UNIVERSIDADE DE SÃO PAULO
ESCOLA DE ENGENHARIA DE LORENA

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Study of new technological alternatives for sugarcane bagasse biorefineries: hydrodynamic cavitation-assisted oxidative pretreatment and sequential and simultaneous hydrolysis and fermentation processes

Estudo de novas alternativas tecnológicas para biorrefinarias de bagaço de cana-de-açúcar: pré-tratamento oxidativo assistido por cavitação hidrodinâmica a processos de hidrólise e fermentação sequenciais e simultâneas

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Thesis presented to Engineering School of Lorena –University of São Paulo to obtain the title of Doctor in Science of the Graduate Program in Biotechnology under the Biomass Conversion Area.

Advisor: PhD. Júlio César dos Santos

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Thesis dedicated to my Family;
in memoriam of my grandmother.

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"The glory of friendship is not the outstretched hand, nor the affectionate smile,

not even the delight of company" Ralph Waldo Emerson

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“Educating is eternalized

in each being educated”

(Paulo Freire)

RESUMO

PRADO, C. A. **Estudo de novas alternativas tecnológicas para biorrefinarias de bagaço de cana-de-açúcar: pré-tratamento oxidativo assistido por cavitação hidrodinâmica e processos de hidrólise e fermentação sequenciais e simultâneos.** 2023. 255p. Tese (Doutorado em Ciências) – Escola de Engenharia de Lorena, Universidade de São Paulo, Lorena, 2023.

No Brasil, o desenvolvimento do processo de produção de etanol a partir do bagaço da cana-de-açúcar é relevante, dada a alta disponibilidade dessa matéria-prima. Entretanto, a viabilidade econômica da produção do etanol 2G requer o aproveitamento integral da matéria-prima em um contexto de biorrefinaria. Diversas alternativas têm sido avaliadas para as diferentes etapas envolvidas como hidrólise e fermentação, as quais podem ser realizadas separadamente, ou de forma simultânea em um sistema SSF (Si*multaneous Saccharification and Fermentation*). Neste último caso, com cofermentação de pentoses e hexoses, o processo é denominado SSCF (*Simultaneous Saccharification and Co-Fermentation*). Neste contexto, foi proposto o desenvolvimento de pré-tratamentos oxidativos assistidos por cavitação hidrodinâmica (CH) para bagaço de cana-de-açúcar, com a utilização do material pré-tratado para produção de etanol em sistemas sequenciais e SSCF com colunas interligadas. Pré-tratamentos oxidativos com O_3 e H_2O_2 assistidos por CH foram avaliados em pH neutro, enquanto que em outra etapa foram testados diferentes valores de pH no pré-tratamento assistido por CH com a presença de O_3. Nesse estudo, para o pH otimizado de 5, foi avaliado também o uso de catalisador de sulfato de ferro (5-15mg/L). Para todos os pré-tratamentos, foram conduzidos experimentos segundo um delineamento estatístico para avaliar a influência de variáveis relevantes. Em todos os casos, nas condições otimizadas, rendimentos de hidrólise de cerca de 90% foram atingidos na hidrólise enzimática subsequente, com tempo de pré-tratamento de 10 min. Empregando o hidrolisado obtido a partir de bagaço pré-tratado por CH, fermentação com *Saccharomyces cerevisiae* IR2 foi realizada para produção de etanol, obtendo-se 50g/L em 12 horas. O etanol foi então separado do caldo fermentado e, na vinhaça obtida, foi realizada a produção de xilitol empregando a levedura *Candida tropicalis* UFMGBX12, obtendo-se 32g/L de xilitol em 48 horas. Para o bagaço pré-tratado por processo oxidativo assistido por CH em pH 5, foi avaliada a produção de etanol em sistema SSCF usando reatores de coluna interligados. Neste processo, a produção de etanol foi estudada usando células de *Scheffersomyces parashehatae* em sistema SSCF em batelada simples e alimentada, os quais apresentaram uma produtividade de 0,35 (g/(L.h)) e 0,45 (g/(L.h)), respectivamente. A cavitação hidrodinâmica mostrou ser uma técnica interessante, sendo testada também na etapa de hidrólise enzimática, resultando na redução do tempo de processo. Neste caso, em condições otimizadas (18 FPU/g e 50°C), 93% e 77% de rendimento de hidrólise da glucana e da xilana foram observados, respectivamente após 12h. Considerando os resultados obtidos, a cavitação hidrodinâmica e as técnicas de fermentação sequencial e simultânea mostraram-se como estratégias promissoras para intensificação dos processos em biorrefinarias.

