}} \tag{1}$$

The yield of amino acids (Eq. 2) was calculated considering the amount of total amino acids produced in the hydrolysate (µg mL$^{-1}$), the amount of proteins presented in BSG (17.72%, dry matter), and the S/F employed in the SWH.

$$\text{Yield}\left(\frac{\text{mg}_{\text{amino acids}}}{\text{g}_{\text{proteins}}}\right)=\frac{\text{Amino acids}_{\text{hydrolysate}}}{\text{Proteins}_{\text{BSG}}}\times\frac{\text{S}}{\text{F}} \tag{2}$$

The yield of organic acids (Eq. 3) and furfural (Eq. 4) was calculated considering the amount of organic acids (mg mL$^{-1}$) and furfural (µg mL$^{-1}$) produced in the hydrolysate, the total carbohydrates content in BSG (71.74%, dry matter), and the S/F employed in the SWH.

$$\text{Yield}\left(\frac{\text{mg}_{\text{organic acids}}}{\text{g}_{\text{carbohydrates}}}\right)=\frac{\text{Organic acids}_{\text{hydrolysate}}}{\text{Carbohydrates}_{\text{BSG}}}\times\frac{\text{S}}{\text{F}} \tag{3}$$

$$\text{Yield}\left(\frac{\text{mg}_{\text{furfurlal}}}{\text{g}_{\text{carbohydrates}}}\right)=\frac{\text{Furfural}_{\text{hydrolysate}}}{\text{Carbohydrates}_{\text{BSG}}}\times\frac{\text{S}}{\text{F}} \tag{4}$$

### *2.5. Characterization of solid residues after SWH*

After the SWH, the solids contained in the hydrolysis vessel were dried in an air convection oven (45 °C, 24 h) until reaching moisture of 9% (w/w), which is equal to the initial moisture of BSG used in the experiment. The amount of solid was determined by the difference between the initial amount of dried biomass used in the SWH and the final dried mass remaining in the reactor at the end of the process. Then, thermogravimetric analysis (TGA) of the initial feedstock and final residues was performed in a thermogravimetric analyzer (PerkinElmer, model STA6000, Akron, Ohio, USA). The analysis consisted of heating the samples (10 mg) at 20 °C min$^{-1}$ from 40 to 700 °C, in a non-oxidizing atmosphere ($N_2$, 99.997% purity) at 20 mL min$^{-1}$. Thermogravimetric data were converted into derivative thermograms (DTG) to determine the lignocellulosic composition, considering the pyrolytic decomposition of semi-volatiles (40–175°C), hemicellulose (175–300 °C), cellulose (300–370 °C), lignin (370–550 °C), and char (550–700 °C) (Carrier et al., 2011).

### *2.6. Statistical analysis*

Analysis of variance (ANOVA) was employed to assess statistically significant factors and interactions between the variables. Significant differences between the samples were evaluated by Tukey's test ($p \leq 0.05$). The statistical analysis was conducted using the Statistica® software (version 10.0, StatSoft Inc., Tulsa, OK, USA).

## 3. Results and discussion

### *3.1. Raw material characterization*

BSG contained 9.03 ± 0.04 g moisture 100 g$^{-1}$, 3.65 ± 0.01 g ashes 100 g$^{-1}$, 19.72 ± 1.02 g crude protein 100 g$^{-1}$, and pH of 6.25 ± 0.03. The amount of total carbohydrates in BSG was 71.74 ± 0.85 g 100 g$^{-1}$, being the lignocellulosic composition composed of 6.12 ± 0.44 g soluble lignin 100 $g^{-1}$, 11.75 ± 0.18 g insoluble lignin 100 $g^{-1}$, 17.90 ± 0.02 g cellulose 100 $g^{-1}$, and 35.70 ± 0.02 g hemicellulose 100 $g^{-1}$. This lignocellulosic composition may be advantageous for SWH since hemicellulose is the macromolecule that can be converted into free sugars, such as xylose and arabinose (Torres-Mayanga et al., 2019). The high protein presented in BSG corroborates with the composition reported in the literature (Qin et al., 2018; Xiros et al., 2008) and can be considered an advantage in recovering amino acids and a protein-rich fraction (Alonso-Riaño et al., 2021). Otherwise, the ash content obtained in BSG may affect the SWH process, especially hindering water access to cellulose and hemicelluloses, undesirably acting as a barrier (Abaide, Mortari, et al., 2019; Lynch et al., 2016). When compared with the literature, differences in the composition of the BSG can be associated with several factors, such as barley species, harvesting time, malting process, and industrial conditions of beer production (Amoriello & Ciccoritti, 2021; Santos et al., 2003).

## *3.2. Hydrolysate characterization*

### *3.2.1. Reducing sugars and total reducing sugars*

**Fig. 2(a-d)** presents the kinetic curves of reducing sugars and total reducing sugars obtained during the hydrolysis of BSG in a single and two sequential flow-through subcritical reactors. The kinetics indicated an increase in sugar concentration in the first 10 min of the hydrolysis, followed by a decrease and stabilization at low sugar concentrations, which indicates a high hydrolytic rate at the beginning of the process that decreases with time. Extending the processing time makes it more expensive and increases the cost, and then the advantage of the SWH designed in this study is associated with the use of a short process time.

The single and two sequential reactors operated at different temperatures resulted in different kinetic behaviors of reducing sugars and total reducing sugars at 40 min (**Table 2**). The use of 180 °C resulted in the highest production of reducing sugar content, statistically equal for the process with single or sequential reactors. The explanation for this fact is associated with the fact that higher temperatures better hydrolyze the biomass. Then, a high amount of oligomers and monomers can be obtained in the hydrolysate. According to Torres-Mayanga et al. (2019), the thermal effects on SWH of BSG dominate at temperatures of 160 °C or greater, corroborating the finding obtained in the current study. This study showed that after 40 min of hydrolysis, the release of sugars in the hydrolysate was stabilized, indicating the optimum time to obtain the highest sugar content regarding the hydrolysate concentration. In this kinetic time, an S/F equal to 20 for the process with a single reactor and 10 for the process with sequential reactors was obtained. At this point, the hydrolysate was collected to measure the profile of monosaccharides, organic acids, and inhibitors.

Additionally, an interesting fact was observed using 130 °C in a process with sequential reactors (SWH 5), where the reducing sugar content was 2.5-fold higher compared to the process using a single reactor (SWH 2) at the same temperature (**Table 2**). The same fact was observed in the content of total reducing sugars, where the process with sequential reactors at 130 °C produced 2.17-fold higher sugar than the SWH 2 at the same temperature.

### *3.2.2. Monosaccharides*

The analysis of monosaccharides indicated that sequential reactors increased the content of cellobiose, glucose, xylose, and arabinose in the hydrolysate (**Table 3**). In total, the concentration of monosaccharides was proportional to the temperature. Using 180 °C in the single reactor produced 1.713 ± 0.127 mg $mL^{-1}$, while the same temperature in the two sequential produced 1.25-fold higher (2.136 ± 0.231 mg $mL^{-1}$). Arabinose was the main sugar released from BSG. Using a single reactor operated at 130 °C produced 0.518 ± 0.024 mg $mL^{-1}$, which increased to 0.914 ± 0.007 mg $mL^{-1}$ when adopting a process with sequential reactors, an increase estimated at 1.76-fold. However, the highest arabinose content (1.039 ± 0.049 mg $mL^{-1}$) was obtained for the process with a single reactor at 180 °C. In the case of xylose, an increase of 1.4-fold higher was obtained for the sequential process operated at 180 °C. The xylose content increased from 0.477 ± 0.004 mg $mL^{-1}$ (single reactor) to 0.647 ± 0.003 mg $mL^{-1}$ (two sequential reactors). These results corroborated with previous studies on SWH of BSG, in which arabinose and xylose represented 88% of the total quantified sugars (Torres-Mayanga et al., 2019).

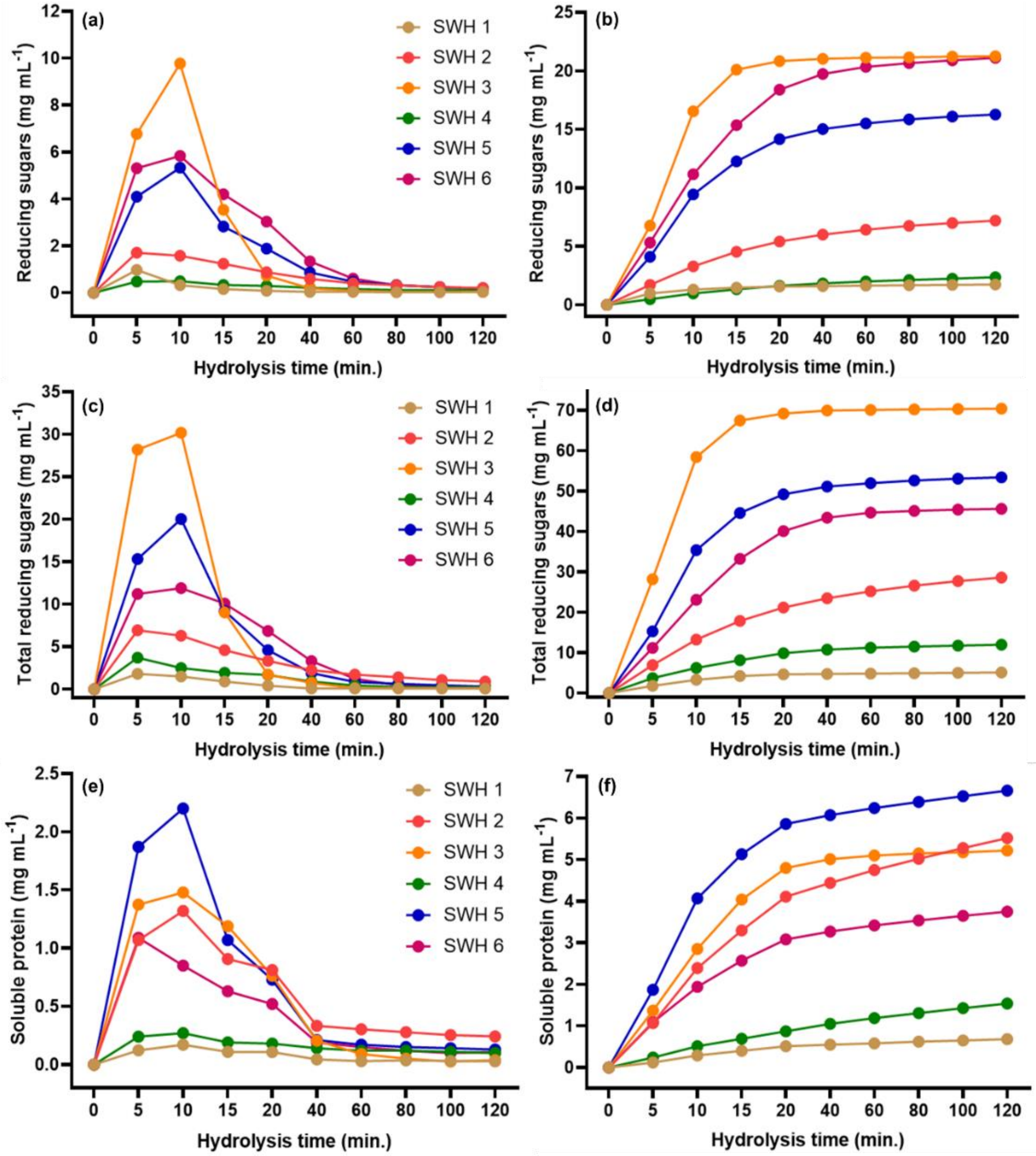

**Figure 2.** Kinetic profile of sugars and proteins during the SWH of BSG in single and sequential reactors. (a) non-accumulated reducing sugars content; (b) accumulated reducing sugars content; (c) non-accumulated total reducing sugars content; (d) accumulated total reducing sugars content; (e) non-accumulated soluble protein content; and (f) accumulated soluble protein content. Legend: SWH 1, 80 °C; SWH 2, 130 °C; SWH 3, 180 °C; SWH 4, 80 °C – 80 °C; SWH 5, 80 °C – 130 °C; SWH 6, 80 °C –180 °C.

**Table 2.** The concentration of reducing sugars (RS), total reducing sugars (TRS), soluble protein (SP), and total amino acids (TAA) for SWH of BSG in single and sequential reactors at 40 min.

| Parameters | | Temperature (°C) | S/F (g water $g^{-1}$ BSG) | RS (mg $mL^{-1}$) | TRS (mg $mL^{-1}$) | SP (mg $mL^{-1}$) | TAA (µg $mL^{-1}$) |
|---|---|---|---|---|---|---|---|
| Single reactor | SWH 1 | R1 80°C | 20 | 1.60 ± $0.02^{d}$ | 4.76 ± $0.45^{f}$ | 0.55 ± $0.09^{e}$ | 348.35 ± $31.05^{b}$ |
| | SWH 2 | R1 130°C | 20 | 6.01 ± $0.68^{c}$ | 23.47 ± $5.24^{d}$ | 4.44 ± $0.91^{c}$ | 318.65 ± $15.25^{c}$ |
| | SWH 3 | R1 180°C | 20 | 21.03 ± $1.92^{a}$ | 69.95 ± $8.24^{a}$ | 5.01 ± $0.30^{b}$ | 386.50 ± $9.50^{a}$ |
| Sequential reactors | SWH 4 | R1 80°C \| R2 80 °C | 10 | 1.84 ± $0.03^{d}$ | 10.80 ± $2.13^{e}$ | 1.05 ± $0.85^{d}$ | 357.12 ± $41.30^{b}$ |
| | SWH 5 | R1 80°C \| R2 130 °C | 10 | 15.03 ± $0.15^{b}$ | 51.10 ± $3.52^{b}$ | 6.07 ± $0.21^{a}$ | 390.72 ± $44.40^{a}$ |
| | SWH 6 | R1 80°C \| R2 180 °C | 10 | 19.74 ± $0.25^{a}$ | 43.42 ± $2.16^{c}$ | 3.27 ± $0.65^{c}$ | 338.60 ± $3.50^{b}$ |

*Results expressed as mean ± standard deviation. Analysis conducted at least in triplicate. Different letters in each column indicate significant differences by Tukey's test at $p \leq 0.05$.

**Table 3.** Sugars, organic acids, alcohol, and inhibitors composition of BSG hydrolysates obtained in single and sequential subcritical reactors.

| Parameters | Single reactor | | | Sequential reactors | | |
|---|---|---|---|---|---|---|
| | SWH 1 (R1 80°C) | SWH 2 (R1 130°C) | SWH 3 (R1 180°C) | SWH 4 (R1 80°C \| R2 80 °C) | SWH 5 (R1 80°C \| R2 130 °C) | SWH 6 (R1 80°C \| R2 180 °C) |
| pH | 5.07 ± 0.12$^{a}$ | 4.39 ± 0.04$^{b}$ | 4.22 ± 0.06$^{b}$ | 4.31 ± 0.07$^{b}$ | 4.19 ± 0.09$^{b}$ | 3.88 ± 0.31$^{c}$ |
| *Sugars (mg mL$^{-1}$)* | | | | | | |
| Cellobiose | 0.275 ± 0.114$^{a}$ | 0.014 ± 0.010$^{d}$ | 0.141 ± 0.013$^{c}$ | 0.182 ± 0.007$^{b}$ | 0.139 ± 0.071$^{c}$ | 0.260 ± 0.071$^{a}$ |
| Glucose | 0.323 ± 0.141$^{b}$ | 0.102 ± 0.002$^{c}$ | 0.056 ± 0.009$^{d}$ | 0.801 ± 0.012$^{a}$ | 0.100 ± 0.018$^{c}$ | 0.340 ± 0.010$^{b}$ |
| Xylose | 0.044 ± 0.013$^{d}$ | 0.037 ± 0.002$^{d}$ | 0.477 ± 0.045$^{b}$ | 0.177 ± 0.005$^{c}$ | 0.407 ± 0.002$^{b}$ | 0.674 ± 0.003$^{a}$ |
| Arabinose | 0.044 ± 0.014$^{f}$ | 0.518 ± 0.024$^{d}$ | 1.039 ± 0.049$^{a}$ | 0.134 ± 0.061$^{e}$ | 0.914 ± 0.007$^{b}$ | 0.862 ± 0.003$^{c}$ |
| Total | 0.686 ± 0.071$^{d}$ | 0.671 ± 0.054$^{d}$ | 1.713 ± 0.127$^{b}$ | 1.294 ± 0.132$^{c}$ | 1.560 ± 0.172$^{b}$ | 2.136 ± 0.231$^{a}$ |
| *Organic acids (mg mL$^{-1}$)* | | | | | | |
| Formic acid | 0.013 ± 0.001$^{d}$ | 0.018 ± 0.001$^{c}$ | 0.092 ± 0.005$^{b}$ | 0.024 ± 0.005$^{c}$ | 0.086 ± 0.036$^{b}$ | 0.146 ± 0.067$^{a}$ |
| Acetic acid | 0.034 ± 0.010$^{c}$ | 0.040 ± 0.001$^{c}$ | 0.251 ± 0.016$^{b}$ | 0.065 ± 0.005$^{c}$ | 0.230 ± 0.092$^{b}$ | 0.431 ± 0.193$^{a}$ |
| Total | 0.047 ± 0.051$^{e}$ | 0.058 ± 0.002$^{d}$ | 0.343 ± 0.019$^{b}$ | 0.089 ± 0.004$^{c}$ | 0.316 ± 0.016$^{b}$ | 0.577 ± 0.091$^{a}$ |
| *Inhibitors (µg mL$^{-1}$)* | | | | | | |
| Furfural | 4.75 ± 0.82$^{d}$ | 7.99 ± 0.33$^{d}$ | 310.71 ± 42.11$^{a}$ | 31.64 ± 3.22$^{c}$ | 162.8 ± 60.2$^{b}$ | 439.66 ± 125.29$^{a}$ |
| 5-HMF | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |

* Results expressed as mean ± standard deviation. Analysis conducted at least in triplicate. Different letters in each column indicate significant differences by Tukey's test at $p \leq 0.05$. Label: n.d., not detected.

The lower thermal stability of hemicellulose compared to cellulose supports the present study’s findings, where hemicellulose sugars (mainly xylose and arabinose) were the main monosaccharides obtained in the hydrolysate. BSG is a feedstock composed of 17.87% lignin, 17.90% of cellulose, and 35.70% of hemicellulose. For the cellulose hydrolysis, temperatures of at least 230 °C are required (Cocero et al., 2018b), which was not evaluated in this study. Process optimization with lower hydrolysis temperature is crucial for the scale-up and economic assessment since the cost of utilities (energy and water) should be reduced to improve the feasibility (Sganzerla, Zabot, et al., 2021).

Considering the composition of BSG used for SWH, the yield of monosaccharides obtained by HPLC per gram of carbohydrates was calculated (**Fig. 3a**). The highest yield was obtained for the process with a single reactor at 180 °C (47.76 mg $g^{-1}$), and this value was similar to the SWH of soybean straw (49.6 mg $g^{-1}$ carbohydrates) and higher than the SWH of soybean hull (15.7 mg $g^{-1}$carbohydrates) operated at 180 °C (Vedovatto et al., 2021). However, regarding using the hydrolysate for a different technological route, the monosaccharides concentration is too low for application as a fermentation medium, resulting also unfeasible from an economic perspective if the objective is to recover the sugars from the liquor. Hence, the concentration of sugars in the liquor is crucial step for application in biotechnological process. For instance, it is possible to apply evaporation, membrane-based and simulated moving bed processes to concentrate biomass-derived sugars (Sievers et al., 2017; Xie et al., 2005). The scalability of the process for sugars production using SWH was simulated in a flow-through subcritical water hydrolysis reactor with capacity of 500 L. The scale-up process from pilot to industrial scale reduced the cost of manufacturing by approximately 80%, and when the sugars were separated, the cost of manufacturing arabinose decreased from 64.10 USD $kg^{-1}$ (pilot-plant) to 7.22 USD $kg^{-1}$ (industrial-plant), which means that the process can be profitable (Sganzerla, Zabot, et al., 2021). Regarding the energetic requirements on the industrial scale, the heat required for the reactor was 610 kJ $kg^{-1}$. For the continuous operation of SWH, a high amount of energy is required, and future studies should be addressed to optimize the heat required for an industrial reactor (Sganzerla, Zabot, et al., 2021).

In a previous study, the arabinose yield (31.3 g $kg^{-1}$) was optimized at 160 °C and S/F of 112 (Torres-Mayanga et al., 2019). In the present study, S/F of 20 and 10 were adopted with a single and sequential reactor, respectively. This difference is associated with both reactors containing the same amount of feedstock for the hydrolysis, consequently decreasing the S/F for the sequential process. This optimization in S/F was conducted because, in industrial projects, it is more suitable to use low instead of high S/F, since this parameter impacts process instrument performance, such as pumping and heat exchanger efficiency (Hatami et al., 2020). For industrial applications, processes with low utility costs (i.e., feedstock, energy, and water) should be optimized to decrease the cost of manufacturing. Hence, the biomass fractionation into individual building blocks poses a significant challenge to a biorefinery. Using subcritical water technology in a single reactor can be an alternative to the hydrolysis of BSG and recovery of sugars, especially xylose and arabinose. Further optimization is necessary for the process with two sequential reactors to produce a hydrolysate with a high sugar yield.

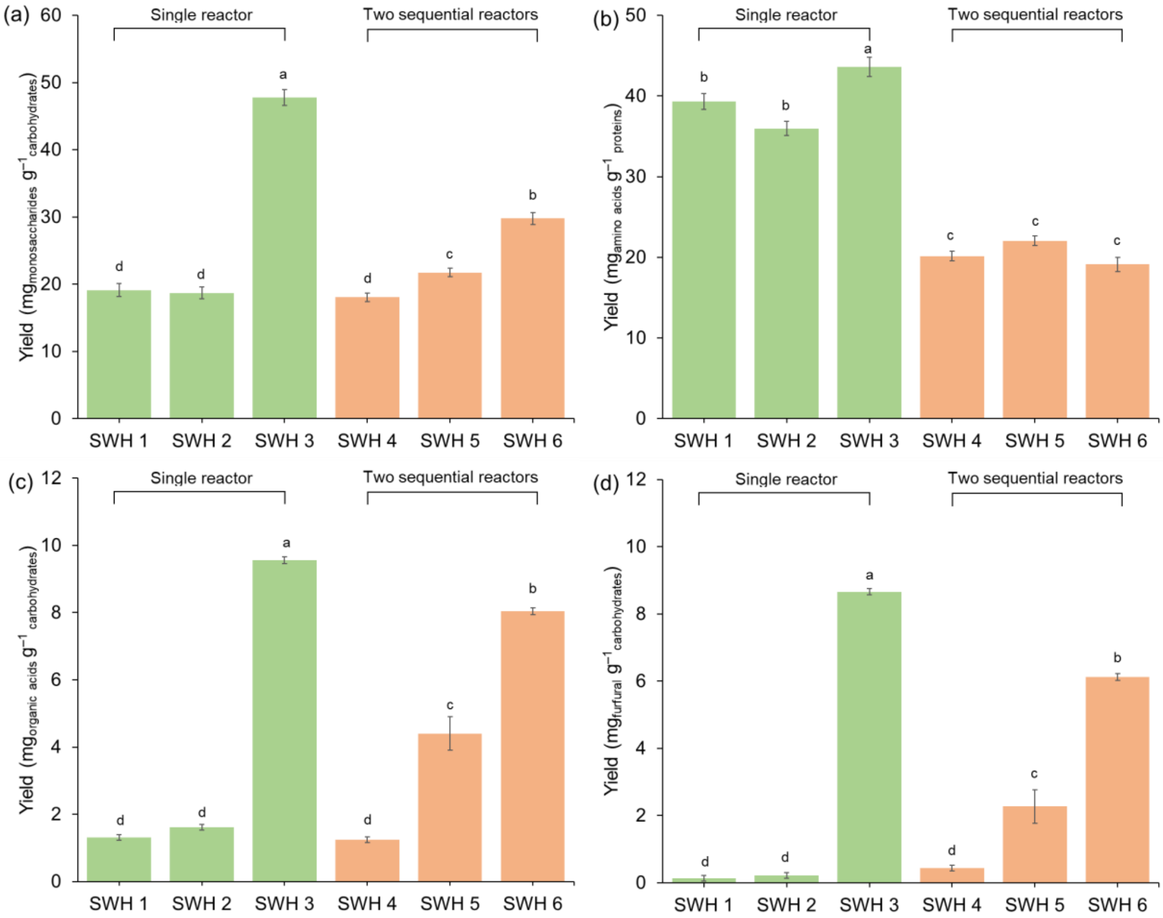

**Figure 3.** The yield of (a) monosaccharides, (b) amino acids, (c) organic acids, and (d) furfural after 40 min of SWH of BSG. Legend: SWH 1, 80 °C; SWH 2, 130 °C; SWH 3, 180 °C; SWH 4, 80 °C – 80 °C; SWH 5, 80 °C – 130 °C; SWH 6, 80 °C –180 °C. Different letters indicate significant differences by Tukey's test ($p \leq 0.05$).

### *3.2.3. Soluble protein and amino acids*

**Fig. 2(e-f)** shows the kinetic behavior of soluble proteins during the hydrolysis of BSG in a single and two sequential flow-through subcritical reactors. The highest soluble protein content was obtained in the SWH 5 (130 °C, two sequential reactors), followed by SWH 3 (180 °C, single reactor) and SWH 2 (130 °C, single reactor). Notably, 130 °C (intermediate temperature) was favorable for recovering proteins from BSG in two sequential reactors. After 40 min (S/F of 20 and 10, respectively, to the process with single and sequential reactors), a plateau of hydrolysis was observed. In this S/F, the hydrolysates were collected and quantified in terms of free amino acids (Table 4). In this study, valine (66.1 µg mL$^{-1}$), tryptophan (223.1 µg mL$^{-1}$), and lysine (26.7 µg mL$^{-1}$) were the majoritarian essential amino acids produced from BSG. The maximum values were found using sequential reactors under the condition of 80 ºC. According to Alonso-Riaño (2021), 53% of the total BSG amino acids were essential amino acids, valine, leucine, and lysine, being the three most abundant. Under subcritical conditions, the ionic product of water increases, favoring biomass hydrolysis (Cocero et al., 2018a). Moreover, the dielectric constant decreases allowing water to interact with non-polar substances, thus decreasing their binding force and dissolving (Cheng et al., 2021). Therefore, protein-rich feedstocks can be broken down into valuable peptides and free amino acids (Li et al., 2018).

The recovery of non-essential amino acids was dependent on the hydrolysis temperature. For instance, arginine, glutamic acid, glutamine, tyrosine, and proline were produced in a higher amount at 80 °C. On the other hand, aspartic acid, asparagine, and serine were produced in higher concentrations at 180 °C. Such behavior clearly shows that the reaction temperature can be tuned for recovery of different amino acids, with the differences attributable either to the thermal stability of the monomer, amino acid solubility, the accessibility of the monomer in the protein itself, or the thermal stability of the peptide bonds associated with a given amino acid (Weiss et al., 2018).

When comparing both processes with a single or two sequential reactors, in the case of valine, the process operated with a single reactor at 80 °C produced 49.45 µg mL$^{-1}$, and this yield increased to 66.1 µg mL$^{-1}$ for the process with sequential reactors at the same temperature, which means an increase in 1.33-fold higher. This behavior occurs during the extraction of phenylalanine and leucine at 80 °C. Moreover, according to the literature, the highest protein level (138 g kg$^{-1}$ BSG, 78% of the total protein in the BSG) was achieved at 185 °C. In contrast, the highest amino acid yield was obtained at 160 °C (Alonso-Riaño et al., 2021), indicating that intermediate temperature can be an advantage in recovering amino acids. According to Jeong et al. (Jeong et al., 2021), the highest isoleucine, tryptophan, proline, and tyrosine concentrations were recovered at 240 °C. At the same time, most of the other amino acids degraded significantly as the temperature was elevated in the two sequential reactors. This fact can be associated with the degradation of proteins into amino acids and carbonic acids, amine, and aldehydes, which is a common reaction under thermal treatment (Hellwig & Henle, 2020; Marcet et al., 2016). For instance, the most common degradation reactions occur with alanine, and the main reactions were decarboxylation to ethylamine and deamination to lactic acid. Glycine decomposes by decarboxylation to methylamine. A small degree of deamination of glycine to glycolic acid was reported in SWH (Klingler et al., 2007). Therefore, a fine selection of operational conditions is needed to maximize the extraction yield of proteins and avoid degradation into monomeric units and decomposition products (Álvarez-Viñas et al., 2021; Di Domenico Ziero et al., 2020).

Regarding the amino acid yield (**Fig. 3b**), the highest amount was recovered from the process with a single reactor. The reactor operated at 180 °C produced the highest yield (43.62 mg amino acids g$^{-1}$ proteins), and this value was similar to the literature (31.7 mg amino acids g$^{-1}$ proteins), which obtained amino acids from SHW of BSG at 185 °C (Alonso-Riaño et al., 2021). In the case of two sequential reactors, amino acids yield lower than 25 mg g$^{-1}$ protein was obtained, which is not a suitable yield for a further purification process. Hence, using subcritical water at 180 °C in a single reactor is still the better option to recover amino acids.

**Table 4.** Composition of essential and non-essential amino acids in BSG hydrolysates obtained in single and sequential subcritical reactors.

| Parameters | Single reactor | | | Sequential reactors | | |
|---|---|---|---|---|---|---|
| | SWH 1 (R1 80°C) | SWH 2 (R1 130°C) | SWH 3 (R1 180°C) | SWH 4 (R1 80°C \| R2 80 °C) | SWH 5 (R1 80°C \| R2 130 °C) | SWH 6 (R1 80°C \| R2 180 °C) |
| *Essential amino acids (µg mL$^{-1}$)* | | | | | | |
| Histidine | 2.50 ± 0.60$^{b}$ | 1.60 ± 0.01$^{c}$ | 2.91 ± 0.20$^{b}$ | 4.53 ± 1.61$^{a}$ | 3.01 ± 0.02$^{ab}$ | 1.22 ± 0.01$^{c}$ |
| Threonine | 2.65 ± 0.85$^{b}$ | 1.25 ± 0.05$^{c}$ | 3.45 ± 0.05$^{a}$ | 3.63 ± 1.62$^{a}$ | 3.25 ± 1.05$^{ab}$ | 1.95 ± 0.45$^{c}$ |
| Valine | 49.45 ± 7.85$^{c}$ | 39.95 ± 7.05$^{c}$ | 64.35 ± 1.65$^{a}$ | 66.11 ± 2.60$^{a}$ | 58.45 ± 8.55$^{b}$ | 51.75 ± 2.05$^{b}$ |
| Methionine | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Tryptophan | 206.90 ± 0.50$^{c}$ | 215.76 ± 6.12$^{b}$ | 215.55 ± 3.55$^{b}$ | 223.14 ± 0.01$^{a}$ | 219.80 ± 7.40$^{ab}$ | 220.85 ± 3.05$^{a}$ |
| Phenylalanine | 8.15 ± 2.25$^{b}$ | 6.85 ± 0.25$^{c}$ | 9.35 ± 0.15$^{b}$ | 14.51 ± 5.80$^{a}$ | 11.23 ± 0.45$^{a}$ | 5.02 ± 0.41$^{c}$ |
| Isoleucine | 1.95 ± 0.55$^{c}$ | 1.20 ± 0.01$^{c}$ | 4.21 ± 0.41$^{a}$ | 2.80 ± 0.11$^{b}$ | 3.62 ± 1.64$^{a}$ | 2.05 ± 0.45$^{b}$ |
| Leucine | 7.90 ± 2.20$^{b}$ | 5.40 ± 0.40$^{c}$ | 11.45 ± 0.45$^{a}$ | 11.70 ± 4.20$^{a}$ | 10.71 ± 3.42$^{a}$ | 5.70 ± 0.01$^{c}$ |
| Lysine | 21.95 ± 0.25$^{b}$ | 22.05 ± 0.15$^{b}$ | 16.55 ± 0.35$^{c}$ | 26.70 ± 3.80$^{a}$ | 21.55 ± 1.65$^{b}$ | 13.91 ± 1.50$^{d}$ |
| *Non-essential amino acids (µg mL$^{-1}$)* | | | | | | |
| Aspartic acid | 4.35 ± 1.55$^{e}$ | 10.55 ± 0.65$^{d}$ | 123.35 ± 7.55$^{a}$ | 8.65 ± 2.85$^{d}$ | 74.45 ± 8.22$^{b}$ | 44.10 ± 6.30$^{c}$ |
| Arginine | 9.35 ± 2.75 $^{b}$ | 5.30 ± 0.30$^{c}$ | 4.10 ± 0.60$^{c}$ | 14.15 ± 7.65$^{a}$ | 8.45 ± 3.35$^{b}$ | 3.90 ± 0.10$^{c}$ |
| Glutamic acid | 6.30 ± 2.50$^{b}$ | 2.65 ± 0.25$^{c}$ | 4.60 ± 0.70$^{c}$ | 10.40 ± 4.80$^{a}$ | 5.30 ± 0.50$^{b}$ | 3.55 ± 0.75$^{c}$ |
| Cysteine | n.d. | n.d. | n.d. | n.d. | n.d. | n.d. |
| Asparagine | 1.15 ± 0.25$^{c}$ | 0.85 ± 0.05$^{d}$ | 2.24 ± 0.21$^{a}$ | 1.55 ± 0.52$^{bc}$ | 1.80 ± 0.51$^{b}$ | 0.81 ± 0.03$^{d}$ |
| Serine | 4.20 ± 0.19$^{c}$ | 2.65 ± 0.05$^{d}$ | 7.63 ± 0.12$^{a}$ | 6.05 ± 1.85$^{b}$ | 7.45 ± 2.61$^{a}$ | 4.55 ± 0.85$^{c}$ |
| Glutamine | 6.05 ± 3.35$^{b}$ | 0.76 ± 0.21$^{d}$ | 0.75 ± 0.15$^{d}$ | 12.05 ± 4.74$^{a}$ | 1.28 ± 0.46$^{c}$ | 0.25 ± 0.05$^{e}$ |
| Glycine | 4.50 ± 0.81$^{d}$ | 4.01 ± 0.01$^{d}$ | 16.11 ± 0.40$^{a}$ | 6.54 ± 1.52$^{c}$ | 16.21 ± 4.38$^{a}$ | 10.21 ± 0.93$^{b}$ |
| Alanine | 6.18 ± 1.75$^{b}$ | 3.95 ± 0.15$^{c}$ | 13.21 ± 0.10$^{a}$ | 13.55 ± 5.75$^{a}$ | 13.55 ± 4.55$^{a}$ | 7.71 ± 0.53$^{b}$ |
| Tyrosine | 3.73 ± 1.22$^{b}$ | 2.54 ± 0.22$^{c}$ | 4.65 ± 0.35$^{b}$ | 6.15 ± 3.15$^{a}$ | 4.61 ± 1.42$^{b}$ | 2.23 ± 0.31$^{c}$ |
| Proline | 5.55 ± 2.45$^{b}$ | 2.55 ± 0.15$^{c}$ | 5.40 ± 0.10$^{b}$ | 12.22 ± 5.34$^{a}$ | 6.95 ± 0.65$^{b}$ | 3.25 ± 0.35$^{c}$ |

*Results expressed as mean ± standard deviation. Analysis conducted at least in triplicate. Different letters in each column indicate significant differences by Tukey's test at $p \leq 0.05$. Label: n.d., not detected.

### *3.2.4. pH, organic acids, and inhibitors*

The pH of the hydrolysate during the SWH of BSG was monitored throughout the kinetics (**Fig. 4**). At the beginning of the SWH, a lower pH was recorded. Afterward, the pH gradually increased due to the dilution of the hydrolysate. Additionally, the pH decreased by increasing the hydrolysis temperature, suggesting the presence of more organic acids and inhibitors in the hydrolysate under these conditions (Prado et al., 2016). Such behavior is expected because the operational conditions necessary to break down the lignocellulosic complex are severe enough to simultaneously promote degradation along the hydrolytic process (Prado et al., 2014). Thus, pH values can give us the magnitude of autocatalytic degradation of sugars into acids. For instance, in the case of SWH at 180 °C (**Fig. 3**), after 80 min of hydrolysis, the pH presented a slight reduction, which can be associated with the content of organic acids and inhibitors and with the degradation of amino acids into carbonic acids, amine, and aldehydes. A similar profile of pH during the SWH of lignocellulosic biomass was reported in the literature (Lachos-Perez et al., 2016; Mayanga-Torres et al., 2017; dos Santos et al., 2020).

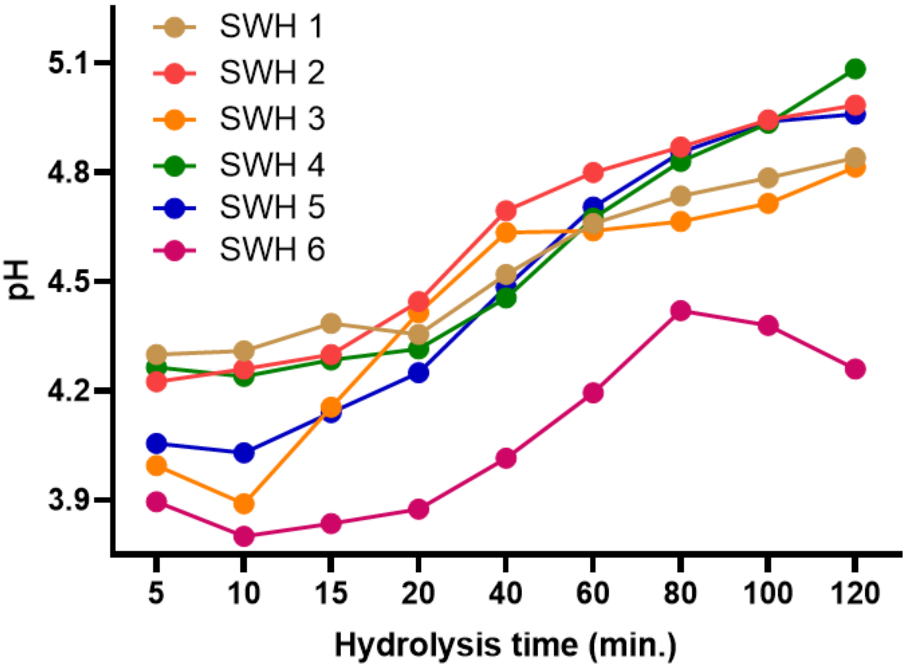

**Figure 4.** Kinetic pH profile during the SWH of BSG in single and sequential reactors. Legend: SWH 1, 80 °C; SWH 2, 130 °C; SWH 3, 180 °C; SWH 4, 80 °C – 80 °C; SWH 5, 80 °C – 130 °C; SWH 6, 80 °C –180 °C

The generation of organic acids and inhibitors has been reported during the SWH of lignocellulosic feedstock. Xylose and arabinose can be transformed into furfural through dehydration. Otherwise, the fructose and glucose can further be transformed into 5-HMF by dehydration reactions. Both furfural and 5-HMF can be degraded into formic acid. However, high temperatures are required for these reactions (Yedro et al., 2017). The formation of by-products such as organic acids and inhibitors could inhibit microbial fermentation and other biological processes (S. Mussatto, 2004; Sarker et al., 2021). In the SWH of BSG, 5-HMF was detected but in concentrations lower than the limit of quantification (<1 mg $L^{-1}$). The content of furfural increased when the temperature was increased. Sequential reactors at 180 °C generated 439.66 µg $mL^{-1}$, 1.41-fold more elevated than the process with a single reactor at the same temperature (310.7 µg $mL^{-1}$). The possible explanation for this increase in the furfural content can be associated with the flow of the hydrolysate obtained from the first reactor to the second one, which concentrates the inhibitor. To further apply hydrolysates obtained by SWH in fermentation, a purification step may be necessary to implement lignocellulosic biorefineries that could transform the hydrolysates into fuels and valuable chemicals (Abaide, Ugalde, et al., 2019). Finally, the inhibitions obtained in this study were lower than reported in the literature for the SWH of sorghum bagasse (Lyu et al., 2019) and rice husks (Abaide, Ugalde, et al., 2019).

Regarding the organic acids, formic and acetic acid were quantified in the hydrolysates. Formic acid was obtained in the lowest concentration, while a significant concentration of acetic acid was obtained in the hydrolysate, proportional to the use of higher temperatures. This fact is in accordance with the literature, which demonstrated that the yield of sugar decreased due to the rapid transformation of monosaccharides into acetic acid in high temperatures (Ishak et al., 2019; Möller et al., 2011). The formation of acetic acid increases the acid strength and facilitates the hydrolytic reactions carried out with subcritical water, benefiting the hydrolysis of lignocellulosic biomass (Sarker et al., 2021). This fact can be explained because the organic acids provide acidic protons to catalyze the subsequent hydrolysis of monomers and oligomers as an autocatalytic process (Abaide, Ugalde, et al., 2019).

The yield of organic acids (**Fig. 3c**) and furfural (**Fig. 3d**) was determined considering the amount of carbohydrates used in the SWH. The use of 180 °C was responsible to the highest yield of organic acids (9.56 mg $g^{-1}$ carbohydrate) and furfural (8.66 mg $g^{-1}$ carbohydrate), which occurred in the process with a single reactor. Using low temperature (80 °C), the yield of these compounds decreased to 1.31 and 0.13 mg $g^{-1}$ carbohydrate, respectively, for organic acids and furfural. Moreover, compared with the better operational condition (SWH 3, 180 °C) for the recovery of monosaccharides (47.76 mg $g^{-1}$), around 30% of the hydrolysate are inhibitors and organic acids. In contrast, 70% are sugars (considering that the sum of monosaccharides, inhibitors, and organic acids are responsible for 100% of the products recovered from the carbohydrates fraction of BSG). This fact indicates that SWH presents a high composition of sugars and a low content of inhibitors, corroborating with the literature (Abaide, Mortari, et al., 2019; Draszewski et al., 2021).

### *3.3. Characterization of the solid residues*

The residual solid obtained after SWH was also determined. **Fig. 5** shows the mass reduction of BSG after SWH. The yield of solid residue decreased with increasing process temperature, as expected for a process involving hydrolysis and solubilization of hemicellulose during the pretreatment. In the case of the process operated at 80 °C, a mass reduction of around 20% was obtained, which increased for 75% of mass reduction in the reactors operated at 180 °C. In this study, a similar biomass removal was observed compared with other feedstocks (Watchararuji et al., 2008).

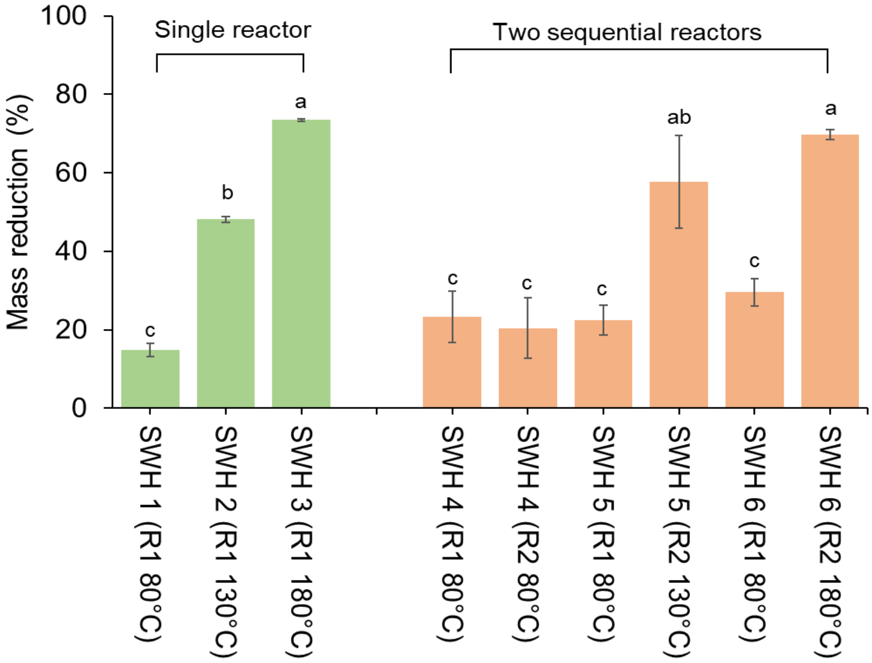

**Figure 5.** Mass reduction (%) of BSG after SWH operated with a single and two sequential reactors. Different letters indicate significant differences by Tukey's test ($p \leq 0.05$).

The initial BSG used for SWH, and the solid residues were analyzed by TGA to determine the lignocellulosic composition. The degradation profile (**Fig. 6a**) and the derivative thermogravimetric analysis (DTG) (**Fig. 6b**) were conducted to determine the contributions of individual components by integration and normalized area (**Fig. 6c**). The semi-volatile components are the remaining water, extractable components, small hydrophilic molecules, and unstable proteins available in the samples. The initial BSG presented by TGA 6.95% semi-volatiles, 27.35% hemicellulose, 40.62% cellulose, and 25.08% lignin. The results obtained by TGA are different from the NREL analysis, which is expected (Gerassimidou et al., 2020). After the SWH, the semi-volatile compounds decreased due to the possible migration from BSG to the hydrolysate, explaining amino acids' presence (Mosteiro-Romero et al., 2014). The lignin content increased as the temperature increased in the SWH process, corroborating with the literature (Abaide, Ugalde, et al., 2019). Moreover, hemicellulose dissociation occurred during the SWH, decreased from 27% (BSG) to 14% (reactor operated at 180 °C), which is in accordance with the results of sugars quantified in the hydrolysate. The remaining solid is richer in lignin and char, which increase according to higher temperatures (Abaide, Ugalde, et al., 2019).

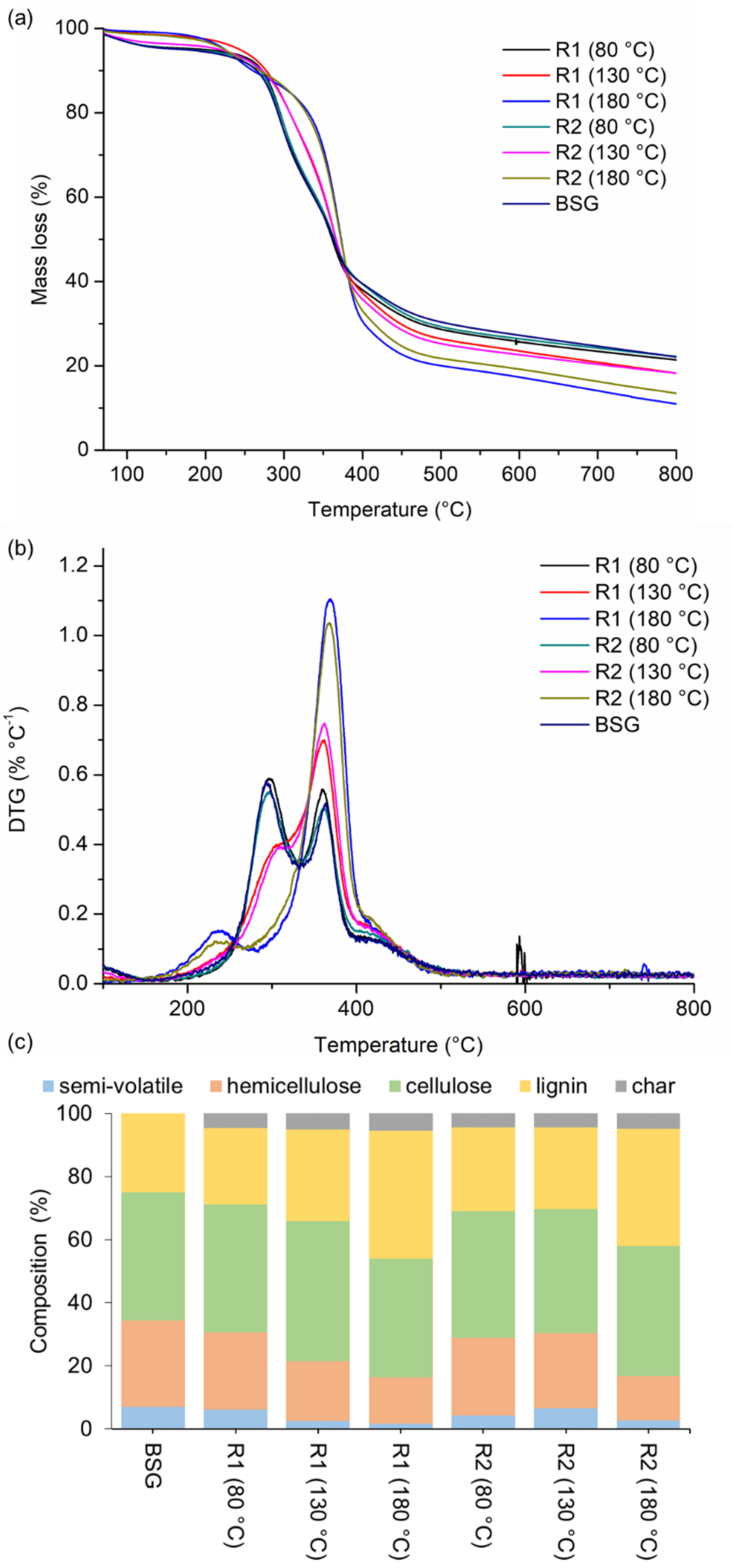

**Figure 6.** TGA of BSG and solid residues after SWH process: (a) degradation profile; (b) derivative thermogravimetric analysis (DTG); and (c) BSG and solid residue composition by TGA.

## 4. Conclusion

This study investigated the subcritical water hydrolysis (SWH) of brewer's spent grain (BSG) in a single and two flow-through sequential reactors to recover sugars and amino acids. The highest release of monosaccharides was obtained at 180 °C using a single reactor (47.76 mg $g^{-1}$ carbohydrates). Xylose (0.477 mg $mL^{-1}$) and arabinose (1.039 mg $mL^{-1}$) were the major sugars in the hydrolysate obtained at 180 °C for 40 min of hydrolysis. The SWH of BSG at the best operational condition (180 °C, single reactor) resulted in low contents of furfural (310.7 µg $mL^{-1}$) and 5-HMF (lower than 1 mg $L^{-1}$). The use of 180 °C in a process with a single reactor was favorable to recover proteins from BSG. The yield under this condition was 43.62 mg amino acids $g^{-1}$ proteins, where tryptophan (215.55 µg $mL^{-1}$), aspartic acid (123.35 µg $mL^{-1}$), valine (64.35 µg $mL^{-1}$), lysine (16.55 µg $mL^{-1}$), and glycine (16.1 µg $mL^{-1}$) were the majoritarian essential amino acids recovered in the hydrolysate. The subcritical process with two sequential reactors is a novel technique for biomass hydrolysis. However, further studies should be conducted to increase the yield of bioproducts. In conclusion, SWH in a single reactor can be adopted as an eco-friendly technology to recover sugars and amino acids from BSG.

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# CHAPTER IV

## Techno-economic assessment of subcritical water hydrolysis process for sugars production from brewer’s spent grains

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# CHAPTER IV: Techno-economic assessment of subcritical water hydrolysis process for sugars production from brewer’s spent grains

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## Abstract

Breweries generate a high amount of brewer’s spent grains (BSG), which is a valuable feedstock for industrial applications. In the current study, a techno-economic assessment of the flow-through subcritical water hydrolysis reactor to produce sugars from BSG was performed. Simulations were done for a sequential hydrolysis process in three-extractor vessels of 10 L (pilot-plant) and 500 L (industrial-plant), coupled or not to a separation system. The sugar separation system was composed of a five-zone simulated moving bed (SMB) process. A study on the cost of manufacturing (COM), profitability indicators, and sensitivity analysis was conducted to verify the project feasibility. Moreover, the mass and energy balance of the industrial process was accomplished to evidence the main operational parameters. Simulation results indicated that the scale-up process from pilot to industrial scale reduced the COM by approximately 80%. In the process coupled with the SMB separation system, arabinose and galactose represented 83.68% of the costs for sugars separation. Arabinose COM decreased from 64.10 USD kg$^{-1}$ in pilot-plant to 7.22 USD kg$^{-1}$ in industrial-plant. The implementation of a separation system recovering six sugars with high added-value can be an advantage in the industrial-plant process when compared to the process without SMB process, which produces a single hydrolysate fraction with low commercial value. Finally, the integrated subcritical water hydrolysis of BSG coupled with a separation system can be a promising alternative to produce different concentrated sugars in a biorefinery concept.

***Keywords:*** *Flow-through hydrolysis reactor; Brewer’s spent grains; Biorefinery; Sugar separation; Subcritical water technology; Scale-up; Techno-economic analysis*

## 1. Introduction

The biorefinery concept is based on the integral conversion of agro-industrial residual biomass into innovative value-added products, minimizing environmental impacts, and maximizing renewable resources (Dragone et al., 2020). Therefore, it is possible to design industrial arrangements for waste valorization integrating bioenergy, biomaterials, and active compounds production towards sustainable growth of the bioeconomy (Ioannidou et al., 2020; Ubando, Felix, & Chen, 2020). From the biorefinery concept, waste generated during the processing should be used as raw material to produce innovative products. Notwithstanding, there are environmental, economic, and commercial interests related to the use of biomass from agro-industrial processes since lignocellulosic residues can be converted into new products, which decrease the volume of residues improperly disposed (Campos et al., 2020; Ubando, Felix, & Chen, 2020).

Nowadays, conventional sugar processing involves complex and distinct unit operations with high energy and chemicals demand. The process can be summarized in raw material processing into raw sugar and refined sugar. In the example of sugar cane, a thousand (1000) tons of feedstock produces a hundred (100) tons of refined sugar with an efficiency of only 10% (El-Haggar, 2007). The electric energy demand is around 440,000 kWh per ton of raw material, which affects the final price of sugar and the greenhouse gas (GHG) emissions (El-Haggar, 2007). In such cases, innovative processes may represent a suitable opportunity to strengthen the economic position of this bioeconomy sector in a biorefinery concept.

Notwithstanding, the use of lignocellulosic materials from agro-industrial waste can be a promising alternative to sugar production. In the case of the breweries, brewer’s spent grains (BSG) are the main lignocellulosic waste generated (Mussatto et al., 2006). The amount of BSG produced by brewing in Brazil is estimated at $2.8\times10^6$ ton $y^{-1}$ (in wet weight) (Sganzerla et al., 2021), with an average production of 20 kg per 100 L of beer (Mussatto et al., 2013). The lignocellulosic material generated by the brewery is destined to landfills, animal feed, or incinerated to produce heat, a fact that affects GHG emissions (Lynch, Steffen, & Arendt, 2016). From an environmental perspective, the reduction of GHG emissions is an advantage when conducting a better destination of solid residues, driving a sustainable production that addresses affordability, accessibility, sustainability, and equity (Dragone et al., 2020; Campos et al., 2020; Ioannidou et al., 2020).

Nonetheless, one of the limiting steps in the depolymerization of lignocellulosic materials is the hydrolysis of cellulose, hemicellulose, and lignin into monosaccharides and disaccharides (Zhang et al., 2020). Recently, several pretreatments (i.e., acid, alkaline, enzymatic, mechanical, and chemical hydrolysis) have been used to promote the rupture of the lignocellulosic material structures (Kamusoko et al., 2019). However, conventional pretreatments have high costs and generate contaminants after the hydrolysis process, requiring additional steps for the post-treatment of the residue generated, resulting in a non-profitable process for industrial implementation (Cheah et al., 2020). Thereby, emerging eco-friendly technologies with low GHG emissions for the recovery of sugars from agro-industrial waste are arising (Freitas et al., 2021), such as subcritical water hydrolysis (SWH) in flow-through reactor (Lachos-Perez et al., 2020a; Oliveira et al., 2020; Abaide et al., 2019). Through the subcritical system, various chemicals and bioproducts can be produced using water as a solvent. Subcritical conditions can be reached when the water is submitted to temperatures between 100 and 374 °C and pressures up to 20 MPa (Knez et al., 2015). Under the conditions mentioned above, the solubility of hydrophobic organic species is significantly increased, which contributes to biomass fractionation into fermentable sugars (Torres-Mayanga et al., 2019).

Previous studies demonstrated the laboratory production of sugars from lignocellulose biomass through SWH in flow-through reactor (Lachos-Perez et al., 2020a; Torres-Mayanga et al., 2019; Abaide et al., 2019; Lachos-Perez et al., 2018; Mayanga-Torres et al., 2018). For instance, orange (*Citrus sinensis*) peel (Lachos-Perez et al., 2020a), sugarcane (*Saccharum officinarum*), bagasse (Zhang et al., 2020), olive (*Olea europaea*), oil pomace (Manzanares et al., 2020), and rice (*Oryza sativa*) husk (Abaide et al., 2019) were used to produce hydrolysates with high sugar content. Sugars produced from SWH of biomass can be used as a substrate for fermentation processes to produce second-generation ethanol (Oliveira et al., 2020) or other platform chemicals (Abaide et al., 2019; Zabot et al., 2019), making SWH a promising technology to industrial implementation in a biorefinery concept. From an industrial perspective, Lachos-Perez et al. (2020b) provided a novel economic evaluation related to using SWH technology to produce flavanones and sugars from orange peel, demonstrating that the implementation of industrial-scale reactors decreases the manufacturing costs and can be feasible for future implementation.

Nonetheless, there are significant technical and economic challenges before the full implementation of SWH process in biorefineries at industrial scale.

A previous study optimized sugar production from BSG under subcritical water conditions (Torres-Mayanga et al., 2019). The findings obtained in the laboratory-scale flow-through reactor provided new insight into the hydrolysis of BSG and recovery of sugars. However, the scale-up project based on economic evaluation should be the next step before the industrial implementation. Based on simulation studies, the industry can observe the main challenges of industrial plants for a real application, especially regarding the cost of manufacturing (COM). Also, COM values should be examined to understand the cost behavior on a laboratory scale considering the current market conditions. The state of the art of subcritical technology is not entirely elucidated for industrial scale. Consequently, simulation studies may be an alternative to demonstrate the most profitable theoretical routes.

Accordingly, to increase the possibility of finding new technological routes for breweries, SWH can be a promising technology to support an integrated biorefinery, especially to convert BSG lignocellulosic biomass into sugars. In an industrial perspective, a technical point of view should be accompanied by the economic study, which contributes to elucidate some bottlenecks to industrial implementation. In this study, a process based on SWH in flow-through reactor was designed and scaled-up for sugar production from BSG. Additionally, techno-economic assessment, mass, and energy balances were performed to elucidate the novelty presented herein. Finally, considering that external conditions can cause moderate changes in the absolute values, a sensitivity analysis was conducted to exam the uncertainty in simulated data.

## 2. Materials and methods

### *2.1 Process description and operational parameters*

Torres-Mayanga et al. (2019) designed a flow-through hydrolysis reactor to produce hemicellulose sugars from BSG. A reactor with tubular geometry with an internal volume of 110 mL was constructed. The reactor was heated by a heating jacket to operate at a maximum temperature of 400 °C. A high-pressure liquid pump was used for pressurization and liquid pumping, which allows us to operate at a maximum pressure of 40 MPa. A filter with 2 μm pores was positioned at the outlet of the reactor to minimize particle spillover. The hot effluent exiting the hydrolysis reactor was cooled in a tube heat exchanger coupled to a recirculating bath to obtain a hydrolysate temperature lower than 27 °C. The laboratory process developed was not coupled with a separation system, and the sugars were obtained in the same fraction (called as hydrolysate). Aiming to simulate an industrial sugar-production process, a five-zone simulated moving bed (SMB) process was coupled in the process to obtain liquid isolated sugars. Xie et al. (2005) developed an SMB process based on poly-4-vinyl pyridine (PVP) to recover sugars from biomass hydrolysates, which allows isolating in a liquid fraction six sugars and four impurities (Xie et al., 2005). In better words, the five-zone non-isocratic SMB process may achieve high multicomponent separation of sugars, reaching a processes' yield of 94% (Xie et al., 2005). The sugars purities can range from 93 to 95% (Xie et al., 2005). Based on the aforementioned processes, **Fig. 1(a)** demonstrates the laboratory SWH process designed without the SMB separation system, and **Fig. 1(b)** shows the SWH process coupled with a SMB separation system.

The laboratory-scale hydrolysis of BSG was performed under the following conditions: temperature of 140–210 °C; water flow rates of 10 and 20 mL $min^{-1}$; solvent/feed (S/F) ratio of 64, 80, and 112 (w/w); constant pressure of 15 MPa; and 5 g of raw material (Torres-Mayanga et al., 2019). Based on the most suitable operational condition to obtain the highest sugar yield, a hydrolysis temperature of 160 °C, the water flow of 10 mL $min^{-1}$, and S/F of 112 g solvent $g^{-1}$ feed was used in the SWH process. These data were used to simulate the scale-up process to sugars production in a pilot-plant and industrial-plant.

For the scale-up process, pilot (3×10 L) and industrial-scale (3×500 L) hydrolysis vessels were considered. **Table 1** demonstrates the base costs for each part of the subcritical water process. The adoption of three hydrolysis vessels was based on the fast feedstock residence time inside the reactor. The simulation was based on a process without the SMB separation system (coded as SWH), and a process coupled with the SMB separation system (coded as SWH-P), in accordance with Torres-Mayanga et al. (2019) and Xie et al. (2005). In the SWH process, the final product is a single hydrolysate composed of five sugars. Otherwise, in the SWH-P process, the final products are six different hydrolysates, composed of one isolated sugar per hydrolysate.

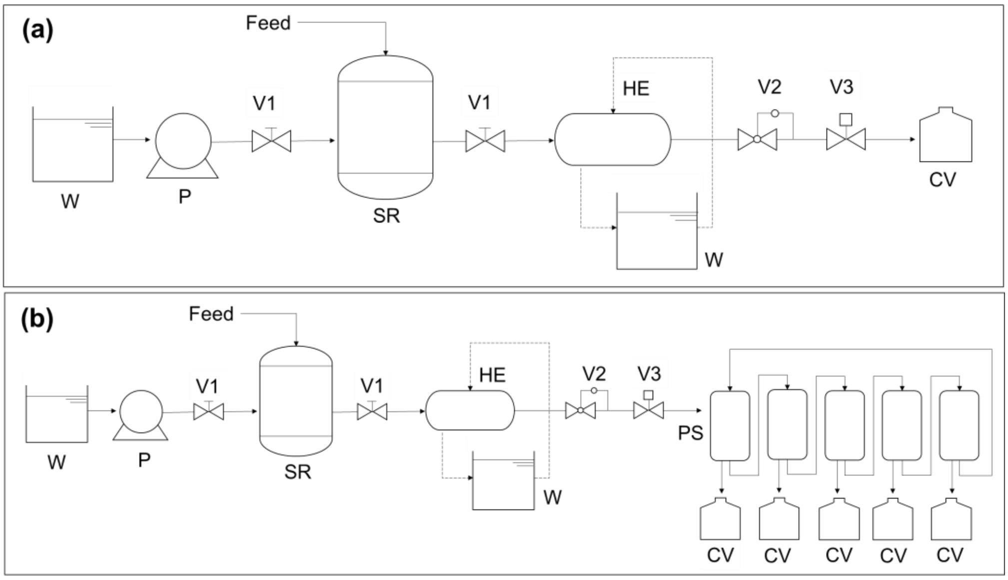

**Figure 1.** Laboratory-scale diagram of the subcritical water unit: (a) process without sugar separation (SWH); and (b) process coupled with a SMB system for sugar separation (SWH-P).

Beyond, an additional assumption in the present scale-up analysis is based on the process simulation with low S/F. In industrial projects, it is most suitable to use low S/F instead of high S/F, since high S/F impacts process instrument performance, such as pumping and heat exchanger efficiency (Hatami et al., 2020). When using S/F of 112 g solvent $g^{-1}$ feed (used in the laboratory-scale experiments), the water demand on an industrial scale is not suitable for real implementation. Based on the concern that in an industrial plant, low S/F is required, the scale-up process was accomplished with an S/F of 4 g solvent $g^{-1}$ feed, and the flow conditions were adjusted to maintain a properly subcritical reactor configuration. The assumption to decrease the S/F from 112 (laboratory process) to 4 g solvent $g^{-1}$ feed (industrial process) is based on a mandatory parameter's optimization from the technical point of view for an industrial process. Additionally, the selection of S/F around 4 g solvent $g^{-1}$ feed is supported by other studies (Abaide et al., 2019; Abaide et al., 2019a; Santos et al., 2020; Vedovatto et al., 2021a; Vedovatto et al., 2021b). For instance, Abaide et al. (2019) tested values of S/F of 15 and 7.5 g solvent $g^{-1}$ feed and concluded that higher yields were obtained when using the lowest S/F. Vedovatto et al (2021a) concluded that lower water flow rates returned higher yields of sugars because the solvent could reach the desired temperature more rapidly in the flow-through reactor, and the residence time was sufficient for having a substantial dissociation of hemicellulose and cellulose.

**Table 1.** Base costs to each part of the subcritical water process for sugar production from brewer's spent grains.

| Component | M [a] | Unit base cost (USD) | Num. | Total base cost (USD) | | |
|---|---|---|---|---|---|---|
| | | | | Laboratory plant | Scale-up (3×10L) | Scale-up (3×500L) |
| HPLC pump | 0.55 | 3,846.00 | 1 | 3,846.00 | 45,945.53 | 395,072.50 |
| Manometer | 0.00 | 93.33 | 6 | 560.00 | 560.00 | 560.00 |
| Structural material | 0.40 | 836.67 | 1 | 836.67 | 5,081.54 | 24,298.72 |
| Exchange heat | 0.59 | 1,116.67 | 1 | 1,116.67 | 15,977.24 | 160,654.97 |
| Blocking valve | 0.40 | 91.67 | 9 | 825.00 | 5,010.68 | 23,959.89 |
| Micrometric valve | 0.40 | 260.25 | 1 | 260.25 | 1,580.63 | 7,558.21 |
| Backpressure valve | 0.40 | 656.24 | 1 | 656.24 | 3,985.71 | 19,058.72 |
| Temperature controller | 0.60 | 33.33 | 3 | 100.00 | 1,496.80 | 15,651.15 |
| Control panel | 0.60 | 533.33 | 1 | 533.33 | 7,982.94 | 83,472.83 |
| Jacketed extraction-hydrolysis vessel | 0.82 | 500.00 | 3 | 1,500.00 | 60,554.90 | 1,497,285.64 |
| Piping, connectors, crossheads, and splitters | 0.60 | 314.43 | 1 | 314.43 | 4,706.44 | 49,212.45 |
| Sugar separation system (Five-Zone PVP SMB) | 0.60 | 38,335.83 | 1 | 38,335.83 | 573,811.50 | 6,000,000.00 |
| Total SWH plant cost (USD) | | | | 10,548.59 | 152,882.43 | 2,276,785.09 |
| Total SWH-P plant cost (USD) | | | | 48,884.42 | 726,693.93 | 8,276,785.09 |

[a] M constant depending on equipment type; SWH, Subcritical water hydrolysis plant without sugar separation; SWH-P, Subcritical water hydrolysis plant coupled with sugar separation in a SMB system.

### *2.2 Simulation flowsheet and Gantt chart*

The simulation flowsheet and Gantt chart were performed using SuperPro Designer 9.0® software (Intelligen Inc., Scotch Plains, NJ, USA). The flowsheet was designed to represent the industrial process without the SMB separation system (SWH) (**Fig. 2(a)**), and the same process coupled with the SMB separation system (SWH-P) (**Fig. 2(b)**). For representation purposes, the Gantt charts for SWH and SWH-P were designed (**Fig. 3).** In this study, the scale-up project was simulated with three hydrolysis vessels and one separation vessel. The flowsheet also contains cooler, mixers, splitter pumps, and piping. For the SWH process, dried and ground BSG was loaded in the SWH vessel (P−4/H−101, P−5/H−102, or P−6/H−103). Water was pumped (P−1/PP−101), heated (P−2/HE−101), and split to the subcritical reactors. After the hydrolysis condition is reached in the extraction vessel, the liquid was cooled (P−8/HX−102) to a temperature lower than 27 °C. A pressure valve was used in the process (P−9/GTV−101), and then, the liquid phase containing the hydrolysate was collected (P−10/V−102). For the SWH-P process (**Fig. 2(b)**), the same method previously mentioned was adopted. After the pressure stabilization (P−9/GTV−101), the hydrolysate was submitted to a five-zone non-isocratic SMB process (separation system) to produce different fractions of sugars. Five vessels were selected to promote the hydrolyzed sugars circulation and separation. The individual recovery of arabinose, galactose, glucose, xylose, fructose, and sucrose was obtained with an operational time of 46.8 minutes per zone of the SMB process. In this simulation, it was assumed that six sugars were isolated in a separation system composed of five-zones, optimized by Xie et al. (2005) and adjusted to the current process. The adoption of the mentioned process has been chosen due to the operational parameters described in the literature (Xie et al., 2005), and the possibility to obtain a similar process on a commercial scale[1] with separation zones adjusted to each process.

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[1] Novasep, Purification processes for cellulosic sugars. Available at www.novasep.com

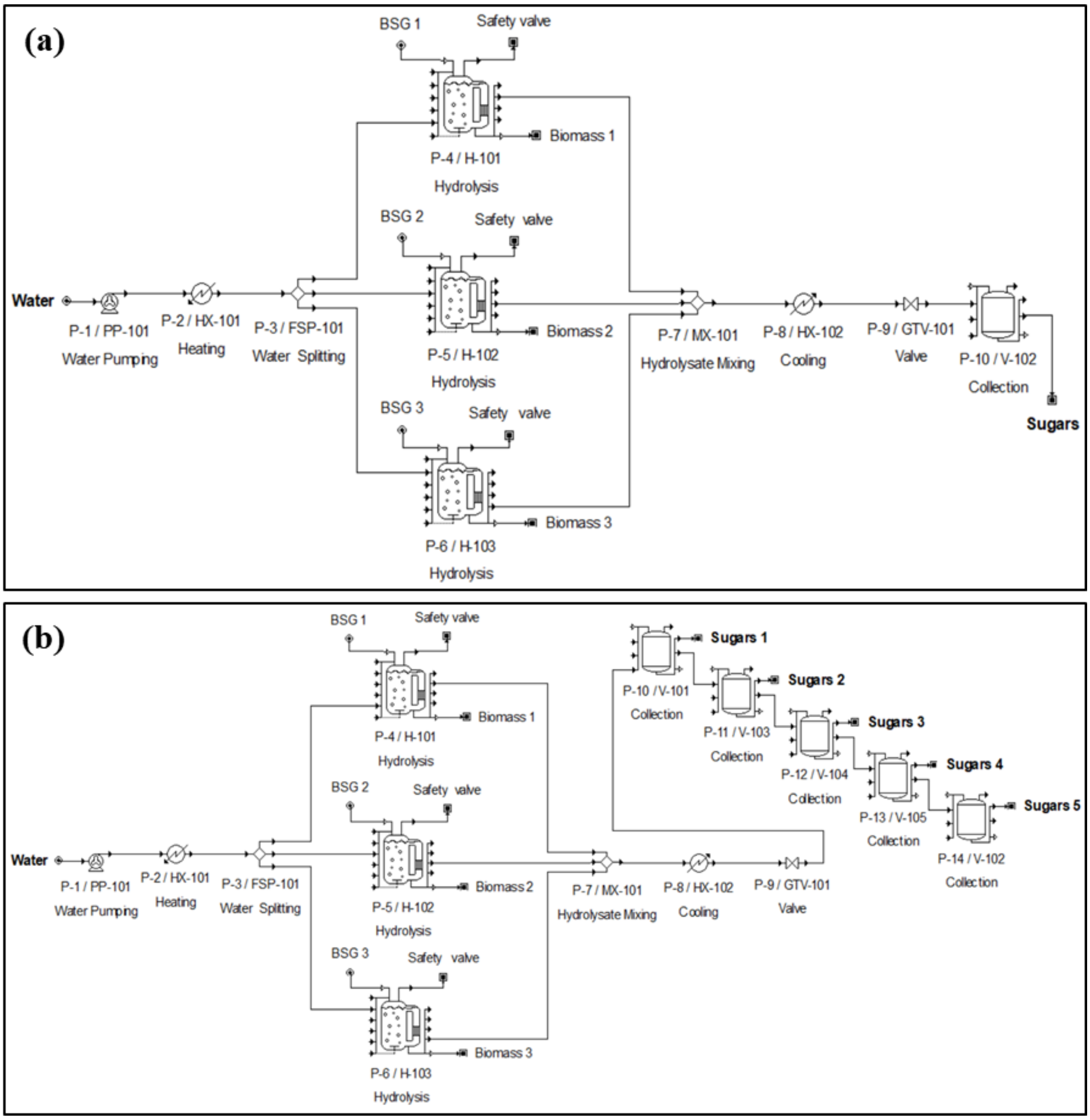

**Figure 2.** Simulation flowsheet designed for sugar production and separation using subcritical water hydrolysis of brewer’s spent grains: (a) SWH and (b) SWH-P.

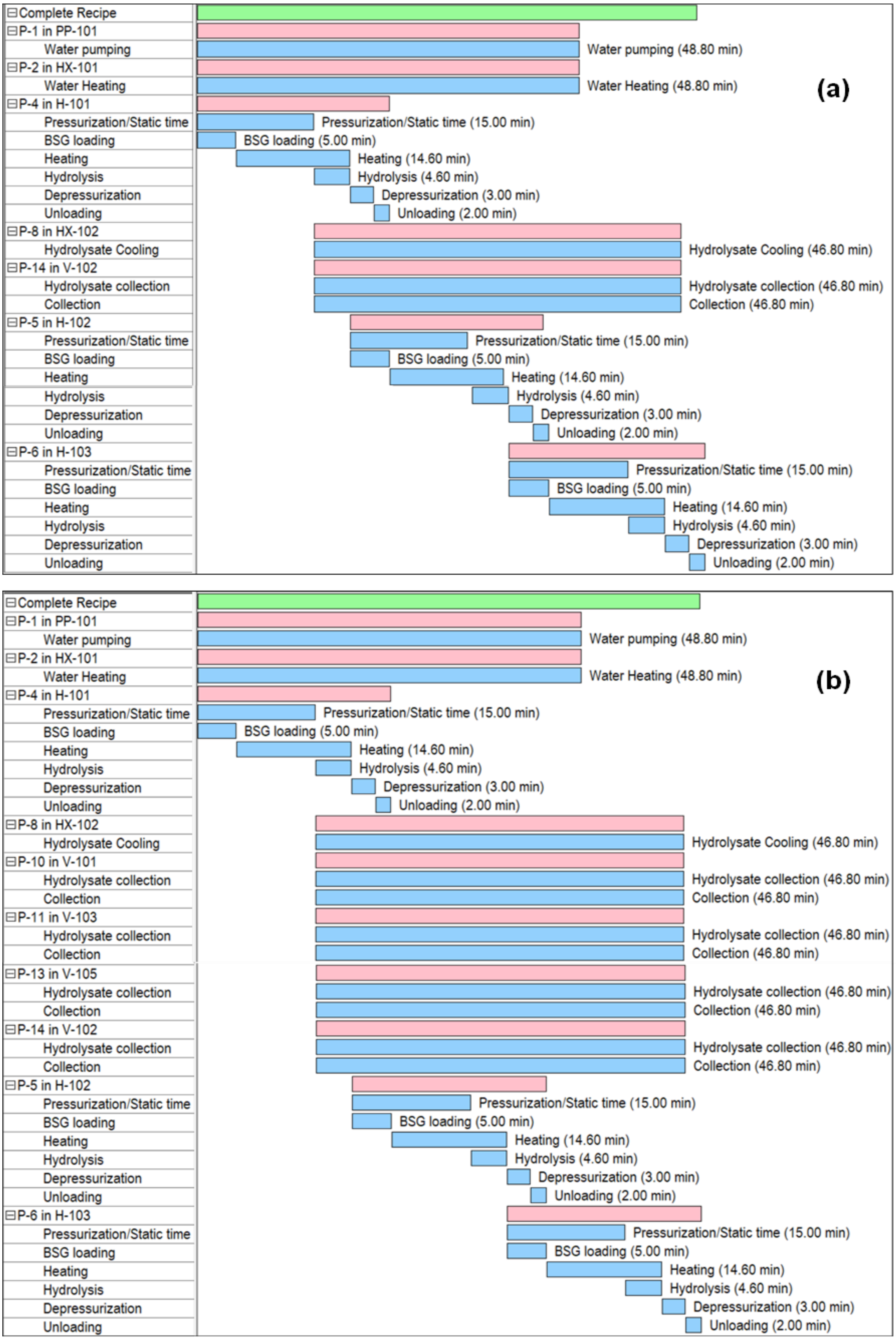

**Figure 3.** Gantt chart designed for sugar production and separation using subcritical water hydrolysis of brewer’s spent grains: (a) SWH and (b) SWH-P.

### 2.3 Economic assessment

The economic assessment was performed using SuperPro Designer 9.0® software (Intelligen Inc., Scotch Plains, NJ, USA). For evaluating the profitability parameters, the selling price of non-concentrated sugars was estimated as 3 USD kg$^{-1}$ (Lachos-Perez et al., 2020b), based on the worldwide average selling price. For individual sugars after separation, the selling price was calculated based on a final product with a commercial-grade higher than 90% purity. The selling price of concentrated sugars was estimated based on the worldwide average selling price of isolated and purified sugars[2]: 59 USD kg$^{-1}$ for arabinose; 21 USD kg$^{-1}$ for galactose; 9 USD kg$^{-1}$ for xylose; 1.2 USD kg$^{-1}$ for glucose and sucrose; and 4.4 USD kg$^{-1}$ for fructose. These values were used to simulate the profitability parameters related to all of the processing scales evaluated in this study. For all the different scales, both labor and production shifts were adequately addressed. **Table 2** shows the parameters used for techno-economic simulation.

#### 2.3.1 Itemized cost estimation

Laboratory equipment installation cost was obtained by the market price. For the scaled-up process (pilot and industrial scale), implementation costs were calculated considering the equipment cost and equipment attribute, as shown in **Eq. 1** (Turton et al., 2009).

$$C_2 = C_1 \times \left(\frac{A_2}{A_1}\right)^M \qquad (1)$$

where: $C_1$ is the reference cost; $C_2$ is the adjusted cost for the proposed process; $A_1$ is the reference equipment production capacity; $A_2$ is the related production capacity; and "M" is a cost parameter collected in the literature to each equipment (Turton et al., 2009).

In addition, to consider the change in equipment cost over time, the chemical engineering plant cost index (CEPCI) was used for the SMB separation system, according to **Eq. 2**.

$$C_2 = C_1 \times \frac{CEPCI_2}{CEPCI_1} \qquad (2)$$

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[2] Gold Biotechnology. Available at: https://www.goldbio.com/

where: $C_1$ is the reference cost obtained from 2005; $C_2$ is the adjusted cost for the proposed process in 2020; $CEPCI_1$ and $CEPCI_2$ were estimated based on the chemical engineering plant cost index, where $CEPCI_2$ /$CEPCI_1$ ratio was considered as 6.

**Table 2.** Input economic parameters used for the economic simulation in the SuperPro Designer 9.0® software**.**

| Parameters | Scale-up plants | | Unit |
|---|---|---|---|
| | 3×10L | 3×500L | |
| BSG | 10.00 | 8.00 | USD ton$^{-1}$ |
| SWH plant cost | 152,882.43 | 2,277,000.00 | USD |
| SWH-P plant cost | 726,693.93 | 8,277,000.00 | USD |
| Water (process) | 2.00 | 2.00 | USD ton$^{-1}$ |
| Water (cooling) | 0.10 | 0.05 | USD ton$^{-1}$ |
| Steam | 12.00 | 4.00 | USD ton$^{-1}$ |
| Electric energy | 0.20 | 0.20 | USD kWh$^{-1}$ |
| Wage (with benefits) | 8.00 | 8.00 | USD wage $^{-1}$ h$^{-1}$ |
| Workers per shift (SWH process) | 1 | 1 | workers shift$^{-1}$ |
| Workers per shift (SWH-P process) | 4 | 4 | workers shift$^{-1}$ |
| Operational time | 7920 | 7920 | h year $^{-1}$ |
| Apparent density | 0.50 | 0.50 | kg BSG L$^{-1}$ reactor |
| S/F | 4 | 4 | g water g$^{-1}$ BSG |
| Project lifetime | 25 | 25 | year |
| Inflation | 4 | 4 | % year$^{-1}$ |
| Low NPV interest | 7 | 7 | % |
| Depreciation period | 15 | 15 | year |
| Loan period for equipment | 12 | 12 | year |
| Loan interest for equipment | 6 | 6 | % year$^{-1}$ |
| Loan | 100 | 100 | % |

Label: BSG, brewer's spent grains; SWH, Subcritical water hydrolysis plant without sugar separation; SWH-P, Subcritical water hydrolysis plant with sugar separation system; S/F, solvent mass to feed mass ratio (kg solvent kg$^{-1}$ feed); NPV, net present value.

The five major components of the cost of manufacturing (COM) were determined based on the sum on the main components in the process (direct costs, fixed costs, and general expenses), according to the method described by Turton et al. (2009) (**Eq. 3**).

$$\text{COM}=0.304\times\text{FCI}+2.73\times\text{COL}+1.23\times(\text{CUT}+\text{CWT}+\text{CRM}) \quad (3)$$

where: FCI is the fixed capital investment; COL is the cost of operational labor; CUT is the cost of utilities; CWT is the cost of waste treatment; and CRM is the cost of raw material (CRM). All the costs were normalized per year of investment to determine the COM. FCI is related to expenses involved in the implementation of the production unit. CRM consists of the costs required to prepare the raw material and the costs of chemicals. COL is related to operators (number and wage) of the unit. CUT considers the energy and cleaning water used in the process, among others. CWT is the residue generated by this process, considered as zero in this study. In this study, more operators were used in the process with separation because this operation requires more activities and control. Consequently, the total cost of operating labor is higher for the SWH-P process. Beyond, in terms of the increase in process size, the same required labor was maintained, assuming similar handling of the processes, with mechanical loading and unloading of reaction vessels.

### *2.3.2 Project feasibility*

Gross profit (USD $\text{year}^{-1}$), annual operating cost (USD $\text{year}^{-1}$), and main revenue (USD $\text{year}^{-1}$) were estimated to SWH and SWH-P processes. To evaluate the feasibility of the proposed scenarios, gross margin (GM), return on investment (ROI), the yearly rate of return (ROR), net present value (NPV), and payback time were evaluated for the project implementation. These parameters are the most common indicators to assess the profitability of a new project. GM is the difference between revenue and cost of goods sold. NPV is the sum of the projected discounted cash flows minus the initial investment. ROI measures the return on each unit of money invested in the project, indicating the investment cost-benefit. Yearly ROR is computed by looking at the value of an investment at the end of one year and comparing it to the value at the beginning of the same year. Payback time is the number of years to recover the initial investment. As soon as the investment is recovered, the project is profitable. Payback occurs at the moment when the sum of the terms of the cash flow is positive.

### *2.3.3 Sensitivity analysis*

A sensitivity analysis was accomplished to evaluate the effects of price uncertainties in the profitability analysis of the best economic scenarios, with a range of ±50% of the established prices. In this study, the SWH-P implementation cost can be a representative factor in the project feasibility. For SWH-P, the price of SMB separation system can affect the project feasibility since it is a high implementation cost. The cost of raw material (BSG) is a parameter with high uncertainties since each brewery designates the content to an appropriate destination, with a fluctuation in the selling price. Otherwise, the number of wages is a difficult factor to estimate since it is possible to develop a process with high automation or manual working.

### *2.4 Mass and energy balance*

To evaluate the main technical parameters of the SWH-P implementation, a mass and energy balance was fulfilled with the industrial proposed process. For sugar production and separation, the balance considers input, output, generation, consumption, and accumulation. Energy balance was used to identify and quantify the energy consumption, accumulation, transformation to another form, and energy loss in the process (Sandler, 2017). A theoretical steady-state design of SWH-P was provided based on the technological route established, considering the separation process and all the parameters used in the simulation. Initially, the mass balance was defined according to **Eq. 4**, assuming a steady-state process, in which the accumulation is null.

$$\text{input} - \text{output} + \text{generation} - \text{consumption} = 0 \tag{4}$$

In the SMB separation system, the mass fractions are isolated into a stream of water, impurities, and isolated sugars (**Eq. 5**). Mass fractions were established based on experimental and simulated conditions.

$$M_7 = \sum_{i=8}^{i=15} M_i \tag{5}$$

For energy balance in the subcritical reactor, the first law of Thermodynamics was adopted at a steady-state, with constant pressure and without shaft work. The variation in kinetic and potential energy was neglected. Thus, the energy balance was expressed according to **Eq. 6.**

$$\sum M_k(H) + Q = 0 \tag{6}$$

Considering the mass constant in the process ($M_{R1} = M_{R2}$ = constant), the heat required in the subcritical reactor (Q) is the difference of enthalpy (H) **(Eq. 7)**:

$$\frac{Q}{M} = H_2 - H_1 \tag{7}$$

Besides, the enthalpy can be calculated based on the specific heat of the mixture in the reactor. For this, the mass of water >> mass of BSG was considered, and then, it was possible to use the specific heat of water ($C_p^*$), which is 4.178 kJ $kg^{-1}$ $K^{-1}$ at 25 °C and 4.285 kJ $kg^{-1}$ $K^{-1}$ at 160 °C (Sandler, 2017). In the current process, the pressure was considered constant since a pressure pump was used to regulate it during hydrolysis. In addition, the subcritical reactor maintains the pressure constant along the time and increases the temperature of water from 25 °C to 160 °C. Thus, enthalpy was calculated based on **Eq. 8**.

$$H\left(\frac{kJ}{kg}\right)=C_p^*\left(\frac{kJ}{kg\ K}\right)\times T(K) \tag{8}$$

## 3. Results and discussion

### *3.1 Previous laboratory studies*

In the subcritical state, water is a promising solvent for biomass depolymerization. Due to the high hemicellulose content (approximately 40%) in BSG, the use of a subcritical state may suggest the BSG as a potential source of sugars. Under laboratory-scale studies, a flow-through hydrothermal treatment was optimized for the hydrolysis of BSG (Torres-Mayanga et al., 2019). Total sugars production was 45.2 g $kg^{-1}$ BSG, and the individual fraction of sugars obtained at the optimal operational condition was: 31.3 g arabinose $kg^{-1}$ BSG; 1.5 g galactose $kg^{-1}$ BSG; 2.1 g glucose $kg^{-1}$ BSG; 8.7 g xylose $kg^{-1}$ BSG; 1.6 g fructose $kg^{-1}$ BSG; and 1.4 g sucrose $kg^{-1}$ BSG. Also, in the SWH process, one impurity (8 g furfural $kg^{-1}$ BSG) and two organic acids (3.7 g formic acid $kg^{-1}$ BSG and 6.1 g acetic acid $kg^{-1}$ BSG) were generated. Consequently, the focus of this study has been put on the production and isolation of sugars.

Generally, from the results obtained by Torres-Mayanga et al. (2019), the reducing sugars increase with hydrolysis temperature increase, independently of flow rate. However, with the S/F effect and mild hydrolysis conditions (160 °C), a minor impact on reducing sugars was obtained. The hydrolysis experiment indicated that temperature and S/F are the most important factor governing the carbohydrates hydrolysis contained in BSG. From the individual sugars production, the lowest temperature promoted the highest productivity of arabinose and xylose, which represents 88% of the total quantified sugar yield.

### *3.2 Costs analysis*

#### *3.2.1 Cost of manufacturing (COM)*

For the proposed scale-up study, eight scenarios were simulated with different residences time of BSG inside the subcritical reactor (ranging from 4.4 to 9.5 minutes) (**Table 3**). The S/F of 4 g $g^{-1}$ (fixed-parameter) showed the total carbohydrate production was directly affected by the residence time. Scenario 7 was operated with 4.6 min and 160 °C of hydrolysis temperature and produced 4.36% of sugars, following the previous laboratory results. In general, scenarios with higher residence time promoted hydrolysate production with a high concentration of active compounds (Hatami et al., 2020; Hatami et al., 2019; Zabot, Moraes, Meireles, 2018). The cost of manufacturing (COM) was calculated at two different production scales (10 L and 500 L), which were coupled or not with a SMB separation system (SWH and SWH-P). When assessing the influence of SMB separation system in the SWH process, the COM increases up to 3-fold, an expected fact since the implementation cost of SMB process is one of the most representative barriers in the adoption of this technology (Matus et al., 2012). For the SWH process, the COM was determined based on producing a single fraction of hydrolysate. Otherwise, COM of the SWH-P process was related to the production of six isolated sugars, and then additional unit operations are required, affecting the COM (**Table 3**). Based on the scenarios simulated, the condition established in Scenario 8 was favorable to produce sugars with the lowest COM. For the pilot-plant scale (3×10 L) the COM obtained was 14.49 USD $kg^{-1}$ (SWH) and 58.88 USD $kg^{-1}$ (SWH-P), an increase in 4-fold comparing to the adoption of a SMB separation system. On an industrial scale (3×500 L), the COM for the SWH process was 2.92 USD $kg^{-1}$, and with the adoption of SMB separation system, the COM increase to 6.63 USD $kg^{-1}$. The 50-fold process increase (3×10 L to 3×500 L), leads to a decrease of approximately 80% of sugars COM. This fact is a consequence of the scale-up process, corroborating with the literature (Hatami et al., 2020; Hatami et al., 2019; Zabot, Moraes, Meireles, 2018).

**Table 3.** Cost of manufacturing (COM, USD kg$^{-1}$) for the different scaled-up plants with and without sugar separation.

| Scenario | Residence time (min) | Total carbohydrates (%) | COM (USD kg$^{-1}$ sugars) | | | |
|---|---|---|---|---|---|---|
| | | | 3×10 L | | 3×500 L | |
| | | | SWH[a] | SWH-P[b] | SWH[a] | SWH-P[b] |
| 1 | 9.5 | 2.11 | 38.98 | 177.07 | 6.67 | 17.08 |
| 2 | 9.3 | 4.52 | 18.21 | 82.22 | 3.18 | 8.02 |
| 3 | 9.1 | 4.41 | 18.66 | 83.67 | 3.33 | 7.56 |
| 4 | 8.8 | 4.61 | 17.87 | 79.39 | 3.29 | 7.96 |
| 5 | 4.8 | 1.78 | 35.24 | 172.43 | 7.22 | 17.5 |
| 6 | 4.7 | 2.50 | 25.21 | 122.43 | 5.27 | 12.58 |
| 7 | 4.6 | 4.36 | 14.49 | 58.88 | 2.92 | 6.63 |
| 8 | 4.4 | 4.13 | 15.38 | 73.33 | 3.39 | 7.77 |

[a] COM calculated based on the production of a single hydrolysate.

[b] COM calculated based on the production of a bulk of isolated sugars.

### *3.2.2 Costs discrimination over the COM*

The contribution of the fixed capital investment (FCI), cost of operational labor (COL), cost of utilities (CUT), and cost of raw material (CRM) discriminated over the COM was determined to evidence the process conditions for the sugar production. Cost's discrimination analysis was conducted with the scale-up plants in all the scenarios tested (**Fig. 4**). For the 3×10 L evaluated scales without the separation system (SWH), the COM contribution was COL> CUT> FCI > CRM, and with the SMB separation process (SWH-P) the contribution was COL>FCI>CUT>CRM. In the 3×500 L industrial process, COM contribution for SWH was CUT>FCI>CRM>COL, and for SWH-P was FCI>CUT>COL>CRM.

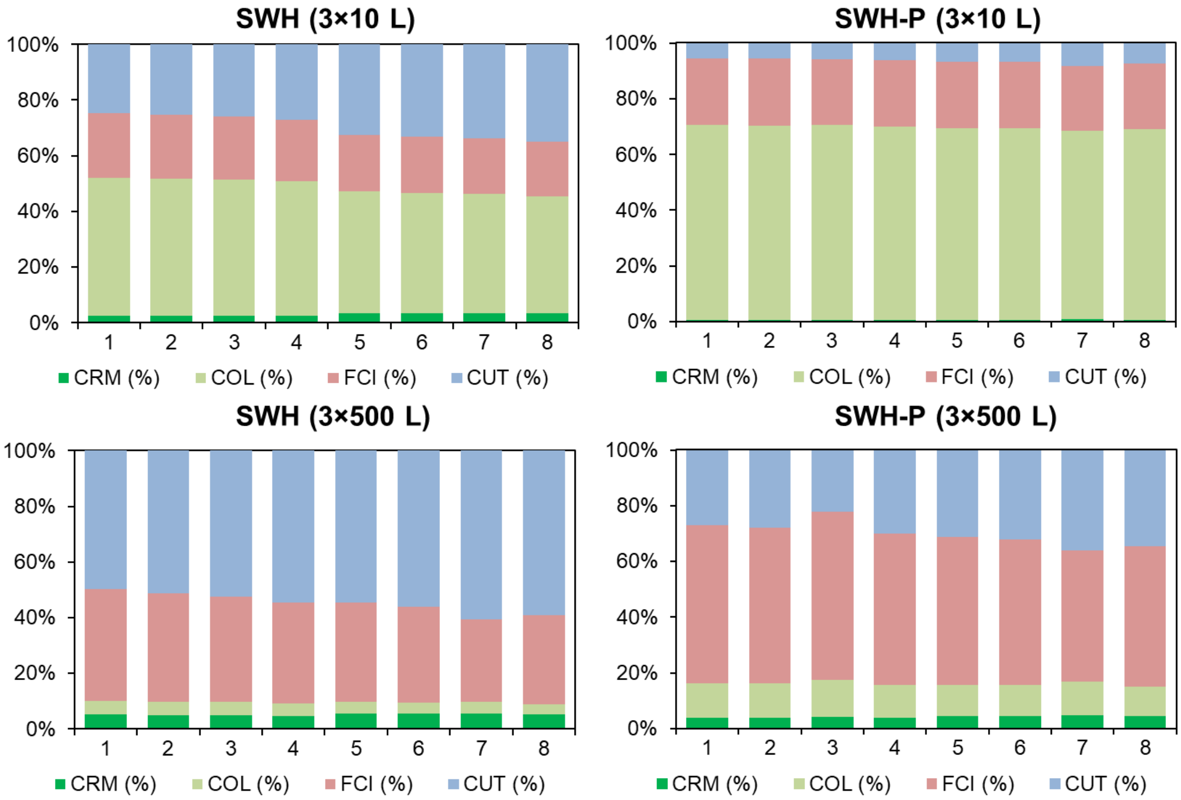

**Figure 4.** Contribution of each cost discriminated over the COM to obtain sugars from subcritical water extraction of brewer's spent grains. Legend: CRM, cost of raw material; COL, cost of operational labor; FCI, fixed capital investment; CUT, cost of utilities.