Palavras-chaves: Bagaço de cana-de-açúcar. Cavitação Hidrodinâmica. Processos Oxidativos avançados. Etanol. Xilitol.

ABSTRACT

PRADO, C. A. **Study of new technological alternatives for sugarcane bagasse biorefineries: hydrodynamic cavitation-assisted oxidative pretreatment and sequential and simultaneous hydrolysis and fermentation processes.** 2023. 255 p. Thesis (Doctoral of Science) – Escola de Engenharia de Lorena, Universidade de São Paulo, Lorena, 2023.

In Brazil, the development of the ethanol production process from sugarcane bagasse is significant given the high availability of this raw material. However, the economic feasibility of producing 2G ethanol requires the full utilization of the raw material in a biorefinery context. Several alternatives have been evaluated for the different steps involved. Hydrolysis and fermentation can be carried out separately or simultaneously, in a SSF system (Simultaneous Saccharification and Fermentation). In the latter case, with co-fermentation of pentoses and hexoses, the process is called SSCF (Simultaneous Saccharification and Co-fermentation). In this context, the development of oxidative pre-treatments for sugarcane bagasse assisted by hydrodynamic cavitation (HC) was proposed, using pre-treated material for ethanol production in sequential systems and SSCF with interconnected columns. Oxidative pre-treatments with O_3 and H_2O_2 assisted by HC were evaluated at neutral pH. In another step, different pH values were tested in the pre-treatment assisted by CH with the presence of O_3. In this study, the use of iron sulfate catalyst (5-15mg/L) was also evaluated for the optimized pH of 5. For all pre-treatments, experiments were conducted using statistical design to assess the influence of relevant variables. Under optimized conditions, hydrolysis yields of around 90% were achieved in the subsequent enzymatic hydrolysis, with pretreatment time of 10 min in all cases. Using the hydrolysate obtained from HC pre-treated bagasse, fermentation with *Saccharomyces cerevisiae* IR2 was carried out to produce ethanol, obtaining 50g/L in 12 hours. The ethanol was then separated from the fermented broth, and xylitol production was carried out in the vinasse obtained using the yeast *Candida tropicalis* UFMGBX12, obtaining 32g/L of xylitol in 48 hours. For bagasse pre-treated by HC-assisted oxidative processes at pH 5, ethanol production was evaluated in a SSCF system using interconnected column reactors. In this process, ethanol production was studied using *Scheffersomyces parashehatae* cells in the SSCF system in single and fed batches, which showed a productivity of 0.35 (g/(L.h)) and 0.45 (g/(L.h)), respectively. Hydrodynamic cavitation was shown as an interesting technique, and also was tested in the enzymatic hydrolysis step, reducing the process time. Under optimized conditions (18 FPU/g and 50°C), a hydrolysis yield of 93% and 77% for glucan and xylan, respectively, was observed after 12h. Considering the results obtained, hydrodynamic cavitation and sequential and simultaneous fermentation techniques proved to be promising strategies for intensifying processes in biorefineries.

Keywords: Sugarcane bagasse. Hydrodynamic cavitation. Advanced Oxidative Processes. Ethanol. Xylitol.

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LIST OF ABBREVIATIONS

AOP	Advanced Oxidative Process
CONAB	National Supply Company
HC	Hydrodynamic Cavitation
HPLC	High-Performance Liquid Chromatography
Lk	Lignin Klason insoluble
LBBSIM	Laboratory of biopolymers, bioreactors and Simulation of process
MA	Mass of dry sample
Mc	Ash mass
Mk	Dry mass of insoluble lignin
Ms	Mass of solid particles
Pf	Final product concentration
Pi	Initial product concentration
Qp	Volumetric productivity in product
Re	Reynolds number
Sf	Final concentration of substrate
Si	Initial concentration of substrate
T	Process temperature
t	Process time
UV	Ultraviolet
vvm	Volume of air per volume of medium per minute
Vt	Useful reactor volume
Xf	Final cell concentration
Xi	Initial cell concentration
$\mathbf{Y}_{P/S}$	Product yield
$\mathbf{Y}_{X/S}$	Biomass yield