In the pilot plant (3×10 L), the COL was the most expressive in all the scenarios, reaching 50% in the SWH process and increasing to 70% in SWH-P. Otherwise, the CUT drastically decreased with the implementation of SMB process, from 25% (SWH) to 6% (SWH-P). In the industrial-scale plant (3×500 L), FCI and CUT were the main costs over the COM. Within the SWH-P adoption on the industrial-scale, the CRM and COL maintain lower than 5% and 15%, respectively. A decrease was observed comparing the COL for the pilot plant and industrial plant, explained by the high degree of instrumentation in industrial plants. The adoption of automation plants requires a relatively lower number of operators to conduct the process, corroborating with the literature (Viganó et al., 2017; Aguiar et al., 2020). In addition, the cost profile obtained in this study is related to the highest equipment cost, where the SMB separation system demands high FCI; and, therefore, high energy cost (CUT) due to the high process temperature adopted, which requires high electric energy.

In the current study, CRM was the lowest significant cost over the COM. However, according to Osorio-Tobón et al. (2016), CRM is the component with the maximum contribution to the COM in their study. In the mentioned study, when the CRM decreased from 7.27 to 1.59 USD kg$^{-1}$, the COM proportionally reduced from 112.70 to 64.97 USD kg$^{-1}$, representing a decrease of 42%. For the proposed process with fast residence time, the amount of feedstock required by the process is too high; however, the market price of BSG can be considered low. Considering that the brewery disposes of the BSG in landfills or animal feeds, this feedstock can be easily implemented in an industrial biorefinery (Sganzerla et al., 2021). In addition, to decrease the COM in the industrial process, an alternative is adopting innovative technologies to increase the recovery of active compounds since higher productivity will decrease the total COM. The adoption of SWH-P decreased the CUT and increased the FCI, an expected fact since the sugars separation system was the most expensive operation.

### *3.3 Implementation of SMB separation system in SWH*

Supercritical technology allows producing a range of active compounds. However, few studies adopted a separation system to isolate the products. As previously mentioned, this simulation was based on a laboratory study that produced sugars by the subcritical process without the sugar's isolation. The individual profile of sugars can be obtained by many analytical techniques; however, the isolation for commercial purposes should be clarified. Thereby, SMB technology can be industrially applied when conventional separation techniques cannot be applied. SMB is a chromatography-based technique that has been successfully used for sugar separation (Azevedo and Rodrigues, 2000). This technology can be operated in a continuous separation method, recovering sugar products from chemical hydrolysates of biomass with a high separation rate (Caes et al., 2013). Then, SMB is a highly efficient separation process with low demand for solvent and energy and a high potential for the separation and isolation of value-added compounds (Lin et al., 2015).

Aiming to address an innovative process to sugars production and separation, a five-zone SMB process was adopted. The SMB process presents a separation performance of 94%, which means that 6% of sugars were lost (Xie et al., 2005). **Table 4** demonstrates the individual sugar productivity for all the scenarios tested, and considering the theoretical yield (100%), and the real yield (94%) in the process.

Adopting a yield lower than 100% represents that it is possible to achieve the sugar concentration obtained in the laboratory-scale; however, the separation process will lose 6% of sugars (Xie et al., 2005). Scenario 7 (with the lowest COM) can produce a maximum of 60.5% of arabinose, 18.2% of xylose, 5.2% of glucose, 3.8% of sucrose, 3.5% of fructose, and 2.9% of galactose for the pilot (3×10 L) and industrial plant (3×500 L).

**Table 4.** Individual sugar productivity for the scenarios tested (SWH-P) scaling 3×10 L and 3×500 L), considering the theoretical yield (100%) and the real processes' yield (94%).

| Scenario | Processes' yield | Arabinose (%) | Galactose (%) | Glucose (%) | Xylose (%) | Fructose (%) | Sucrose (%) |
|---|---|---|---|---|---|---|---|
| 1 | 100% | 71.4 | 2.9 | 2.9 | 15.9 | 3.5 | 3.4 |
| | 94% | 67.1 | 2.7 | 2.7 | 15.0 | 3.3 | 3.2 |
| 2 | 100% | 67.0 | 3.3 | 4.5 | 18.7 | 3.5 | 3.0 |
| | 94% | 63.0 | 3.1 | 4.2 | 17.6 | 3.3 | 2.8 |
| 3 | 100% | 69.1 | 3.1 | 4.5 | 17.6 | 4.1 | 1.6 |
| | 94% | 65.0 | 2.9 | 4.2 | 16.6 | 3.8 | 1.5 |
| 4 | 100% | 41.5 | 4.0 | 10.7 | 33.1 | 9.0 | 1.8 |
| | 94% | 39.0 | 3.7 | 10.0 | 31.1 | 8.5 | 1.7 |
| 5 | 100% | 70.8 | 2.2 | 2.2 | 18.2 | 1.0 | 5.6 |
| | 94% | 66.6 | 2.1 | 2.0 | 17.1 | 0.9 | 5.3 |
| 6 | 100% | 70.6 | 2.6 | 4.4 | 16.1 | 3.2 | 3.1 |
| | 94% | 66.3 | 2.4 | 4.1 | 15.2 | 3.0 | 3.0 |
| 7 | 100% | 64.3 | 3.1 | 5.5 | 19.4 | 3.7 | 4.0 |
| | 94% | 60.5 | 2.9 | 5.2 | 18.2 | 3.5 | 3.8 |
| 8 | 100% | 56.1 | 3.8 | 6.6 | 27.3 | 6.0 | 0.3 |
| | 94% | 52.8 | 3.6 | 6.2 | 25.6 | 5.6 | 0.3 |

In addition, this study focuses on the evaluation of the production of six sugars from BSG. The contribution of each sugar over the COM for the SWH-P can be shown in **Fig. 5**. This proportion was attributed based on the current market price of each sugar, and then, the contribution cost of each sugar can be related to the final selling price obtained in the market. Arabinose (61.87%) and galactose (21.18%) were the sugars most expensive in the market price and then could represent the most expressive in the subcritical process coupled with the separation system. Glucose, fructose, and sucrose were produced in a lower proportion, and the cost attributed to these sugars represent only 16.32%. The values estimated over the selling cost were correlated with the market price of each sugar. Arabinose (59 USD kg$^{-1}$) and galactose (21 USD kg$^{-1}$) represent 83.68% based on the sum of all sugars (**Fig. 5**), in reference to weighted profit. This proportion allows calculating the COM for the SMB separation process, considering 94% of process' yield for the 3×10 L and 3×500 L process (**Table 5**). In the current case (SWH-P), the COM was calculated based on individual sugar productivity and considering a yield of 94%. Following the simulation data presented in **Table 3**, scenario 7 promoted the lowest COM for all individual sugars.

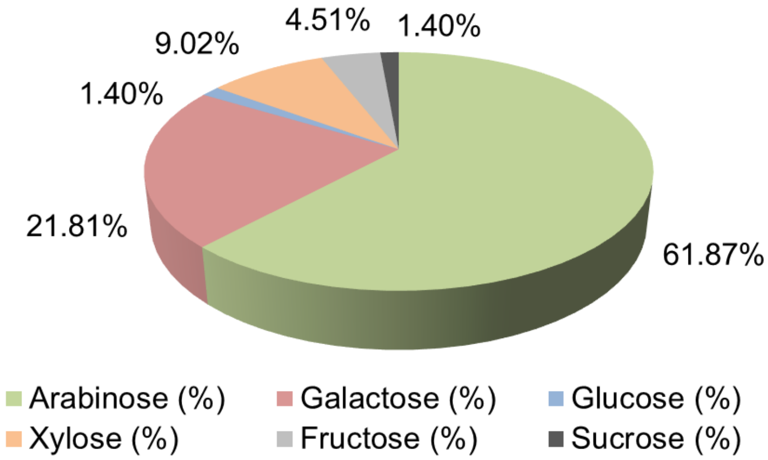

**Figure 5.** Percent value of each sugar obtained in the SWH-P processes calculated based on the current market price.

In the tested scales, galactose was the sugar with the highest COM, explained due to the combination of contribution cost of individual sugar in the separation cost (SWH-P) (**Fig. 5**) with the individual sugar productivity (**Table 4**). Then, arabinose COM was the second one obtained in the hydrolysis process. For the 3×10 L scale in scenario 7, arabinose COM was 64.10 USD kg$^{-1}$, and in the 3×500 L scale, the COM decreased to 7.22 USD kg$^{-1}$. The results shown in **Table 3**, arabinose represented up to 60% in the COM for scenario 7 (SWH-P, 3×500 L), which was 6.63 USD kg$^{-1}$. However, the data presented in **Table 3** did not consider 94% of the SMB separation process. Then, the sum of the COM of **Table 5** is different since the last one considered the separation factor and the COM based on the contribution cost of individual sugar in the SMB separation cost (SWH-P). Focusing on implementing a SMB separation system in SWH based on the COM, it is possible to note that the COM at industrial scale (3×500 L) is lower than the market price for all sugars, which is a positive indicator for the project feasibility.

**Table 5.** Cost of manufacturing (COM, USD $kg^{-1}$) for the different scaled-up plants (SWH-P) considering a processes' yield of 94% and expressed based individual mass of sugar.

| Scenario | Arabinose | Galactose | Glucose | Xylose | Fructose | Sucrose |
|---|---|---|---|---|---|---|
| | 3×10 L (USD $kg^{-1}$ individual sugar) | | | | | |
| 1 | 173.72 | 1500.14 | 96.21 | 113.42 | 260.63 | 82.28 |
| 2 | 85.94 | 622.90 | 28.80 | 44.76 | 120.78 | 43.12 |
| 3 | 84.75 | 675.65 | 29.59 | 48.40 | 104.26 | 82.60 |
| 4 | 133.93 | 492.31 | 11.78 | 24.51 | 44.95 | 70.98 |
| 5 | 170.51 | 1921.78 | 126.88 | 96.70 | 872.26 | 48.53 |
| 6 | 121.45 | 1169.78 | 43.90 | 77.48 | 198.25 | 61.60 |
| 7 | 64.10 | 468.56 | 16.86 | 31.03 | 81.80 | 23.20 |
| 8 | 91.48 | 478.18 | 17.66 | 27.46 | 62.82 | 374.74 |
| | 3×500 L (USD $kg^{-1}$ individual sugar) | | | | | |
| 1 | 16.76 | 144.70 | 9.28 | 10.94 | 25.14 | 7.94 |
| 2 | 8.38 | 60.76 | 2.81 | 4.37 | 11.78 | 4.21 |
| 3 | 7.66 | 61.05 | 2.67 | 4.37 | 9.42 | 7.46 |
| 4 | 13.43 | 49.36 | 1.18 | 2.46 | 4.51 | 7.12 |
| 5 | 17.31 | 195.04 | 12.88 | 9.81 | 88.53 | 4.92 |
| 6 | 12.48 | 120.20 | 4.51 | 7.96 | 20.37 | 6.33 |
| 7 | 7.22 | 52.76 | 1.90 | 3.49 | 9.21 | 2.61 |
| 8 | 9.69 | 50.67 | 1.87 | 2.91 | 6.66 | 39.71 |

### *3.4 Profitability and sensitivity analysis*

#### *3.4.1 Profitability analysis*

The evaluation of gross profit, annual operating cost, main revenue, and profitable indicators are the initial parameters to observe the behavior of different scales and designs for the subcritical hydrolysis of BSG (**Table 6**). For both SWH plants (3×10L and 3×500L), a negative gross profit was obtained per year, which indicated that after all the maintenance and implementation costs, the plant would not return the initial investment. Otherwise, plants with SMB separation process (SWH-P) presented a positive gross profit only for industrial scale (3×500L). For instance, in the SWH-P industrial scale (3×500L), the gross profit ranged from 2,160,000.00 USD (Scenario 1) to 9,921,000.00 (Scenario 7). In addition, from the annual cost, it was clear that Scenario 7 required the significant costs to the operational labor. This cost increased with the scale-up and the adoption of a separation system. On an industrial scale (SWH-P, 3×500L), the annual operational cost was higher than 1.5 million USD for all the scenarios.

Notwithstanding, the main revenue was obtained from the sum between gross profit and annual operating cost since a fraction of the revenues are designated to the operating costs, and the other is the gross profit. Thus, the best revenue condition was obtained for the process with SMB separation system on the industrial scale. This fact can be explained by the sugar isolation in the SMB system since the market price increases with the production of isolated sugars, when comparing to the single fraction of sugars obtained in SWH process. The difference in the scenarios and profits are related mainly to yields and the composition of hydrolysates for different reaction times.

Among the eight (08) scenarios evaluated, the plants that produced one hydrolysate (one market product) were not profitable. In the SWH (3×10 L and 3×500 L), payback time was up to 90 years, and negative GM, ROI, yearly ROR, and NPV were obtained. The current market price of the hydrolysate (3 USD $kg^{-1}$) does not allow achieving a profitable condition and should not be implemented. Otherwise, with the implementation of a SMB separation system, the profitability indicators were positive on industrial scale. Scenario 7 was the most profitable one, corroborating the COM analysis since the lowest productivity cost may increase profitability. The SWH-P industrial-scale (3×500 L) of this scenario (SWH-P, Scenario 7) can improve the profitability indicators, which can be considered an advantage to implementation to produce isolated sugars from BSG.

The profitability results obtained in this study are in accordance when comparing with other scale-up studies. For instance, Viganó et al. (2017) studied the scale-up of supercritical fluid and pressurized liquid extraction of phytonutrients from passion fruit (*Passiflora edulis*) by-products. In the mentioned study, the payback time ranged from 0.6 (industrial scale) to 14.9 years (laboratory scale), and the NPV increased to approximately 109 million USD in the industrial scale. Lachos-Perez et al. (2021) evaluated sugars and flavanones production from orange peel using a sequential subcritical water process. The process simulation was conducted at the laboratory (2×5 L), pilot (3×10 L), and industrial (3×500 L) scales. The results demonstrate that the scale-up process decreased the COM and improved the profitability parameters. Zabot et al. (2017) simulated the scale-up process of different supercritical reactors to obtain quercetin-rich powdered extracts from onion (*Allium cepa*) peels. The laboratory-scale obtained negative returns; however, with the implementation of pilot and industrial plant, a satisfactory IRR, ROI, NPV, and payback were obtained, attributing a profitable process. Beyond, Zabot et al. (2018) evaluated the economic feasibility of supercritical fluid extraction coupled to low-pressure solvent extraction to produce tocotrienols oil and bixin extract from annatto (*Bixa orellana*) seeds. On industrial scale, the ROI, GM, and payback obtained were respectively 389.7%, 66.5%, and 0.26 years, which suggest an implementation in new production lines.

### *3.4.2 Sensitivity analysis*

The effect of sugar production and purification in the subcritical water process was evaluated by a sensitivity analysis. In the current scale-up project, implementation cost, BSG cost, and wage per shift were evaluated by a sensitivity analysis in the industrial-scale process (3×500 L) coupled with the SMB separation system. These variables were adopted since they were the most influencing variables on an industrial scale and directly affected the project feasibility. Thereby, the project condition was evaluated to identify the effects of significant variations on ROI and NPV (**Fig. 6**). With a variation of ±50% in the input data, it is possible to observe that plant cost is the most sensitive parameter. This fact can be associated with the highest implementation cost of the industrial process. Otherwise, BSG cost cannot be considered a sensitive parameter since non-significant differences were obtained with a variation in ±50% for the SWH-P process on the industrial-scale. Notwithstanding, the wage per shift directly affects the SWH-P process since this plant requires a high number of wages to implement the process. The condition evaluated herein may be an alternative to the implementation since the investor can choose the best operational conditions and the market price of the material to construct the most profitable industrial plant.

**Table 6.** Results of profitability parameters to obtain sugars from different scaled-up plants (3×10L and 3×500L) considering SWH and SWH-P.

| Scenario | GM (%) | ROI (%) | Yearly ROR (%) | Payback (years) | NPV (USD) | Gross profit (USD $year^{-1}$) | Annual operating cost (USD $year^{-1}$) | Main revenue (USD $year^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| 3×10L (SWH) | | | | | | | | |
| 1 | -1193.44 | -46.44 | -1233 | >99 | -950,000.00 | -74,000.00 | 80,000.00 | 6000.00 |
| 2 | -510.04 | -42.39 | -567 | >99 | -871,000.00 | -68,000.00 | 80,000.00 | 12,000.00 |
| 3 | -519.67 | -42.88 | -575 | >99 | -881,000.00 | -69,000.00 | 81,000.00 | 12,000.00 |
| 4 | -497.41 | -43.11 | -531 | >99 | -886,000.00 | -69,000.00 | 82,000.00 | 13,000.00 |
| 5 | -1079.81 | -51.90 | -1186 | >99 | -1,0066,000.00 | -83,000.00 | 90,000.00 | 7000.00 |
| 6 | -738,34 | -50.43 | -810 | >99 | -1,037,000.00 | -81,000.00 | 91,000.00 | 10,000.00 |
| 7 | -383.14 | -45.84 | -411 | >99 | -946,000.00 | -74,000.00 | 92,000.00 | 18,000.00 |
| 8 | -413.20 | -47.31 | -422 | >99 | -976,000.00 | -76,000.00 | 94,000.00 | 18,000.00 |
| 3×10L (SWH-P) | | | | | | | | |
| 1 | -363.77 | -37.81 | -365 | >99 | -3,695,000.00 | -285,000.00 | 363,000.00 | 78,000.00 |
| 2 | -109.85 | -25.28 | -111 | >99 | -2,519,000.00 | -191,000.00 | 363,000.00 | 172,000.00 |
| 3 | -119.13 | -26.30 | -119 | >99 | -2,616,000.00 | -198,000.00 | 364,000.00 | 166,000.00 |
| 4 | -107.93 | -25.11 | -108 | >99 | -2,503,000.00 | -189,000.00 | 364,000.00 | 175,000.00 |
| 5 | -351.63 | -37.91 | -357 | >99 | -3,705,000.00 | -286,000.00 | 366,000.00 | 80,000.00 |
| 6 | -220.67 | -33.57 | -221 | >99 | -3,299,000.00 | -253,000.00 | 367,000.00 | 114,000.00 |
| 7 | -54.22 | -17.51 | -54 | >99 | -1,794,000.00 | -132,000.00 | 375,000.00 | 243,000.00 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 8 | -92.06 | -23.50 | -92 | >99 | -2,355,000.00 | -177,000.00 | 369,000.00 | 192,000.00 |
| 3×500L (SWH) | | | | | | | | |
| 1 | -122.37 | -16.24 | -122 | >99 | -5,108,000.00 | -376,000.00 | 683,000.00 | 307,000.00 |
| 2 | -6.04 | -1.73 | -6 | >99 | -923,000.00 | -41,000.00 | 703,000.00 | 662,000.00 |
| 3 | -10.93 | -3.08 | -11 | >99 | -1,315,000.00 | -72,000.00 | 724,000.00 | 652,000.00 |
| 4 | -9.52 | -2.83 | -9 | >99 | -1.249,000.00 | -66,000.00 | 756,000.00 | 690,000.00 |
| 5 | -140.73 | -19.41 | -140 | >99 | -6,050,000.00 | -451,000.00 | 771,000.00 | 320,000.00 |
| 6 | -75.82 | -14.74 | -75 | >99 | -4,706,000.00 | -343,000.00 | 795,000.00 | 452,000.00 |
| 7 | 2.69 | 1.10 | 2.72 | 90.85 | -125,000.00 | 26,000.00 | 930,000.00 | 956,000.00 |
| 8 | 12.92 | -4.21 | -13 | >99 | -1,661,000.00 | -99,000.00 | 857,000.00 | 758,000.00 |
| 3×500L (SWH-P) | | | | | | | | |
| 1 | 55.26 | 25.88 | 79.26 | 3.86 | 25,471,000.00 | 2,160,000.00 | 1,749,000.00 | 8,432,000.00 |
| 2 | 78.99 | 79.70 | 78.67 | 1.25 | 81,593,000.00 | 6,661,000.00 | 1,771,000.00 | 8,303,000.00 |
| 3 | 80.20 | 79.88 | 81.29 | 1.25 | 81,578,000.00 | 6,659,000.00 | 1,644,000.00 | 8,785,000.00 |
| 4 | 79.14 | 83.24 | 55.05 | 1.20 | 85,229,000.00 | 6,953,000.00 | 1,832,000.00 | 4,076,000.00 |
| 5 | 54.17 | 26.42 | 67.53 | 3.79 | 26,056,000.00 | 2,208,000.00 | 1,868,000.00 | 5,753,000.00 |
| 6 | 67.06 | 46.15 | 84.25 | 2.17 | 46,626,000.00 | 3,858,000.00 | 1,895,000.00 | 12,032,000.00 |
| 7 | 81.59 | 118.25 | 78.14 | 0.85 | 121,474,000.00 | 9,921,000.00 | 2,111,000.00 | 9,656,000.00 |
| 8 | 79.65 | 91.94 | 76.70 | 1.09 | 94,420,000.00 | 7,691,000.00 | 1,965,000.00 | 8,432,000.00 |

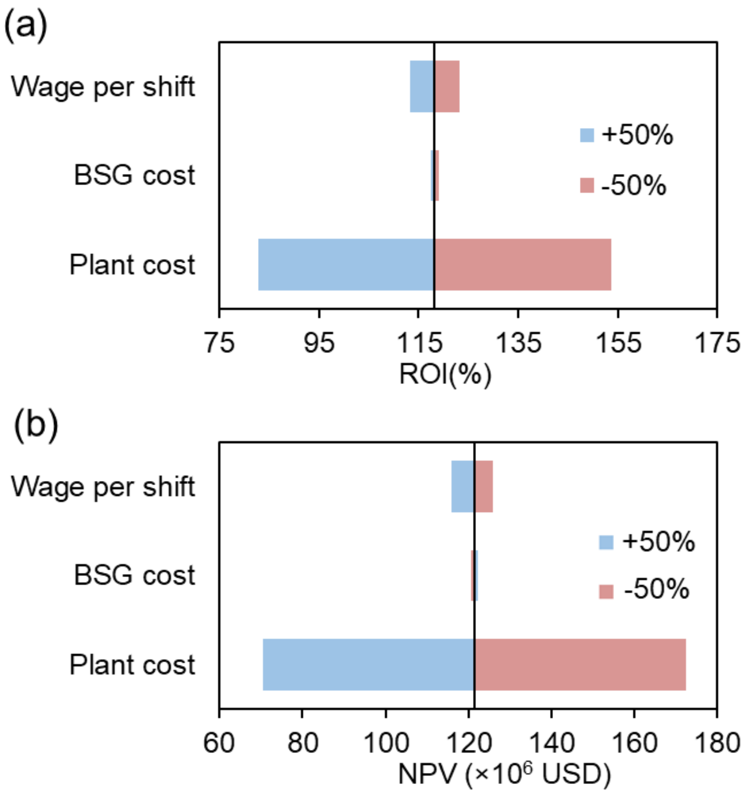

**Figure 6.** Sensitivity analysis for the industrial-scale (3×500L) process coupled with the SMB purification system (SWH-P) on the ROI (a) and NPV (b).

The adoption of simulated scale-up projects to produce a new product may be accomplished with an economic assessment and a deep sensitivity study. After choosing the best operational conditions on the laboratory scale, the simulated pilot and industrial plant provide an insight related to the adoption of this technology. Therefore, the next step of this study is to conduct the pilot-plant of SWH and extend the technology scope to evidence the operational conditions for an industrial process, transferring successful smaller-scale initiatives to larger-scale processes.

### *3.5 Mass and energy balance of the SWH-P process*

In the current study, the mass balance was evaluated to obtain equations that observe the mass flow in each step of SWH-P. Energy balance was used to identify and quantify all the energy that flows in the process. A theoretical steady-state design of SWH was provided based on the technological route established, considering the SMB separation process (**Fig. 7**). Thus, the lists of conventional equipment for the present technical routes were the subcritical reactor, heat exchange, and the separation system.

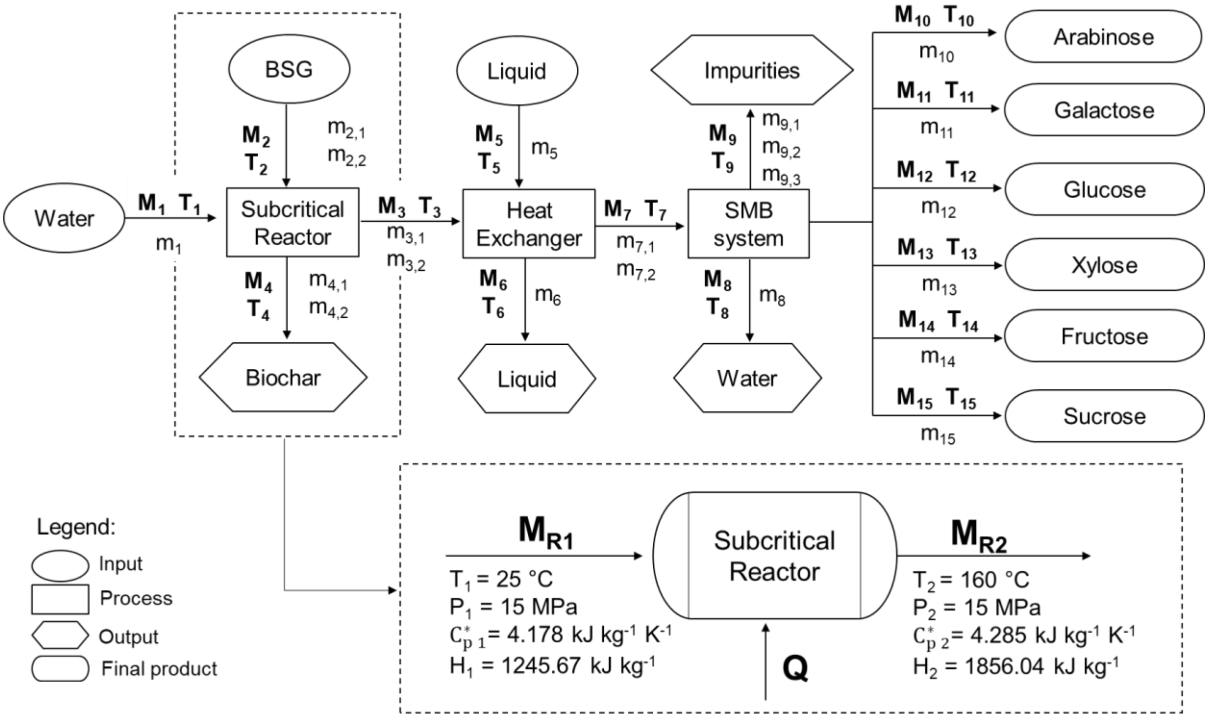

**Figure 7.** Representation of the mass and energy balance for sugar production from BSG.

Additionally, it was still possible to describe some considerations for the current process, considering the laboratory scheme established in **Fig. 1** and demonstrated in previous studies (Mayanga-Torres et al., 2017; Lachos-Perez et al., 2017; Lachos-Perez et al., 2018). The mass balance of the process can be observed in **Table 7**. For its interpretation, the considerations described herein, and the equation described in the **Section 2.4.** were the basis to conduct the mass and energy balance of the process:

1) Knowing "$M_2$" grams of BSG fed into the reactor and knowing the S/F, it is possible to determine the amount of water required using **Eq. 9.** In the case of the subcritical reactor of 500 L, the amount of BSG used for hydrolysis is 250 kg. Considering the same S/F used in this study (4 g solvent $g^{-1}$ feed) the amount of water per reactor on an industrial scale is approximately one (1) $m^3$.

$$M_1 = M_2 \times \frac{S}{F} \tag{9}$$

2) The process presents a net extract yield of 56.63%, and then, "$M_3$" can be calculated using **Eq. 10**. The volume of hydrolysate produced in the industrial subcritical reactor of 500 L is 707 L per batch.

$$M_3=0.5663\times(M_1+M_2) \tag{10}$$

3) The process generates a fraction of waste in the reactor (known as biochar) which can be calculated using **Eq. 11.** Through the global balance in the reactor, the amount of biochar produced in the industrial reactor is 543 kg.

$$M_4=0.4337\times(M_1+M_2)=M_1+M_2-M_3 \tag{11}$$

4) Under the laboratory experiment, it is known that 5 g of the sample introduced in the reactor produces 2.68 g of residue that remains in the reactor, that is, 53.6%. Thus, the $m_{4,1}$ fraction should be approximately 2.68 g, which corresponds to a solid mass fraction of 0.011 w/w, and therefore, 0.989 w/w is the water fraction ($m_{4,2}$) (**Table 7**).

5) In the heat exchanger, it is possible to assume that there is no degradation of the compounds, only the cooling of the solution, with constant mass. The mass balance can be expressed according to **Eq. 12.** However, the cooling solution is continuous along the time (**Eq. 13**), and then, the liquid is constant along in the heat exchanger (**Eq. 14**):

$$M_3+M_5=M_6+M_7 \tag{12}$$

$$M_5= M_6 \tag{13}$$

$$M_3= M_7 \tag{14}$$

6) Also, if 5 g of sample was fed into the laboratory reactor, it is possible to obtain the composition of sugars (**Eq. 15**) and impurities (**Eq. 16)**, where **Eq. 15** is related to the mass entering the reactor with the sugars generated. **Eq. 16** is associated with the sample mass entering the reactor with the impurities generated. Thus, the sum of sugars produced on is industrial scale is 11.65 kg, and the impurities mass is 4.45 kg.

$$M_2\times0.0466=\sum_{i=10}^{i=15} M_i \tag{15}$$

$$M_9=M_2\times0.0178 \tag{16}$$

The evaluation of energetic efficiency in a scale-up project is necessary to optimize future implementation in an industrial process. The heat required for the reactor was calculated as 610 kJ kg$^{-1}$. Generally, in the industrial-scale (reactor capacity of 500 L), the pump operated with a power of 38.36 kW, and an efficiency of 50%. The heating agent with high pressure was adopted with a rate of 679.65 kg h$^{-1}$, with an engine's effectiveness in units of work done per unit of fuel as $285\times10^3$ kcal h$^-$

$^{1}$, and an efficiency of 70%. Finally, in the colling system (heat exchanger), colling water is using at a rate of 27×10$^{3}$ kg h$^{-1}$, assuming a heat transfer coefficient of 1 kW m² K$^{-1}$, and an efficiency of 90%. For continuous operation of SWH-P process, a high amount of energy is required; however, futures studies should be addressed to improve the heat required for an industrial reactor. With the adoption of a mass and energy balance demonstrated herein, it is possible to decrease the COM with the improvement of energetic efficiency drastically.

**Table 7.** Mass and energy balance for the integrated process for sugar production from BSG.

| Flow established | Mass fraction | | Unit |
|---|---|---|---|
| $M_1$ | $m_1$ | 1 | g water g$^{-1}$ water |
| $M_2$ | $m_{2,1}$ | 0.95 | g solids g$^{-1}$ solids |
| | $m_{2,2}$ | 0.05 | g water g$^{-1}$ water |
| $M_3$ | $m_{3,1}$ | 0.007 | g solids g$^{-1}$ solids |
| | $m_{3,2}$ | 0.993 | g water g$^{-1}$ water |
| $M_4$ | $m_{4,1}$ | 0.011 | g solids g$^{-1}$ solids |
| | $m_{4,2}$ | 0.989 | g water g$^{-1}$ water |
| $M_5$ | $m_5$ | 1 | g water g$^{-1}$ water |
| $M_6$ | $m_6$ | 1 | g water g$^{-1}$ water |
| $M_7$ | $m_{7,1}$ | 0.007 | g solids g$^{-1}$ solids |
| | $m_{7,2}$ | 0.993 | g water g$^{-1}$ water |
| $M_8$ | $m_8$ | 1 | g water g$^{-1}$ water |
| $M_9$ | $m_{9,1}$ | 0.45 | g furfural g$^{-1}$ furfural |
| | $m_{9,2}$ | 0.21 | g formic acid g$^{-1}$ formic acid |
| | $m_{9,3}$ | 0.34 | g acetic acid g$^{-1}$ acetic acid |
| $M_{10}$ | $m_{10}$ | 0.67 | g arabinose g$^{-1}$ arabinose |
| $M_{11}$ | $m_{11}$ | 0.03 | g galactose g$^{-1}$ galactose |
| $M_{12}$ | $m_{12}$ | 0.05 | g glucose g$^{-1}$ glucose |
| $M_{13}$ | $m_{13}$ | 0.19 | g xylose g $^{-1}$ xylose |
| $M_{14}$ | $m_{14}$ | 0.03 | g fructose g$^{-1}$ fructose |
| $M_{15}$ | $m_{15}$ | 0.03 | g sucrose g$^{-1}$ sucrose |

## 4. Conclusion

This study allowed to conclude that the flow-through subcritical water hydrolysis of brewer's spent grains followed by a SMB separation system can be a promising technology to produce isolated sugars on industrial scale. The lowest COM was obtained for the process with a residence time of 4.6 minutes and operating at 15 MPa and 160 ºC. The COM for sugars can address a perspective for a future application in a biorefinery. In an industrial-scale (3×500L) of SWH-P, the project implementation is economically applicable, and from the sensitivity analysis, the plant cost is the most affecting parameter. The process efficiency can be intensified from mass and energy balance to produce isolated sugars using subcritical water hydrolysis. Therefore, the technological route simulated and scaled-up is economically viable at the industrial scale. The biorefinery approach can be an alternative for the destination of brewer's spent grains generated by breweries.

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# CHAPTER V

## Techno-economic assessment of subcritical water hydrolysis of brewer’s spent grains to recover xylo-oligosaccharides

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# CHAPTER V: Techno-economic assessment of subcritical water hydrolysis of brewer's spent grains to recover xylo-oligosaccharides

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## Abstract

This study evaluated the techno-economic assessment of xylo-oligosaccharides (XOs) production from brewer's spent grains (BSG) in a single and two sequential flow-through subcritical water hydrolysis (SWH) reactors at different temperatures (80 – 180 °C). The process with a single reactor produced the highest yield (72.83 mg XOs $g^{-1}$ BSG) at 180 °C. The SWH in two sequential reactors produced up to 52.47 mg XOs $g^{-1}$ BSG for the process operated at 80 °C followed by 130 °C. The lowest cost of manufacturing (18.36 USD $kg^{-1}$ XOs) was obtained for the process with two sequential reactors. Although the fixed capital investment was 0.82-fold lower for the process with a single reactor, the gross profit of the process with two sequential reactors was 30% higher, which resulted in a return on investment of 54.26% and a payback of 1.84 years. In conclusion, SWH is a potential eco-friendly process to produce XOs from BSG.

***Keywords:*** *Lignocellulosic Biomass; Brewery by-products; Biorefinery; Value-added products; Biobased products; Pretreatment.*

## 1. Introduction

Oligosaccharides have been reported as functional ingredients due to their positive impact on human health [1]. Xylo-oligosaccharides (XOs) are short-chain carbohydrates that can be produced from lignocellulosic biomass [2]. XOs currently dominate the prebiotic category and are recognized for their functional food effects, which bring health benefits to the gastrointestinal tract [3,4]. Considering the remarkable functional effects of oligosaccharides, XOs promote the growth of probiotic cultures in the final gastrointestinal tract, such as those of the genus *Lactobacillus* spp. and *Bifidobacterium* spp., which mainly help to combat intestinal constipation, in addition to inhibiting the development of potentially pathogenic bacteria, such as *Clostridium perfringens*, *Escherichia coli*, and *Staphylococcus aureus* [5,6]. These prebiotics also have antioxidant activity and inhibit the development of cancer cells, especially in the small intestine, colon, and liver tissues; they promote the reduction of blood glucose, triglycerides, and cholesterol levels, helping to combat diabetes mellitus and obesity, and can also act as immunomodulating agents, helping to strengthen the immune system, especially in combating infectious diseases [7].

Generally, XOs are oligomers composed of xylose units containing β 1–4 bonds that are naturally found in vegetables and have been used to formulate functional foods [8]. However, there is a lack of literature regarding sustainable methods to produce XOs from lignocellulosic biomass. The exploration of an affordable strategy for lignocellulosic biomass conversion into value-added chemicals is urgently needed [9]. The global market for XOs is growing due to their wide application in animal feed, human food additives, and medicine [10]. The global prebiotics market reached 1,084 kilotons in 2020 [11]. The worldwide market for prebiotic XOs was valued at 102.1 million USD in 2020 and is expected to reach 135.7 million USD by the end of 2026 [12].

In recent years, the production of biobased products derived from lignocellulosic biomass has gained increased attention due to new sustainability policies for industry decarbonization [13]. The design of strategic biorefineries for the processing of biomass into value-added products and bioenergy acts as a promising mechanism for the circular economy transition [14]. Otherwise, the technical viewpoint for the conversion of lignocellulosic biomass remains a challenging task, requiring further development of eco-friendly technologies to produce biobased products [15].

Therefore, several pretreatments (i.e., acid, alkaline, enzymatic, mechanical, and chemical hydrolysis) have been proposed to promote the rupture of lignocellulosic materials [16,17]. However, conventional pretreatments have high costs and generate contaminants after the hydrolysis process, requiring additional steps for the post-treatment of the waste generated, resulting in a nonprofitable process for industrial implementation [18]. Subcritical water hydrolysis (SWH) can convert lignocellulosic biomass into chemicals and value-added bioproducts [19]. SWH consists of an eco-friendly process without the generation of contaminants and wastes and can be considered a promising technology for industrial implementation in biorefineries [20]. SWH is gaining popularity as an environmentally friendly technology to obtain reducing sugars; however, this technology needs further studies to optimize the process to produce XOs from lignocellulosic biomass [21].

Brewer’s spent grains (BSG) are the main solid waste remaining after the brewing process, accounting for 20 kg of wet BSG per 100 L of beer produced [22]. Brazilian beer production reached $14\times10^{6}$ L in 2019, which resulted in the generation of $2.8\times10^{6}$ ton $y^{-1}$ BSG (in wet weight) [23]. BSG is considered lignocellulosic biomass because of its chemical composition, which is based on cellulose (17.9%), hemicelluloses (35.7%), and lignin (17.8%) as major components [24]. Due to its abundance and low cost, BSG is considered a feedstock of interest to be used in a range of bioprocess applications after an appropriate pretreatment, including the recovery of XOs in a biorefinery concept [25].

In previous studies, the SWH of BSG was evaluated [24,26]. Sganzerla et al. [26] developed and optimized a semi-continuous process using SWH in a single and two sequential flow-through reactors for the recovery of sugars and amino acids. The process was designed for the extraction of bioproducts (such as bioactive compounds and proteins) at low temperature in the first reactor, followed by an additional extraction/hydrolysis in the second reactor, which can be operated at higher temperatures. The results obtained highlighted that the recovery of monosaccharides (7.76 mg $g^{-1}$ carbohydrates) and amino acids (43.62 mg $g^{-1}$ proteins) from BSG was higher with the use of a single subcritical reactor operated at 180 °C [26]. However, there is a lack of literature regarding the recovery of XOs with the use of a single and two sequential flow-through subcritical water reactors.

Based on the abovementioned, this study aimed to evaluate the production of XOs from a laboratory-scale semi-continuous flow-through SWH process with a single and two-sequential reactors. Furthermore, this study addressed the economic analysis of large-scale XOs production based on the data obtained in the laboratory-scale process. The novelty of this study lies in the technical and economic analysis related to the use of a single and two sequential flow-through subcritical reactors, which contributes to elucidating some bottlenecks in the industrial production of XOs from the SWH of BSG.

## 2. Materials and methods

### *2.1. Raw material*

BSG was supplied by Ambev brewery (Jaguariúna, SP, Brazil). The raw material was dried in an air convection oven (45 °C, 24 h) until reaching a moisture of 9% (w/w). The size reduction was carried out in a multiprocessor until it reached an average particle size of 0.41 mm [27]. The BSG used for SWH presents 9.03 ± 0.04% moisture, 3.65 ± 0.01% ash, 19.72 ± 1.02% crude protein, a pH of 6.25 ± 0.03, 6.12 ± 0.44% soluble lignin, 11.75 ± 0.18% insoluble lignin, 17.90 ± 0.02% cellulose, and 35.70 ± 0.02% hemicellulose, characterized according to the methodologies established by the National Renewable Energy Laboratory (NREL) [28–30].

### *2.2. Subcritical water hydrolysis of brewer's spent grains*

The process for SWH of BSG was conducted in single and two sequential reactors (patent pending BR1020150314019) [31,32]. The system is composed of a high-pressure liquid pump (double piston pump, Model 36 preparation pump, Apple Valley, MN, USA) to pressurize and feed water to the subcritical reactor. The hydrolysis vessels consist of 316-stainless steel tubes with an internal volume of 110 mL. A thermal jacket was used to deliver 1500 W heat to the reactor, which was insulated by a ceramic fiber. The temperature was controlled using two thermocouples (type K) located in the entrance and outlet of the reactor. The product exiting the hydrolysis reactor is cooled in a serpentine coupled to a thermostatic bath (Marconi, model MA-184). The system pressure is controlled by a micrometer valve (Autoclave Engineers, model 10VRMM2812) located after the liquid exchanger. The pressure in the system is measured by pressure gauges from WIKA company (0 – 50 MPa), with an accuracy of up to 0.1%. **Fig. 1** presents the laboratory-scale diagram of the subcritical water unit with single and two sequential reactors.

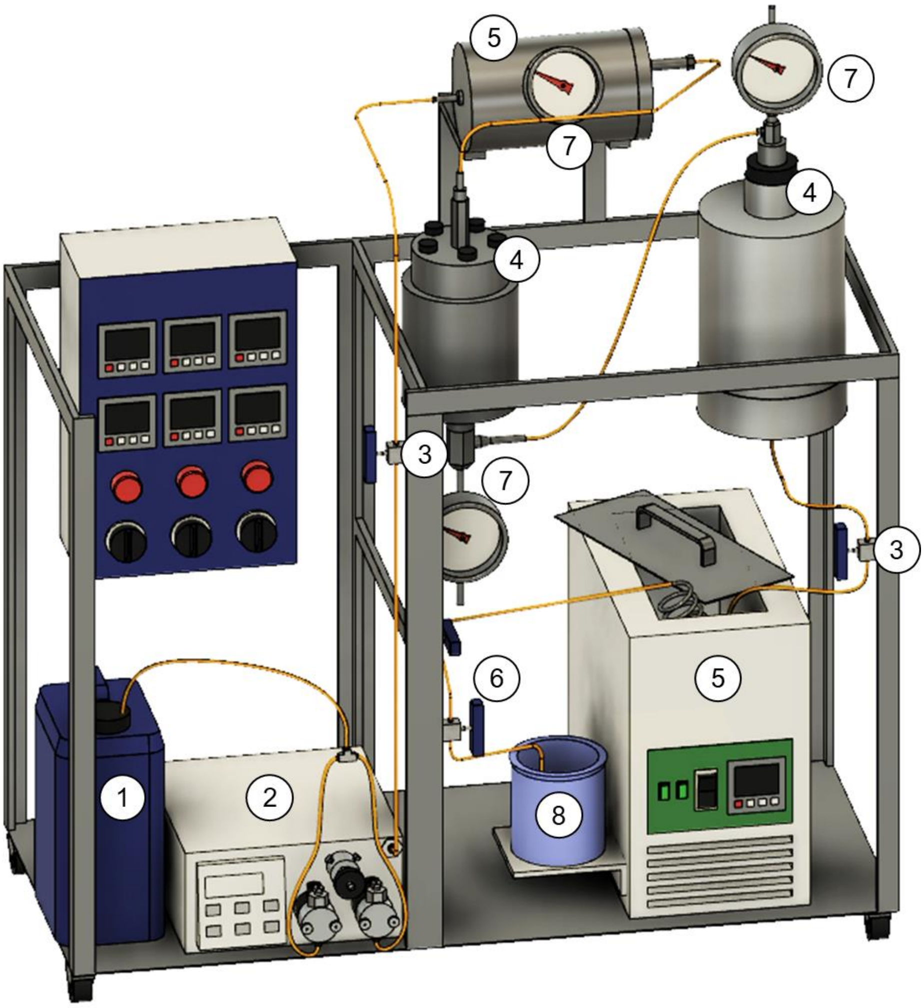

**Figure 1.** Laboratory-scale subcritical water hydrolysis process. Adapted from Barroso et al [32]. Label: 1, water tank; 2, high-pressure pump; 3, block valve; 4, subcritical reactor; 5, heat exchanger; 6, micrometric valve; 7, manometer; and 8, collecting vessel.

In each experiment, 10 g of BSG was loaded into each reactor, which was operated in semi-continuous mode. The reactor was filled with water from the pump to reach a pressure of 15 MPa, which was constant for all experiments. The hydrolysis was performed with a water flow of 5 mL min$^{-1}$ for 60 min (collect times of 5, 10, 15, 20, 30, 40, and 60 min). The influence of the hydrolysis temperature (80, 130, and 180 °C) on the subcritical process was studied. In the case of two sequential processes, the first reactor was always operated at 80 °C, and the hydrolysate of this reactor was used in the second reactor, which was operated at 80, 130, and 180 °C. The residence time of the solvent in the process was calculated according to **Eq. 1**.

$$\tau = \frac{V_R\ \rho_R\ (T,P)}{\vartheta_0\ \rho_0} \quad (1)$$

*where* $\tau$ is the residence time of the solvent, $V_R$ is the reactor fluid volume (110 mL), $\rho_R$ is the density of the fluid at the temperature and pressure used in the process, $\vartheta_0$ is the flow rate of the solvent at 25 °C (5 mL $\text{min}^{-1}$), and $\rho_0$ is the density of the solvent at 25 °C (1.08 g $\text{mL}^{-1}$). The $\rho_R$ was estimated using NIST steam tables [33]. The $\rho_R$ was 0.978, 0.942, and 0.896 g $\text{mL}^{-1}$ for the use of water at 80, 130, and 180 °C with a pressure of 15 MPa, respectively. In this experiment, the residence time of the fluid in the reactors was 19.92, 19.18, and 18.25 min for the use of 80, 130, and 180 °C, respectively.