SUMMARY

CHAPTER I

Introduction, objectives, and thesis overview

1.1 Introduction

In the context of sustainability, Brazil stands out as the Second-largest producer of ethanol in the world, following only the USA, with both countries being responsible for 70% of the world´s production of this alcohol (CONAB; 2023). Brazil is the largest producer of ethanol from sugarcane with on average production of 400,000 liters per day (EMBRAPA; 2023). It has been producing this biofuel since 1970 when Proálcool program was implemented. This program was driven by the oil crisis, with the aim of replacing gasoline with ethanol (Moreno et al., 2017). The biofuel sector has also received other incentives over time, such as the introduction of flex-fuel vehicles and the mandatory addition of ethanol to gasoline. According to the resolution 75 of MAPA, the percentage of biofuel in premium and regular gasoline is 25% and 27%, respectively (MAPA, 2015).

Brazilian ethanol production generated 26.35 billion liters of fuel in the 2021/2022 harvest, according to Agência Brasil (2023). First-generation ethanol production is well-established in the country, and second-generation ethanol production plants have also been installed, particularly by Raízen and Granbio (Santos et al., 2016). However, these 2G ethanol plants do not operate at full production capacity due to difficulties with process steps such as pre-treatment and enzymatic hydrolysis. The development of lignocellulosic material biorefineries is a promising solution to increase the production of this biofuel, mainly considering the abundant availability of sugarcane bagasse, a by-product of the national sugar-alcohol sector. Nevertheless, the hydrolysis of its carbohydrate fractions is necessary to release fermentable sugars that can be transformed into ethanol or other products by fermentation processes.

Indeed, in Brazil, the development of an ethanol production process from sugarcane bagasse is essential. Most of the sugarcane bagasse generated in the mills, is used in the industry itself to produce electricity; however, there are many studies on interesting alternatives for the use of bagasse, including the production of second-generation ethanol (Kumar et al., 2020). However, the economic feasibility of producing 2G ethanol requires the utilization of all fractions of the raw material in a biorefinery context.

Currently, the technology under development includes enzymatic hydrolysis of the cellulose present in biomass. This requires a pre-treatment step to modify the structure or composition of the material and facilitate access of cellulases to their substrate. Several pre-treatment alternatives exist, including physical, chemical, physical-chemical, and biological methods (Gonzalez et al., 2012; Ubano et al., 2020). Despite numerous studies conducted over the past few decades, pretreatment remains an important bottleneck for the viability of lignocellulosic biorefineries.

Recently, hydrodynamic cavitation-assisted processes have shown promise in biomass pretreatment. Cavitation refers to the process of cavity or microbubble formation, growth, and collapse, releasing a significant amount of local energy (M'arimi et al., 2020; Zielinski et al., 2019). Collapsed bubbles induce localized supercritical conditions with high temperature and pressure. Hydrodynamic cavitation has been shown to be efficient in accelerating chemical processes of degradation and product synthesis. However, since this technology has only more recently been applied in biomass pretreatment (Hilares et al., 2020; Prado et al., 2022; Bimestre et al., 2022), other options need to be evaluated to maximize the process's performance. Possibilities to consider include advanced oxidative processes (AOPs) and catalytic processes.

Advanced oxidative processes (AOPs) are low-cost technologies that have been used to degrade high molecular weight compounds. In these, HO• (hydroxyl) is used as an oxidizing agent. This radical can be generated by the reaction of ozone in the presence of water, or with peroxide action. Indeed, catalytic processes can also be used, with the addition of metals in the process. For example, the presence of iron catalysts has been found to increase effectiveness in some systems, while also being cost-effective (Carbajo, et al., 2018; Manef et al., 2018).

After pretreatment, the solid material is subjected to the biological steps of enzymatic hydrolysis and fermentation. In hydrolysis, a broth rich in glucose is produced, which can be transformed into ethanol by the action of yeasts, such as *Saccharomyces cerevisiae*, traditionally used in the production of 1G ethanol, followed by the distillation of alcohol. The hemicellulosic fraction is also rich in sugars (mainly pentoses), which can be obtained in their monomeric form by hydrolysis in the pretreatment or in the enzymatic hydrolysis step, depending on the pretreatment method used (Parada et al., 2017). If there is hemicellulose in the pre-treated solid material, it can be hydrolyzed biotechnologically, since the presence of xylanolytic enzymes in enzymatic preparations of commercial cellulases is common (Pérez-Bibbins et al., 2016).