In total, six experiments were randomly performed in duplicate to estimate the experimental uncertainty and establish reproducibility. The following codes were used to identify the hydrolysates: SWH 1, single reactor operated at 80 °C; SWH 2, single reactor operated at 130 °C; SWH 3, single reactor operated at 180 °C; SWH 4, two sequential reactors operated at 80 °C (first reactor) and 80 °C (second reactor); SWH 5, two sequential reactors operated at 80 °C (first reactor) and 130 °C (second reactor); and SWH 6, two sequential reactors operated at 80 °C (first reactor) and 180 °C (second reactor).

### *2.3. Chemical composition of the hydrolysate*

#### *2.3.1. Quantification of xylose and XOs*

Xylose and XOs with degrees of polymerization (DPs) between 2 and 6 were quantified using high-performance anion exchange-pulsed amperometric detection chromatography (HPAE–PAD). The chromatographic analysis was conducted on a Dionex DX-500 System (Sunnyvale, CA, EUA) with a CarboPac PA1 column (4 mm × 250 mm), a CarboPac PA1 guard column (4 mm × 50 mm), and an electrochemical detector, adopting a linear gradient of A (NaOH 100 mmol $\text{L}^{-1}$) and B (NaOAc 300 mmol $\text{L}^{-1}$; NaOH 100 mmol $\text{L}^{-1}$). The flow rate was constant at 1 mL $\text{min}^{-1}$ and the running time was 23 min. For the analysis, the hydrolysates were centrifuged (10,000 × g) and filtered (nylon 0.22 µm). The injection volume was 10.0 µL. The integrated peak areas were adjusted based on standards purchased from Megazyme® (Bray, County Wicklow, Ireland): xylose ($X_1$), xylobiose ($X_2$), xylotriose ($X_3$), xylotetraose ($X_4$), xylopentaose ($X_5$), and xylohexaose ($X_6$). Xylose and XOs were quantified at all kinetic points, and at 40 min, the hydrolysate was used to quantify the global yield of the process.

### *2.3.2. Calculations*

The yield of XOs (**Eq. 2**) was calculated considering the amount of $X_2$-$X_6$ produced in the hydrolysate (mg $L^{-1}$), the total hemicellulose content in BSG (0.357 g $g^{-1}$), and the solvent-to-feed ratio (S/F, g water $g^{-1}$ BSG) employed in the SWH, which was equal to 20 for the process with a single reactor and 10 for the process with two sequential reactors.

$$\text{XOs yield}\left(\frac{mg_{XOs}}{g}\right)=\frac{X_2+X_3+X_4+X_5+X_6}{\text{Hemicellulose}}\times\frac{S}{F} \qquad (2)$$

The XOs purity (**Eq. 3**) was determined considering the total XOs produced in the hydrolysate ($X_2$-$X_6$, mg $L^{-1}$) in relation to the concentration of XOs and xylose (mg $L^{-1}$).

$$\text{XOs purity}(\%)=\frac{\text{XOs}}{\text{Xylose+XOs}}\times100 \quad (3)$$

### *2.3.3. Quantification of antioxidant activity*

The antioxidant activity of the hydrolysates was determined by the inhibition of the DPPH free radical [34]. The reaction was composed of 150 µL of hydrolysate and 2850 µL of DPPH 0.1 mM solution. The measurement was performed at 515 nm after 30 min of incubation (UV–Vis spectrophotometer, model 752D, Labman, China). The antioxidant capacity was also obtained through ferric reduction antioxidant power (FRAP) [35,36]. The reaction was composed of 100 µL of hydrolysate, 100 µL of ferric chloride (3 mmol $L^{-1}$), and 1800 µL of TPTZ (1 mmol $L^{-1}$). After 30 min in a water bath (37 °C), the absorbance was measured at 620 nm (UV–Vis spectrophotometer, model 752D, Labman, China). The results of antioxidant activity (DPPH and FRAP assays) were expressed as mg of Trolox equivalent antioxidant capacity (TEAC) per mL hydrolysate (mg TEAC $mL^{-1}$).

### *2.3.4. Statistical analysis*

Analysis of variance (ANOVA) was employed to assess statistically significant factors and interactions between the variables. Significant differences between the samples were evaluated by Tukey's test (*p* ≤ 0.05). The statistical analysis was conducted using Statistica® software (version 10.0, StatSoft Inc., Tulsa, OK, USA).

## *2.4. Economic analysis*

### *2.4.1 Simulation flowsheet and Gantt chart*

The simulation flowsheet and Gantt chart were generated using SuperPro Designer 9.0® software (Intelligen Inc., Scotch Plains, NJ, USA). The flowsheet and Gantt charts were designed to represent the industrial process of SWH in a single reactor (**Fig. 2a**) and two sequential reactors (**Fig. 2b**).

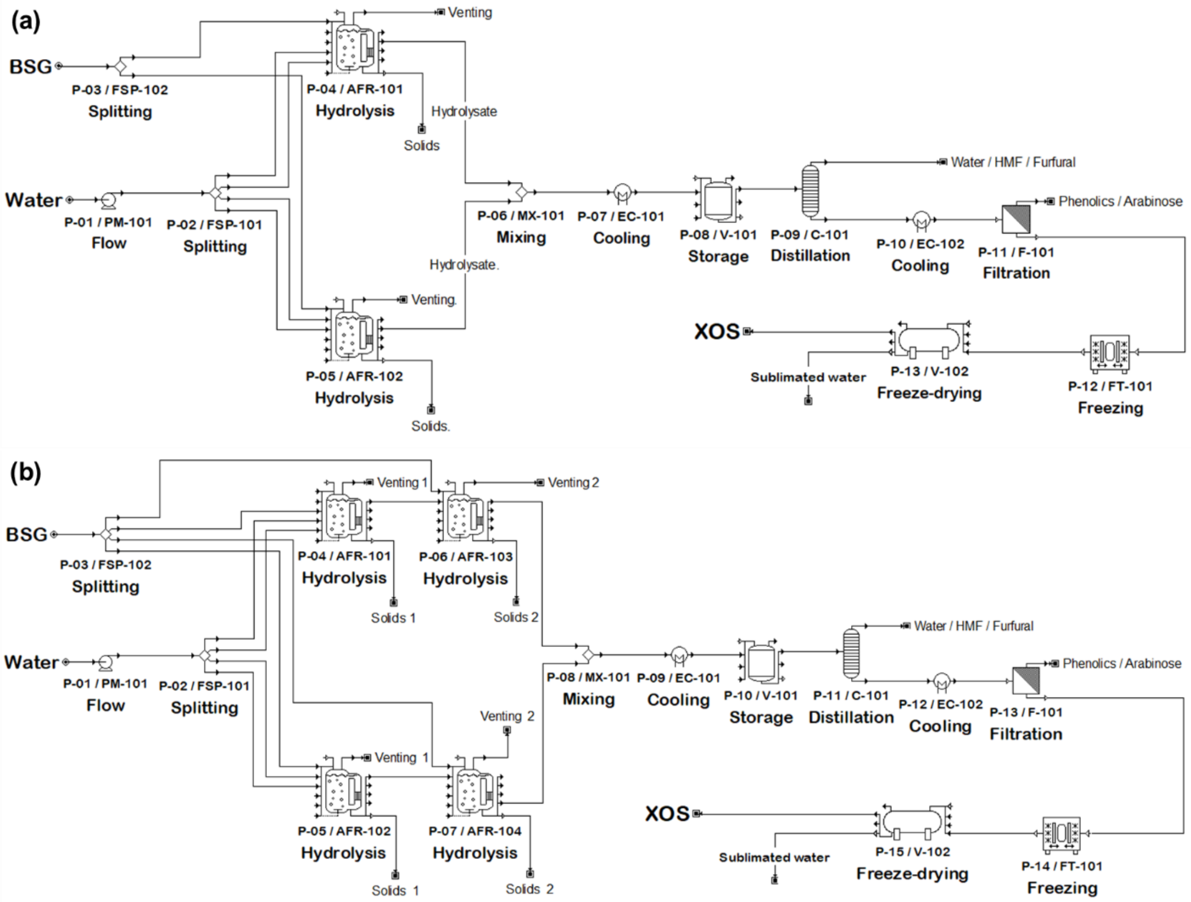

**Figure 2.** Simulation flowsheet designed for XOs production from BSG with SWH. (a) Process intensification with a single reactor. (b) Process intensification with two sequential reactors.

The process starts with the flow of water and BSG and splits into subcritical reactors (P−4/AFR−101 and P−5/AFR−102 for the process with a single reactor and P−4/AFR−101, P−5/AFR−102, P−6/AFR−103, and P−7/AFR−104 for the process with two sequential reactors). After the hydrolysis condition was reached, the hydrolysate was mixed, cooled to a temperature of 25 °C, and stored. Then, the hydrolysate was distilled to separate water, 5-hydroxymethylfurfural, and furfural. In the simulation presented, the distillation process was applied because it is considered a robust and efficient method that can be easily implemented on a large scale [37]. The cooled distilled hydrolysate was filtered through a gel filtration process to eliminate the remaining formic acid, phenols, and sugars. Finally, this hydrolysate was freeze-dried to produce a powder rich in XOs. The Supplementary Material presents the detailed flowsheet and Gantt charts for all the scenarios studied. The flow diagram and Gantt chart of scenarios 1, 2, and 3 are the same, except that the process presents different temperatures in the subcritical reactor. In the case of the process with two sequential subcritical reactors, the temperature is 80 °C in the first reactor, and the second reactor presents different temperatures (80, 130, and 180 °C), which refer to the experimental design adopted and described in the methodology.

### *2.4.2. Economic assessment*

The economic assessment was performed using SuperPro Designer 9.0® software (Intelligen Inc., Scotch Plains, NJ, USA). In the scaled-up process, an economic analysis was conducted based on the annual production of XOs, which was determined based on the experimental production of XOs in the laboratory-scale semi-continuous flow-through SWH process. To evaluate the profitability parameters, the selling price of XOs was estimated as 50 USD kg$^{-1}$ [38]. In this study, the following parameters were used for techno-economic simulation: cost of BSG, 8 USD ton$^{-1}$; cost of water for the process, 2 USD ton$^{-1}$; cost of water for cooling, 0.05 USD ton$^{-1}$; cost of steam, 4 USD ton$^{-1}$; cost of electricity, 0.2 USD kWh$^{-1}$; cost of $CaCl_2$ brine, 0.25 USD ton$^{-1}$; cost of wage (with benefits), being 2 operators for hydrolysis, 1 for distillation, 1 for filtration, 1 for drying, 1 for vacation, and 1 supervisor, 8 USD wage$^{-1}$ h$^{-1}$; operation time, 7920 h year$^{-1}$; project lifetime, 25 years; inflation, 4% year$^{-1}$; low-interest rate, 7%; depreciation period, 15 years; loan period for equipment, one year; loan interest for equipment, 6% year$^{-1}$; and loan, 100% [39–41]. The equipment installation cost of SWH was obtained by the market price and considering the scale-up to an industrial scale [42]. Furthermore, the cost regarding the purification of XOs was obtained by considering the default data already established in the SuperPro Designer 9.0® software and adjusted based on the Brazilian environment [38].

For the proposed process, the five major components of the cost of manufacturing (COM) were determined based on the sum of the main components in the process (direct costs, fixed costs, and general expenses), according to the method described by Turton et al. [43] (**Eq. 4**).

$$\mathrm{COM}=0.304\times \mathrm{FCI}+2.73\times \mathrm{COL}+1.23\times(\mathrm{CUT}+\mathrm{CWT}+\mathrm{CRM}) \quad (4)$$

*where* FCI is the fixed capital investment; COL is the cost of operational labor; CUT is the cost of utilities; CWT is the cost of waste treatment; and CRM is the cost of raw material. All the costs were normalized per year of investment to determine the COM. FCI is related to expenses involved in the implementation of the production unit. CRM consists of the costs required to prepare the raw material and the costs of chemicals. COL is related to operators (number and wage) of the unit. CUT considers the energy and cleaning water used in the process, among others. CWT is the residue generated by this process, considered zero in this study since the by-products generated can be used in additional processes.

### *2.4.3. Project feasibility*

The project feasibility was analyzed from the following indicators: gross margin (GM); net margin (NM); net present value (NPV); return on investment (ROI); and payback. GM is the difference between the revenue and the costs of the goods sold, while NM is the GM minus operational expenses and all other expenses. NPV is the difference between the present value of cash inflows and outflows over a period. NPV is used to determine the profitability of a project, and it can be calculated by **Eq. 5** [43].

$$\mathrm{NPV}=\sum_{t=1}^{n}\frac{\mathrm{FC}_t}{(1+i)^t}-I_0 \quad (5)$$

*where* $\mathrm{FC}_t$ is the cash flow in “t” time; “t” is the time in which the money will be invested (year); “n” is the project lifetime; “i” is the cost of the capital; and “$I_0$” is the initial investment.

ROI is a decision tool from a business perspective used for capital budgeting and to evaluate the performance of an investment project, as estimated by **Eq. 6** [44].

$$\mathrm{ROI}\ (\%)=\frac{\text{Annual net profit}}{\text{Total capital investment}} \quad (6)$$

Payback is the time in years required to recover the original investment (**Eq. 7**) [43].

$$\text{Payback (year)}= \frac{\text{Total capital investment}}{\text{Annual net profit}} \quad (7)$$

*2.4.4. Sensitivity analysis*

The effect of XOs production with SWH was evaluated by a sensitivity analysis. In the current scale-up project, the FCI, COL, CUT, CRM, and selling price of XOs were evaluated by a sensitivity analysis, with a variation of ±30%. These variables were adopted since they were the most influential variables on an industrial scale and directly affected the project's feasibility. The cash flow was modeled to obtain the ROI and NPV.

## 3. Results and discussion

### *3.1. Hydrolysis kinetics of xylose and XOs*

XOs are obtained from the depolymerization of xylan present in the hemicellulose fraction of lignocellulosic biomass [45]. Kinetic assays were performed to evaluate the evolution of the release of XOs from the SWH of BSG (**Fig. 3**). Time and temperature are among the main parameters to be evaluated to obtain maximum efficiency in the conversion of SWH processes [21]. Moreover, the concentration of total XOs (sum of $X_2$ - $X_6$) released as a function of time in the hydrolysis process and the accumulated concentration of oligosaccharides throughout the process were monitored (**Fig. 4a-b**). In all assays, an increase in the production rate of XOs was observed during the first 5 min of the process, decreasing after 10 min. The reaction temperature significantly affected the concentrations of XOs. Among the processes with a single reactor, SWH 3 (180 °C), with the highest temperature, presented a higher release rate of XOs. In the processes with two sequential reactors, SWH 5 (80 °C in the first reactor followed by 130 °C in the second reactor) presented the highest hydrolysis efficiency and consequently the highest accumulated concentration of XOs (5246.75 mg $L^{-1}$) among all the tests performed. In hydrothermal processes, the action of pressure and temperature for a certain residence time promotes the liberation of acetyl groups from hemicellulose, acidifying the medium through the release of hydronium ions and promoting the depolymerization of xylan [21]. Hydrolysis conditions by this method must be moderate to avoid excessive production of xylose, in addition to inhibitors such as furfural and 5-hydroxymethylfurfural [45].

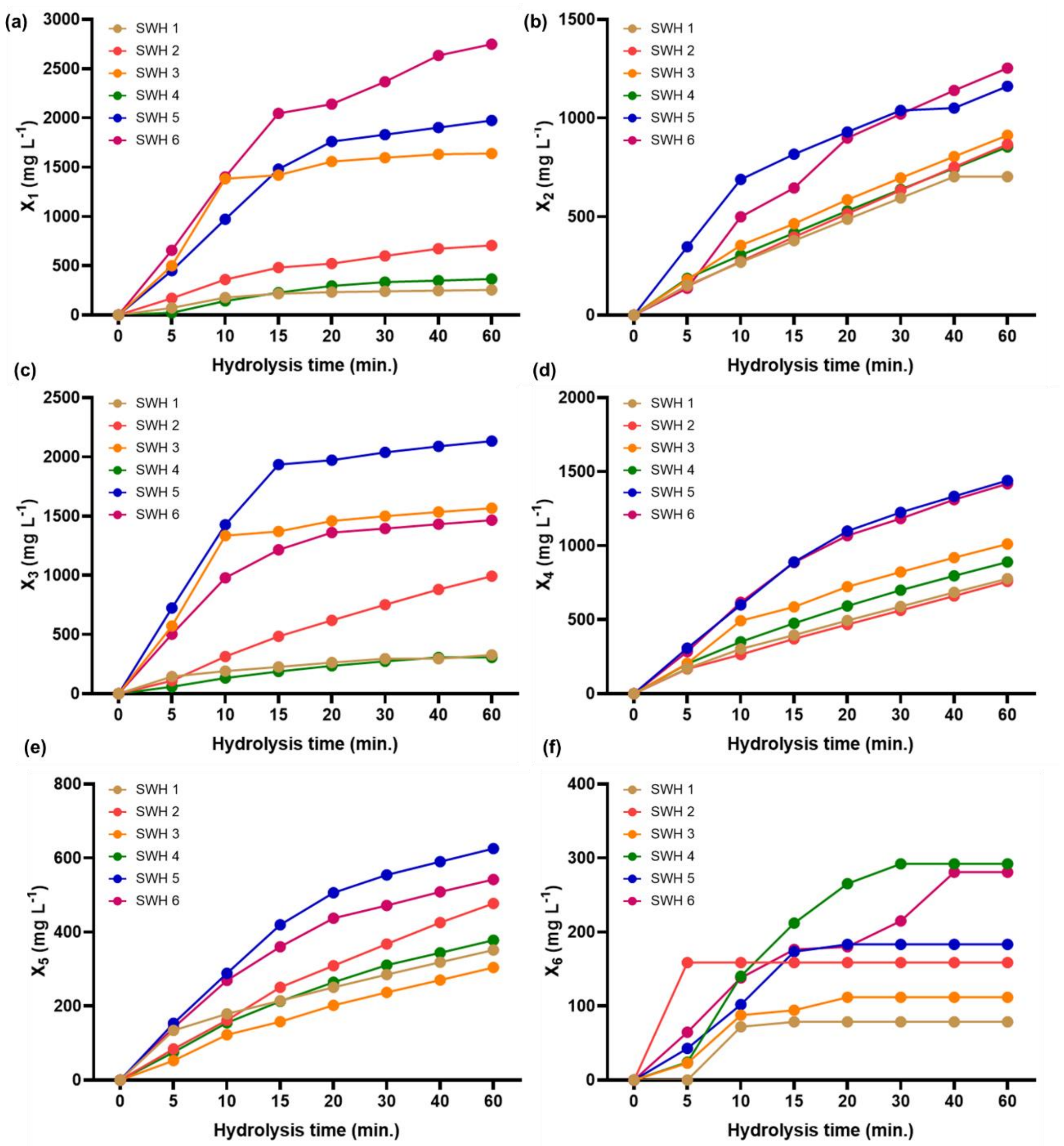

**Figure 3.** Kinetic profile of xylose ($X_1$), xylobiose ($X_2$), xylotriose ($X_3$), xylotetraose ($X_4$), xylopentaose ($X_5$), and xylohexaose ($X_6$) obtained from the SWH of BSG.

The conversion of BSG to XOs (**Fig. 4c**) and the hydrolysis efficiency of hemicellulose (**Fig. 4d**) were calculated to estimate the global yield of the process. For the process with a single reactor, the highest yield (72.83 mg XOs g$^{-1}$ BSG and 204.01 mg XOs g$^{-1}$ hemicellulose) was obtained for SWH 3 (180 °C). However, for the process with two sequential reactors, the yield reached the highest value of 52.47 mg XOs g$^{-1}$ BSG and 146.97 mg XOs g$^{-1}$ hemicellulose for the process operated with the first reactor at 80 °C, followed by the second reactor at 130 °C. Monteiro et al. [21] studied the application of hydrothermal treatment in the depolymerization of hemicellulose from mango seed husks to produce XOs, obtaining a maximum yield of 393.44 mg XOs g$^{-1}$ hemicellulose at 2.5 MPa, 15 min, and 180 °C. Sing et al. [46] evaluated the production of XOs from almond shells using hydrothermal treatment at 180 °C for 10 min and 2.5 MPa, obtaining a yield of 540 mg XOs g$^{-1}$ hemicellulose. The results suggest that the yield of XOs depends directly on the composition of the lignocellulosic biomass and the operational conditions used during the hydrothermal pretreatment [47]. Therefore, BSG applied under the conditions of SWH 3 (single reactor) and SWH 5 (two sequential reactors) can become advantageous for the recovery of XOs because of the high yields obtained.

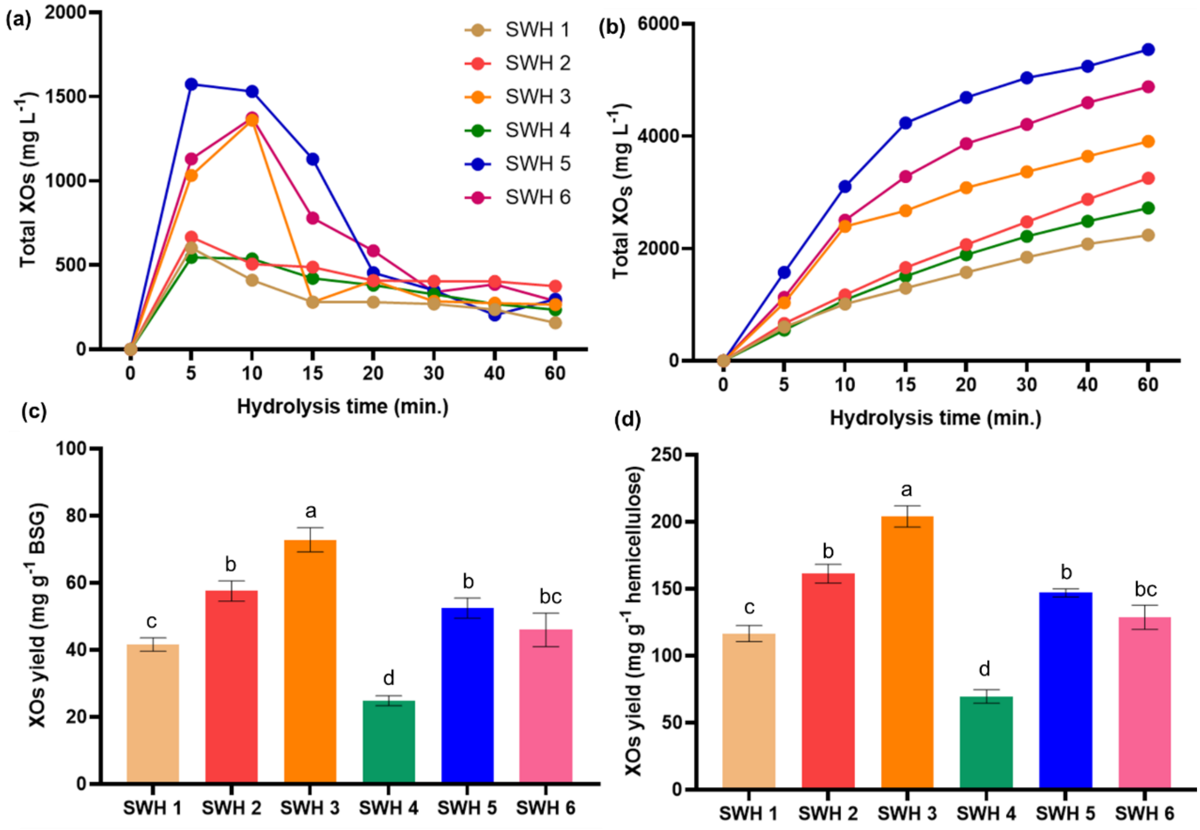

**Figure 4.** Kinetics and yield of total XOs (X$_2$-X$_6$) obtained from the SWH of BSG. (a) the non-accumulated concentration of XOs in the hydrolysate; (b) accumulated concentration of XOs in the hydrolysate; (c) yield of XOs in relation to the mass of biomass used in the SWH; and (d) yield of XOs in relation to the mass of hemicellulose used in the SWH.

Knowledge of the profile and composition of XOs in the hydrolysate is an indispensable and significant factor in determining the functional and nutraceutical properties of the oligomers. **Table 1** shows the accumulated concentrations of XOs and xylose (mg $L^{-1}$) in the hydrolysates obtained within 40 min of the process. From the kinetic point of view, except for xylohexose ($X_6$), a gradual increase in the concentration of XOs and xylose was observed in all assays. The longest chain XOs ($X_5$ and $X_6$) were obtained at lower concentrations, ranging from 270.67 to 590.6 mg $X_5$ $L^{-1}$ and 78.77 to 292.22 mg $X_6$ $L^{-1}$. This fact can be explained by the use of high temperature breaking the xylan bonds and releasing the long-chain XOs (e.g., $X_5$ and $X_6$), which during the SWH can undergo autohydrolysis and release short-chain XOs (e.g., $X_2$, $X_3$, and $X_4$) and xylose [45]. Xylotriose ($X_3$) was the short-chain XOs with the highest concentration observed, ranging from 295.65 – 2088.82 mg $L^{-1}$, corresponding to approximately 40% of the XOs produced in SWH 5. In addition, xilotetrose ($X_4$) ranged from 660.38 to 1333.32 mg $L^{-1}$, and xylobiose ($X_2$) ranged from 702.75 – 1140.03 mg $L^{-1}$. Similar profiles of short-chain XOs were reported when evaluating the production of XOs from the almond shell; using hydrothermal treatment, 42% of $X_2$ and $X_3$ were obtained [46]. The concentration of xylose ranged from 248.72 to 2634.08 mg $L^{-1}$. The purity of XOs obtained in this study was higher than 60% (**Table 1**). The use of lower temperatures promoted the highest purity. For instance, the process operated at 80 °C promoted a purity higher than 87%, and the purity decreased to 63% for 180 °C, which is a desirable purity for a commercial production of XOs [48,49]. Higher temperatures favored greater monomer release, especially in SWH 6, in which the use of sequential reactors at 180 °C promoted the highest release of monosaccharides [31]. This operational condition is excessive, promoting the hydrolysis of XOs into xylose. Depending on the desired degree of purity of the XOs produced, the hydrolysate could go through a nanofiltration system for the separation of xylose and inhibitors, being able to redirect and apply this monomer as a carbon source for other bioprocesses [50]. Therefore, to improve the XOs purity by removing the xylose, the application of nanofiltration for downstream processing can be considered a future alternative. However, purification of XOs with nanofiltration is considered an expensive process that demands further optimization for large-scale applications [51].

**Table 1.** Concentration of XOs in the hydrolysates obtained in a single and two sequential subcritical reactors collected at 40 min.

| Parameters | Single reactor | | | Sequential reactors | | | Unit |
|---|---|---|---|---|---|---|---|
| | SWH 1 | SWH 2 | SWH 3 | SWH 4 | SWH 5 | SWH 6 | |
| $X_1$ | $248.72 \pm 4.25^{e}$ | $672.97 \pm 8.34^{d}$ | $1630.88 \pm 12.87^{c}$ | $349.72 \pm 4.21^{e}$ | $1902.05 \pm 2.47^{b}$ | $2634.08 \pm 4.96^{a}$ | mg $L^{-1}$ |
| $X_2$ | $702.75 \pm 3.25^{e}$ | $751.65 \pm 3.75^{d}$ | $804.92 \pm 7.36^{c}$ | $745.58 \pm 2.67^{d}$ | $1050.46 \pm 2.94^{b}$ | $1140.03 \pm 3.48^{a}$ | mg $L^{-1}$ |
| $X_3$ | $295.65 \pm 5.28^{d}$ | $881.44 \pm 5.24^{c}$ | $1534.66 \pm 9.25^{b}$ | $306.98 \pm 4.72^{d}$ | $2088.82 \pm 3.98^{a}$ | $1431.61 \pm 4.27^{b}$ | mg $L^{-1}$ |
| $X_4$ | $685.2 \pm 7.31^{d}$ | $660.38 \pm 3.93^{d}$ | $919.45 \pm 5.82^{b}$ | $796.35 \pm 3.85^{c}$ | $1333.32 \pm 1.28^{a}$ | $1311.95 \pm 10.45^{a}$ | mg $L^{-1}$ |
| $X_5$ | $318.51 \pm 1.35^{e}$ | $425.77 \pm 5.82^{c}$ | $270.67 \pm 2.45^{f}$ | $344.2 \pm 3.27^{d}$ | $590.65 \pm 3.29^{a}$ | $508.81 \pm 0.98^{b}$ | mg $L^{-1}$ |
| $X_6$ | $78.77 \pm 2.35^{d}$ | $158.95 \pm 5.96^{b}$ | $111.86 \pm 3.76^{a}$ | $292.22 \pm 1.83^{a}$ | $183.5 \pm 4.26^{b}$ | $280.95 \pm 0.93^{a}$ | mg $L^{-1}$ |
| Total XOs | $2080.88 \pm 3.32^{e}$ | $2878.19 \pm 4.28^{d}$ | $3641.56 \pm 6.93^{c}$ | $2485.33 \pm 5.29^{d}$ | $5246.75 \pm 4.97^{a}$ | $4595.83 \pm 5.39^{b}$ | mg $L^{-1}$ |
| XOs purity | $89.32 \pm 2.18^{a}$ | $81.05 \pm 3.17^{b}$ | $69.07 \pm 1.82^{c}$ | $87.66 \pm 2.64^{a}$ | $73.39 \pm 3.18^{c}$ | $63.57 \pm 2.73^{d}$ | % |

The results are expressed as the mean ± standard deviation. Analysis was conducted at least in triplicate. Different letters in each line indicate significant differences by Tukey's test at $p \leq 0.05$. Label: SWH 1, R1 at 80 °C; SWH 2, R1 at 130 °C; SWH 3, R1 at 180 °C; SWH 4, R1 at 80 °C and R2 at 80 °C; SWH 5, R1 at 80 °C and R2 at 130 °C; SWH 6, R1 at 80 °C and R2 at 180 °C; $X_1$, xylose; $X_2$, xylobiose; $X_3$, xylotriose; $X_4$, xylotetraose; $X_5$, xylopentaose; $X_6$, xylohexaose; total XOs represent the sum of $X_2$ until $X_6$.

Nevertheless, the use of SWH in a single reactors at 180 °C resulted in a hydrolysate composed by cellobiose (0.14 ± 0.01 g $L^{-1}$), glucose (0.05 ± 0.01 g $L^{-1}$), xylose (0.47 ± 0.04 g $L^{-1}$), arabinose (1.04 ± 0.05 g $L^{-1}$), formic acid (0.09 ± 0.01 g $L^{-1}$), acetic acid (0.25 ± 0.01 g $L^{-1}$), furfural (310.71 ± 42.11 mg $L^{-1}$), histidine (2.91 ± 0.20 mg $L^{-1}$), threonine (3.45 ± 0.05 mg $L^{-1}$), valine (64.35 ± 1.65 mg $L^{-1}$), tryptophan (215.55 ± 3.55 mg $L^{-1}$), phenylalanine (9.35 ± 0.15 mg $L^{-1}$), isoleucine (4.21 ± 0.41 mg $L^{-1}$), leucine (11.45 ± 0.45 mg $L^{-1}$), lysine (16.55 ± 0.35 mg $L^{-1}$), aspartic acid (123.35 ± 7.55 mg $L^{-1}$), arginine (4.10 ± 0.60 mg $L^{-1}$), glutamic acid (4.60 ± 0.70 mg $L^{-1}$), asparagine (2.24 ± 0.21 mg $L^{-1}$), serine (7.63 ± 0.12 mg $L^{-1}$), glutamine (0.75 ± 0.15 mg $L^{-1}$), glycine (16.11 ± 0.40 mg $L^{-1}$), alanine (13.21 ± 0.10 mg $L^{-1}$), tyrosine (4.65 ± 0.35 mg $L^{-1}$), and proline (5.40 ± 0.10 mg $L^{-1}$) [31].

After SWH, the solid fraction remaining in the reactor was lower for the process operated at a higher temperature. The mass reduction of BSG after SWH was 20.4 ± 7.7, 57.8 ± 8.2, and 69.8 ± 1.3% for the reactors operated at 80, 130, and 180 °C, respectively. The results obtained are expected for a process involving the solubilization of hemicellulose during pretreatment. The chemical composition of the solid residue after the SWH of BSG was determined in previous studies [24,26]. After the SWH, the semi-volatile compounds decreased due to the possible migration from BSG to the hydrolysate. In addition, hemicellulose dissociation occurred during the SWH, decreasing from 27% (BSG) to 14% (reactor operated at 180 °C). In a circular economy, the designed process can be an alternative to treat lignocellulosic by-products, especially due to the high mass reduction obtained. The solid residues remaining in the reactor after hydrolysis can be applied to produce silica [52], biochar [53], oxygenated hydrocarbons [54], and adsorbent materials [55]. Finally, the development of an eco-friendly process with zero waste generation that can produce a hydrolysate rich in short-chain XOs is desirable for further industrial implementation.

### *3.2. Antioxidant activity*

The *in vitro* antioxidant potential of hydrolysates containing XOs obtained from the SWH process was evaluated by DPPH and FRAP assays (**Fig. 5).** The DPPH free radical scavenging assay is based on the measurement of the discoloration resulting from the reduction of the DPPH free radical by an antioxidant. The reaction mechanism involves the transfer of electrons by the reducing agent to the DPPH radical. The FRAP method is based on iron reduction. Both methods are valid, easy, accurate, sensitive, and economical for assessing the sequestering activity of antioxidant plant extracts [4]. Regarding the results, the same profile of antioxidant activity was obtained between the two assays. The antioxidant activity obtained by the DPPH ranged from 0.03 to 0.24 mg TEAC mL$^{-1}$, while the FRAP assays ranged from 0.04 to 0.37 mg TEAC mL$^{-1}$. SWH 1 and SWH 4 showed the lowest values of antioxidant activity and corresponded to assays with lower concentrations of XOs. The antioxidant activity increased proportionally with increasing temperature. This can be attributed to the presence of XOs in the hydrolysate. However, the increase in antioxidant activity is also associated with Maillard reaction products due to the caramelization of sugar and amino acid breakdowns [56]. Finally, the presence of phenolic compounds affects the antioxidant capacity of the hydrolysate [57].

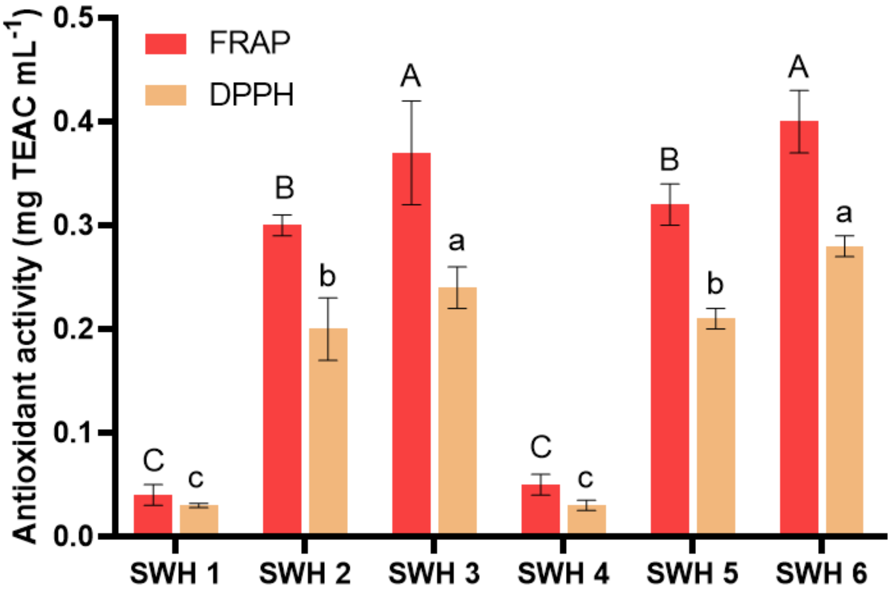

**Figure 5.** Total antioxidant activity of the hydrolysate obtained by the SWH of BSG. Label: Different letters (lowercase for DPPH reactor and uppercase for FRAP) indicate significant differences by Tukey's test at $p \leq 0.05$.

Compared with the literature, the *in vitro* antioxidant potential of XOs obtained in this study is similar to the antioxidant activity of XOs produced from coffee husks and sugarcane straw [4]. In addition, the antioxidant activity of the XOs is dependent on the concentration and reached saturation at 2 g L$^{-1}$ [4], which corroborates the present study for the SWH 2, SWH 3, SWH 5, and SWH 6 hydrolysates that presented concentrations higher than 2 g XOs L$^{-1}$. The assay with the highest concentration of

XOs (SWH 5) showed a total antioxidant activity of 0.21 mg TEAC $mL^{-1}$ for DPPH and 0.32 mg TEAC $mL^{-1}$ for FRAP.

### *3.3. Economic analysis*

#### *3.3.1. Cost analysis*

**Table 2** presents the itemized costs and FCI for industrial process implementation [38,40,58].

**Table 2.** Base costs to each part of the subcritical water process for XOs production from BSG.

| Component | Number or capacity | Individual cost (USD)* | Total Cost (USD) |
|---|---|---|---|
| Pump | 1 | 395,072.50 | 395,072.50 |
| Manometer | 6 | 93.33 | 559.98 |
| Structural material | - | 24,298.72 | 24,298.72 |
| Exchange heat | 1 | 160,654.97 | 160,654.97 |
| Blocking valve | 2 | 2,662.21 | 5,324.42 |
| Micrometric valve | 1 | 7,558.21 | 7,558.21 |
| Backpressure valve | 1 | 19,058.72 | 19,058.72 |
| Temperature controller | 1 | 5,217.05 | 5,217.05 |
| Control panel | 1 | 83,472.83 | 83,472.83 |
| Subcritical reactor | 2 | 499,095.21 | 998,190.43 |
| Piping, connectors, crossheads, and splitters | 1 | 49,212.45 | 49,212.45 |
| Flat bottom tank | 300 L | 32,000.00 | 65,898.76 |
| Batch distillation vessel | 89.9 L | 455,000.00 | 1,273,906.59 |
| Gel filtration column | 132.82 L | 224,000.00 | 327,381.35 |
| Freeze dryer | 66.06 kg | 537,000.00 | 1,193,370.09 |
| Heat exchange (cooler) | 2 | 3,000.00 | 6,000.00 |
| Freezer | 20 | 3,000.00 | 60,000.00 |
| Total plat cost (scenario 1, 2, and 3) | | | 4,675,177.07 |
| Total plat cost (scenario 4, 5, and 6) | | | 5,673,367.49 |

*Individual cost of the equipment obtained from Sganzerla et al. [40], Barbosa et al. [38], and Lachos-Perez et al. [58].

The itemized cost revealed that for the hydrolysis of biomass, the subcritical reactor (998,190.43 USD), pump (395,072.50 USD), and heat exchange (160,654.97 USD) were the most expensive operations. For XO purification, a batch distillation vessel (1,273,906.59 USD) and freeze dryer (1,193,370.09 USD) are the operations with the highest implementation cost. Both processes were considered, with a single and two sequential reactors, and the estimated total plant cost was 4,675,177.07 and 5,673,367.49 USD, respectively.

The processes were simulated to determine the best operational and economic conditions for producing XOs on a large scale. The COM to produce XOs from the SWH of BSG is presented in **Fig. 6**. The COM ranged from 18.36 to 37.54 USD kg$^{-1}$ XOs. In the case of the process operated with a single reactor, the use of high temperature (180 °C) promoted the lowest COM (24.53 USD kg$^{-1}$ XOs). In contrast, in the two sequential reactors, the use of 130 °C (18.36 USD kg$^{-1}$ XOs) and 180 °C (21.12 USD kg$^{-1}$ XOs) in the second reactor presented a small difference in the COM. In SWH 5, the highest productivity (146,619 kg year$^{-1}$) of XOs was obtained. Moreover, by selling the XOs at 50 USD kg$^{-1}$ it is possible to recover up to 7 million USD. This behavior is a consequence of the combination of productivity and operational costs [59]. The literature reported that the use of SWH on an industrial scale could produce purified sugars at 6.63 USD kg$^{-1}$ [42], flavanones at 25.72 USD kg$^{-1}$ [58], and now, there is an additional option, the XOs.

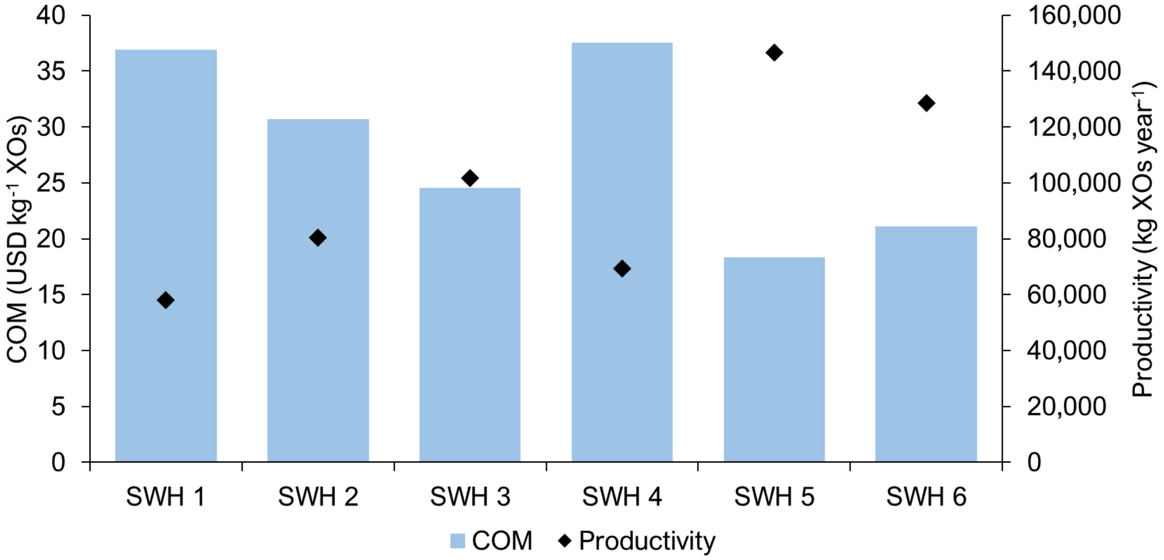

**Figure 6.** Cost of manufacturing (COM, USD kg$^{-1}$ XOs) and productivity (kg XOs year$^{-1}$) for the proposed industrial process to recover XOs from BSG.

At an industrial scale, the predominant costs are the cost of utilities (CUT), raw materials (CRM), operational labor (COL), and initial investment (FCI). Therefore, the major costs were discriminated over the COM of XOs to observe the main contribution to the process scaling up (**Fig. 7**). In all the scenarios tested, the FCI was the main contributor to the COM since the installation of the subcritical process is considered an expensive process. In the current study, the FCI for the process operated with a single reactor was 4,675,177 USD, and for the process with two sequential reactors, the FCI increased to 5,673,367 USD due to the necessity of an additional reactor. The literature reported that the SWH process presents an FCI ranging from 2.2 to 8.2 million USD [42].

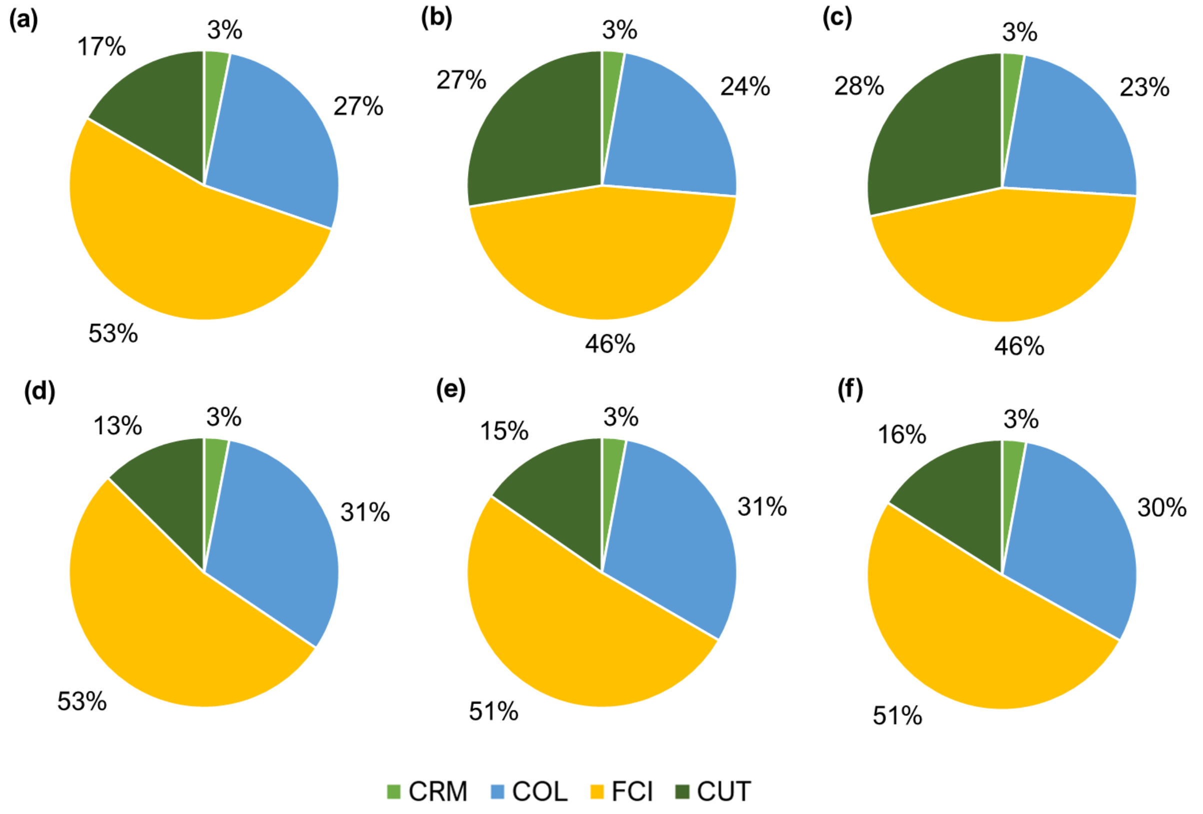

**Figure 7**. Cost analysis over the COM for the industrial process to recover XOs from BSG. (a) SWH 1, 80 °C; (b) SWH 2, 130 °C; (c) SWH 3, 180 °C; (d) SWH 4, 80 °C – 80 °C; (e) SWH 5, 80 °C – 130 °C; (f) SWH 6, 80 °C –180 °C.

The operating costs are controlled by facility-dependent costs, defined as the cost of maintenance, depreciation, and other miscellaneous costs [59]. In this study, the cost discrimination over the COM followed the sequence FCI > COL > CUT > CRM.

The SWH process operated with a single reactor presented a COL of approximately 25%, while the process with two sequential reactors increased the COL to 31%. Annually, the COL represents approximately 581 thousand USD (process with a single reactor) and 819 thousand USD (process with two sequential reactors) (**Table 3**). This increase is associated with the demand for a higher wage per shift in the process. Regarding the CUT, which is associated with the demands of electricity, steam, and water, a contribution of 17–28% (356 to 708 thousand USD) was obtained for the process with a single reactor, which decreased to 13–16% (328 to 434 thousand USD) for the two sequential reactors (**Fig. 5**). Finally, the CRM presented the lowest contributor over the COM. For all the simulated scenarios, the CRM was 3%, representing 67 thousand USD and 78 thousand USD for the process with a single and two sequential reactors, respectively. Therefore, the annual operating cost of the process ranged from 2.1 million USD (process with a single reactor) to 2.7 million USD (process with two sequential reactors).

### *3.3.2. Profitability analysis*

The condition of best economic profitability can be defined by the processing time in which the economic indexes assumed their maximum (GM, ROI, NPV, and IRR) and minimum values (payback time) [59]. The evaluation of gross profit, annual operating cost, main revenue, and profitable indicators are the initial parameters used to observe the behavior of the different processes for XOs production. **Table 4** presents the results of profitability parameters to obtain XOs from the SWH of BSG. In all the scenarios tested, positive values of GM, ROI, and NPV were obtained. The highest GM and ROI were 63.28 and 54.26%, respectively, for SWH 5. Notwithstanding, the main revenue is classified into gross profit and annual operating cost since a fraction of the revenue is designated to the operating costs, and the other is the gross profit. Thus, the best revenue condition was obtained for SWH 5, reaching 7,330,950 USD year$^{-1}$ (4,639,000 USD of gross profit and 2,692,000 USD of annual operation cost) (**Table 4**). Hence, the best payback was 1.84 years, demonstrating that the adoption of two sequential subcritical water reactors can be a profitable option to produce XOs from BSG.

**Table 4.** Results of profitability parameters to obtain XOs from SWH of BSG.

| Scenario | GM (%) | ROI (%) | Payback (years) | NPV (USD) | Gross profit (USD year$^{-1}$) | Main revenue (USD year$^{-1}$) |
|---|---|---|---|---|---|---|
| SWH 1 | 26.19 | 15.78 | 6.34 | 4,179,000 | 760,000 | 2,902,900 |
| SWH 2 | 38.64 | 25.60 | 3.91 | 10,010,000 | 1,552,000 | 4,016,250 |
| SWH 3 | 50.94 | 38.56 | 2.59 | 17,569,000 | 2,590,000 | 5,083,900 |
| SWH 4 | 24.93 | 15.19 | 6.58 | 4,592,000 | 866,000 | 3,472,300 |
| SWH 5 | 63.28 | 54.26 | 1.84 | 32,045,000 | 4,639,000 | 7,330,950 |
| SWH 6 | 57.76 | 44.63 | 2.24 | 25,377,000 | 3,710,000 | 6,423,500 |

### *3.3.3. Sensitivity analysis*

The effect of XOs production with SWH was evaluated by sensitivity analysis (**Fig. 8**). The effects of FCI, COL, CUT, CRM, and selling price of XOs were evaluated on ROI and NPV. These variables were adopted since they were the most influential variables on an industrial scale and directly affected the project feasibility. With a variation of ±30%, it is possible to observe that the FCI and selling price of XOs are the most sensitive parameters, which can be associated with the highest implementation cost of the industrial process. The CRM and CUT were not significantly affected by the variation in the input data, indicating that oscillation in the market price of raw materials and utilities does not affect the feasibility of the project.

## 4. Conclusion

The semi-continuous flow-through SWH process in a single and two sequential reactors can be a promising technology to produce XOs from BSG. The highest concentration of XOs in the hydrolysate (5246.75 mg L$^{-1}$) was obtained for the process operated with two sequential reactors (80 °C in the first reactor, followed by 130 °C in the second reactor). Under these operating conditions, xylotriose was the short-chain XOs with the highest concentration (2088.82 mg L$^{-1}$), corresponding to approximately 40% of the XOs produced. Regarding the yield, the process with two sequential reactors could produce 52.47 mg XOs g$^{-1}$ BSG and 146.97 mg XOs g$^{-1}$ hemicellulose for the process operated with the first reactor at 80 °C followed by the second reactor at 130 °C. The economic analysis revealed that the estimated total plant cost at the industrial scale (reactor volume of 500 L) was 4,675,177.07 and 5,673,367.49 USD, respectively, for the process with a single and two sequential reactors. The annual operating cost of the process ranged from 2.1 million USD (process with a single reactor) to 2.7 million USD (process with two sequential reactors), where the fixed capital investment and the cost of operational labor were the major costs. The lowest cost of manufacturing (18.36 USD kg$^{-1}$ XOs) was obtained for the process with two sequential reactors, with a productivity of 146,619 kg year$^{-1}$. In all the scenarios, positive profitability indicators were obtained. The process operated with two sequential reactors presented the highest gross margin (63.28%), with a payback of 1.84 years and a net present value of 32,045,000 USD. In conclusion, SWH is an eco-friendly technological process that can be used to recover XOs from BSG.

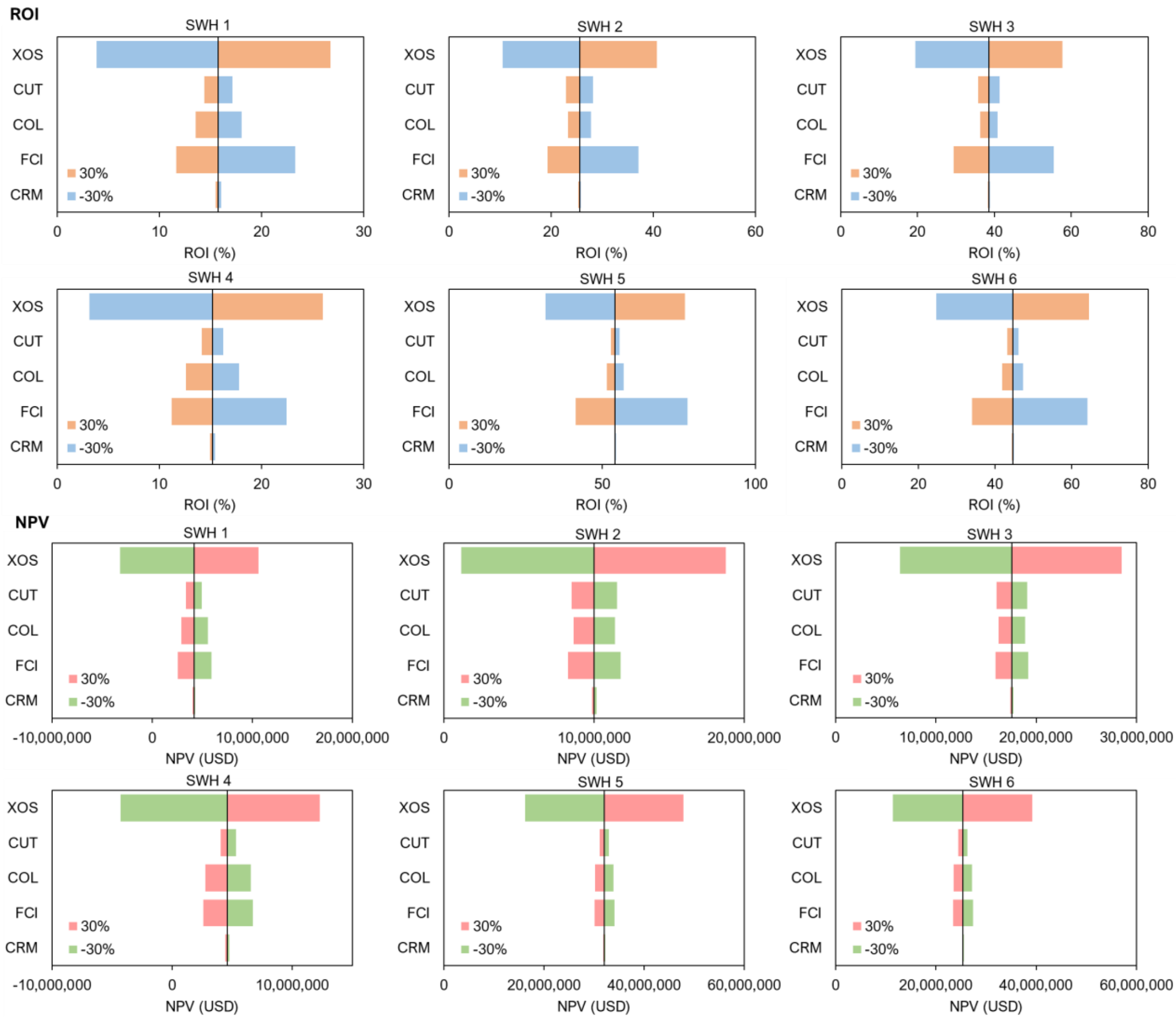

**Figure 8.** Sensitivity analysis to obtain XOs from the SWH of BSG. Label: XOs, selling price of xylo-oligosaccharides; CUT, cost of utilities; COL, cost of operational labor; FCI, fixed capital investment; CRM, cost of raw material.

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# CHAPTER VI

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## Subcritical water pretreatment enhanced methane-rich biogas production from the anaerobic digestion of brewer's spent grains

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# CHAPTER VI: Subcritical water pretreatment enhanced methane-rich biogas production from the anaerobic digestion of brewer's spent grains

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## Abstract

This study evaluated the effectiveness of a semi-continuous flow-through subcritical water hydrolysis (SWH) pretreatment of brewer's spent grains (BSG) for subsequent application in the anaerobic digestion (AD) process. BSG pretreatment was conducted at 160 °C and 15 MPa with a flow rate of 10 mL water $min^{-1}$ and 15 g water $g^{-1}$ BSG. The results revealed that SWH attacked the hemicellulose structure, releasing arabinose (46.54 mg $g^{-1}$) and xylose (39.90 mg $g^{-1}$) sugars, and proteins (34.89 mg $g^{-1}$). The start-up of anaerobic reactors using pretreated BSG (747.71 L $CH_4$ $kg^{-1}$ TVS) increased the methane yield compared with the reactor without pretreatment (53.21 L $CH_4$ $kg^{-1}$ TVS). For the process with pretreatment, the generation of electricity (134 kWh $t^{-1}$ BSG) and heat (604 MJ $t^{-1}$) are responsible for the mitigation of 43.90 kg $CO_{2\,eq}$ $t^{-1}$ BSG. The adoption of SWH as an eco-friendly pretreatment of biomass for AD could be a technological route to increase methane-rich biogas and bioenergy production, supporting the circular economy transition by reducing the carbon footprint of the beer industry.

***Keywords:*** *Brewer's spent grains; Subcritical water hydrolysis; Lignocellulosic biomass; Anaerobic digestion; Biorefinery*

## 1. Introduction

The exhaustive use of fossil fuels and the massive greenhouse gas (GHG) emissions have motivated the worldwide interest in finding sustainable energy sources [1]. Additionally, there is international concern associated with the correct management of lignocellulosic biomass, which requires appropriate final disposal [2]. The development of sustainable "waste to energy" processes raises interest in a biobased economy, which involves the production of goods, services, and energy, advocating the circular economy and biorefinery concepts [3]. Recently, the circular economy has been connected with the cleaner production of biobased products, expanding knowledge of biomaterial circulation and technologies to recover value-added products [4].

Biofuel based on lignocellulosic biomass has been considered a promising alternative to fossil fuels [1,5]. Therefore, developing innovative renewable technologies based on lignocellulosic wastes for bioenergy production is an excellent approach to decrease GHG emissions [6]. Additionally, lignocellulosic biomass presents competitive advantages for biofuel production due to its abundance, high yield, low price, and lack of intervention with the food supply [7].

Among various technological routes for biofuel production from lignocellulosic wastes, anaerobic digestion (AD) is a sustainable, profitable, and well-consolidated technology globally implemented for the management and correct disposal of waste [8]. The main product generated from methanogenic AD is methane, which is an adequate fuel to replace fossil fuels. Additionally, from the AD process, it is possible to recover an organic fertilizer composed of nitrogen, phosphorus, and potassium (NPK), replacing fossil fertilizer and decreasing the carbon footprint of the food production chain [9].

In the beer industry, the main solid waste is brewer's spent grains (BSG), accounting for the generation of 20 kg per 100 L of beer produced [10]. For instance, 14 billion L of beer was produced in Brazil in 2019 [11], which corresponds to a generation of $2.8\times10^{6}$ t BSG $y^{-1}$ [12]. BSG is a lignocellulosic biomass composed of approximately (wt%) protein, 19; cellulose, 18; hemicellulose, 35; and lignin, 11 [13]. Currently, BSG is usually discarded in landfills, incineration, composting, or animal feed. However, BSG is a feasible feedstock for the sustainable manufacturing of biobased products and bioenergy [14]. The valorization of BSG in a biorefinery is an effective strategy for the circular economy transition [14]. In addition, several studies evaluated different alternatives for the management of BSG with AD, such as the use of a silicone membrane reactor [15], the addition of biochar [16], co-digestion with sewage sludge [17,18], co-digestion with brewery wastewater [19], and ultrasonic pretreatment for AD [20]. However, a stronger pretreatment should be applied in BSG to release soluble molecules from the lignocellulosic biomass and increase methane production.

Notwithstanding, there are many challenges in obtaining biogas from the AD of lignocellulosic feedstocks, especially due to the poor accessibility between substrate and microorganisms, resulting in a lower biogas yield [7]. The complex structures of lignocellulosic biomass require pretreatment methods to improve anaerobic biodegradability [21]. Various pretreatments have been tested for the hydrolysis of lignocellulosic feedstocks before AD, such as biological, mechanical, thermal, and chemical methods [1,22]. Currently, one of the most eco-friendly processes studied to enhance AD by the hydrolysis of lignocellulosic biomass is hydrothermal technology [1,5,8,21,23].

Hydrothermal pretreatment under subcritical conditions promotes the degradation of lignocellulosic materials into short-chain sugars, which are easily catabolized molecules for AD [24]. Subcritical water hydrolysis (SWH) occurs by applying high pressure (1–22.1 MPa) and temperature (100–374 °C) conditions, which results in a greater self-ionization potential of water [25]. The SWH process has been widely investigated for many advantages, such as a fast reaction time, the use of only water as a solvent, and the non-generation of toxic residues [26]. Previous studies have demonstrated that there are several applications of SWH to produce biobased products from lignocellulosic biomass, including bamboo [27], coffee residues [28], rice husks [29], pecan wastes [30], soybean straw, and hulls [26]. In addition, SWH can be a promising pretreatment to enhance methane production from the AD of lignocellulosic biomass, such as macaúba husks [31], açaí seeds [32], and soybean straw [24]. The subcritical pretreatment of BSG for AD has not been studied previously. Therefore, studies should be conducted to elucidate the gaps in the industrial implementation of SWH before the AD of this brewery by-product.

Therefore, this study investigated the SWH pretreatment of BSG for methane production by AD technology. The methanogenic reactors were evaluated by the analysis of biogas production, methane composition, volatile fatty acids, and operational parameters. The reactor's performance was also assessed regarding energy balance, electricity, heat productivity, and avoided GHG emissions. Hence, this study highlighted the prospects for the future implementation of the SWH of lignocellulosic biomass for AD as a promising alternative for bioenergy recovery in a biorefinery.

## 2. Materials and methods

### *2.1. Raw materials and inoculum*

BSG (wet basis) was supplied by the Ambev brewery (Lages, SC, Brazil). The inoculum used in this study consisted of mesophilic sludge obtained after the treatment of brewery wastewater (Ambev brewery, Jaguariúna, SP, Brazil). The methods for the characterization of the raw materials used for SWH pretreatment and AD are described in **Section 2.5**.

### *2.2. Experimental setup for SWH pretreatment of BSG*

**Figure 1** describes the semi-continuous process used for the SWH pretreatment of BSG. The subcritical process is based on the hydrolysis of lignocellulosic biomass in a 110 mL hydrolysis reactor. The system was equipped with a water pump (high-pressure double piston pump, Model 36, Apple Valley, USA). The water fed into the reactor was heated by a preheater. The subcritical reactor (316-stainless steel tubes) was insulated by ceramic fiber. The temperature was monitored by thermocouples (type K). The pressure was monitored by manometers (WIKA company, 0 – 7.500 psi). The hydrolysate obtained in the process was cooled in a thermostatic bath (Marconi, model MA-184, São Paulo, SP, Brazil). A micrometer valve positioned at the end of the process was used to control the pressure of the process.

Previous studies optimized the conditions for the SWH pretreatment of BSG [13,33]. The following conditions were applied: 30 g of wet BSG; hydrolysis temperature of 160 °C; pressure of 15 MPa; flow rate of 10 mL water min$^{-1}$; solvent-to-feed ratio of 15 g water g$^{-1}$ BSG; and total reaction time of 45 min. The hydrolysate was collected every 5 min to perform the hydrolysis kinetics.

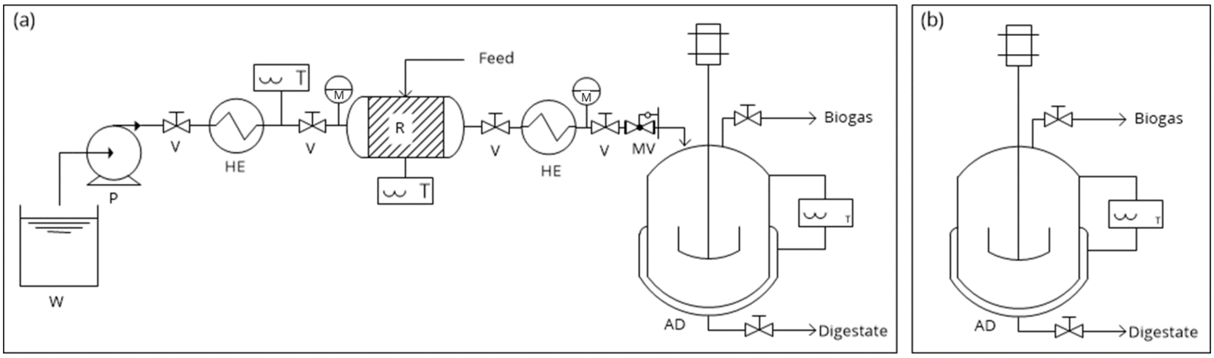

**Figure 1.** Laboratory-scale diagram of the process for subcritical water pretreatment and anaerobic digestion. (a) SWH+AD reactor and (b) AD reactor. Reproduced from Rosa et al. [37], with permission from Elsevier.

### *2.3. Analysis of the hydrolysate obtained by SWH*

#### *2.3.1. Reducing sugars and total reducing sugars*

The colorimetric Somogyi-Nelson method [34] was applied to quantify the content of reducing sugars and total reducing sugars, with modifications. For reducing sugars, the raw hydrolysate was used. For total reducing sugars, 200 µL of hydrolysate was submitted to acid hydrolysis with 200 µL HCl (2 mol $L^{-1}$) at 100 °C for 10 min, followed by neutralization with 200 µL NaOH (2 mol $L^{-1}$). Aliquots of 100 µL were used for the analysis of total reducing sugars. The reaction was composed of 100 µL sample (deionized water was used as a control) and 200 µL SN-I (aqueous solution composed of 4 g $CuSO_4$ $L^{-1}$, 24 g $Na_2CO_3$ $L^{-1}$, 16 g $NaHCO_3$ $L^{-1}$, 12 g $KNaC_4H_4O_6.4H_2O$ $L^{-1}$, and 18 g $Na_2SO_4$ $L^{-1}$). The reaction was homogenized in a vortex, heated in a water bath (100 °C, 6 min), and cooled in an ice bath (5 min). Then, 200 µL SN-II (aqueous solution composed of 50 g $(NH_4)6Mo_7O_{24}$ $L^{-1}$, 42 mL $H_2SO_4$ $L^{-1}$, and 6 g $Na_2HaSO_4$ $L^{-1}$) and 2.5 mL water were added to the reaction. The absorbance was measured at 540 nm (Hach, model DR 4000U, SP, Brazil). A calibration curve of glucose (0.005 to 0.1 g $L^{-1}$) was used to determine the sugar content. The results were expressed as mg glucose $g^{-1}$ BSG (dry weight, d.w.).

#### *2.3.2. Soluble protein*

The Bradford [35] method was used to determine the soluble protein, with modifications. The hydrolysate (100 µL) was diluted with deionized water (900 µL) and reacted with 1000 µL of Bradford reagent (aqueous solution composed of 100 mg Coomassie Brilliant Blue G-250 $L^{-1}$, 50 mL ethanol 95% $L^{-1}$, and 100 mL of $H_3PO_4$

85% $L^{-1}$). The reaction was homogenized in a vortex and rested for 5 min. The absorbance was measured at 595 nm (Hach, model DR 4000U, SP, Brazil). A calibration curve of bovine serum albumin (0.005 to 0.1 g $L^{-1}$) was used to determine the soluble protein content. The results were expressed as mg albumin $g^{-1}$ BSG (dry weight, d.w.).

### *2.3.3. Sugar monomers, organic acids, and inhibitors*

The concentrations of sugars (cellobiose, glucose, xylose, and arabinose), organic acids (formic acid and acetic acid), and inhibitors (furfural and 5-hydroxymethilfurfural) were analyzed by high-performance liquid chromatography using a refractive index detector. For this, the hydrolysate was centrifuged (10,000×*g*) and filtered (nylon 0.22 µm). The chromatographic conditions were described in a previous study [33]. A calibration curve was used for each standard (0.01 to 1 g $L^{-1}$). The results were expressed as mg $g^{-1}$ BSG (dry weight, d.w.).

### *2.3.4. Characterization of solid residues after SWH*

The mass reduction during SWH was determined by the difference between the initial mass of BSG (dry matter) and the remaining solids after hydrolysis (dried at 45 °C for 24 h). Thermogravimetric analysis (TGA) of samples was performed in a thermogravimetric analyzer (PerkinElmer, model STA6000, Akron, Ohio, USA). Thermogravimetric data were converted into derivative thermograms (DTG) to determine the lignocellulosic composition, according to Carrier et al. [36].

## ***2.4. Experimental setup for the AD of BSG***

The AD process was started-up in a 4.3 L stirred tank reactor and operated in semi-continuous mode for 50 days. The reactor was designed as 40% for headspace (1.72 L) and 60% for substrate (2.58 L). The following reactors were started: *i) AD reactor:* substrate composed of 45% wet BSG (476.93 g) and 55% liquid phase (0.7095 L inoculum and 0.7095 L of water); *ii) SWH+AD reactor:* substrate composed of 70% hydrolysate obtained from SWH (1.806 L) and 30% inoculum (0.774 L inoculum).

The AD was operated at mesophilic temperature (36 °C) with a thermostatic bath (Tecnal, TE-2005) attached in the reactors. The pH was held between 7 and 8.5 by adding NaOH (6 mol $L^{-1}$) to support the methanogenic reactions. The reactors were homogenized through a system of mechanical stirrers. Homogeneization was applied for 10 min, 5 min before the removal of digestate and 5 min after the addition of substrate and NaOH for pH control [37]. The biogas produced in the reactor was collected in a Tedlar bag (Supelco Analytical, Darmstadt, Germany). The digestate collected was analyzed to control the process efficiency.

The reactors were operated with the same hydraulic retention time (HRT) (**Eq. 1**), organic load rate (OLR) (**Eq. 2**), and volatile solids loading rate (VSR) (**Eq. 2**). For this, the daily feed flow rate used in both reactors (AD and SWH+AD) consisted of 18.49 g of BSG and 55 mL water (total flow of 100 mL $d^{-1}$).

$$\text{HRT (d)}= \frac{V_{\text{reactor}}}{Q_{\text{feed}}} \qquad (1)$$

$$\text{OLR}\left(\frac{\text{g COD}}{\text{L} \cdot \text{d}}\right)= \frac{Q_{\text{feed}} \times S_{\text{feed}}}{V_{\text{reactor}}} \qquad (2)$$

$$\text{VSR}\left(\frac{\text{g TVS}}{\text{L} \cdot \text{d}}\right)= \frac{Q_{\text{feed}} \times S_{\text{feed}}}{V_{\text{reactor}}} \qquad (3)$$

*where* $Q_{feed}$ is the flow of feed ($g_{BSG}$ $d^{-1}$); $S_{feed}$ is the concentration of COD or TVS in the substrate (g $g^{-1}_{BSG}$); and $V_{reactor}$ is the volume of the substrate in the reactor (L).

In this experiment, $V_{\text{reactor}}$ was 2.58 L, $Q_{\text{feed}}$ was 18.49 $g_{BSG}$ $d^{-1}$, and $S_{\text{feed}}$ was 1.52 g COD $g^{-1}_{BSG}$ and 0.5517 g TVS $g^{-1}_{BSG}$. Therefore, the HRT was calculated as 25.2 d for both reactors. The OLR and VSR were calculated as 10.89 g COD $L^{-1}$ $d^{-1}$ and 3.95 g TVS $L^{-1}$ $d^{-1}$, respectively.

### *2.5. Operational performance of AD*

#### *2.5.1. Determination of physicochemical parameters*

The physicochemical characteristics of the reactors were evaluated by determining the pH, alkalinity, Kjeldahl protein, ammonia nitrogen, total nitrogen, soluble chemical oxygen demand (COD), total solids (TS), fixed solids (TFS), and volatile solids (TVS) according to the Standard Methods for the Examination of Water and Wastewater [38]. The volatile fatty acids (VFA) were determined by gas chromatography coupled with a flame ionization detector, according to the methods described by Ampese et al. [39]. All analyses were conducted at least in triplicate. The TVS (**Eq. 4**) and COD (**Eq. 5**) removal were determined.

$$\text{TVS removal (\%)}=\frac{\text{TVS}_{\text{initial; day 0}}\text{-TVS}_{\text{final; day 50}}}{\text{TVS}_{\text{initial}}}\times 100 \quad (4)$$

$$\text{COD removal (\%)} = \frac{\text{COD}_{\text{initial; day 0}} - \text{COD}_{\text{final; day 0}}}{\text{COD}_{\text{initial; day 0}}} \times 100 \quad (5)$$

### *2.5.2. Determination of phosphorus in the digestate*

The determination of phosphorus in the digestate was conducted by spectrophotometry [40,41]. First, the phosphorus was extracted by solubilizing the digestate (2.5 g, wet basis) with 25 mL of Mehlich-1 (aqueous solution composed of 0.05 mol HCl $L^{-1}$ and 0.0125 mol $H_2SO_4$ $L^{-1}$) at 125 rpm (10 min, 25 °C). The sample was rested for 24 h and filtered. Then, the filtered solution (1 mL) was reacted with 2 mL Mehlich-2 (aqueous solution composed of 0.6 g $Bi_2O_2(CO_3)$ $L^{-1}$, 45 mL $H_2SO_4$ $L^{-1}$, 6 g $(NH_4)_6Mo_7O_{24}$ $L^{-1}$ and 6 g ascorbic acid $L^{-1}$). The reaction was homogenized in a vortex and rested for 1 h. The absorbance was measured at 660 nm (Hach, model DR 4000U, SP, Brazil). A calibration curve of $KH_2PO_4$ (1 to 25 g $L^{-1}$) was used to determine phosphorus. The results were expressed as g phosphorus per L (g $L^{-1}$).

## ***2.6. Biogas volume, composition, and methane yield***

The biogas produced was collected from the Tedlar bag. Quantification of the volume produced was conducted with the adoption of a syringe. The accumulated biogas volume was quantified by the sum of daily biogas produced. The composition of oxygen ($O_2$), hydrogen ($H_2$), methane ($CH_4$), and carbon dioxide ($CO_2$) in the biogas was determined by a gas chromatograph coupled with a thermal conductivity detector [42]. **Eq. 6** was applied to determine the experimental methane.

$$\text{Methane yield}\left(\frac{\text{L CH}_4}{\text{kg TVS}_{\text{added}}}\right) = \frac{V_{\text{biogas}} \times C_m}{\text{TVS}} \qquad (6)$$

*where* $Q_{biogas}$ is the accumulated biogas volume (L) obtained at the end of the experiment, $C_m$ is the methane composition in the biogas (%), and *TVS* is the initial content of total volatile solids.

## ***2.7. Energy production and avoided GHG emissions from biogas***

Based on the experimental production of methane, the potential for energy production (electricity and thermal energy) was estimated (**Eqs. 7** and **8**). For this, the on-site burning of biogas in a co-generator was assumed [43].

$$EG_{CH_4} = \frac{Q_{\text{biogas}} \times LCV_{CH_4} \times C_m \times \eta_e \times F}{M_{BSG}} \qquad (7)$$

$$HG_{CH_4}=\frac{Q_{biogas}\times LCV_{CH_4}\times C_m\times \eta_e}{M_{BSG}} \qquad (8)$$

*where* $EG_{CH_4}$ is the electricity produced (MWh $t^{-1}$ BSG); $HG_{CH_4}$ is the heat produced (MJ $t^{-1}$ BSG); $M_{BSG}$ is the mass of BSG used during the AD (start-up and feed); $Q_{biogas}$ is the biogas volume; and $LCV_{CH_4}$ is the lower calorific value of methane (35.59 MJ $m^{-3}$); $C_m$ is the methane composition in the biogas (%); $\eta_e$ is the engine efficiency of the co-generator (electricity, 40%; heat, 50%) [44]; and *F* is necessary for the conversion from MJ to MWh.

The generation of renewable energy and the substitution of non-renewable energy implies the avoidance of GHG emissions (**Eqs. 9** and **10**) [45].

$$GHG_{electricity}=EF_{CO_2\text{-}EG}\times EG_{CH_4} \qquad (9)$$

$$GHG_{heat}=EF_{CO_2\text{-}HG}\times HG_{CH_4} \qquad (10)$$

*where* $EF_{CO_2-EG}$ is the emission factor of $CO_{2\text{-eq}}$ for Brazilian electricity (0.075 t $CO_{2eq}$ $MWh^{-1}$ [46]) and $EF_{CO_2-HG}$ is the emission factor of $CO_{2\text{-eq}}$ for natural gas in a boiler (0.056 $tCO_{2eq}$ $GJ^{-1}$ [47]).

### *2.8. Energy balance*

Finally, a global industrial energy balance was calculated to determine the energy demand of SWH pretreatment based on a subcritical water laboratory plant used for pretreatment [48]. For the energy balance of the subcritical reactor (**Eq. 11**), the following conditions were assumed: i) steady state; ii) constant pressure; iii) without shaft work; iv) without kinetic energy; v) variation in potential energy neglected; and vi) constant mass [49]. Therefore, the heat required (Q) is the difference in enthalpy (H) **(Eq. 12)**:

$$\sum M_k(H)+Q=0 \qquad (11)$$

$$\frac{Q}{M}=H_2-H_1 \qquad (12)$$

The enthalpy can be calculated considering that the mass of water >> mass of BSG (**Eq. 13**). Hence, the specific heat of water ($C_p^*$) was determined to be 4.178 kJ $kg^{-1}$ $K^{-1}$ at 25 °C and 4.285 kJ $kg^{-1}$ $K^{-1}$ at 160 °C (Sandler, 2017), considering constant pressure.

$$H\left(\frac{kJ}{kg}\right)=C_p^*\left(\frac{kJ}{kg\cdot K}\right)\times T(K) \qquad (13)$$

### *2.9 Statistical analysis*

All the data obtained were analyzed at least in triplicate. The results are expressed as the average ± standard deviation. The results were statistically analyzed by one-way analysis of variance (ANOVA) and Tukey's test ($p \leq 0.05$) (Statistica®, StatSoft Inc., version 10.0, Tulsa, OK, USA).

## 3. Results and discussion

### *3.1. SWH pretreatment of BSG*

**Figure 2** shows the kinetics of sugar (reducing and total), protein, and pH of the hydrolysate obtained from the SWH of BSG. The reducing sugar yields at the first (5 min) and second collection points (10 min) reached 5.56 mg $g^{-1}$ and 5.61 mg $g^{-1}$, respectively. After 15 min of hydrolysis, a considerable increase was noticed when the reducing sugars reached the highest yield (11.37 mg $g^{-1}$) (**Figure 2a**). The accumulated total reducing sugars reached 36.05 mg $g^{-1}$. In addition, the total reducing sugar yield presented a similar kinetic behavior. However, it reached 92.68 mg $g^{-1}$ at the end of the SWH pretreatment (**Figure 2b**). Similar temperature conditions (180 °C) were tested in the SWH of rice husks at 15 mL $min^{-1}$, 25 MPa, and 15 min of hydrolysis [25]. The sugar yields were 8 mg $g^{-1}$ rice husks, suggesting that SWH pretreatment of BSG presents a more significant potential to produce sugars when compared with rice husks [25].

The monosaccharide profile revealed arabinose (46.54 mg $g^{-1}$) and xylose (39.90 mg $g^{-1}$) as the main monosaccharides obtained in the hydrolysate, followed by cellobiose (4.78 mg $g^{-1}$) and glucose (5.51 mg $g^{-1}$). Formic (34.47 mg $g^{-1}$) and acetic acids (85.27 mg $g^{-1}$) were the organic acids obtained from the SWH of BSG. Low concentrations of 5-hydroxymethylfurfural and furfural were found in the hydrolysate (<0.01 mg $mL^{-1}$), which is a desired feature for the application of hydrolysate in fermentation processes [50].

Regarding the production of soluble protein from SWH pretreatment of BSG, a noticeable increase was observed until 20 min of hydrolysis (**Figure 2c**). At the end of the SWH, the accumulated hydrolysate presented 34.89 mg albumin $g^{-1}$, which means that 21% of the total protein in the BSG (165 mg $g^{-1}$, **Table 1**) was hydrolyzed. This study achieved a higher extraction when compared to Du et al. [51], who obtained 6.7% of protein by SWH of BSG at 200 °C.

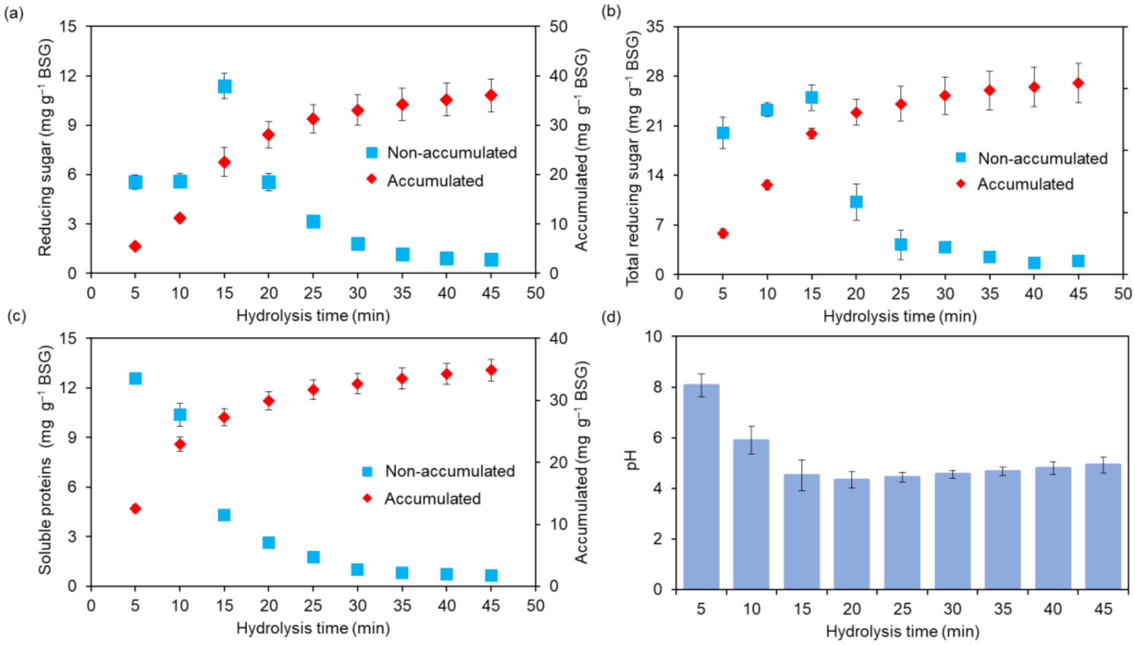

**Figure 2.** Kinetic profile during the SWH of the BSG. (a) reducing sugars (RS); (b) total reducing sugars (TRS); (c) soluble protein; and (d) pH.

In the SWH of BSG, the pH values were initially alkaline (8.1), decreasing to 5.9 after 10 min and stabilizing between 4.4 and 4.9 after 15 min (**Figure 2d**). During the hydrolysis of lignocellulosic biomass, the concentrations of organic acids and phenolic compounds (including some phenolic acids) increase in the hydrolysate, promoting an acidic pH [52]. Therefore, the most substantial reduction in pH occurred between 10 and 15 min of reaction at the beginning of hydrolysis, indicating the release of acidic compounds from hemicellulose and lignin. Similar behavior was reported for the SWH of sugarcane bagasse [52] and soybean residues [26].

The SWH of BSH resulted in a final fraction inside the reactor composed of 42% dry mass. From the 17.22 g of dry BSG initially loaded in the reactor, which corresponded to 30 g (wet weight), approximately 9.8 g of dry BSG was hydrolyzed and released in the hydrolysate used for AD, and 7.42 g of dry solid residue remained in the reactor. This fact has been previously reported, for example, in the SWH of sugarcane straw [53] and BSG [13]. The final fraction obtained inside the reactor can be applied to obtain silica [54], biochar [55], biocrude (mixture of oxygenated hydrocarbons) [56], and adsorbent materials [57]. **Figure 3** shows the individual components in the raw and pretreated biomass, as determined by thermal analysis. It is possible to observe that hemicellulose was degraded since the peak available between 175–300 °C disappeared when comparing the residue of SWH with the raw BSG used in the hydrolysis. The composition of raw BSG by thermal analysis was 33.41% semi-volatiles, 31.64% hemicellulose, 22.01% cellulose, and 12.92% lignin. After SWH, this composition changed to 1.61% semi-volatiles, 24.57% hemicellulose, 24.75% cellulose, 42.53% lignin, and 6.52% char. An external char layer has been detected during the SWH, and the char increases proportionally to temperature increases [58].

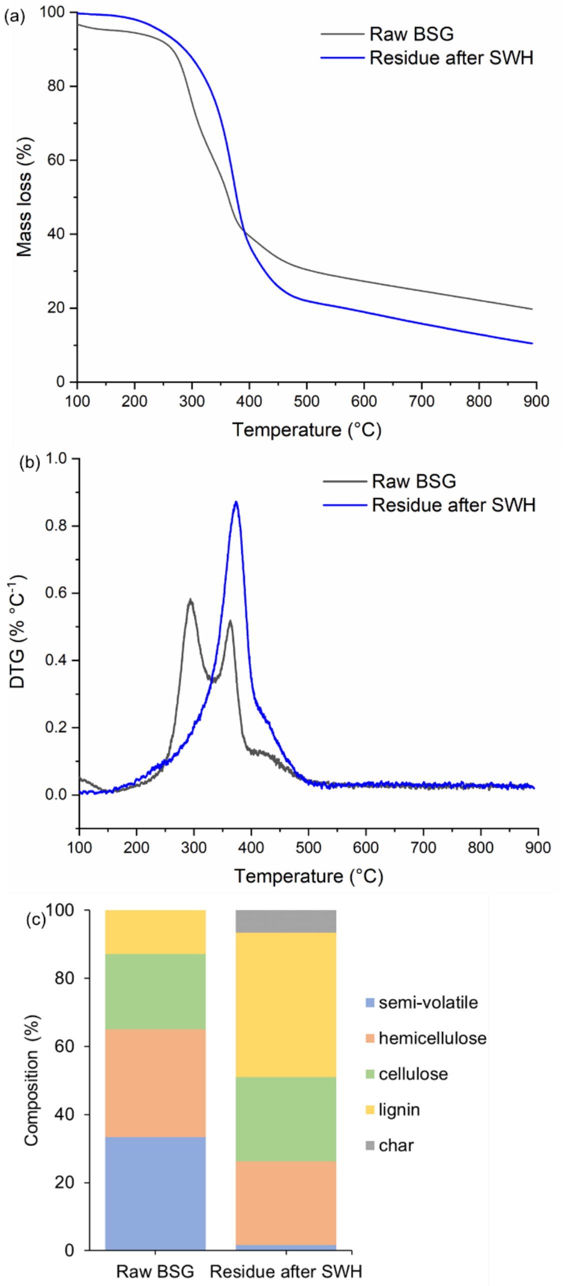

**Figure 3.** TGA of raw BSG and solid residues after the SWH process: (a) degradation profile; (b) derivative thermogravimetric analysis (DTG); and (c) raw BSG and solid residue composition by TGA.

Finally, the results revealed that SWH can be applied as a pretreatment to recover arabinose, xylose, and soluble proteins from BSG. Based on this composition and taking advantage that SWH is an eco-friendly pretreatment, this hydrolysate should be investigated in fermentative routes to produce biofuels, for example, by AD technology.

### *3.2. Characterization of raw materials*

The initial characterization of BSG, hydrolysate, inoculum, and feed is presented in **Table 1**. Some parameters deserve special attention, such as the pH value. BSG and the hydrolysate obtained by SWH presented pH values of 6.65 and 6.25, respectively, revealing that the pretreatment did not cause a significant reduction in the pH of the final hydrolysate. The pH values of the inoculum and feed were in the neutral range, which is favorable for AD, since the desirable pH range for use in AD should be from 7.5 to 8.5 to promote methanogenic reactions [59]. Regarding the TVS, BSG presented 55.17% and hydrolysate 1.56%, representing a removal yield of 97.2% after pretreatment with subcritical water. The applied pretreatment also reduced the alkalinity, total nitrogen, ammonia nitrogen, and phosphorus. The COD of BSG (1519.04 g $O_2$ $kg^{-1}$) and hydrolysate (694.29 g $O_2$ $kg^{-1}$) demonstrate suitable values for AD [60]. In addition, the hydrolysate presented a low nitrogen content (1.65 g $kg^{-1}$) and should be supplemented with BSG to provide nutrients for the methanogenic microbiota [61]. The protein content in the hydrolysate (10.34 ± 0.34 g $kg^{-1}$) may be relevant to enhance the buffer capacity of AD and meet methanogenic bacteria's nutritional requirements. The phosphorus content in BSG (1.14 ± 0.02 g $L^{-1}$) decreased after the application of SWH pretreatment (0.11 ± 0.01 g $L^{-1}$), indicating that phosphorus was retained in the solid residue fraction after SWH, corroborating with Tu et al. [62]. Overall, the evaluated parameters indicate the effectiveness of SWH in converting BSG solids into soluble organic molecules of interest for application in AD.

### *3.3. Operational performance of AD reactors*

The physicochemical characterization of the AD and SWH+AD reactors was monitored. The evolution of each parameter (first and last day of AD) is presented in **Table 2**. **Figure 4** shows the evolution of pH, alkalinity, TS, TVS, TFS, ammonia nitrogen, COD, and phosphorus during the 50 days of AD.

**Table 1.** Characterization of raw materials.

| Parameters | BSG | Hydrolysate | Inoculum | Feed* | Unit |
|---|---|---|---|---|---|
| pH | 6.65 ± 0.01 | 6.25 ± 0.03 | 7.57 ± 0.01 | 7.06 ± 0.02 | – |
| Density | 0.41 ± 0.03 | 1.01 ± 0.04 | 1.01 ± 0.02 | 0.41 ± 0.01 | g $mL^{-1}$ |
| Moisture | 42.59 ± 2.38 | 98.40 ± 0.01 | 89.57 ± 0.56 | 79.11 ± 0.12 | % |
| Total solids | 57.41 ± 2.38 | 1.60 ± 0.01 | 10.43 ± 0.56 | 20.89 ± 0.12 | % |
| Total fixed solid | 2.24 ± 0.12 | 0.04 ± 0.02 | 1.33 ± 0.08 | 0.59 ± 0.01 | % |
| Total volatile solid | 55.17 ± 2.27 | 1.56 ± 0.03 | 9.10 ± 0.48 | 20.30 ± 0.11 | % |
| Alkalinity | 707.75 ± 2.77 | 518.23 ± 1.42 | 194.75 ± 4.75 | 251.75 ± 4.75 | mg $CaCO_3$ $L^{-1}$ |
| Ammonium nitrogen | 364.42 ± 2.66 | 18.62 ± 2.66 | 23.94 ± 2.66 | 111.72 ± 5.32 | mg $NH_3$ $L^{-1}$ |
| Chemical oxygen demand | 1519.04 ± 23.82 | 694.29 ± 9.52 | 15.77 ± 2.00 | 537.13 ± 39.8 | g $O_2$ $kg^{-1}$ |
| Total nitrogen | 26.49 ± 0.55 | 1.65 ± 0.05 | 9.73 ± 2.18 | 6.77 ± 0.31 | g N $kg^{-1}$ |
| Proteins | 165.54 ± 3.44 | 10.34 ± 0.34 | 60.80 ± 13.60 | 42.31 ± 1.92 | g $kg^{-1}$ |
| Total phosphorus | 1.14 ± 0.02 | 0.11 ± 0.01 | 0.99 ± 0.02 | 0.35 ± 0.03 | g $L^{-1}$ |

The results are expressed as the mean ± standard deviation. Analysis conducted in triplicate (n=3). * Feed for AD and SWH+AD reactors.