Enzymatic hydrolysis can be performed sequentially or simultaneously with fermentation (Ask et al., 2012; Ramos, 2019). The alterntives may include simultaneous saccharification and fermentation (SSF) of hexoses or simultaneous saccharification and co-fermentation (SSCF) of hexoses and pentoses. SSF/SSCF configurations have advantages over separate processes, such as reducing problems related to enzymatic activity per product and reducing the number of required equipment (c). However, considering the most usual system where hydrolysis and fermentation take place in the same vessel, one of the biggest drawbacks is the use of non-optimized temperatures for hydrolysis and fermentation, which can result in an inefficient process (Sahara et al., 2017). As an alternative, the process can be carried out using an interconnected reactor system. This system has been developed in LBBSIM, and in it an enzymatic hydrolysis column reactor is coupled to a reactor with immobilized cells. This SSF or SSCF system allows each of the biological steps to be carried out in its optimal temperature (Hilares et al., 2017). The results of the literature indicate that this technique needs further studies, including other possibilities for the production of different products.

1.1.1 Sugarcane bagasse and Biorefinery

The search for renewable energy sources to replace fossil fuels has become a relevant issue. It is necessary to reduce the environmental impact and greenhouse gas emissions due to the use of petroleum derivatives (Cherubini, 2010; Boudet, 2011; Silva et al., 2019). In this sense, Brazil has stood out on the world due to its high use of renewable energy. According to Matos et al. (2022), Brazil used 82% of renewable energy in its energy matrix in 2022. The share of renewables in the energy matrix of the rest of the world and the countries of OECD (Organization for Economic Cooperation and Development) were only 13% and 8.1%, respectively.

According to research presented in Matos et al. (2022), sugarcane derivatives are the main source of renewable energy used in Brazil, followed by hydropower, firewood, charcoal, and lye, among others (Figure 1.1). Among the non-renewable energies, oil and its derivatives remain the main sources, followed by natural gas and natural coal (IEA, 2020).

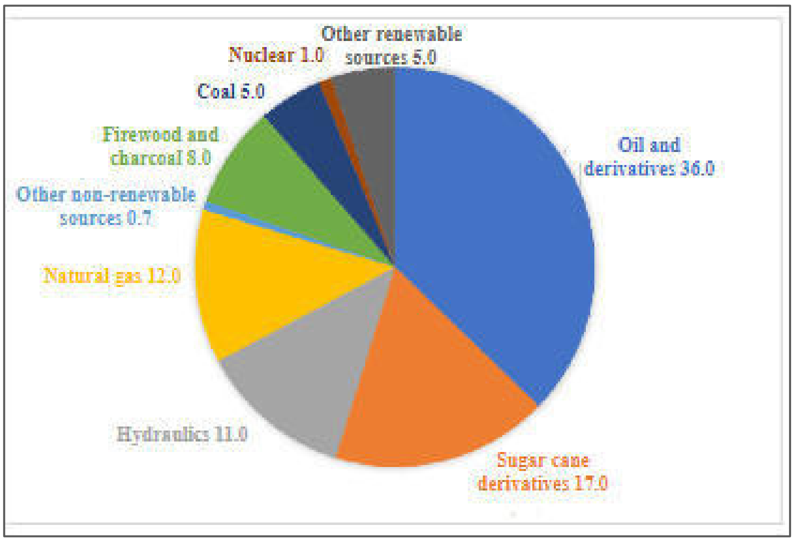

Figura 1.1 Brazilian energy matrix. Adapted from Matos et al. (2022)

Among the biofuel options, ethanol deserves special attention. Brazil is the second-largest producer and the largest exporter of ethanol in the world, mainly derived from sugarcane juice. The technology used in the country is a world reference regarding alternatives to oil (Da Silva et al., 2020; Ubano et al., 2020).