**Table 2.** General parameters recorded during the continuous AD and SWH+AD of BSG.

| Parameters | AD reactor | | SWH+AD reactor | | Unit |
|---|---|---|---|---|---|
| | Day 0 | Day 50 | Day 0 | Day 50 | |
| pH | 7.15 ± 0.09$^{b}$ | 8.49 ± 0.12$^{a}$ | 6.86 ± 0.05$^{B}$ | 8.34 ± 0.21$^{A}$ | – |
| Total solids | 18.55 ± 1.30$^{a}$ | 10.52 ± 0.06$^{b}$ | 1.94 ± 0.35$^{B}$ | 9.07 ± 0.03$^{A}$ | % |
| Total fixed solid | 0.81 ± 0.03$^{b}$ | 2.35 ± 0.03$^{a}$ | 0.24 ± 0.06$^{B}$ | 2.39 ± 0.03$^{A}$ | % |
| Total volatile solid | 17.74 ± 1.33$^{a}$ | 8.17 ± 0.09$^{b}$ | 1.70 ± 0.40$^{B}$ | 6.68 ± 0.00$^{A}$ | % |
| Alkalinity | 356.25 ± 4.75$^{b}$ | 2403.50 ± 19.00$^{a}$ | 128.25 ± 4.75$^{B}$ | 2398.75 ± 23.75$^{A}$ | mg CaCO$_3$ L$^{-1}$ |
| Ammonium nitrogen | 106.40 ± 1.28$^{b}$ | 260.68 ± 5.32$^{a}$ | 23.94 ± 2.66$^{B}$ | 244.72 ± 10.64$^{A}$ | mg NH$_3$ L$^{-1}$ |
| Chemical oxygen demand | 9.39 ± 0.47$^{a}$ | 7.42 ± 0.00$^{b}$ | 1.66 ± 0.13$^{B}$ | 5.48 ± 0.12$^{A}$ | g O$_2$ L$^{-1}$ |
| Total nitrogen | 10.57 ± 0.04$^{a}$ | 4.60 ± 0.01$^{b}$ | 3.83 ± 0.07$^{B}$ | 4.38 ± 0.09$^{A}$ | g kg$^{-1}$ |
| Proteins | 66.03 ± 0.26$^{a}$ | 28.78 ± 0.09$^{b}$ | 23.93 ± 0.45$^{B}$ | 27.35 ± 0.54$^{A}$ | g kg$^{-1}$ |
| Total phosphorus | 0.68 ± 0.01$^{a}$ | 0.69 ± 0.01$^{a}$ | 0.37 ± 0.01$^{B}$ | 0.68 ± 0.01$^{A}$ | g L$^{-1}$ |

The results are expressed as the mean ± standard deviation. Analysis conducted in triplicate (n=3). Different letters in each line (lowercase for the AD reactor and uppercase for the SWH+AD reactor) indicate significant differences by Tukey's test at $p \leq 0.05$.

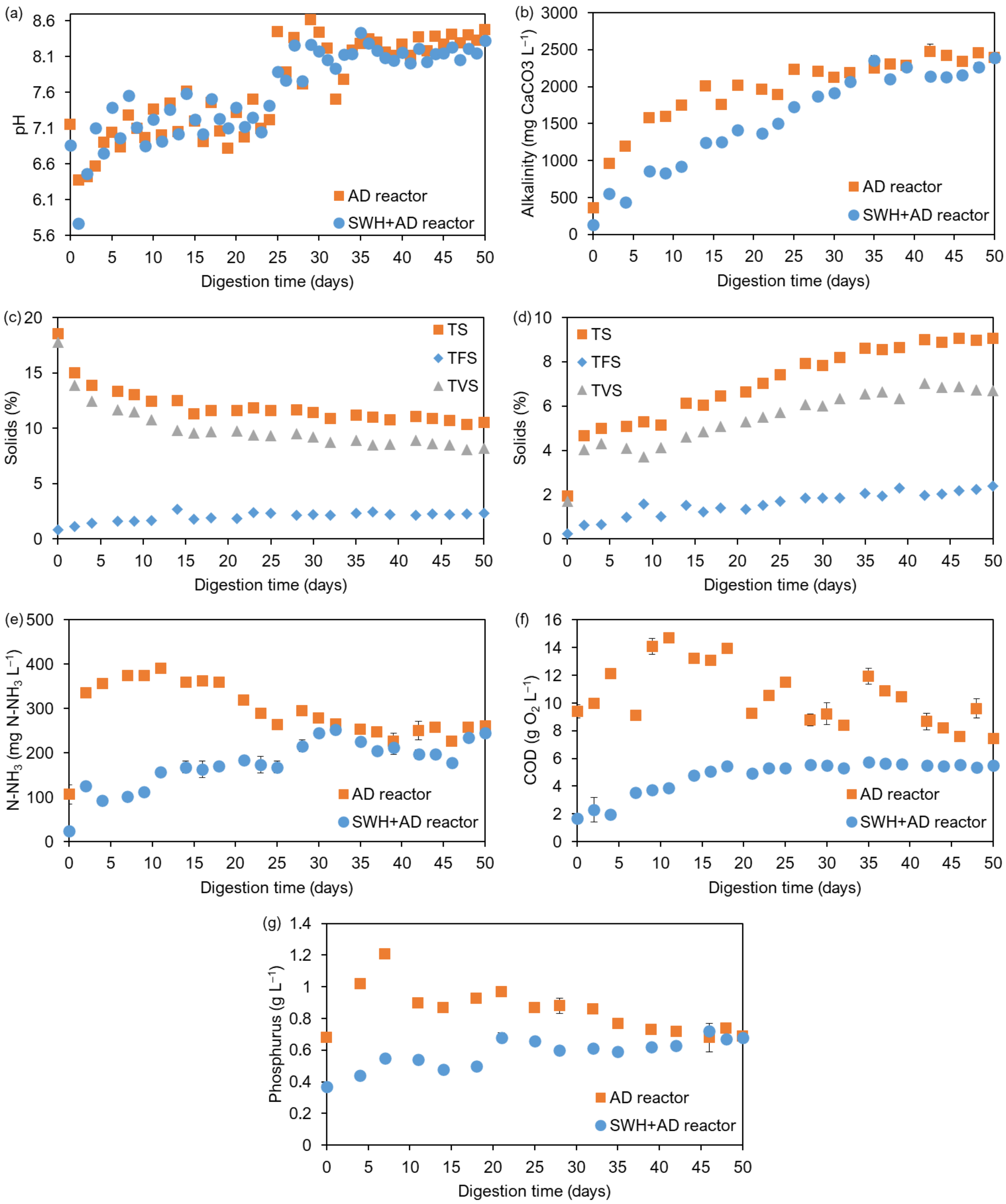

**Figure 4.** Operational parameters during the continuous AD and SWH+AD of BSG. (a) pH; (b) alkalinity (mg $CaCO_3$ $L^{-1}$); (c) solids (%) for the AD reactor; (d) solids (%) for the SWH+AD reactor; (e) ammonia nitrogen (mg N-$NH_3$ $L^{-1}$); (f) chemical oxygen demand (g $O_2$ $L^{-1}$); and (g) phosphorus (g $L^{-1}$).

### *3.3.1. pH and alkalinity*

**Figure 4a** presents the pH measurements (before NaOH addition) during the AD of BSG. At the beginning of AD, both reactors presented an oscillating behavior for pH, corresponding to the predominance of hydrolysis and acidogenic phases. In this stage, the pH remained between 6.4 and 7.5, which was an advantage for the hydrolysis of the lignocellulose content in BSG. On the other hand, after day 25, the occurrence of acetogenesis and methanogenesis phases was predominant, which can be associated with the pH stabilizing between 7.5 and 8.5. In the hydrolysis phase, the bacteria convert carbohydrates into sugars, proteins into amino acids, and lipids into fatty acids [37]. In the acidogenic phase, sugars, amino acids, and lipids are converted into VFA, causing decreases in pH values [60,63,64]. In this study, approximately 400 mL of NaOH (6 mol $L^{-1}$) was used in each reactor over 50 days of AD to keep the pH around the optimum range for methane production.

The alkalinity evolution (**Figure 4b**) indicates the buffering capacity of the system, i.e., the resistance to pH variations [65]. AD and SWH+AD showed an increasing behavior for alkalinity, faster for the AD reactor, while for the SWH+AD, it was more constant. These results could explain the formation of carbonates and bicarbonates during the reaction [32]. The increase in alkalinity has been reported as a positive effect during AD, accelerating the removal of pollutants and improving the buffering capacity, which helped decrease methanogenesis inhibition [66]. The alkalinity range considered favorable for anaerobic microorganisms ranges between 1 and 5 g $CaCO_3$ $L^{-1}$ [67]. This range was reached on days 4 and 14 for the AD and SWH+AD reactors, respectively. After day 25, both reactors presented an alkalinity between 2200 mg $CaCO_3$ $L^{-1}$, allowing suitable operational conditions for developing the methanogenic microbiota.

### *3.3.2. Solids*

The evolution of solids was determined for the AD reactor (**Figure 4c**) and the SWH+AD reactor (**Figure 4d**). The initial TVS of the AD reactor was 17.74%, characterizing this reactor as a dry AD [68]. The dry AD was studied to demonstrate that BSG without pretreatment can be used for AD with high solid content, which is an advantage for further industrial application. In addition, the TVS decreased from 13.86% to 12.44% for the 2nd and 4th days of AD, respectively. After 16 days of AD, an average value of 8.9% was obtained until the end of the study. Regarding the AD reactor, the removal of 53.95% of TVS was observed.

On the other hand, the SWH+AD reactor started up with 1.7% TVS and was characterized as wet AD. After 16 days of AD, an average of 6.16% was obtained until the end of the study. The TVS behavior differed among the reactors: while the AD reactor presented a decreasing trend, the SWH+AD showed the opposite trend. This fact can result from the feed with high VSR (3.95 g TVS $L^{-1}$ $d^{-1}$). This configuration was adopted in the SWH+AD reactor since it is necessary nutrients for the microbiota. In the case of operating the reactor without BSG addition, a low yield of biogas would probably be obtained since the hydrolysate presented only soluble compounds.

### *3.3.3. Ammonia nitrogen*

**Figure 4e** shows the profile of ammonia nitrogen. During AD, the proteins and other nitrogen compounds from the feedstock are catabolized and transformed into ammonia [69]. Consequently, the solids loaded into each reactor affect the nitrogen concentration. Ammonia in its free form ($NH_3$) has been considered an essential nutrient for bacterial growth. However, excess concentrations of ammonia can inhibit methane production [70]. In this study, ammonia nitrogen increased during AD, which is associated with the degradation of proteins from BSG [19,71]. The ammonia nitrogen in the AD reactor ranged from 106.4 (day 0) to 260.68 mg N-$NH_3$ $L^{-1}$ (day 50), while the SWH+AD ranged from 23.94 (day 0) to 244.72 mg N-$NH_3$ $L^{-1}$ (day 50).

### *3.3.4. Chemical oxygen demand*

**Figure 4f** shows the evolution of COD. COD represents the amount of oxygen needed to oxidize the organic matter completely, and its removal is related to methane production [72,73]. In the present study, the greatest and lowest COD values for the AD reactor were obtained on the 11th and 50th days (14.72 and 7.42 g $O_2$ $L^{-1}$, respectively). For the AD reactor, it is possible to notice an increase in COD values from day 0 to day 18, corresponding to the hydrolysis and acidogenesis phase [31]. The respective maximum and minimum values for the SWH+AD reactor were 5.74 g $O_2$ $L^{-1}$ (day 35) and 1.66 g $O_2$ $L^{-1}$ (day 0), and the COD in this reactor was almost constant after day 14, with an average of 5.39 g $O_2$ $L^{-1}$ until the last day of digestion. Hence, the COD removal reached 20.98% for the AD reactor, while for SWH+AD, it showed an increase of 3.3-fold. This fact can be explained by the high OLR (10.89 g

$O_2$ $L^{-1}$ $d^{-1}$) used in the reactors. The methanogenic microorganisms that consume organic matter were more activated in the AD reactor, and then, the COD removal was higher. In addition, COD can be transformed into soluble compounds and VFA for further conversion into methane [74].

### *3.3.5. Phosphorus*

The reactors operated with and without pretreatment presented a stable phosphorus content (**Figure 4g**). The phosphorus content of the AD reactor ranged from 0.68 (day 0) to 0.69 g $kg^{-1}$ (day 50), while the content in the SWH+AD reactor ranged from 0.37 to 0.68 g $kg^{-1}$. BSG presented an initial composition of 1.14 g phosphorus $kg^{-1}$. After the subcritical pretreatment, this value decreased to 0.11 g $kg^{-1}$.

Phosphorus is a key compound in fertilizers. Therefore, one of the routes for digestate valorization is its use as organic fertilizer in agriculture [75], especially if the digestate presents a high phosphorus content [76]. Studies have demonstrated better soil qualities after using digestate as fertilizer [77,78]. The digestate is composed of a liquid and a solid fraction. The solid fraction can be used as fertilizer since it contains 60–80% phosphorus [79], while the liquid fraction can be used to recover struvite (i.e., magnesium ammonium phosphate) [80]. Struvite is a slow-releasing fertilizer with similar properties to conventional fertilizer obtained from fossil rock [81].

### *3.3.6. Volatile fatty acids*

**Figure 5** presents the VFA produced along the AD of BSG. These short-chain organic acids are intermediate products during anaerobic reactions and are considered important precursors for methane production [82]. Acetic acid was the most concentrated volatile fatty acid produced in both reactors, with averages of 12.69 and 7.73 g $L^{-1}$, respectively, for the AD and SWH+AD reactors. The second most abundant VFA during all the experiments was butyric acid, while for the SWH+BSG reactor, the concentration of isovaleric acid exceeded the butyric acid in the last days of the experiment.

When analyzing the alkalinity data (**Figure 4b**) with the concentration of VFA (**Figure 5**), it is possible to notice that VFA production occurred at higher concentrations in the AD reactor. In the case of the SWH+AD reactor, a constant increase in the VFA was observed when the pH dropped, which required more addition of basis and consequently increased alkalinity. Additionally, the higher concentration of acetic acid was directly related to methane production in the reactors, and the peak of acetic acid occurred on the same day that the lowest alkalinity values were recorded (at the 14th and 46th days, respectively, for the AD and SWH+AD reactors).

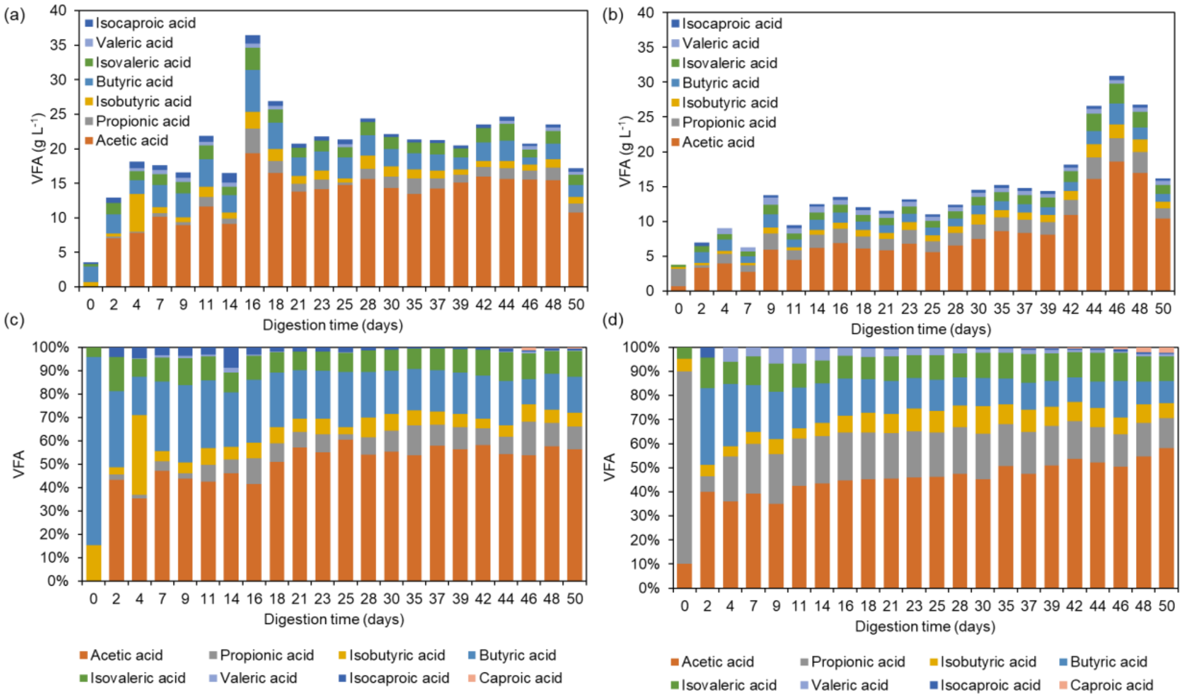

**Figure 5.** Production of volatile fatty acids during the continuous AD and SWH+AD of BSG. (a) The concentration of VFA in the AD reactor (g $L^{-1}$); (b) concentration of VFA in the SWH+AD reactor (g $L^{-1}$); (c) the percentage of VFA in the AD reactor (%); and (d) the percentage of VFA in the SWH+AD reactor (%).

The main applications of VFAs are as food additives, plasticizers, dyes (acetic acid), perfumes, textiles, varnishes, plastics (butyric acid), pharmaceuticals, and fungicides (isovaleric acid) [83]. Furthermore, studies have evaluated the possibility of conducting AD not for biogas production but for the recovery of VFA from the digestate, which can be an advantage for a biorefinery [84]. The feedstock composition and reactor conditions (temperature, pH, and organic loading rate) are important parameters to be considered to increase the VFA yield during the AD process [85].

### *3.4. Production of methane-rich biogas*

**Figure 6** shows the biogas volume and composition for the anaerobic reactors. The daily and accumulated volumes of biogas were higher for the SWH+AD reactor. The control reactor produced 38.1 L, while SWH+AD reached 47.1 L, which represents an increase of 23.7% due to SWH pretreatment. Fluctuations in biogas production and composition are expected until reactor stabilization since each stage of digestion is conducted by a different group of microorganisms, presenting distinct optimal conditions [60]. For the SWH+AD reactor, the initial measurement of biogas showed a composition consisting mainly of $CO_2$ (65.91%) and $CH_4$ (34.09%), while for the AD reactor, the composition was $CO_2$ (70.59%) and $CH_4$ (29.41%). Both reactors presented a rapid increase in methane content, reaching an equivalent methane composition of 63% after five days of digestion. The methane content in biogas was more stable for the SWH+AD reactor, demonstrating that subcritical pretreatment positively increases the methane content. The peak of $CH_4$ was obtained on day 25 for the AD reactor (84.53% $CH_4$). In the case of the SWH+AD reactor, the maximum methane content was obtained on day 50 (89.77% $CH_4$). This fact can be explained by the easy accessibility of the microbial community to convert the substrate into methane [55]. SWH pretreatment promoted the generation of smaller molecules (e.g., sugars, organic acids, and amino acids), which enhanced the metabolism of methanogenic microorganisms [86]. The methane composition obtained corroborates previous studies on the dry AD of BSG [87]. In addition, several variables are responsible for increasing the methane content in biogas. For instance, pH, HRT, density, TVS, temperature, consistency of feed material, OLR, and particle size are the most important parameters that should be continuously evaluated to produce methane-rich biogas [31]. Hence, lignocellulosic biomass pretreatment could be an advantage to boost biogas production.

Regarding the methane yield, the dry AD of BSG in continuous mode produced 53.21 L $CH_4$ $kg^{-1}$ TVS. This value is higher than the dry AD of the same feedstock in batch mode (39.5 L $CH_4$ $kg^{-1}$ TVS) [87]. Bougrier et al. [88] reported a yield of 374 L $CH_4$ $kg^{-1}$ TVS for continuous digestion of BSG using a VSR of 2.23 g TVS $L^{-1}$ $d^{-1,}$ and Szaja et al. [89] reported that 210 L $CH_4$ $kg^{-1}$ TVS could be produced from a VSR of 1.98 g TVS $L^{-1}$ $d^{-1}$. Hence, the present study adopted a VSR of 3.95 g TVS $L^{-1}$ $d^{-1}$ and resulted in lower methane production for the AD reactor (53.21 L $CH_4$ $kg^{-1}$ TVS). That is, the use of a higher organic loading rate negatively affected the methane yield [90]. However, after the subcritical water pretreatment of BSG to start-up AD reactors, the yield increased to 747.71 L $CH_4$ $kg^{-1}$ TVS. This value is consistent with the yield reported by Maciel-Silva [32], who obtained 791.81 L $CH_4$ $kg^{-1}$ TVS for the continuous AD of açaí seeds pretreated with SWH and increased 10-fold when compared with the dry reactor. Finally, the methane yield obtained from the AD of thermally pretreated BSG (121 °C, 1.45 atm for 30 min) (289.1 L $CH_4$ $kg^{-1}$ TVS) [91] was lower than the yield obtained in the present study, indicating that SWH is an effective pretreatment to breakdown biomass and produce biogas with high methane content.

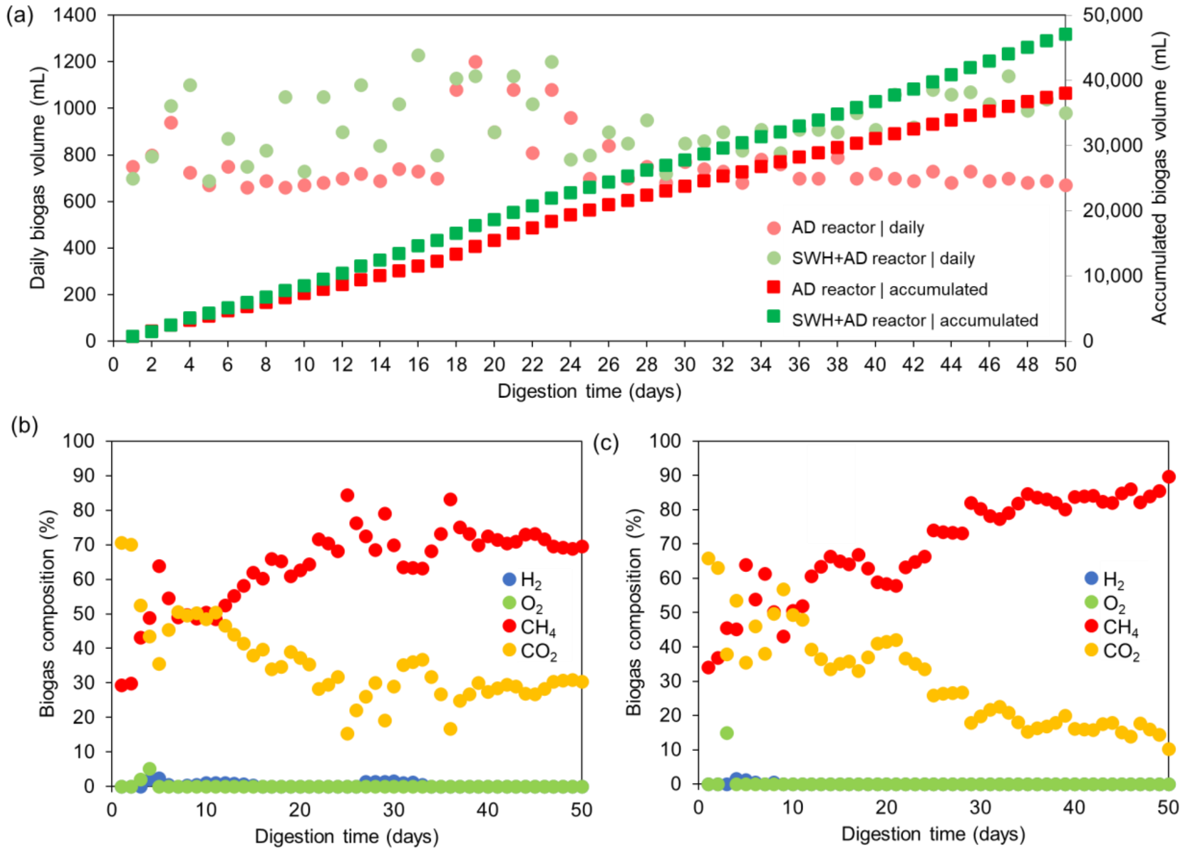

**Figure 6.** Production of methane-rich biogas during the continuous AD and SWH+AD of BSG. (a) Volume of biogas produced (daily and accumulated); (b) biogas composition for the AD reactor; and (c) biogas composition for the SWH+AD reactor.

### *3.5. Bioenergy potential, GHG mitigation, and energy balance*

The biomethane generated from AD is considered a renewable energy source that reduces GHG emissions, especially when applying biomethane for heat and electricity generation [92]. The development of biogas plants has been encouraged to produce bioenergy and establish neutral climate policies [93]. Hence, modern anaerobic digestors convert methane into electricity and heat through combined heat and power plants [94]. Therefore, based on the volume of biogas, methane composition, and mass of BSG, the estimations for electricity and heat were calculated **(Table 3**).

**Table 3.** Methane yield, the potential of electric energy, heat, and avoided GHG emissions for the continuous AD and SWH+AD of BSG.

| Parameters | AD reactor | SWH+AD reactor | Unit |
|---|---|---|---|
| Biogas productivity | 26.39 | 48.76 | L biogas $kg^{-1}$ BSG |
| Methane productivity | 16.87 | 33.95 | L $CH_4$ $kg^{-1}$ BSG |
| Methane yield | 53.21 | 747.71 | L $CH_4$ $kg^{-1}$ $TVS_{added}$ |
| $EG_{CH_4}$ | 66.70 | 134.00 | kWh $t^{-1}$ BSG |
| $HG_{CH_4}$ | 300.30 | 604.14 | MJ $t^{-1}$ BSG |
| $GHG_{electricity}$ | 5.01 | 10.06 | kg $CO_{2-eq}$ $t^{-1}$ BSG |
| $GHG_{heat}$ | 16.82 | 33.83 | kg $CO_{2-eq}$ $t^{-1}$ BSG |
| $GHG_{total}$ | 21.82 | 43.90 | kg $CO_{2-eq}$ $t^{-1}$ BSG |

The AD of 1 ton BSG could produce 0.0667 MWh of electricity and 300.3 MJ thermal energy. Beyond, for the process with pretreatment, there is an increase in the production of electricity (0.134 MWh $t^{-1}$ BSG) and heat (604.14 MJ $t^{-1}$ BSG), considering the mass of BSG added during the feed and not the mass used for the pretreatment. In a previous study, the electricity and heat potential of BSG were estimated at 63.9 kWh $t^{-1}$ and 239.31 MJ $t^{-1}$ for a plant with a treatment capacity of 137 t BSG $d^{-1}$ [12]. Positive economic indicators for the industrial AD implementation of BSG were obtained, with a payback of 3.76 y, return on investment of 23.68%, and net present value up to 1.5 million USD [12]. Hence, in the present study, an increase of 2.1-fold for electricity and 2.5-fold for heat was obtained with the adoption of pretreatment, demonstrating that the industrial process operated under the operational conditions established in this study (for the AD reactor) may be profitable for industrial implementation.

From an environmental perspective, the electricity generated from biogas can be used on-site by facilities. In the case of thermal energy, the heat recovered could replace natural gas in the boiler, avoiding the use of natural gas and decreasing GHG emissions. The biomethane-rich biogas generated in the AD reactor could mitigate a total of 21.82 kg $CO_{2\ eq}$ $t^{-1}$ BSG (5.01 and 16.82 kg $CO_{2\ eq}$ $t^{-1}$ BSG, respectively, for electricity and heat). Moreover, with the implementation of SWH pretreatment, methane production increased. Consequently, the GHG mitigation increased to 43.90 kg $CO_{2\ eq}$ $t^{-1}$ BSG (10.06 and 33.83 kg $CO_{2\ eq}$ $t^{-1}$ BSG, respectively, for electricity and heat) (**Table 3**). Therefore, the adoption of the AD system can reduce GHG emissions compared with hydroelectric and petroleum-based energy [95], supporting the biorefinery concept.

Notwithstanding, the specific energy demand for processing beer is estimated according to the production capacity [96], ranging from 8 to 25 kWh electricity $hL^{-1}$ and 49 to 24 kWh heat $hL^{-1}$. In general, the brewery demands 40% of electric energy for refrigeration, 6% for air compression, 13% for other industrial processing stages, and 23% for administration and illumination [96]. Then, the application of bioenergy from biogas can support the brewery energy demand, supporting circularity with economic and environmental benefits [97]. Therefore, the anaerobic management of BSG can increase the energy efficiency of breweries, reducing the consumption of fossil fuels and decreasing GHG emissions [97].

The energy balance of SWH and AD was determined by specific electricity and associated with the energy recovered from methane. For both processes, estimates were based on the operational condition established in the methodology framework. **Figure 7** shows a global industrial balance with the adoption of AD and SWH+AD reactors. Both scenarios were studied considering the production of 5000 L of beer and the generation of 1 t BSG. In addition, electricity (29.84 kWh $hL^{-1}$ beer produced) and heat (154.05 MJ $hL^{-1}$ beer produced) demand were estimated for a conventional and well-managed brewery [98,99]. The results showed that 1.49 MWh electricity and 7,702.5 MJ of heat are required for the production of 5000 L of beer. In **Figure 7a**, the AD of BSG without pretreatment could produce 26.39 $m^3$ biogas. The biogas should be purified into biomethane. The purified biogas can be upgraded into electric and thermal energies in a co-generator. However, upgrading biogas into biomethane demands 0.301 kWh $m^{-3}$ biogas upgraded [100], which means that the current scenario demands 7.94 kWh of electricity for biogas purification. In addition, the energy balance considered that a standard anaerobic mesophilic reactor requires electricity (10 kWh $t^{-1}$ feedstock) and heat (69.84 MJ $t^{-1}$ feedstock) [101]. This energy can be used from the energy generated in the co-generator. Hence, the net electricity (49.76

kWh) and heat (230.46 MJ) generated from the AD reactor can be used in the industrial process for beer production.

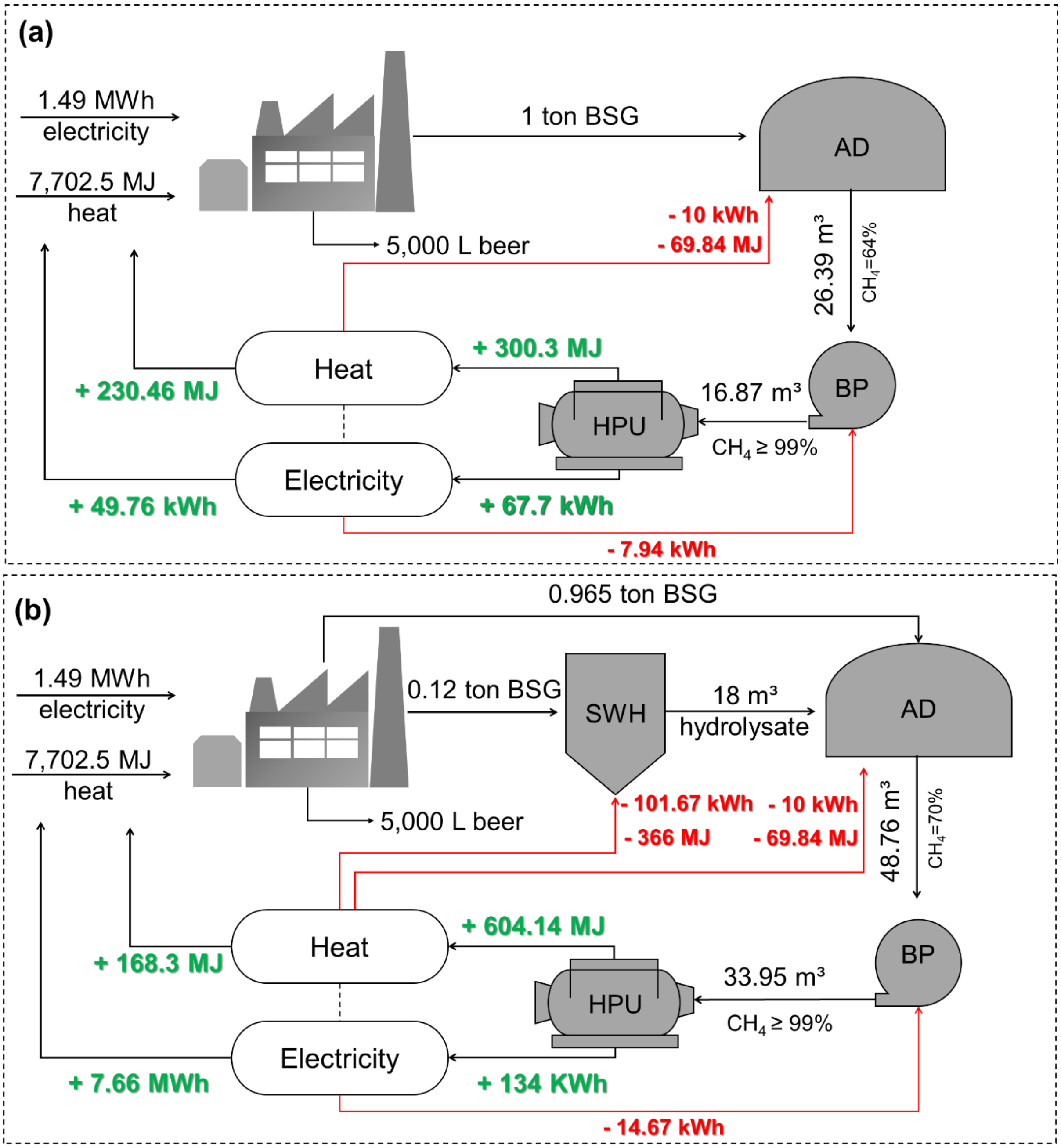

**Figure 7.** Industrial mass and energy balance for the AD of BSG with and without pretreatment. (a) AD reactor and (b) SWH+AD reactor.

In the case of the process operated with SWH, it is necessary to determine the energy demand for the pretreatment. For this, the energy balance based on the laboratory conditions of hydrolysis showed that the heat required for the reactor was 610 J $g^{-1}$. Considering that 18 m³ of hydrolysate can be produced for a scenario where

0.12 t BSG is destined for the pretreatment and 0.956 t is fed in the reactor during the HRT of 25.2 d, a total of 732 MJ is necessary for the pretreatment. In the current energy balance, it was assumed that 50% of the energy in the reactor was provided by thermal energy, and the other 50% was supplied by electricity [37]. That is, the SWH demanded heat (366 MJ) and electricity (101.67 kWh) for the pretreatment of BSG used in the SWH+AD reactor (**Figure 7b**). The energy for SWH, AD, and biogas purification can be used from the energy generated in the co-generator. In this scenario, the surplus for the industry was calculated at 7.66 kWh and 168.3 MJ. This surplus is low since the pretreatment requires high energy; however, the AD can supply the energy necessary for the pretreatment and is an alternative for the implementation of this waste management system.

Therefore, based on the industrial waste management system for BSG treatment, the proposed scheme would be an initial approach to promote the beer industry's circular economy transition since the designed industrial process can be a technological route to be applied in a biorefinery [102].

## 4. Conclusion

This study evaluated the effectiveness of semi-continuous flow-through subcritical water hydrolysis (SWH) pretreatment of brewer's spent grains (BSG) for AD. The monosaccharide profile of the hydrolysate obtained through the SWH of BSG demonstrates a high concentration of arabinose (46.54 mg $g^{-1}$) and xylose (39.90 mg $g^{-1}$) and low production of 5-hydroxymethylfurfural and furfural, which are desired characteristics for application of the hydrolysate in fermentation processes. During AD, the COD removal reached 20.98% for the reactor operated with raw BSG without pretreatment. Regarding the methane yield, the dry AD reactor produced 53.21 L CH4 $kg^{-1}$ TVS, and SWH+AD showed a yield of 747.71 L $CH_4$ $kg^{-1}$ TVS, indicating that SWH is an effective pretreatment to breakdown biomass and produce methane-rich biogas. The AD of 1 ton BSG could produce 0.0667 MWh of electricity and 300.3 MJ heat. Beyond that, the recovery of electricity (0.134 MWh $t^{-1}$ BSG) and heat (604.14 MJ $t^{-1}$ BSG) increased with the adoption of pretreatment. Hence, it was possible to increase the bioenergy productivity by 2.1-fold for electricity and 2.5-fold for heat. Finally, it was concluded that the adoption of SWH pretreatment of biomass for AD could be a sustainable technological route to increase methane-rich biogas and bioenergy production, contributing to reducing the environmental impacts and advocating circular economy transition of the beer industry.

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# CHAPTER VII

## Techno-economic assessment of bioenergy and fertilizer production by anaerobic digestion of brewer’s spent grains in a biorefinery concept

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# CHAPTER VII: Techno-economic assessment of bioenergy and fertilizer production by anaerobic digestion of brewer’s spent grains in a biorefinery concept

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## Abstract

This study presents a techno-economic assessment of bioenergy and fertilizer production by anaerobic digestion (AD) of brewer's spent grains (BSG). Simulations were performed by integrating the production of biomethane, electricity, thermal energy, and fertilizer for five revenue conditions. BSG generation from different brewery industrial scales was considered. Results showed that investing one million USD for a treatment capacity of 137 t BSG $d^{-1}$ makes it possible to recover up to 350,000 USD $y^{-1}$ when selling electric energy to the grid, using the thermal energy for the facility self-consumption, and selling the fertilizer for agriculture application. The economic analysis for this scale resulted in a payback time of 3.76 y, ROI of 23.68%, and NPV up to 1.5 million USD. The revenue considering biomethane and fertilizer was profitable and amenable for a real application, with a payback time of 3.67 y and IRR up to 20%. From the sensitivity analysis, the selling price of fertilizer, biomethane, electricity, and heat were the most important variables affecting the project's economic viability. Beyond, project implementation from equity could upgrade the feasibility. Ultimately, the waste management system with bioenergy recovery and fertilizer production from the AD of BSG is profitable and could be suitable for an oncoming implementation in breweries.

***Keywords**: Brewer's spent grain; Electrical Energy; Methane; Fertilizer; Biorefinery; Techno-economic assessment*

## 1. Introduction

Strategic integration of food and energy supply chains offers several opportunities to improve productivity (from field to the industry) while mitigating losses and environmental side-effects. The agro-industrial sector contributes to the generation of a large amount of organic wastes and faces the challenge of developing strategies for sustainable energy, food, and materials production to meet the growing global demands of humanity (Pérez-Flores et al., 2019). Hence, appropriate organic waste treatment is necessary for an environmentally friendly final disposal and energy recovery (Winquist et al., 2019). The integration between waste treatment and bioenergy production could be a solution to reconcile energy production and environmental conservation within the Sustainable Development Goals (SDGs) established by the United Nations Framework Convention on Climate Change (UNFCCC) (UN, 2020). SDGs are strategic targets to overcome the economic crisis caused by water scarcity risk, energy security issues, and climate change, among other factors, to support the development of public policies towards sustainable development. For instance, the 6th (clean water and sanitation), the 7th (affordable and clean energy), and the 13th (climate action) SDGs can be directly associated with the implementation of waste management systems for bioenergy recovery aiming to underpin a circular economy, which opposes to the current economic logic (Geng et al., 2019). Moreover, due to environmental concerns and social policies to reduce the environmental impacts of industrial activities, the replacement of fossil raw materials by more sustainable options is receiving special attention in recent years, and the focus is on the use of residual biomass from agro-industrial processes (Pérez-Flores et al., 2019). Nowadays, converting food waste into energy and other useful platform chemicals is a growing research area to develop new opportunities to decrease carbon dioxide emissions for more sustainable industrial processing (Dragone et al., 2020).

Biotransformation of organic wastes into biogas can be considered a rousing alternative with many benefits (Maciel-Silva et al., 2019). Anaerobic digestion (AD) is a well-consolidated low-cost technology for treating organic wastes (Garcia et al., 2019). AD produces clean energy due to the high concentration of methane in the biogas, which can reach 50 to 75%, according to the organic material employed (Nathia-Neves et al., 2018). Moreover, AD is considered one of the most promising techniques to produce biofuels because of its easy implementation, versatility, and low investment (Leme & Seabra, 2017). From an economic perspective, the global biogas market is expected to reach $50 billion by 2026 (Waste Management World, 2017). Considering these points and the growing energy demand, biogas production can be seen as an attractive, economical, and ecological alternative to support a sustainable industrial development (Jiménez-Castro et al., 2020). Moreover, due to its methane content, biogas is suitable for burning in heat and power units to produce electric and thermal energies to supply the own food industry requirements (Garcia et al., 2019). The virtuous loop of energy inside the production chain fits the circular economy concept and places AD in an even more critical outlook to overcome the current food-energy trade-off (Freitas et al., 2019).

The global electricity demand is expected to grow at a rate of 2.1% per year until 2040, reaching more than 40 thousand TWh (IEA, 2020). The world greenhouse gas (GHG) emissions from the power sector reached 13.8 Gt of $CO_{2eq}$ in 2018, and, specifically in Brazil, the power required to the industry was responsible for 90 Mt of $CO_{2eq}$ (IEA, 2020). The production of biogas from the AD of industrial wastes and by-products, followed by its conversion into electric energy, may reduce GHG emissions and the associated environmental side-effects (Campello et al., 2020). Furthermore, the Brazilian energy laws (ANEEL, 2017) and the new state policy “RenovaBio” (13.576/2017) encourage biogas production and allow the non-centralized output of electric energy. AD can be a suitable alternative to industries that present a high amount of solid wastes and high-energy requirements (Ferreira et al., 2018), likewise the brewing industry. Brazilian beer production reached 14 billion liters in 2019, with an income of approximately 6.34 billion USD (IBGE, 2017). This industrial sector grows at an average rate of 20% per year in Brazil (MAPA, 2020). Consequently, the high production of beer generates high amount of brewer’s spent grains (BSG), the solid waste remaining after the brewing process (Mussatto, 2014). An estimate for the brewing process is that 20 kg of wet BSG is generated per 100 L of beer produced (Mussatto et al., 2006). Therefore, Brazilian annual BSG production can be estimated at $2.8\times10^{6}$ t $y^{-1}$ (in wet weight). BSG, available from large and small breweries with low cost and large quantities throughout the year (Mussatto et al., 2013), is suitable to *in loco* application by the industry, such as for AD treatment.

The design of systems for energy recovery and materials recycling in supply chains can be addressed from a biorefinery perspective, which consists of an integral conversion of agro-industrial wastes into value-added products, minimizing environmental side-effects, and maximizing the use of renewable resources (Dragone et al., 2020). From a feedstock, it is possible to integrate the production of bioenergy, biomaterials, and new food inputs or products for a flourishing bioeconomy. So far, BSG has been mainly used as animal feed or disposed of in landfills (Hassan et al., 2020). However, several studies have reported the possibility of producing diverse biomaterials, including xylitol (Mussatto & Roberto, 2008), bioactive compounds (Socaci et al., 2018), lactic acid (Mussatto et al., 2007), enzymes (Hassan et al., 2020), arabinoxylan (Pérez-Flores et al., 2019), activated carbon (Mussatto et al., 2010), among others. BSG can also produce biogas to be converted into heat and power for the brewing industry self-consumption. An eventual energy surplus can also be sold to the grid, contributing to increased energy efficiency and GHG emissions mitigation (Winquist et al., 2019).

A growing interest in AD to treat wastewater and solid wastes generated from breweries is noticeable. Mainardis et al. (2019) evaluated the biochemical methane potential of the co-digestion of solid and liquid wastes from a small brewery (beer production capacity ranging from 2,940 to 4,628 hL $y^{-1}$) and highlighted a net electricity (53.9% of the total plant demand) and heat production (64.4% of the total industrial thermal energy requirement) for a 65 $m^3$ up-flow anaerobic sludge blanket (UASB) reactor. Otherwise, for a large capacity brewery (90,000 hL $y^{-1}$), Weber and Stadlbauer (2017) observed a biogas production from liquid effluents treatment in a 21 $m^3$ CSTR (continuous stirred tank reactor), enough to save 20% of the electricity and 9% of the thermal energy requirements. Despite the previous studies about AD application in breweries, there is a knowledge gap in the scientific literature regarding the scale-up of continuous AD reactors to treat solid wastes, specifically BSG, at an industrial-scale level. The assessment of different industrial scales for BSG treatment could support future decision-making for a local and decentralized energy generation to meet the industrial energy requirements (Karger & Hennings, 2009). Along with the potential economic savings, waste energy recovery can be a strategy to decrease brewery's carbon footprint towards a sustainable transition to a circular economy. Furthermore, such a strategy is valuable to accomplish SDGs.

In a biorefinery concept, processes including an AD to produce bioenergy could be economically profitable and environmentally sustainable (Dragone et al., 2020), consequently improving the energy cycling of agro-industrial chains (Campos et al., 2020). Hereupon, this case study presents a techno-economic assessment of bioenergy and fertilizer production from BSG-AD. Different revenues were considered for biomethane, electricity, thermal energy, and fertilizer production to identify the most profitable option for industrial implementation. The results present economic indicators for different breweries' industrial capacities and could subsidize decision-making processes towards the broad adoption of waste management systems based on AD and energy cycling from solid wastes. Finally, the research findings are intimately related to future biorefinery implementation strategies to support a transition to a circular economy for the beer industry.

## 2. Materials and methods

### *2.1 AD implementation and biogas production*

#### *2.1.1 Description of the AD system*

This case study was based on energy recovery and fertilizer production from BSG generated from breweries with an AD treatment system included in the facility. The process flowsheet (block diagram) is presented in **Fig. 1a,** while **Fig. 1b** demonstrates the industrial arrangement for bioenergy and fertilizer production from BSG-AD. BSG is generated from the barley used in the brewing process, with average moisture of 85% (Mussatto, 2014). After brewing, the final solid waste is collected and destined to an equalizer tank to achieve ideal water and pH values for AD. With water addition, the total flow required for the AD at an industrial scale was adjusted. BSG pH was corrected. In this case study, no pre-treatment was applied to BSG previously to the experimental assay, being the material directly used in the proposed waste management system (Sayana and Sánchez, 2019). After digestion, the biogas obtained can be destined to produce biomethane (after a purifying step), electrical and thermal energies. Additional information about the purifying step was included in the **Supplementary Material**. The digestate obtained from AD can be treated in a circular decanter to recover water and produce a fertilizer rich in nitrogen, phosphorus, and potassium (NPK) for agricultural use (Buller et al., 2020).

In the laboratory AD assays, the reactor temperature was kept at mesophilic conditions (35 °C ± 2) in a neutral pH achieved by the addition of sodium hydroxide (NaOH), which amount was estimated for an industrial scale. The initial lab-scale reactor was fed by 65% of the substrate, with a headspace of 35% (this proportion was kept in the scale-up analyzes). From the 65% initial substrate, 28% was dry BSG, 39% inoculum, and 33% liquid phase. In the industrial AD, the BSG can be introduced on a wet basis without pre-treatment (Weber and Stadlbauer, 2017). For the simulations, the operational parameters of the lab-scale AD were adjusted to the large-scale.