However, ethanol production in the country could be expanded without the need to increase the agricultural area if the potential of producing this alcohol from residues of agro-industrial by-products, known as 2G ethanol, is taken advantage of (Patil and Gogate, 2012; Bechara et al., 2018). According to Bonassa et al. (2018), Brazil has the potential to produce more than 298 million tons per year of residues and lignocellulosic by-products from the processing of crops such as rice, sugarcane, corn, soybeans, and wheat, in addition to the approximately 6 million m³ of waste generated during pine and eucalyptus forest harvests. Actually, Brazil stands out for being a country that has a great agricultural and forestry production, generating large amounts of waste that require proper disposal. These residues can be used to increase the use of biomass in the energy matrix for global supply (EMBRAPA, 2023). Among the main Brazilian agro-industrial by-products, sugarcane bagasse can be highlighted due to its abundance in the country.

1.1.1.1 Sugarcane: general aspects, bagasse and its composition

Sugarcane is a plant known worldwide, mainly for being raw material in the production of ethanol and sugar. The sugarcane cycle has an average of six years, in which there are four or five harvests. Sugarcane belongs to a group of tall perennial grass species of the genus *Saccharum*. This plant is native to tropical regions, such as southern Asia and northern Brazil (Pereira et al. 2011; Antunes, 2015). Its stems are robust, articulated, and fibrous. The height of the cane can be from two to six meters.

There are researchers who have developed complex hybrids from different sugarcane species, with the aim of improving their performance as a raw material in different areas (Candido and Gonçalves, 2019). Sucrose is the main sugar obtained from sugarcane juice and can be extracted and purified in biorefineries for later use as a raw material in the food industry or fermented to produce ethanol (EMBRAPA, 2023). In 2022, sugarcane grew on about 24.0 million hectares of agricultural land in more than 90 countries, with a world harvest of 594 million tons (IEA, 2023).

The main by-product of the national sugar and alcohol industry is sugarcane bagasse. Every year, between 5 and 12 million tons of sugarcane bagasse are generated in Brazil from the 1G ethanol and sugar production process (EMBRAPA, 2023). The amount produced depends on the fiber content of the processed sugarcane, with bagasse representing about 14% of the ground sugarcane (EMBRAPA, 2023).

Sugar and alcohol plants use 60% to 90% of this bagasse for energy production, replacing fuel oil in the boiler heating process (CONAB, 2020), to generate steam and electricity. Recently, sugarcane straw has also been used for energy production. Other applications of sugarcane bagasse include the production of cellulose and its use in feed for confined cattle (Song et al., 2019; Silva et al., 2020). Although the sugar-alcohol industry uses bagasse to produce electricity, there are studies aimed at using this raw material to obtain other products of interest (Oliveira, 2012; Arruda et al., 2017; Ramos, 2019; Hilares et al., 2020). However, there are limitations in the use of this plant biomass due to its composition, which poses a challenge for researchers due to its recalcitrance to processing.

Lignocellulosic materials are mainly composed of three macromolecule fractions: cellulose, hemicellulose, and lignin. Cellulose (Figure 1.2) is a linear polymer of high molecular weight, insoluble in water, and constituted by repeating units of cellobiose, with glucose residues connected by beta-1-4 glycosidic bonds. The degree of polymerization, determined by the number of glucose residues, varies from 1000 to 50000, depending on the origin (Silva et al., 2013). It has amorphous and crystalline parts, which are difficult for cellulases to access during the hydrolysis process.

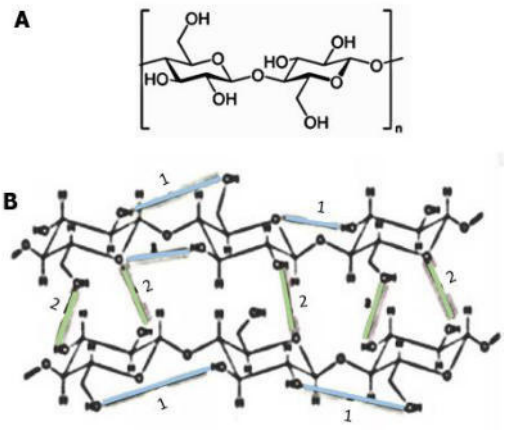

Figure 1.2. Structure of cellulose (A) and hydrogen bonds (B) intramolecular (1) and intermolecular (2). Adapted Roweel et al. (2005)

Due to their high degree of polymerization, cellulose molecules can bond together through hydrogen bonds (Figure 1.3) (Sjostrom, 1993; Caballa et al., 1981). Cellulose produced by plants is usually composed of highly amorphous regions, containing large voids and other irregularities, as well as crystalline regions, with greater density and more resistance to most forms of degradation. The degree of polymerization, as well as crystallinity, are significant factors in the cellulose hydrolysis process (Zhang and Lynd, 2004).

Hemicellulose is another group of polysaccharides present in plant biomass, serving as a reserve and support material. This biomass fraction is a complex group of polymers in terms of components and molecular structure (Harmsen et al., 2009). It is made up of branched polysaccharides, classified as heteropolymers, since they contain different monomeric sugars, including pentoses (β-D-xylose and α-L-arabinose), hexoses (β-D-mannose, β-D-glucose, and α-D-galactose) and/or uronic acids (α-D-glucuronic acid, α-D-4-O-methylgalacturonic acid, and α-D-galacturonic acid) (Gírio et al., 2010; Mahmood et al., 2019; Ramos, 2019). Hemicellulose has a lower molecular weight than cellulose and, due to its amorphous branched structure, is more prone to hydrolysis. It is classified according to the predominant carbohydrate in the main chain and side branch. Among the components of hemicelluloses, xylans (Figure 1.3), 4-methyl-glucuromo-xylans, galactosans, and galacto-arabino-glucuronide-xylan stand out (Biely, 1985; Han and Rowell, 1996; Sjostrom, 1993; Rowell et al., 2005).

In the composition of hemicellulosic hydrolysates from lignocellulosic materials, compounds such as D-xylose, D-maltose, D-glucose, D-galactose, and L-arabinose, D-glucuronic acid, and D-galacturonic acid are found, among others. Among these compounds, hemicellulosic hydrolysates of sugarcane bagasse contain D-xylose in greater proportion, typically above 95% (Kanauf and Moniruzzaman, 2004; Xiong et al., 2019).

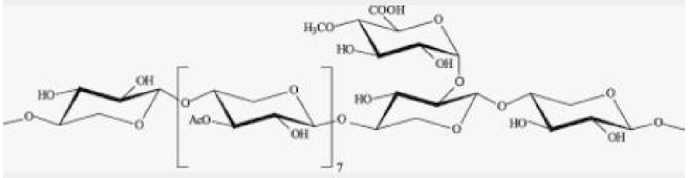

Figure 1.3. Representative structure of xylan (Harmsen et al., 2009).

Lignin is an amorphous and water-insoluble component, and one of the most abundant and important organic macromolecules in the biosphere (Brunow, et al., 1999; Fengel & Wegener, 1983). It makes up 20 to 30% of lignocellulosic biomass and is structurally linked to cellulose and hemicelluloses, providing rigidity and low reactivity to the plant fibers and preventing their degradation (Brunow, et al., 1999). Lignin also enhances the mechanical resistance of plants, contributing to their rigidity and verticality. Additionally, lignified tissues resist microbial attacks, preventing destructive enzymes from penetrating the cell tissue, and provide protection against oxidative stress (Fengel & Wegener, 1983).

Normally, lignins are classified based on their structural elements, and there is compositional variation among different species. For example, hardwoods have a lignin percentage composition of 18% to 25%, while softwoods have 25% to 35% (Rowell et al., 2005).

Lignin has a polyphenolic structure composed mainly of phenylpropane units that cover the material's polysaccharides (Pérez et al., 2002; Canilha et al., 2010). It is synthesized from three radicals: coniferyl alcohol, p-coumaryl alcohol, and sinapyl alcohol (see Figure 1.4). Depending on the degree of methoxylation, the basic subunit coniferyl alcohol results in guaiacyl, p-coumaryl alcohol in p-hydroxyphenyl, and sinapyl alcohol in syringyl. The end result of this polymerization is a heterogeneous structure (Carrot and Carrot, 2007; Budziak et al., 2004). It is important to emphasize that the proportion of these monomers varies among plant species, and this relationship has been used for taxonomic purposes.

Since lignin is not a polysaccharide, it is not intended for the production of ethanol via fermentation. Therefore, biorefineries use pre-treatment methods that usually remove this material from plant biomass. Lignin can be used as a raw material for the production of electricity and steam, among other possible applications (Patel and Shah., 2021; Paramasivan et al., 2021).