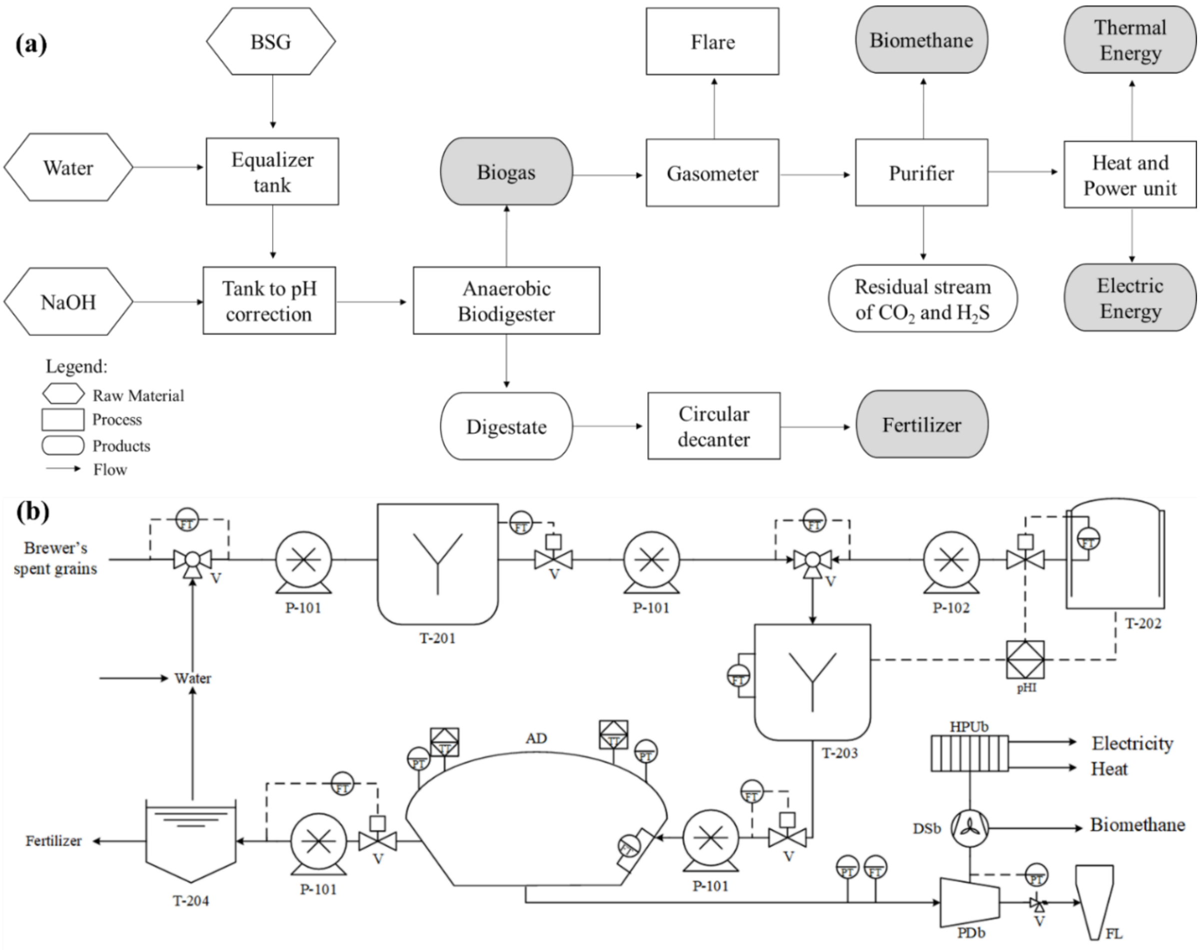

**Figure 1.** (a) Block diagram flowsheet and (b) process flow diagram of the industrial process arrangement for bioenergy and fertilizer production by anaerobic digestion of BSG.

Experimental data for BSG physicochemical composition and for the lab-scale BSG-AD reactor were summarized in **Table 1**. The lab-scale reactor was fed with 179.45 g of dried BSG, and, to simulate a continuous reactor, an additional 5 g of BSG was fed in a daily basis. The reactor was started-up with an initial solids content of 13.35%, characterizing as a wet process (Kothari et al., 2014). The AD reactor was operated with a volatile solid loading rate (VSR) of 0.54 g Total Volatile Solids (TVS) $L^{-1}$ $d^{-1}$, which promoted 76% of solids biodegradation after 30 d digestion. After completing the AD, 31.36 L of biogas, composed of 45% methane, were produced, i.e., from 1 t of BSG (wet basis), 30.76 m$^3$ of biogas were obtained. Additionally, the accumulated yield of 5.05 L $CH_4$ $kg^{-1}$ TVS follows the results from the AD of other lignocellulosic feedstocks (dos Santos et al., 2018; Maciel-Silva et al., 2019; Jiménez-Castro et al., 2020).

The potential of electrical and thermal energies that could be generated by the biogas burning in a co-generator was estimated according to Eqs. 1 and 2, respectively (Campello et al., 2020):

$$EG_{CH_4} = Q_{biogas} \times LCV_{CH_4} \times C_m \times \eta_e \quad (1)$$

$$HG_{CH_4} = Q_{biogas} \times LCV_{CH_4} \times C_m \times \eta_e \quad (2)$$

*where:* $EG_{CH_4}$ is the potential electricity generation from experimental biogas yield; $HG_{CH_4}$ is the potential heat generation from experimental biogas yield; $Q_{biogas}$ is the experimental biogas volume produced ($m^3$); $LCV_{CH_4}$ is the lower calorific value of methane (35.59 MJ $m^{-3}$); $C_m$ is the percentage of methane in biogas (%); and $\eta_e$ is the engine efficiency (%), assumed as 40% for electric energy and 50% for thermal energy.

By theoretical conversion (Eqs. 1 and 2), biogas with 45% of methane produces 2.13 kWh of electric energy and 7.78 MJ of thermal energy per $m^3$ of biogas burned in commercial generators. Based on the experimental data and operational conditions, biogas production was scaled-up for different brewery's capacities selected for the current case study (for small to large industrial sizes).

After the biogas production, an industrial section for the digestate upgrade and fertilizer production was designed (**Fig. 1**). The mixture obtained flows through a solid-liquid separation step (a circular decanter) to obtain solid and liquid fractions. The use of a circular decanter allows a faster separation of the liquid fraction with high efficiency. The separated water can be reused in the process, while the remaining fraction from digestate is the fertilizer, which can be used in agriculture to replace mineral NPK (Buller et al., 2020). The fertilizer composition was assumed as a mixture of 30% of dry matter and 70% of the liquid phase, i.e., from 1 t of wet BSG fed in the reactor, 436.66 kg of fertilizer was produced. Beyond, it was assumed that no NPK was lost during the AD process since the amount of NPK in the fertilizer is proportional to BSG initial contents, considering non-degradation (Li et al., 2020). The NPK composition in BSG was analyzed by Mussatto (2014) and summarized in **Table 1**. Additional information about the fertilizer can be found in the **Supplementary Material**. In the current case study, the fertilizer was assumed as "liquid organic fertilizer" according to the EU (2019/1009), containing at least 1% by mass of total nitrogen (N), 1% by mass of total phosphorus pentoxide ($P_2O_5$), or 1 % by mass of total potassium oxide ($K_2O$). A liquid fertilizer within the limits of pathogens (*Salmonella* spp. and *Escherichia coli)* for agricultural use, according to the established by the regulation (EU, 2019/1009), was assumed. The final composition of the fertilizer was 7.48 g N $kg^{-1}$, 1.55 g $P_2O_5$ $kg^{-1}$, and 0.0774 g $K_2O$ $kg^{-1}$.

Based on the estimations for bioenergy and fertilizer production from BSG-AD, **Fig. 2** shows the industrial mass and energy balance obtained for this study considering the digestion of 1 t wet BSG. The flows established were assumed as the basis for the economic simulation for bioenergy and fertilizer recovery. The AD of 1 t BSG requires 0.76 kg NaOH, and 1.27 $m^3$ water. After the digestion, 30.76 $m^3$ biogas and 1353.83 kg digestate can be produced. The biogas can be upgraded into biomethane (13.84 $m^3$ $t^{-1}$ BSG) or burnt to generate electricity (63.9 kWh $t^{-1}$ BSG) and thermal energy (239.31 MJ $t^{-1}$ BSG). A residual stream of $CO_2$ and $H_2S$ is also produced (16.92 $m^3$ $t^{-1}$ BSG). The digestate can be upgraded in a circular decanter to recover liquid fertilizer (436.66 kg $t^{-1}$ BSG) and re-use water for the AD process (917.17 L $t^{-1}$ BSG).

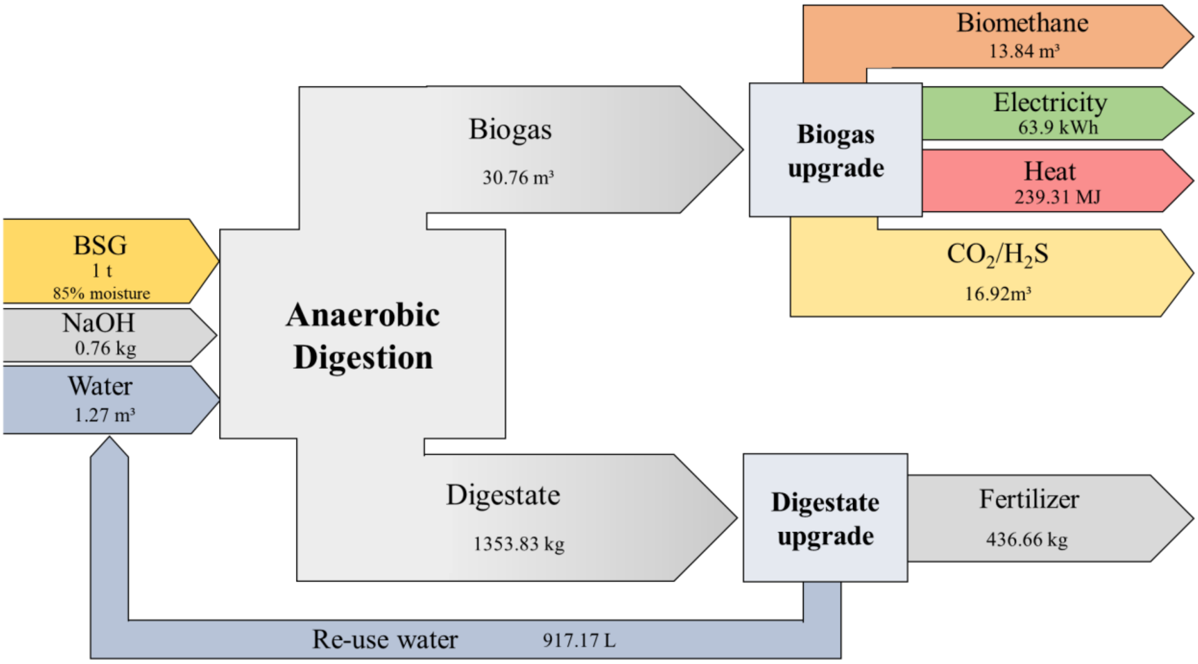

**Figure 2.** Industrial mass and energy balance for bioenergy and fertilizer production from BSG.

**Table 1.** BSG physicochemical composition and general parameters recorded for the AD of BSG in a lab-scale reactor.

| Parameters | Unit | BSG composition | Parameters | Unit | BSG-AD | |
|---|---|---|---|---|---|---|
| | | | | | Digestate in the reactor (Initial) | Digestate in the reactor (Final) |
| Protein[a] | % | 19.2 | pH[b] | – | 6.76 | 7.50 |
| Total Lignin[a] | % | 17.8 | Chemical oxygen demand[b] | mg $O_2$ $L^{-1}$ | 5449.58 | 25,290.81 |
| Cellulose[a] | % | 17.9 | Ammonia nitrogen[b] | mg $NH_3$ $L^{-1}$ | 79.10 | 1227.27 |
| Hemicellulose[a] | % | 35.7 | Total solids[b] | % | 13.35 | 3.16 |
| Total solids[b] | % | 98.1 | Total volatile solids[b] | % | 12.70 | 2.71 |
| Total volatile solids[b] | % | 73.5 | Total fixed solids[b] | % | 0.65 | 0.46 |
| pH[b] | – | 5.96 | Alkalinity[b] | mg $CaCO_3$ $L^{-1}$ | 745.76 | 8990.19 |
| Ammonia nitrogen[b] | mg $NH_3$ $L^{-1}$ | 185.3 | Acetic acid[b] | mg $L^{-1}$ | 100.00 | 1291.22 |
| Chemical oxygen demand[c] | g $O_2$ $g^{-1}$ TS | 1.48 | Propionic acid[b] | mg $L^{-1}$ | 11.00 | 13.63 |
| Anaerobic biodegradability[c] | % | 86.9 | Isobutyric acid[b] | mg $L^{-1}$ | 2.00 | 111.62 |
| Density (wet basis)[d] | kg $m^{-3}$ | 219.0 | Butyric acid[b] | mg $L^{-1}$ | 13.02 | 106.10 |
| Nitrogen (N)[e] | g $kg^{-1}$ | 24.86 | Isovaleric acid[b] | mg $L^{-1}$ | 0.00 | 115.73 |
| Phosphorus (P)[e] | g $kg^{-1}$ | 5.186 | Valeric acid[b] | mg $L^{-1}$ | 0.00 | 5.89 |
| Potassium (K)[e] | g $kg^{-1}$ | 0.258 | Hexanoic acid[b] | mg $L^{-1}$ | 0.00 | 0.78 |

[a] Torres-Mayanga et al. (2019); [b] Analysis previously conducted by authors; [c] Vitanza et al. (2016); [d] Cordeiro et al. (2013); [e] Mussatto (2014).

### *2.1.2 Industrial scales and revenues established*

Three BSG processing capacities were simulated (t wet BSG $d^{-1}$): (i) 137 (Scale 1), (ii) 34 (Scale 2), and (iii) 9 (Scale 3), respectively to large, medium, and small industrial sizes. With the addition of the necessary water for the AD, the total flows were established as: (i) 800 (Scale 1), (ii) 200 (Scale 2), and (iii) 50 $m^3$ $d^{-1}$ (Scale 3). From the AD proposed scheme (**Fig. 1**), biogas conversion routes into biomethane, electrical and thermal energies were evaluated.

Revenues from the energy conversion can be combined to achieve a better management of BSG-AD co-products. In this study, the biomethane selling price was estimated in 0.28 USD $m^{-3}$ based on the replacement of natural gas by biogas (Ferreira et al., 2019). Electric energy price was assumed in 60.56 USD $MWh^{-1}$ (Watanabe et al., 2020) based on the average cost of the Brazilian grid. The selling price of thermal energy was estimated in 0.0082 USD $MJ^{-1}$ based on the heat replacement in a biomass boiler, in accordance with Rahimi & Shafiei (2019). Fertilizer selling price was estimated in 6.16 USD $t^{-1}$ based on the NPK composition (Li et al., 2020; Koido et al., 2018). According to these assumptions, five *ex-ante* revenues were established for the three scales simulated, aiming to address different market conditions to support an *ex-post* analysis of the most suitable investment, as follows:

(i) **Revenue 1:** 100% of the fertilizer produced was sold for agricultural use, and 100% of the biomethane produced was sold as natural gas;

(ii) **Revenue 2:** 100% of the fertilizer produced was sold for agricultural use, and 100% of the electric energy generated was sold to the grid;

(iii) **Revenue 3:** 100% of the fertilizer produced was sold for agricultural use; 50% of the biomethane produced was sold as natural gas, and 50% was converted into electric energy in a generator and sold to the grid;

(iv) **Revenue 4:** 100% of the fertilizer produced was sold for agricultural use; 100% of the electric energy produced in a co-generator was sold to the grid, and 100% of the thermal energy produced in the co-generator was sold to the industry.

(v) **Revenue 5:** 100% of the fertilizer produced was sold for agricultural use; 50% of the biomethane produced was sold as natural gas, and 50% was converted into electric and thermal energy.

For the revenues above, different implementation costs were obtained according to the necessary equipment and sales options. After the purification, biomethane can be directly sold, and for electrical and thermal energy production, a generator/co-generator is needed, which promoted several sales options.

### *2.1.3 AD operational configurations*

A covered lagoon anaerobic digester is considered a robust biological reactor with simple construction (Leme & Seabra, 2017). Additionally, this biodigester type presents a rapid stabilization time and produces less biomass per unit of organic material processed (EPA, 2002). The anaerobic lagoons do not require electric energy, heat, and mixing systems to treat organic wastes (EPA, 2002). Therefore, a covered lagoon digester was scaled to promote the treatment of BSG and biogas production, taking into account the flow of the scales 1, 2, and 3 previously established. The biodigester volume was calculated according to **Eq. 3** (Van der Lubbe and Van Haandel, 2019).

$$\text{Biodigester volume}\left(\mathrm{m}^3\right)=\mathrm{HRT}\ (\mathrm{d})\times \mathrm{Q}\ \left(\frac{\mathrm{m}^3}{\mathrm{d}}\right)=\frac{\mathrm{S}_0\left(\frac{\mathrm{kg}}{\mathrm{m}^3}\right)\times \mathrm{Q}\left(\frac{\mathrm{m}^3}{\mathrm{d}}\right)}{\mathrm{VSR}\ \left(\frac{\mathrm{kg}}{\mathrm{m}^3\ \mathrm{d}}\right)} \quad (3)$$

*where:* the hydraulic retention time (HRT) was set as 22 days (based on the experimental results); Q is the flow of BSG fed in the reactor ($m^3\ d^{-1}$); $S_0$ is the initial concentration of substrate (kg $m^{-3}$); and VSR is the volatile solid loading rate (kg $m^{-3}\ d^{-1}$).

The AD sizes were determined for each scale. **Fig. 3a** shows the covered lagoon digester and its size variables, the front (**Fig. 3b**), and the superior view (**Fig. 3c**).

## *2.2 Economic analysis of biogas production from AD*

The economic performance of biogas production was evaluated by estimating the capital investment, operation cost, and revenue generation. Profitability and sensitivity analysis were assessed. The economic parameters were calculated for the current Brazilian market. A 100% of external capital (loan finance) was assumed. The external capital bank financing was established for 10 y, with an annual interest rate of 8.5%. The project lifetime was established as 25 y, with an annual depreciation rate of 10%. An annual tax rate of 25% and an attractiveness rate of 15% were assumed. The plant's annual time operation was set in 7,680 h (320 d $y^{-1}$). The economic analysis was conducted considering an average exchange rate of 3.94 USD/BRL for the year 2019 (IPEA, 2020).

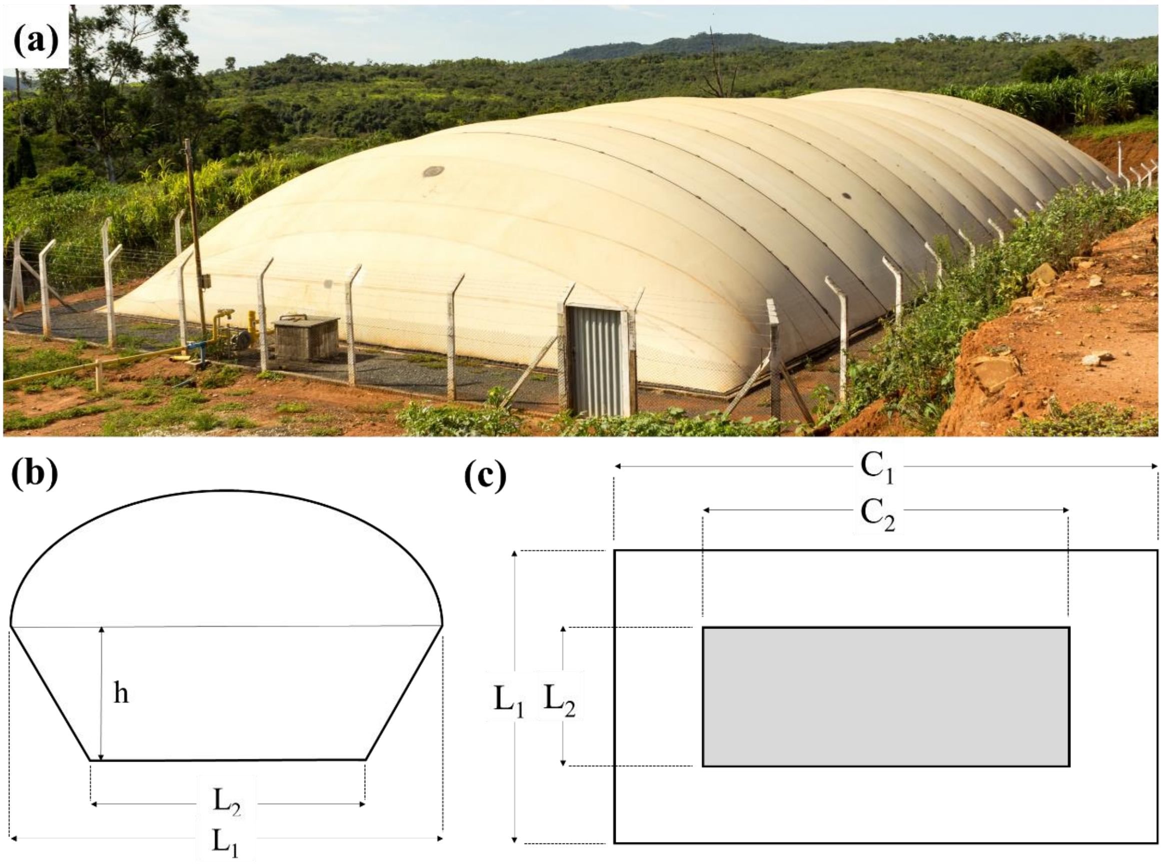

**Figure 3.** Covered lagoon anaerobic digester scaled for BSG anaerobic digestion (a) and the front view (b) and superior view (c) of its variables.

### *2.2.1 Costs estimation*

The itemized cost estimation of each economic parameter was discriminated against the fixed capital costs. Equipment installation costs were collected from the current Brazilian market. Based on the industrial design (**Fig. 1b**) and revenues established (**Section 2.1.2**), the total fixed capital investment (FCI) was evaluated. The five major components of the cost of manufacturing (COM) were determined as the sum of the main process' components (Turton et al., 2009), according to **Eq. 4.**

$$COM=(0.304\times FCI)+(2.73\times COL)+[1.23\times(CUT+CWT+CRM)] \quad (4)$$

*where:* FCI is the fixed capital investment; COL the cost of operational labor; CUT the cost of utilities; CWT the cost of waste treatment, and CRM the cost of raw material.

FCI is related to the expenses for the implementation of the production unit. CRM consists of the costs required to prepare the raw material and the costs of the chemicals. COL is related to labor (manpower number and wages). CUT considers the energy used for processing and cleaning. CWT is the residue generated by the process, which was established as the capital necessary for the implementation of the process for fertilizer production.

For Scale 1, it was assumed 3 operational shifts with 1 worker/shift (3 workers $d^{-1}$). In Scale 2 the AD plant was operated in 2 shifts (2 workers $d^{-1}$), while in Scale 3, 1 shift (1 worker $d^{-1}$) was assumed. CRM of BSG, water, and NaOH were estimated as 0.5 USD $t^{-1}$, 0.35 USD $m^{-3}$, and 0.53 USD $kg^{-1}$, respectively. COL was assumed as 3 USD $h^{-1}$ worked. CUT refers to the water demand for cleaning (0.35 USD $m^{-3}$) and energy demand for the whole process (0.06 USD $kWh^{-1}$), both considering the current Brazilian market prices.

### *2.3 Profitability analysis*

The feasibility of the proposed scales was analyzed from the following indicators: (i) gross margin (GM); (ii) net margin (NM); (iii) return on investment (ROI); (iv) net present value (NPV); (v) internal rate of return (IRR); and (vi) payback time. These indicators are the most commonly used for industrial projects' profitability analysis. GM is the difference between the revenue and the costs of the goods sold. NM is similar to GM, but it also takes into account all the discounts. NPV is the sum of the projected discounted cash flows minus the initial investment and can be calculated according to **Eq. 5.**

$$\mathrm{NPV}=\sum_{t=1}^{n}\frac{\mathrm{FC}_t}{(1+i)^t}-\mathrm{I}_0 (5)$$

*where*: $\mathrm{FC}_t$ is the cash flow in time period "t"; "t" is the investment lifespan; "n" is the number of time periods; "i" is the cost of capital; and $I_0$ is the initial investment.

IRR is the discount rate when NPV reaches zero and can be calculated through **Eq. 6.** The project with the highest IRR is considered the most attractive.

$$\mathrm{NPV}=0=\sum_{t=1}^{n}\frac{\mathrm{FC}_t}{(1+\mathrm{IRR})^t} \quad (6)$$

*where*: FC is the cash flow in time period "t"; "t" is the investment lifespan; "n" is the number of periods; "i" is the cost of capital.

ROI **(Eq. 7)** measures the return of the money invested in the project, indicating the investment cost-benefit. Payback time **(Eq. 8)** is the number of years to recover the initial investment.

$$\text{ROI}(\%)=\frac{\text{Annual net profit}}{\text{Total capital investment}}\quad(7)$$

$$\text{Payback time (y)}=\frac{\text{Total capital investment}}{\text{Annual net profit}}\quad(8)$$

### *2.4 Sensitivity analysis*

A sensitivity analysis was conducted to evaluate the effects of price uncertainties in the profitability analysis of the established scales. Initially, for the best economic case (Scale 1, Revenues 1 and 4) the sensitivity for the itemized costs prices, external capital, and co-products selling price were analyzed with a variation in ±50%. IRR and NPV were modeled in the cash flow for these variables' conditions. These variables were selected due to the market fluctuations, which are usually highly uncertain. For the external capital (loan finance), the profitability indicators were determined to analyze the best financial condition to address different investors' profiles, from external capital contributions from 0% to 100%. Beyond, the selling prices of fertilizer, biomethane, electrical, and thermal energies can influence the techno-economic results and then should be evaluated by sensitivity analysis to propose an effective and real market condition for the co-products commercialization.

### *2.5 Environmental benefits from GHG mitigation*

From an environmental perspective, it is possible to avoid GHG emissions from replacing usual electricity and heat sources for those obtained from the biogas burning. From **Eqs. 9** and **10** it is possible to calculate the avoided GHG emissions from electricity ($\mathrm{A_{GHG-Electricity}}$) and heat ($\mathrm{A_{GHG-Heat}}$) (dos Santos et al., 2018).

$$\mathrm{A_{GHG\text{-}Electricity}=EF_{CO_2\text{-}EG}\times EG_{CH_4}}\quad(9)$$

$$\mathrm{A_{GHG\text{-}Heat}=EF_{CO_2\text{-}HG}\times HG_{CH_4}}\quad(10)$$

*where:* $\mathrm{EG_{CH_4}}$ is the annual electricity generated (previously calculated in **Eq. 1**); $\mathrm{HG_{CH_4}}$ is the annual heat generated (previously calculated in **Eq. 2**); $\mathrm{EF_{CO_2-EG}}$ is the emission factor of $CO_{2eq}$ for 2019 national electric energy generation, assumed as 0.075 $tCO_{2eq}$ $MWh^{-1}$ (MCTIC, 2019); and $\mathrm{EF_{CO_2-HG}}$ is the emission factor of heat energy, assumed as 0.056 $tCO_{2eq}$ $GJ^{-1}$ (IPCC, 2006).

## 3. Results and discussion

### *3.1 Technological parameters for AD implementation and biogas production*

The covered lagoon was scaled for BSG-AD and bioenergy recovery. The dimensions calculated for each scale are summarized in **Table 2**. From the HRT of 22 days, the biodigester volume was estimated in 17,600, 4,400, and 1,100 $m^3$, respectively, to the flows of 800, 200, and 50 $m^3\ d^{-1}$. By establishing the $C_1/L_1$ and $C_2/L_2$ ratios as 3 and 5, respectively, it was possible to estimate the biodigester dimensions (**Fig. 3**). The height was calculated to 7 (Scale 1), 4 (Scale 2), and 3.5 m (Scale 3) to obtain the exact total volume of the reactor. For the largest size, the biodigester presents an external length of 100 m and a width of 35 m. In general, length and width proportionally decreased according to the biodigester volume. The technical variables can be considered the first step to estimate the biodigester implementation costs since a high precision is desirable to obtain real data for biogas production to achieve acceptable profitability (Akbulut, 2012).

After AD implementation, all the demands were determined for biogas production (**Table 3**). BSG, water to flow correction, NaOH, operational labor, water for cleaning, and electric energy were assumed for the whole process. Firstly, the feedstock needs an amount of water to equilibrate the flow. The amount of water for Scales 1, 2, and 3 are shown in **Table 3.** An input of 30% of water was estimated, according to the final flow of BSG for the different scales, as follows: 240, 60, and 15 $m^3\ d^{-1}$ of water to achieve BSG flows of 800, 200, and 50 $m^3\ d^{-1}$, respectively, in the AD process. In addition, around 5% of the water used in AD is used for cleaning, which is a low quantity since cleaning is sporadic in this type of treatment system (Mutungwazi et al., 2018). Further, before being fed to the digester, BSG needs a pH correction. The NaOH flow was calculated to keep the pH in 7, a condition that yields an adequate production of methane (Mao et al., 2017). For this, 52.56, 13.12, and 3.28 kg NaOH $d^{-1}$ were necessary for Scales 1, 2, and 3, respectively.

The technical results obtained for the scale-up process were summarized in **Table 4**. Thereby, 1 ton of wet BSG produces 30.76 $m^3$ biogas $d^{-1}$. By evaluating the BSG flows, the biogas production was estimated in 4,210.28 $m^3\ d^{-1}$ (Scale 1), 1,052.57 $m^3\ d^{-1}$ (Scale 2), and 262.72 $m^3\ d^{-1}$ (Scale 3). This means that for the highest flow capacity (800 $m^3\ d^{-1}$), during a yearly continuous operation, $40\times10^6$ $m^3$ of biogas could be produced. From this biogas yield, the electrical and thermal energy generation was estimated as 8,975.67 kWh $d^{-1}$ and 32,766.89 MJ $d^{-1}$. Considering that the whole biogas produced was purified, biomethane production was estimated in 1,894.62 m$^3$ d$^{-1}$ (Scale 1), 473.66 m$^3$ d$^{-1}$ (Scale 2), and 118.22 m$^3$ d$^{-1}$ (Scale 3).

**Table 2.** Covered lagoon anaerobic digester designed to BSG anaerobic digestion and technical scale-up variables.

| Parameters* | Unit | Scale 1 | Scale 2 | Scale 3 |
|---|---|---|---|---|
| BSG mass (web basis) | t d$^{-1}$ | 137 | 34 | 9 |
| Total flow (water + BSG) | m$^3$ d$^{-1}$ | 800 | 200 | 50 |
| HTR | d | 22 | 22 | 22 |
| Biodigester volume | m$^3$ | 17,600 | 4,400 | 1,100 |
| Length – $C_1$ | m | 100 | 70 | 35 |
| Length – $C_2$ | m | 80 | 55 | 30 |
| Width – $L_1$ | m | 35 | 25 | 12 |
| Width – $L_2$ | m | 15 | 10 | 7 |
| Height – h | m | 7 | 4 | 3.5 |
| $C_1/L_1$ | – | 3 | 3 | 3 |
| $C_2/L_2$ | – | 5 | 5 | 5 |

*The codes established are associated with the Fig. 3.

**Table 3.** Technical parameters regarding the process consumptions in the AD process.

| Parameters | Unit | Scale 1 | Scale 2 | Scale 3 |
|---|---|---|---|---|
| | | BSG-AD consumptions | | |
| BSG | t y$^{-1}$ | 43,800 | 10,950 | 2,733 |
| Water to flow correction | m$^3$ y$^{-1}$ | 86,400 | 21,600 | 5,400 |
| NaOH | kg y$^{-1}$ | 16,819.20 | 4,198.40 | 1,049.60 |
| Operational Labor | h y$^{-1}$ | 7,680 | 5,120 | 2,560 |
| Water for cleaning | m$^3$ y$^{-1}$ | 3,024.00 | 756.00 | 189.00 |
| Electric energy | kWh y$^{-1}$ | 80,000 | 48,000 | 25,600 |

**Table 4.** Technical parameters regarding the production of biogas, biomethane, electricity, thermal energy, and fertilizer.

| Parameters | Unit | Scale 1 | Scale 2 | Scale 3 |
|---|---|---|---|---|
| | | BSG-AD co-products | | |
| Biogas | $m^3\ y^{-1}$ | 40,418,640.00 | 10,104,660.00 | 2,522,123.14 |
| Biomethane | $m^3\ y^{-1}$ | 18,188,388.00 | 4,547,097.00 | 1,134,955.41 |
| Electricity | $kWh\ y^{-1}$ | 2,872,215.92 | 718,053.98 | 179,226.27 |
| Thermal Energy | $MJ\ y^{-1}$ | 10,485,403.59 | 2,621,350.90 | 654,289.18 |
| Fertilizer | $t\ y^{-1}$ | 19,125.71 | 4,781.43 | 1,193.44 |

### *3.2 Implementation cost of the AD system*

#### *3.2.1 Itemized costs*

**Table 5** presents the itemized cost estimations for the whole BSG-AD process according to the system described in **Fig. 1b**. BSG is a feedstock that requires pumping systems, which was assumed as a gear pump (P-101). A centrifugal pump (P-102) was used to transfer a NaOH concentrated solution to the tank for pH stabilization (T-203). In all the initial steps, automatic valves were used for flow control. A pH transmitter connected to the centrifugal pump and the NaOH valve to keep the pH at 7 during the operational time was included. In the anaerobic biodigester, temperature (TT) and pressure transmitters (PT) were used to maintain the operating conditions with a flow transmitter to keep the HRT in 22 days.

**Table 5** also presents the prices for plant construction. For the biodigester, the price refers to all the equipment parts, including soil membranes, safety valves, etc. The total cost of the digester is lower than 50,000.00 USD (Scale 1), which can be considered a minor cost for the project implementation, due to the type of biodigester selected (covered lagoon), which is built solely by a soil hole protected by polymeric membranes to avoid liquid leaching and gas leakage (Mutungwazi et al., 2018). In Brazil, this type of digester is commonly used for the treatment of organic materials from pig farming without automation (Freitas et al., 2019). However, this digester's implementation in a brewery for solid waste treatment requires an automated process including pumps, valves, and transmitters, as evidenced herein.

After biogas production, a treatment system is required (Adnan et al., 2019). In this study, biogas was initially pressurized, dehumidified, and then purified. After this step, the biogas can be sold as biomethane (Revenue 1) since all hydrogen sulfide ($H_2S$) and carbon dioxide ($CO_2$) were removed (Adnan et al., 2019). However, an electric energy generator (Revenue 2) and a co-generator for electricity and heat (Revenue 4) can be implemented. The generator's cost was the most expressive in all the scales evaluated, reaching almost 350,000 USD in Scale 1. Based on this analysis, other revenues should be estimated to obtain the project implementation's best profitability indicators.

### *3.2.2 Total fixed capital investment (FCI) to the AD systems*

Considering the three scales and the five revenues considered in this study, three different arrangements of treatment plants were necessary, especially regarding the implementation of the generator (**Table 6**). Based on the established revenues, biogas production, purification, and selling as biomethane (Revenue 1) is a route that avoids the acquisition of a generator with a reduction in the total FCI (706,587.53 USD; 287,217.17 USD, and 147,476.63 USD, for scales 1, 2, and 3, respectively). Another possibility is the acquisition of a generator to produce electricity (Revenue 2). However, in this case, the estimated FCI was ~25% higher than Revenue 1. Otherwise, a combined selling of biomethane (50%) and electricity (50%) (Revenue 3) was possible. For this condition, half of the biogas produced was converted into electricity, and both were sold at their respective market prices. The FCI was equal when compared to Revenue 2 because of the generator. Beyond, an alternative to use the potential heat was the same engine's co-generation (Revenue 4). However, the FCI, in this case, was higher. Finally, similar to Revenue 3, it was possible to implement a co-generator and sell biogas, electricity, and heat (Revenue 5). For this case, it was assumed that 50% of biogas were sold as natural gas, and 50% was converted into electricity and thermal energy, generating additional revenue compared to Revenue 3. Based on the itemized data and addition of the generator or co-generator, the highest FCI reached 1,038,016.10 USD (Scale1, Revenues 4 and 5), and this price decreased according to the reduction of the plant capacity. Also, for all the revenue conditions, the digestate can be upgraded into fertilizer and then commercialized for agricultural use. The implementation cost (FCI) for all the processes includes the construction and operation of the waste management system.

**Table 5.** Itemized costs estimation to the anaerobic digester implementation.

| Code* | Description | Scale 1 |  | Scale 2 |  | Scale 3 |  |
|---|---|---|---|---|---|---|---|
|  |  | Base cost/ amount | Total price (USD) | Base cost/ amount | Total price (USD) | Base cost/ amount | Total price (USD) |
| P-101 | Gear pump | 4 | 37,656.01 | 4 | 17,266.07 | 4 | 12,634.61 |
| P-102 | Centrifugal pump | 1 | 1,785.06 | 1 | 1,705.06 | 1 | 1,620.83 |
| V | Automatic valves | 7 | 6,362.84 | 7 | 3,811.11 | 7 | 2,818.61 |
| TT | Temperature transmitter | 2 | 348.22 | 2 | 348.22 | 2 | 348.22 |
| FT | Flow transmitter | 9 | 23,136.77 | 9 | 23,136.77 | 9 | 23,136.77 |
| PT | Pressure transmitter | 4 | 4,702.73 | 4 | 4,702.73 | 4 | 4,702.73 |
| pHI | pH transmitter | 1 | 1,110.10 | 1 | 1,110.10 | 1 | 1,110.10 |
| T-201 | Equalization tank | 2400 $m^3$ | 96,000 | 600 $m^3$ | 24,000 | 150 $m^3$ | 6,000 |
| T-202 | NaOH tank | 10 $m^3$ | 29,309 | 6 $m^3$ | 17,585.40 | 3 $m^3$ | 8,792.70 |
| T-203 | Tank to pH stabilization | 2400 $m^3$ | 96,000 | 600 $m^3$ | 24,000 | 150 $m^3$ | 6,000 |
| - | Pipe | 1000 m | 10,876.80 | 750 m | 7,920 | 500 m | 5,280 |
| AD | Anaerobic digester | 1 | 46,800 | 1 | 30,000 | 1 | 12,000 |
| - | Landscaping services | 50 h | 1,500 | 40 h | 1,200 | 30 h | 900 |
| FL | Flare | 1 | 7,000 | 1 | 5,000 | 1 | 3,000 |
| PDb | Biogas pressurizer | 1 | 68,000 | 1 | 28,000 | 1 | 12,000 |
| DSb | Biogas desulfurizer | 1 | 36,000 | 1 | 30,000 | 1 | 26,000 |
| T-204 | Circular decanter | 2400 $m^3$ | 240,000 | 600 $m^3$ | 60,000 | 150 $m^3$ | 15,000 |
| HPUb | Co-generator | 5 | 331,428.57 | 2 | 136,666.67 | 1 | 44,000.00 |
| HPUb | Generator | 5 | 238,095.24 | 2 | 99,333.33 | 1 | 26,000.00 |

*The codes established are associated with the **Fig. 1b**.

**Table 6.** Total FCI to the industrial process proposed for bioenergy production and estimated annual sales for the scales and revenues established.

| Revenues | | Scale 1 | Scale 2 | Scale 3 |
|---|---|---|---|---|
| | | Total FCI (USD) | | |
| 1 | Fertilizer + Biomethane | 706,587.53 | 287,217.17 | 147,476.63 |
| 2 | Fertilizer + Electric energy | 944,682.77 | 386,550.50 | 173,476.63 |
| 3 | Fertilizer + Biomethane + Electric energy | 944,682.77 | 386,550.50 | 173,476.63 |
| 4 | Fertilizer + Electric energy + Thermal Energy | 1,038,016.10 | 423,883.83 | 191,476.63 |
| 5 | Fertilizer +Biomethane + Electric energy + Thermal Energy | 1,038,016.10 | 423,883.83 | 191,476.63 |
| | | Annual sales (USD y$^{-1}$) | | |
| 1 | Fertilizer | 117,812.45 | 29,453.11 | 7,351.50 |
| | Biomethane | 169,758.29 | 42,439.57 | 10,592.92 |
| | Total (Fertilizer + Biomethane) | 287,570.74 | 71,892.68 | 17,944.41 |
| 2 | Fertilizer | 117,812.45 | 29,453.11 | 7,351.50 |
| | Electric energy | 173,941.40 | 43,485.35 | 10,853.94 |
| | Total (Fertilizer + Electric energy) | 291,753.84 | 72,938.46 | 18,205.44 |
| 3 | Fertilizer | 117,812.45 | 29,453.11 | 7,351.50 |
| | Biomethane | 84,879.14 | 21,219.79 | 5,296.46 |
| | Electric energy | 86,970.70 | 21,742.67 | 5,426.97 |
| | Total (Fertilizer + Biomethane + Electric energy) | 289,662.29 | 72,415.57 | 18,074.93 |
| 4 | Fertilizer | 117,812.45 | 29,453.11 | 7,351.50 |
| | Electric energy | 173,941.40 | 43,485.35 | 10,853.94 |
| | Thermal Energy | 86,398.68 | 21,599.67 | 5,391.28 |
| | Total (Fertilizer + Electric energy + Thermal Energy) | 378,152.52 | 94,538.13 | 23,596.72 |
| 5 | Fertilizer | 117,812.45 | 29,453.11 | 7,351.50 |
| | Biomethane | 84,879.14 | 21,219.79 | 5,296.46 |
| | Electric energy | 86,970.70 | 21,742.67 | 5,426.97 |
| | Thermal Energy | 43,199.34 | 10,799.83 | 2,695.64 |
| | Total (Fertilizer + Biomethane + Electric energy + Thermal Energy) | 332,861.63 | 83,215.41 | 20,770.57 |

### *3.2.3 Costs discrimination*

The annual costs related to the project implementation and operation (COL, CRM, and FCI) were the most significant, with differences between the scales and selling conditions (**Fig. 4**). For the plant designed to produce biomethane (Revenue 1), a moto-generator was not necessary, and FCI was lower. In Scale 1, FCI increased from 28.86% (Revenue 1) to 41.25% (Revenues 4 and 5). The plant with the lowest capacity (Scale 3), operating with high total FCI (Scale 3) presented 53.17% of FCI. Higher CRM contribution was obtained for the highest plant capacity, which decreased proportionally to the decrease in plant capacity, as observed in other scale-up studies (Viganó et al., 2017; Zabot et al., 2018). Usually, CRM and FCI are the main contributors to COM (Hatami et al., 2019), which is in agreement with the present study. Related to the COL and CUT, a lower contribution was obtained for the scales with high operational capacity. CUT ranged from 3.04% (Scale 1, Revenues 4 and 5) to 3.20% (Scale 1, Revenue 1), meaning that the projected plant demands low consumable expenses, such as electricity and water. In contrast, COL had a similar behavior for the different revenues, indicating that the AD plants require a minimum operational team. However, high COL contribution was obtained for the plant with the lowest treatment capacity, with a contribution of up to 20%, which is significant for the project operation. Beyond, the CWT was higher for Scale 1 (16.09%), since this plant condition generates more digestate, which demands a treatment system for the fertilizer recovery.

With an operating AD plant, COM was estimated for each product, taking into account the previously established scales and revenues. COM was evaluated for the production of biogas, biomethane, electricity, thermal energy, and fertilizer (**Fig. 5**). Biogas presented the highest production in Scale 1 (40,418,640.00 $m^3$ $y^{-1}$) due to the highest treatment capacity. With an FCI similar to the Revenue 1, biogas COM was obtained as 0.017, 0.028, and 0.058 USD $m^{-3}$, for Scales 1, 2, and 3, respectively (**Fig. 5a**), in agreement with Cervi et al. (2010).

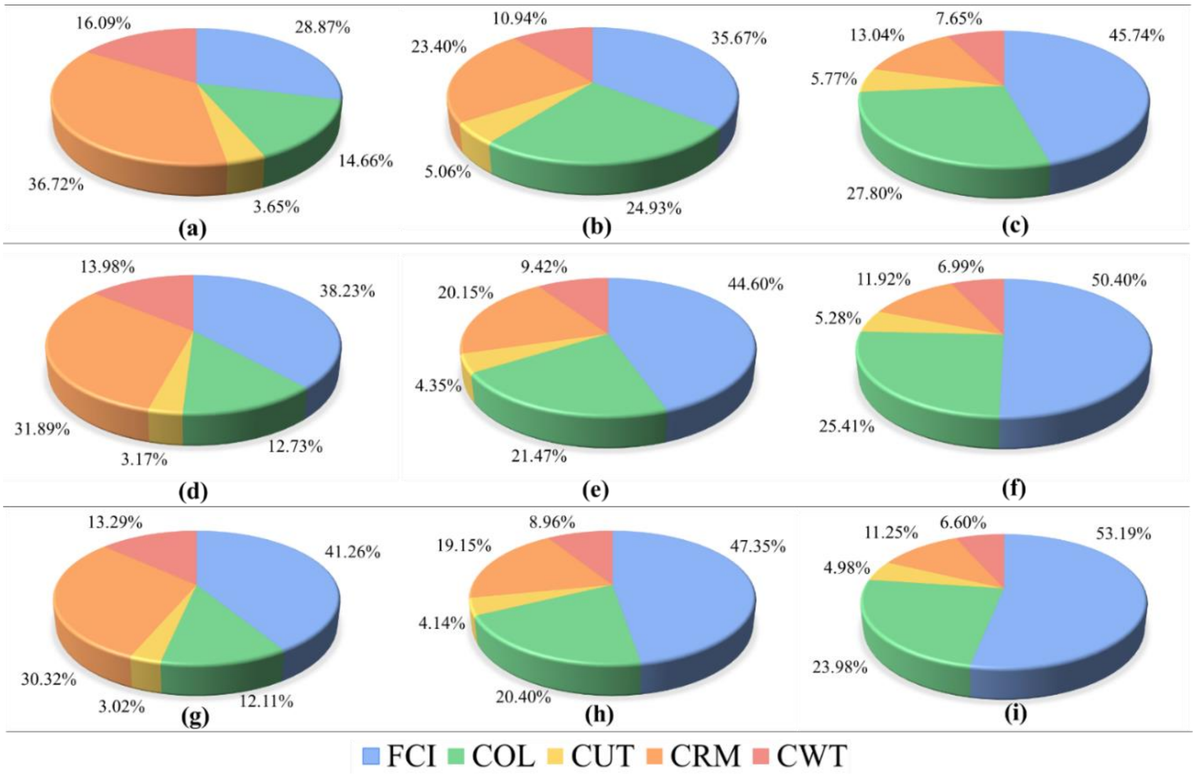

**Figure 4.** Contribution of each cost discriminated over the COM. (a) Scale 1 – Revenue 1; (b) Scale 2 – Revenue 1; (c) Scale 3 – Revenue 1; (d) Scale 1 – Revenue 2 and 3; (e) Scale 2 – Revenue 2 and 3; (f) Scale 3 – Revenue 2 and 3; (g) Scale 1 – Revenue 4 and 5; (h) Scale 2 – Revenue 4 and 5; (i) Scale 3 – Revenue 4 and 5; CRM, cost of raw material; COL, cost of operational labor; FCI, fixed capital investment; CUT, cost of utilities; CWT, cost of waste treatment.