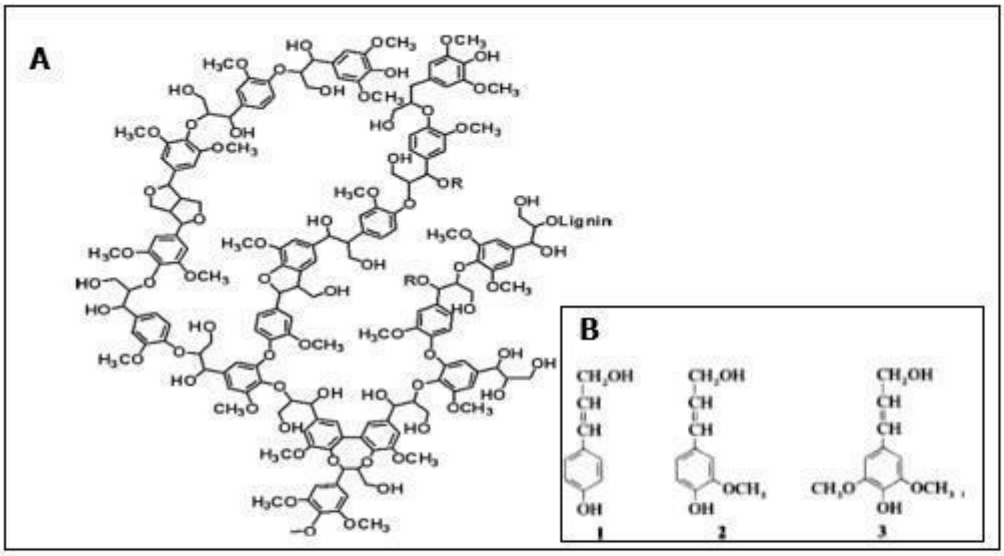

Figure 1.4. A- Representative scheme of the chemical structure of lignin B- Representative structure of elements that make up lignin (Harmsean et al., 2009)

1.1.1.2 Biorefinery of lignocellulosic materials

The biorefinery can be defined as an integrated complex capable of producing different products (such as fuels, chemicals, and electricity) based on various biomasses. It processes them entirely, generating products of economic and social interest (Parada, et al., 2017; Hu, et al., 2018). There are, in fact, several biorefinery concepts that are all similar to one another. The IEA Bioenergy (2013) defines a biorefinery as the sustainable processing of biomass into a range of marketable products (such as food, feed, materials, and chemicals) and/or energy (such as fuels, electricity, and heat). The NREL - National Renewable Energy Laboratory (2012), in turn, defines a biorefinery as a facility that integrates biomass conversion processes and equipment for the production of fuels, energy, and chemicals. An example of a biorefinery is a sugarcane plant that combines and integrates the production of sugar, bioethanol, electricity, and other products.

The raw material must be renewable and have a regular supply for the biorefinery. It can be found in agriculture, forestry, industrial or domestic waste. This biomass can be divided into three categories: 1. carbohydrates and lignin, 2. triglycerides, and 3. mixed organic waste (Bittencourt et al., 2019). Carbohydrates are the most common component in biorefinery raw materials, including five- and six-carbon sugar monosaccharides. The most common pentoses are xylose and arabinose, while the hexoses are glucose, galactose, and mannose. These monomers polymerize into starch or lignocellulosic chains (Antunes et al., 2015).

The definition of a biorefinery is to have an ideal model for the integral processing of biomass (Mondenbach and Nokes, 2013; Dragone et al., 2020). In the case of using lignocellulosic materials, biorefineries must be developed in a way that favors the full utilization of their constituent fractions, which can be used directly in energy generation or serve as raw material for chemical, enzymatic, or fermentative processes. Thus, the deconstruction of the recalcitrant lignocellulosic structure is required (Candido, et al., 2019).

Currently, to produce ethanol and other products of interest, the technology under development has been directed towards a process whose initial step corresponds to pre-treatment. The function of pre-treatment is to increase the digestibility of cellulose in a subsequent enzymatic hydrolysis (Sun et al., 2016). Depending on the type of pre-treatment used, the hemicellulose can be hydrolyzed at this stage or remain in the pretreated material and can be enzymatically hydrolyzed along with cellulose (Menon and Rao, 2012). The hydrolysates rich in sugars from the carbohydrate fractions of biomass can be used in several processes, resulting in the production of compounds such as ethanol and xylitol, which were evaluated as study models in the present project, and their importance will be below described (Mood et al., 2013).