On the other hand, with the purification step, only 45% of the biogas could be destined for sales as biomethane (Adnan et al., 2019). Therefore, the productivity decreased to 18,188,388.00 m$^3$ y$^{-1}$ for Scale 1. Hence, a lower COM was obtained for this product (which increased with the plant capacity) (**Fig. 5b**). This difference in prices can be explained by the productivity increase associated with the COM decrease (Zabot et al., 2018). When evaluating the COM to produce electricity (**Fig. 5c**), 0.067 USD kWh$^{-1}$ was obtained for Scale 1 with an annual productivity of 2,872,215.92 kWh. COM is the initial parameter considered for a project feasibility analysis. If the market price is higher than the COM obtained, positive profitability can be achieved. In this study, the market price for biomethane (0.28 USD m$^{-3}$) was higher than COM, which is a good initial economic indicator (since the production price was lower than the market ones). However, for electricity, only the highest scale presented COM values

(0.067 USD kWh$^{-1}$) equivalent to the market price (0.06 USD kWh$^{-1}$). The COM for thermal energy (**Fig. 5d**) was calculated based on the adoption of co-generation, and 0.0187, 0.0315, and 0.0537 USD MJ$^{-1}$ were obtained for Scales 1, 2, and 3, respectively. Otherwise, high fertilizer recovery was obtained for the highest plant capacity (19,125.71 t y$^{-1}$), which resulted in a COM lower than 2 USD t$^{-1}$. A lower COM value for the fertilizer production could be promising for its commercialization to mineral NPK replacement in agriculture (Li et al., 2020), which usually present high acquisition costs (Koido et al., 2018).

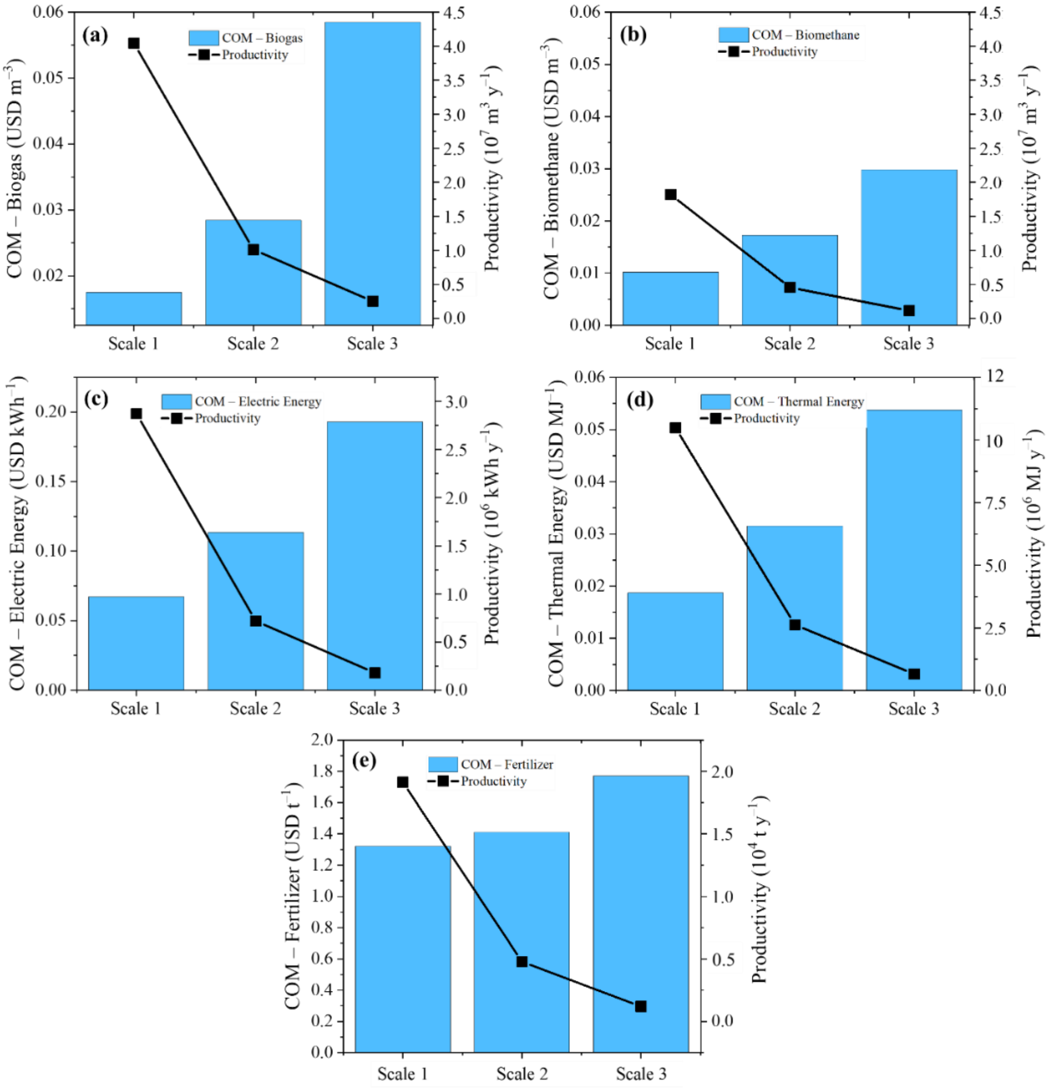

**Figure 5.** COM and productivity of biogas, biomethane, electricity, thermal energy, and fertilizer from the proposed AD process. (a) COM – biogas (USD m–3); (b) COM – biomethane (USD m–3); (c) COM – electricity (USD kWh–1); (d) COM – thermal energy (USD MJ–1); and (e) COM – fertilizer (USD t–1).

## *3.3 Profitability analysis*

### *3.3.1 Annual sales and cash flow*

For the economic evaluation, the initial step was to determine the annual sales for the project. In this study, it was considered that in the year 0, the plant was implemented and started until the biodigester stabilization. After this, it was possible to achieve a complete digestion in the first year of operation, producing 100% of the theoretical capacity. Productivity was assumed constant during the following years. Considering the market prices of biomethane, electrical and thermal energies, it was possible to determine the annual sales for the established revenues (**Table 6**). As reported in the technical parameters, productivity was proportional to the plant capacity, and the annual sales were assumed to follow the same behavior. Scale 1 was the most advantageous, and Revenue 4 presented the highest annual sales (378,152.52 USD, digestate, electric, and thermal energy), followed by Revenue 5 (332,861.63 USD, fertilizer, biomethane, electric and thermal energy), and Revenue 2 (291,753.84 USD, fertilizer and electric energy). Revenues 1 and 3 were similar and less attractive, with annual sales of 287,570.74 USD and 289,662.29 USD, respectively.

From the annual sales, it was necessary to discount the implementation costs (FCI, CRM, CWM, COL, and CUT), as well as depreciation, interest rate, and income tax to obtain the real cash flow. The project cash flow (data not showed) was accomplished to all the scales and revenues, and the current assets (cash balance) were considered after the discounts. An opening cash balance equivalent to 30% of the initial sales of the first year was considered. Moreover, in the implementation year (time 0), total FCI was considered negative (start-up investment). In addition, it was considered that 100% of the FCI value was financed by the National Bank for Economic and Social Development (BNDES) in Brazil under an interest rate of 8.5% per year. From the cash balance, Scale 1 presented positive current assets for all the revenues. Plant operating at the condition of Scale 1 showed the highest value in the tenth year of the cash balance. Also, the conditions mentioned above were the most suitable because payback occurs in a shorter time. Based on the cash flow, the profitability indicators were calculated to demonstrate the liquid return and interest after revenues.

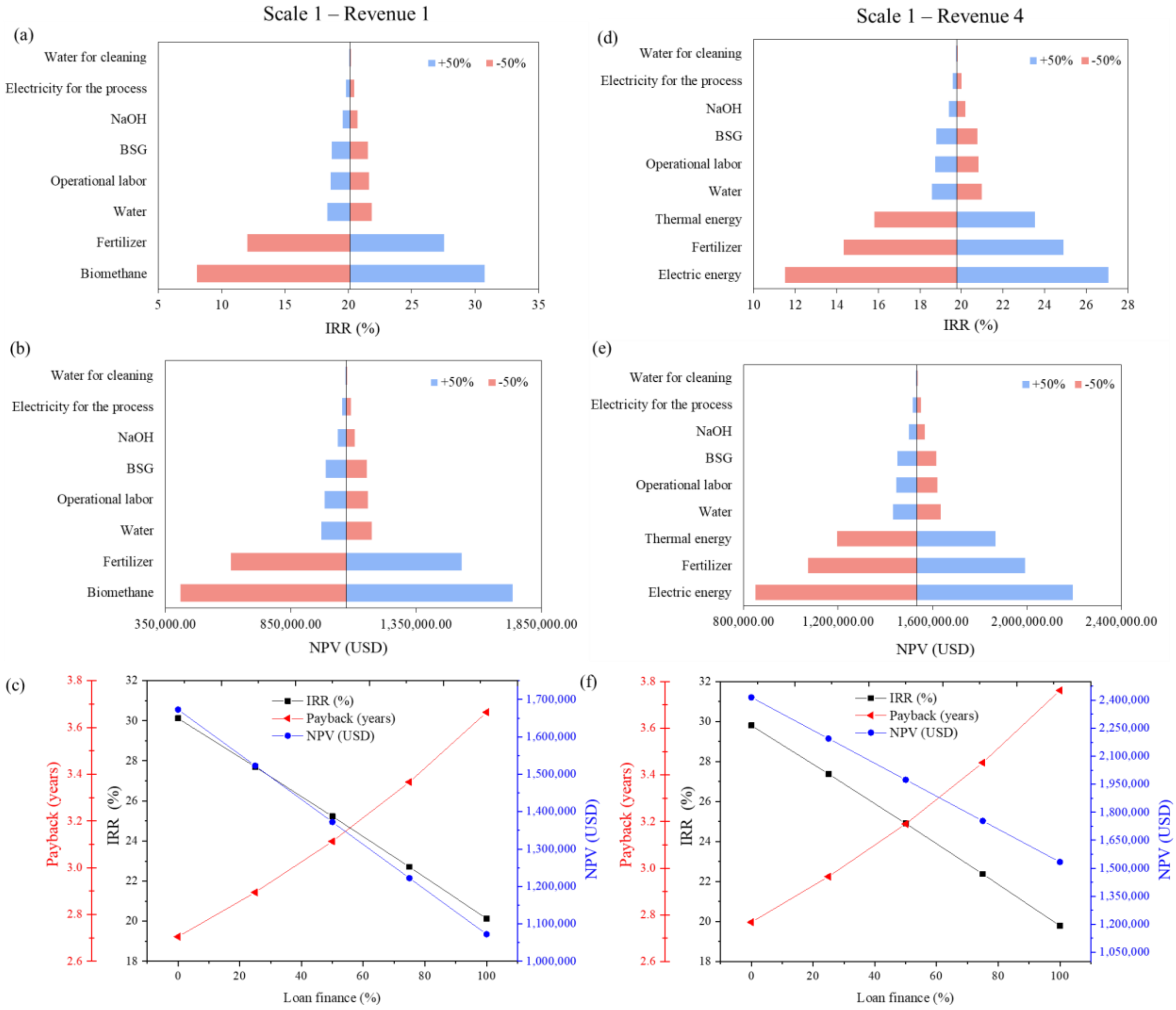

**Figure 6.** Sensitivity analysis for the most profitable BGS-AD project (Scale 1, Revenues 1 and 4). (a) Revenue 1 on IRR; (b) Revenue 1 on NPV; (c) Scale 1 – Revenue 1 on loan finance; (d) Revenue 4 on IRR; (e) Revenue 4 on NPV; (f) Scale 1 – Revenue 4 on loan finance.

### *3.3.2 Profitability indicators*

A combined analysis of the technical and economic aspects is essential to define a project implementation strategy, with the primary objective of obtaining a feasible technology for industrial application (Akbulut, 2012; Campos et al., 2020). In this study, the evaluation of profitability indicators was accomplished for all the scales and revenues. The profitability analysis was more advantageous for the highest plant capacity **(Table 7)**. Scale 3 (lowest capacity) presented a payback time between 14 and 20 y, with negative NPV in the tenth year. In addition, Scale 2 showed more positive results when compared to Scale 3. By using a co-generator (Revenue 4), a GM of 65.18% was obtained. However, the NM was 10.77%, demonstrating that the process presents high annual costs. Also, the payback time was 6.91 y, and in the tenth year, the NPV is equivalent to 177,230.53 USD. The most attractive scale was for the highest plant capacity (Scale 1). The five revenues presented payback time lower than 5 y. Scale 1 was more advantageous to Revenues 1 and 4, with NM up to 70%, 23% ROI, and 20% IRR. Moreover, Revenues 1 (3.67 y) and 4 (3.76 y) presented a similar payback time. However, the NPV after 10 y was almost 30% higher than Revenue 4 (1,533,597.46 USD). Briefly, for the approach adopted in this study, Scale 1 was the most financially attractive, reaching outstanding results for Revenues 1 and 4. Even requiring a high initial investment (FCI of 1,038,016.10 USD), a positive cost-benefit could be obtained and could be a suitable investment for industrial application.

**Table 7.** Profitability indicators for bioenergy production from BSG anaerobic digestion.

| Plant capacity | Revenues | GM (%) | NM (%) | ROI (%) | IRR (%) | Payback (y) | NPV (USD) |
|---|---|---|---|---|---|---|---|
| Scale 1 | 1 | 69.91 | 31.55 | 23.95 | 20.12 | 3.67 | 1,071,985.24 |
| | 2 | 70.34 | 25.24 | 18.90 | 13.64 | 4.80 | 928,661.11 |
| | 3 | 70.13 | 24.88 | 18.74 | 13.42 | 4.85 | 912,346.99 |
| | 4 | 77.12 | 34.51 | 23.68 | 19.79 | 3.76 | 1,533,597.46 |
| | 5 | 74.01 | 29.00 | 20.41 | 15.63 | 4.43 | 1,180,328.49 |
| Scale 2 | 1 | 54.21 | 6.70 | 12.79 | 4.74 | 7.23 | 101,592.71 |
| | 2 | 54.86 | -3.90 | 10.37 | 0.67 | 9.09 | 36,342.44 |
| | 3 | 54.54 | -4.47 | 10.27 | 0.49 | 9.19 | 32,263.91 |
| | 4 | 65.18 | 10.77 | 13.51 | 5.88 | 6.91 | 177,230.53 |
| | 5 | 60.44 | 2.03 | 11.51 | 2.64 | 8.18 | 88,913.29 |
| Scale 3 | 1 | 28.16 | -48.74 | 5.18 | -10.47 | 18.60 | -65,708.68 |
| | 2 | 29.19 | -59.11 | 4.91 | -11.21 | 19.74 | -82,886.68 |
| | 3 | 28.67 | -60.07 | 4.85 | -11.37 | 19.97 | -83,904.68 |
| | 4 | 45.37 | -34.95 | 6.80 | -6.45 | 14.16 | -54,136.71 |
| | 5 | 37.93 | -49.91 | 5.70 | -9.11 | 16.98 | -76,180.69 |

### *3.4 Sensitivity analysis*

Since Revenues 1 and 4 in Scale 1 were identified as the best case (**Table 7**), such project conditions were subject to the sensitivity analysis to determine the effects of significant variations of prices and external capital on IRR and NPV (**Fig. 6**). For the revenues evaluated, CRM, COL, and CUT were reduced by 50%, and no significant changes in IRR and NPV were observed. Then, only a slight difference was obtained in comparison to the data of the project feasibility (**Table 7**). Based on these data, the studied costs did not change the project profitability, which can be a decisive factor for practical implementation under the conditions evaluated.

Regarding the loan finance, an expressive sensitivity result was observed for Revenues 1 (**Fig. 6c**) and 4 (**Fig. 6f**). Regarding Revenue 1, under an external financing contribution of 100%, 20.12% of IRR was obtained, with a payback time of 3.67 y (**Table 7**). By applying an external contribution of 25% and 75% of equity, an IRR up to 25% and a payback time of 2.89 y were obtained. On the other hand, for 75% and 25% of equity, an IRR of 22% and a payback time of 3.36 y were obtained. Finally, for 100% of equity, the IRR increased to 30%, and the payback time was reduced to 2.7 y, with an NPV of 1,672,786.38 USD (**Fig. 5c**). The same behavior was obtained for Scale 1 – Revenue 4 (**Fig. 5f**); however, the NPV for this condition reached 2,415,303.19 USD for 100% equity, equivalent to 1.5-fold higher in comparison to the Revenue 1.

It is noteworthy that the market prices of energy are seasonal and should be evaluated in a sensitivity analysis (Watanabe et al., 2020). The effect of fertilizer, biomethane, electricity, and thermal energy was evaluated for price variations of ±50%. Revenue 1 was not tested by the selling price of electric and thermal energies. Beyond, biomethane price was not evaluated in Revenue 4, since this market condition sells fertilizer, electric and thermal energies. **Fig. 6a and 6b** show the Scale 1 - Revenue 1 on IRR and NPV, respectively. In general, a higher biomethane price improved the economic feasibility, reaching 30% of IRR and NPV of 1,734,378.57 USD when applying a biomethane price of 0.42 USD m$^{-3}$. As biomethane presents lower COM, the conditions established with this revenue could be suitable for an industrial implementation (commercializing biomethane to replace natural gas). On the other hand, higher electricity prices improved the project feasibility for Revenue 4 (**Fig. 6d** and **6e)**. With an electric energy price of 0.09 USD kWh$^{-1}$, an NPV of 2,193,486.14 USD was obtained with an IRR of 27%. Compared to the average market price (0.06 USD kWh$^{-1}$), the NPV increased in 1.5-fold, which is a feasible advantage to support an industrial application. The thermal energy was another essential factor that could upgrade the project feasibility; however, with a lower impact than fertilizer and electricity.

Nonetheless, the fertilizer selling price affected the revenues. In Scale 1 – Revenue 1, the IRR ranged between 12% and 27% when a rate of ±50% was applied for the fertilizer price, with the same behavior for the NPV. In Scale 1 – Revenue 4, the NPV could reach almost 2 million USD for a fertilizer selling price of 9.24 USD $t^{-1}$. Therefore, fertilizer, biomethane, electric energy, and heat selling prices were considered the most critical parameters affecting the project feasibility in a brewery.

### *3.5 Environmental benefits of BSG-AD*

From an environmental perspective, the use of AD, a consolidated waste management technology, could contribute to the mitigation of GHG emissions, reducing the carbon footprint of the brewery. The electric energy generation could mitigate $2.15\times10^5$, $5.38\times10^4$, and $1.33\times10^4$ t $CO_{2eq}$ $y^{-1}$, respectively, to the plant capacities of 137 (Scale 1), 34 (Scale 2), and 9 t wet BSG $d^{-1}$ (Scale 3). Additionally, from the thermal energy, the use of heat in a boiler to replace natural gas could mitigate $5.87\times10^2$, $1.46\times10^2$, and $0.36\times10^2$ t $CO_{2eq}$ $y^{-1}$, respectively, to the Scales 1, 2, and 3. Taking advantage of the potential energy of BSG, a biogas economy could diversify and contribute to a country's energy matrix renewability. The use of AD for the treatment of BSG presents lower impacts than wastes landfilling or incineration (Slorach et al., 2019). AD also displays net savings in typical Life Cycle Analysis usual impact categories, likewise human and ecotoxicity potential, depletion of metals, water, fossil fuels, and ozone layer (Slorach et al., 2019). Climate change intimately associated to the fossil-based economy is a worldwide concern. The recent growing research on renewable energy aims to introduce cleaner energy sources in the fossil-based economy to support a worldwide Clean Development Mechanism. Ultimately, biogas could be a promising biofuel source to replace fossil fuels, reducing environmental side-effects, and contributing to the beer industry decarbonization.

## 4. Conclusions and future prospects

This study demonstrates an economically feasible approach for bioenergy and fertilizer production from the AD of BSG. By investing 1 million USD and operating with a treatment capacity of 137 ton $d^{-1}$, it is possible to recover up to 350,000 USD $y^{-1}$, selling the fertilizer for agriculture, using biomethane to replace natural gas, and generating electricity for the own facility and eventually for sales to the grid, as well as thermal energy. The scale with the highest treatment capacity presented the most positive profitability indicators, with payback lower than 5 y, IRR around 20%, and NPV up to 1 million USD. The sensitivity analysis revealed that the operational costs did not affect the project feasibility. However, the selling price of fertilizer, biomethane, electricity, and heat affected the project feasibility. Beyond, the adoption of an investment form of equity could upgrade the profitability indicators. The proposed waste management system is sustainable and profitable for large-scale BSG treatment. The adoption of the AD technology avoids the BSG final disposal in landfills, incinerators, or use as animal feed, and a noble destination is possible. AD can be one of the industrial routes to support a brewery biorefinery. A biorefinery for BSG valorization is a strategy towards a circular economy and a powerful way to decrease environmental burdens, especially when replacing fossil energy and mineral fertilizers. For futures studies, attention could be given to the use of eco-friendly methods for BSG pre-treatment before the AD in order to improve the methane yield.

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# CHAPTER VIII

## Discussion

# CHAPTER VIII: Discussion

The implementation of a biorefinery for the management of agri-food by-products can be an interesting strategy to support the circular economy transition of the beer industry. In a biorefinery, brewer's spent grains valorization can be maximized, contributing to environmental, economic, and social benefits since greenhouse gas emissions are avoided, and the costs are reduced, contributing toward a sustainable industry. Beyond, in the circular economy transition, the beer industry should adopt waste management processes with the perspective of achieving sustainable development with the generation of value-added products and bioenergy. From an industrial perspective, brewer's spent grains is still considered a low-value by-product without an established valorization pathway. However, this scenario should change in the future due to environmental concerns regarding the appropriate destination of solid waste, allowing a more relevant destination for brewer's spent grains to produce value-added biobased products and bioenergy. Brewer's spent grains is a lignocellulosic biomass composed mainly of hemicellulose (around 35%). Then, a pretreatment process can be applied to produce a hydrolysate containing sugars, amino acids, and other molecules.

This PhD thesis investigated the use of subcritical water hydrolysis, as an eco-friendly pretreatment of brewer's spent grains, in a single and two flow-through sequential reactors to recover sugars and amino acids (**Chapter III**) and xylo-oligosaccharides (**Chapter V**). The process using 180 °C in a single reactor was responsible for the highest production of sugars (47.76 mg $g^{-1}$ carbohydrates). Xylose (0.477 mg $mL^{-1}$) and arabinose (1.039 mg $mL^{-1}$) were the major sugars in the hydrolysate at 180 °C. In addition, subcritical water hydrolysis of brewer's spent grains at the best operational condition (180 °C, single reactor) resulted in low furfural (310.7 µg $mL^{-1}$) and 5-hydroxymethylfurfural, contents (<1 mg $L^{-1}$). The use of 180 °C in a process with a single reactor was also favorable for the recovery of proteins from brewer's spent grains. The yield at this condition was 43.62 mg amino acids $g^{-1}$ proteins, where tryptophan (215.55 µg $mL^{-1}$), aspartic acid (123.35 µg $mL^{-1}$), valine (64.35 µg $mL^{-1}$), lysine (16.55 µg $mL^{-1}$), and glycine (16.1 µg $mL^{-1}$) were the majoritarian essential amino acids recovered in the hydrolysate. Xylo-oligosaccharides were also produced in a single subcritical water hydrolysis reactor at 180 °C (72.83 mg g$^{-1}$). These results allow to conclude that subcritical water hydrolysis can be adopted as an eco-friendly technology to produce a hydrolysate containing sugars, xylo-oligosaccharides, and amino acids.

The economic study of subcritical water hydrolysis of brewer's spent grains was assessed to determine the industrial production of sugars (**Chapter IV**) and xylo-oligosaccharides (**Chapter V**). For this, the sugars could be separated in a simulated moved-bed separation system. An industrial scale (3 reactors of 500 L) coupled with the separation system demonstrated that the project implementation could be economically applicable. In the process coupled with the separation system, arabinose and galactose represented 83.68% of the costs for sugar separation. The cost of manufacturing of arabinose decreased from 64.10 USD kg$^{-1}$ in the pilot-plant to 7.22 USD kg$^{-1}$ in the industrial-plant. The implementation of a separation system recovering six sugars with high value-added can be an advantage in the industrial-plant process when compared to the process without the separation process, which produces a single hydrolysate fraction with low commercial value. In addition, the hydrolysate presents a low cost of manufacturing, which could be an advantage for application as a fermentative medium. Regarding the economic analysis of xylo-oligosaccharides production, the lowest cost of manufacturing (18.36 USD kg$^{-1}$ xylo-oligosaccharides) was obtained for the process with two sequential reactors. Although the fixed capital investment was 0.82-fold lower for the process with a single reactor, the gross profit of the process with two sequential reactors was 30% higher, which resulted in a return on investment of 54.26% and a payback time of 1.84 years.

The subcritical water hydrolysis of brewer's spent grains can be profitable to produce value-added products. The pretreatment was applied as a previous step for anaerobic digestion (**Chapter VI**). This pretreatment is associated with the fact that anaerobic digestion of lignocellulosic biomass is a challenge since hydrolysis can be a limiting stage given that the polymeric structure of the material makes it difficult to convert into more biodegradable molecules. Then, a semi-continuous subcritical water hydrolysis was adopted for anaerobic digestion. The pretreatment was conducted at 160 °C, 15 MPa, a water flow rate of 10 mL min$^{-1}$, and a solvent to feed of 15 g water g$^{-1}$ feedstock. The anaerobic digestion process was operated with a hydraulic retention time of 25.2 days, organic loading rate of 10.89 g $O_2$ L$^{-1}$ d$^{-1}$, and volatile solids loading rate of 3.95 g TVS L$^{-1}$ d$^{-1}$. The results showed that the anaerobic reactors that started with pretreated brewer's spent grains (747.71 L $CH_4$ kg$^{-1}$ TVS) increased the methane yield compared to the dry reactor (53.21 L $CH_4$ $kg^{-1}$ TVS) without pretreatment. Subcritical water hydrolysis broke the hemicellulose structure, releasing arabinose (46.54 mg $g^{-1}$), xylose (39.90 mg $g^{-1}$), and proteins (34.89 mg $g^{-1}$), which improved the anaerobic digestion. During the anaerobic digestion of the reactor without pretreatment, the solids biodegradation and chemical oxygen demand removal reached 53.95% and 20.98%, respectively. The methane produced in the pretreated reactor can generate 134 kWh $ton^{-1}$ of electricity and 604 MJ $ton^{-1}$ of heat, mitigating a total of 43.90 kg $CO_{2\text{-eq}}$ $ton^{-1}$ brewer's spent grains. That is, subcritical water hydrolysis as pretreatment has the benefit of increasing methane-rich biogas production, reducing the industry's carbon footprint, and advocating a circular economy transition of the beer industry. Beyond this, only the reactor composed of brewer's spent grains without pretreatment demonstrated a high methane yield and could be an option for industrial implementation. Then, the economically feasible bioenergy and fertilizer production from the anaerobic digestion of brewer's spent grains (**Chapter VII**), without subcritical water hydrolysis pretreatment. By investing 1 million USD and operating with a brewer's spent grains treatment capacity of 137 ton $day^{-1}$, it is possible to recover up to 350 thousand USD $year^{-1}$, selling the fertilizer for agriculture, using biomethane to replace natural gas, and generating electricity for the own facility and eventually for sales to the grid, as well as thermal energy. The scale with the highest treatment capacity presented the most positive profitability indicators, with payback lower than 5 years, internal rate of return around 20%, and net present value up to 1 million USD. The sensitivity analysis revealed that the operational costs did not affect the project's feasibility. However, the selling price of fertilizer, biomethane, electricity, and heat affected the project's feasibility. Moreover, adopting an investment form of equity could upgrade the profitability indicators. The proposed waste management system is sustainable and profitable for large-scale brewer's spent grains treatment. The adoption of the anaerobic digestion technology avoids the brewer's spent grains final disposal in landfills, incinerators, or use as animal feed, and a noble destination is possible. Anaerobic digestion can be one of the industrial routes to support a brewery biorefinery. A biorefinery for brewer's spent grains valorization is a strategy towards a circular economy and a powerful way to decrease environmental burdens, especially when replacing fossil energy and mineral fertilizers.

Finally, the pretreatment of brewer's spent grains with subcritical water hydrolysis can be a sustainable and cost-effective approach for producing bio-based products and bioenergy. The valorization of brewer’s spent grains in a biorefinery is a powerful strategy to promote the circular economy transition of the beer industry.

# CHAPTER IX

## Conclusion

# CHAPTER IX: Conclusion

This PhD thesis evaluated the application of subcritical water hydrolysis of brewer's spent grains as a technological process to produce biobased products and bioenergy in a biorefinery concept. The pretreatment was conducted in a semi-continuous subcritical reactor to obtain a hydrolysate, which was characterized in terms of sugars, xylo-oligosaccharides, and amino acids. In addition, the integration of subcritical water hydrolysis and anaerobic digestion was conducted to produce biomethane, electricity, heat, and fertilizer. Finally, in a biorefinery concept, the process was evaluated to demonstrate the profitability of producing isolated sugars (xylose and arabinose), xylo-oligosaccharides, and bioenergy, aiming to identify the most profitable option for industrial implementation.

The following conclusions were obtained from this PhD thesis:

**Chapter III**

1) The highest release of monosaccharides was obtained at 180 °C in a single reactor (47.76 mg $g^{-1}$ carbohydrates). Xylose (0.477 mg $mL^{-1}$) and arabinose (1.039 mg $mL^{-1}$) were the major sugars in the hydrolysate at 180 °C and after 40 min of hydrolysis.
2) The use of 180 °C in a process with a single reactor was favorable for the recovery of proteins from brewer's spent grains. The yield at this condition was 43.62 mg amino acids $g^{-1}$ proteins, where tryptophan (215.55 µg $mL^{-1}$), aspartic acid (123.35 µg $mL^{-1}$), valine (64.35 µg $mL^{-1}$), lysine (16.55 µg $mL^{-1}$), and glycine (16.1 µg $mL^{-1}$) were the majoritarian essential amino acids recovered in the hydrolysate.
3) Subcritical water hydrolysis in a single reactor can be adopted as an eco-friendly technology to recover sugars and amino acids from lignocellulosic biomass.

**Chapter IV**

4) The subcritical water hydrolysis of brewer's spent grains followed by a separation system was evaluated as a promising technology to produce isolated sugars on an industrial scale.

5) The lowest cost of manufacturing of arabinose was 7.22 USD kg$^{-1}$ at industrial scale.
6) In an industrial scale (3 reactors of 500 L) of subcritical water hydrolysis with purification of sugars, the project implementation is economically applicable, and from the sensitivity analysis, the plant cost is the most affecting parameter.
7) The technological route simulated and scaled up is economically viable at the industrial scale.

**Chapter V**

8) The highest concentration of xylo-oligosaccharides in the hydrolysate (5246.75 mg L$^{-1}$) was obtained for the process operated with two sequential reactors (80 °C in the first reactor, followed by 130 °C in the second reactor).
9) Xylotriose was the short-chain xylo-oligosaccharide with the highest concentration (2088.82 mg L$^{-1}$), corresponding to approximately 40% of the xylo-oligosaccharides produced.
10) The process with two sequential reactors could produce 52.47 mg xylo-oligosaccharides g$^{-1}$ brewer's spent grains and 146.97 mg xylo-oligosaccharides g$^{-1}$ hemicellulose for the process operated with the first reactor at 80 °C followed by the second reactor at 130 °C.
11) The economic analysis revealed that the estimated total plant cost at the industrial scale (reactor volume of 500 L) was 4,675,177.07 and 5,673,367.49 USD, respectively, for the process with single and two sequential reactors.
12) The lowest cost of manufacturing of xylo-oligosaccharides (18.36 USD kg$^{-1}$) was obtained for the process with two sequential reactors, with a productivity of 146,619 kg year$^{-1}$.
13) The process operated with two sequential reactors presented the highest gross margin (63.28%), with a payback of 1.84 years and a net present value of 32,045,000 USD.

**Chapter VI**

14) The semi-continuous flow-through subcritical water hydrolysis pretreatment of brewer's spent grains for anaerobic digestion was evaluated.
15) During the anaerobic digestion, the chemical oxygen removal reached 20.98% for the reactor operated only raw brewer's spent grains without pretreatment.

16) The dry reactor (without pretreatment) produced 53.21 L $CH_4$ $kg^{-1}$ TVS, and the reactor operated with pretreated brewer's spent grains showed a yield of 747.71 L CH4 $kg^{-1}$ TVS, indicating that subcritical water hydrolysis is an effective pretreatment in breaking down the biomass and producing methane-rich biogas.
17) Each ton of brewer's spent grains submitted to the anaerobic digestion reactor could produce 0.0667 MWh of electricity and 300.3 MJ of heat. This value increased to 0.134 MWh $ton^{-1}$ BSG of electricity and 604.14 MJ $ton^{-1}$ BSG of heat with the adoption of pretreatment.
18) With the adoption of pretreatment, it was possible to increase the bioenergy productivity by 2.1-fold higher for electricity and 2.5-fold higher for heat.
19) The adoption of subcritical water hydrolysis as a pretreatment of biomass for anaerobic digestion could be a technological route to increase methane-rich biogas and bioenergy production.

**Chapter VII**

20) The economic analysis of anaerobic digestion of brewer's spent grains (without pretreatment) demonstrates an economically feasible approach for bioenergy and fertilizer production.
21) Investing 1 million USD and operating with a treatment capacity of 137 ton $day^{-1}$ could recover up to 350 thousand USD $year^{-1}$, selling the fertilizer for agriculture, using biomethane to replace natural gas, and generating electricity for the own facility and eventually for sales to the grid, as well as thermal energy.
22) The scale with the highest treatment capacity presented the most positive profitability indicators, with payback lower than 5 years, internal rate of return around 20%, and net present value up to 1 million USD.
23) A biorefinery for brewer's spent grains valorization is a strategy towards a circular economy and a powerful way to decrease environmental burdens, especially when replacing fossil energy and mineral fertilizer.

Therefore, it can be concluded that subcritical water hydrolysis is an eco-friendly technological process that can be used in a biorefinery to produce sugars, xylo-oligosaccharides, and amino acids from brewer's spent grains, being a profitable process for industrial implementation to produce purified sugars and xylo-oligosaccharides. In addition, the integration of subcritical water hydrolysis and anaerobic digestion increases the methane yield and bioenergy recovery and can decrease the emission of greenhouse gases. However, the application of anaerobic digestion of brewer’s spent grains without pretreatment is also a feasible approach for bioenergy and fertilizer production. Finally, the application of subcritical water hydrolysis of brewer's spent grains is a sustainable pretreatment to produce value-added products and bioenergy, supporting the circular economy transition by reducing the carbon footprint of the beer industry.

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# Appendices

# Appendices

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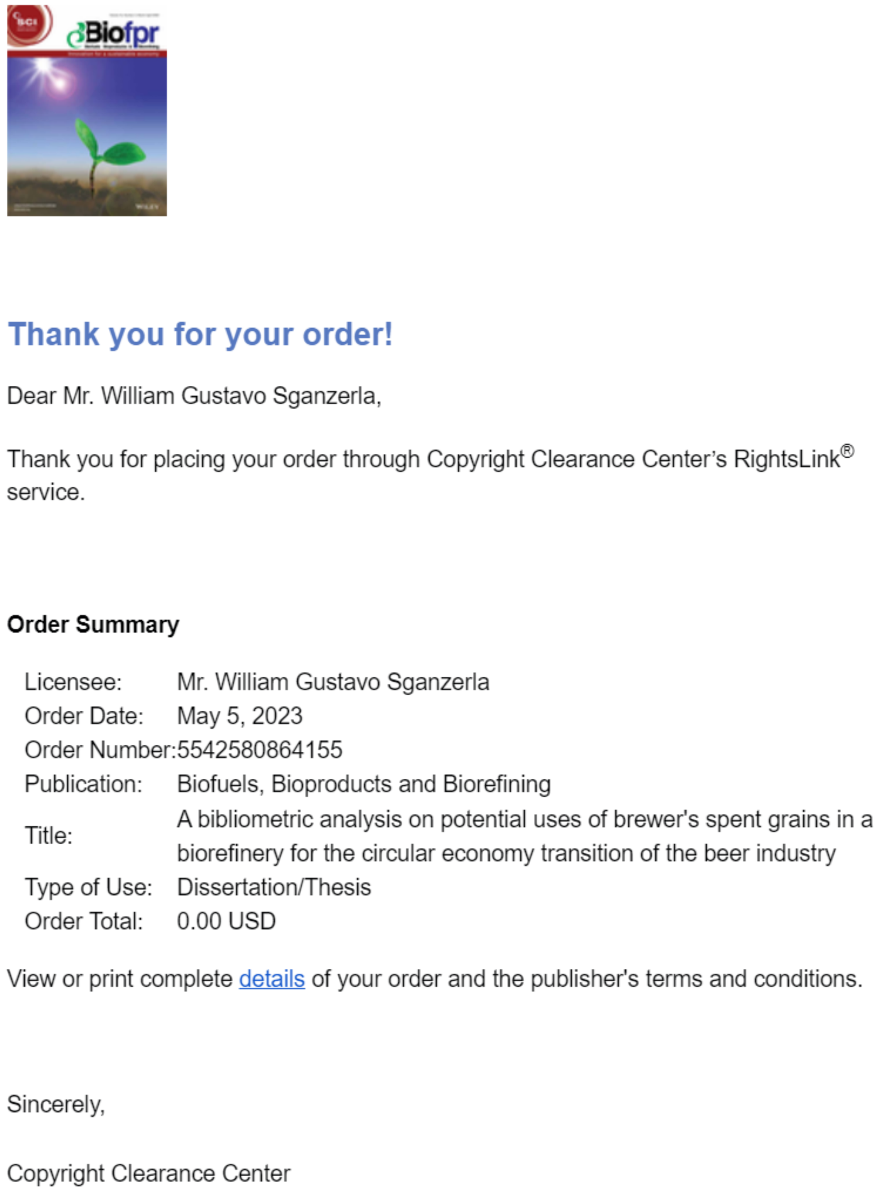

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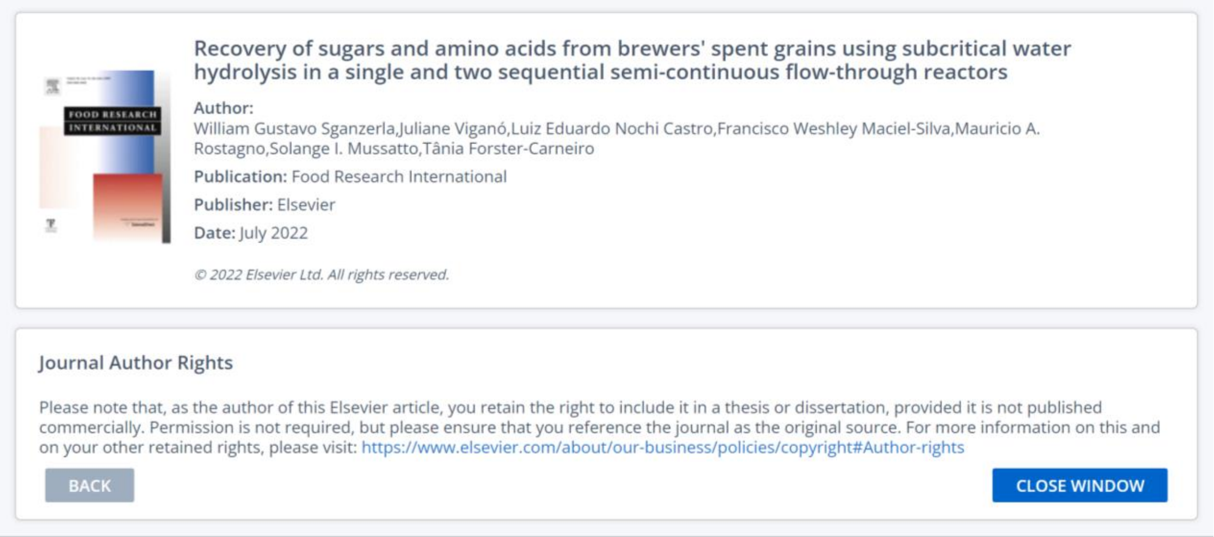

**Recovery of sugars and amino acids from brewers' spent grains using subcritical water hydrolysis in a single and two sequential semi-continuous flow-through reactors**

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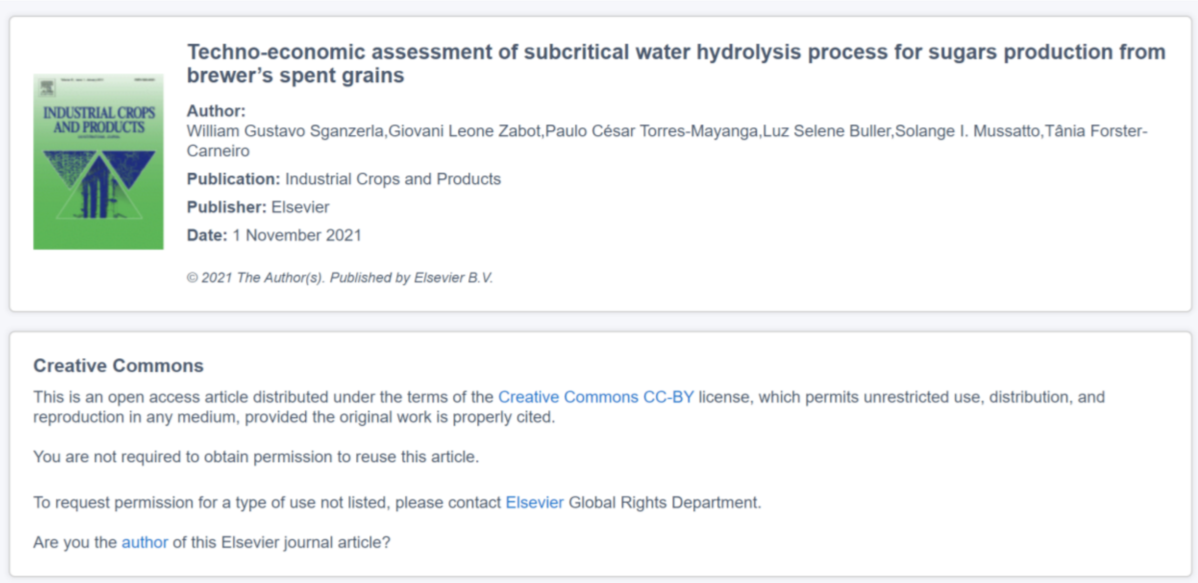

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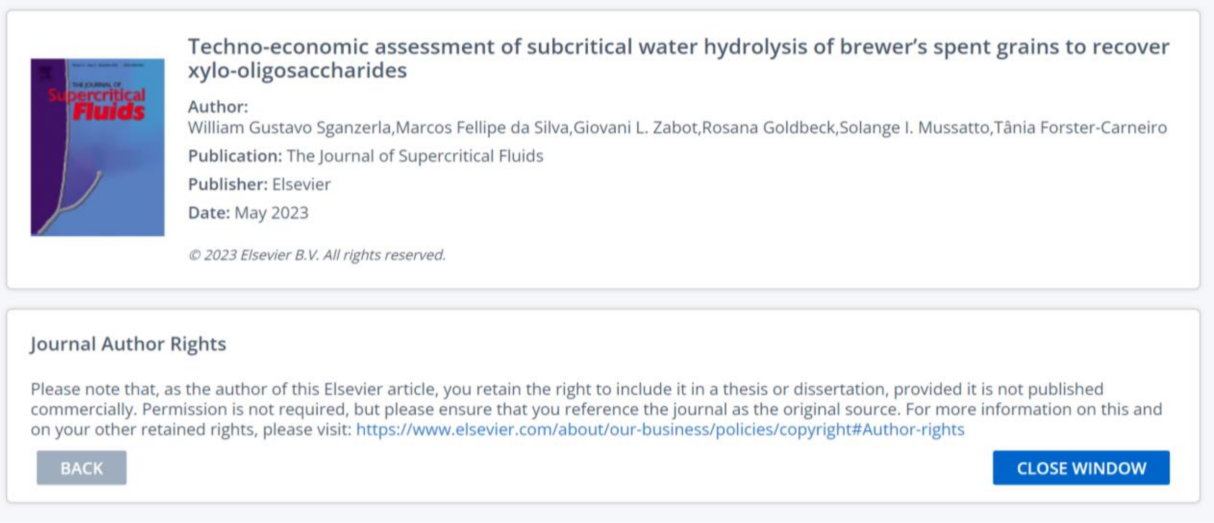

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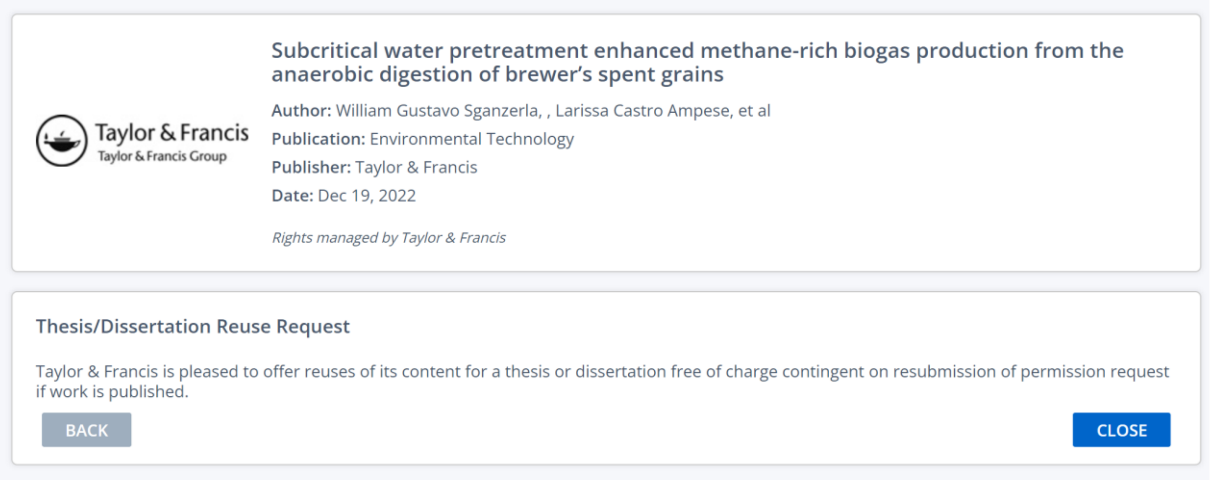

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