1.1.2 Products of interest from biomass: ethanol and xylitol

First-generation ethanol production began on a large scale in Brazil with Proálcool, a program created by the federal government due to the oil crisis. Its use peaked in 1986, and it remained important throughout the 1990s (Venturi and Vincenzini, 2012; Ubano et al., 2020). In the early 2000s, alcohol once again became widely used in the country due to new oil prices and the Brazilian government's efforts to become less dependent on oil as a source of energy. The use of ethanol during this period was also driven by the development of flexible-fuel vehicles (Ferreira et al., 2009).

The technology used in Brazil is a world reference in terms of alternatives to oil, and Brazil is the second-largest producer of ethanol, mainly using sugarcane as raw material. One advantage of producing first-generation biofuels is the high sugar content present in the biomass and the ease of converting it into fuel (Ubano et al., 2020).

Second-generation biofuels have been extensively studied in recent decades (Bagder et al., 2002; Bai et al., 2008; Akram et al., 2018; Artrith et al., 2020), but the technology is still immature for industrial scale production. To produce 2G ethanol, lignocellulosic raw materials are used, which do not compete with the food industry, corresponding mainly to agro-industrial residues and by-products (Parada et al., 2017; Mahmood et al., 2019).

Some advantages of second-generation bioethanol over first generation (mainly when using starchy raw materials) include not only the high availability of raw materials but also a greater potential for reducing CO_2 emissions when considering the global carbon balance, and the fact that its production does not imply competition with the food production chain (BNDS and CGEE, 2008; Gonzalez et al., 2012; Bryngemark., 2019). In fact, the use of corn as a raw material, as in the United States, has been identified as a factor that reduces availability and leads to an increase in food prices. Furthermore, expectations show that the corn produced is not sufficient to meet the growing demand for fuel (Gonzalez et al., 2012; Bryngemark , 2019).

In the case of ethanol from Brazil, an established technology is used based on traditional Brazilian culture, and the Brazilian product has economic and environmental advantages (BNDS and CGEE, 2018). Although the process of producing ethanol from sugarcane juice in Brazil is efficient, it is estimated that using bagasse and some straw of sugarcane could increase alcohol production by 30 to 40% for the same planted area (Pantil and Gogate., 2012). Other raw materials for which processing technologies are sought, such as elephant grass, brachiaria, panicuns, and fast-growing trees, can represent competitive and efficient alternatives for places where it is not possible to grow sugarcane (Medina et al., 2013). In the specific case of sugarcane bagasse, its use for ethanol production could compete with the production of electricity, and the plants could follow a competition for the market (Proskuryakova et al., 2018; Haldar et al., 2020).

Another product with potential for production in biorefineries is xylitol. This is a polyalcohol as sweet as sucrose and has been used in the food, dental, pharmaceutical, and medical industries, in addition to presenting significant potential for application in other industrial segments, such as the textile and chemical sectors (Almeida et al., 2007; Haldar et al., 2020)

Xylitol is also found in human and mammalian organisms, produced in small amounts as an intermediate product of carbohydrate metabolism. The daily endogenous production is on average 20 grams in a normal adult person (Mussatto and Roberto, 2002; Pandiyan et al., 2019). Xylitol has interesting properties, such as being anticariogenic and can be used in post-surgical infusions. It also improves the biomechanical properties of bones in case of osteoporosis and can be used to prevent acute otitis media in children (Chaud et al., 2010).

In fact, there are several characteristics that make xylitol an attractive substance for use in different commercial products. Its use is indicated for diabetic people since its metabolism is insulin-independent (Makinen, 2000; Pérez et al., 2019). Xylitol is also used in the formulation of dermatological cream or gel to combat skin lesions such as dermatitis. According to the work by Ferreira, et al., (2009), *in vivo* tests were carried out to verify the safe application of xylitol on the skin. The authors observed that the cream with 10% xylitol did not induce the formation of erythema or edema and resulted in the improvement of skin lesions. Xylitol is also indicated for patients with glucose-6-phosphate dehydrogenase deficiency (Makinen, 2000) and as a dietary sweetener (Mussatto and Roberto, 2002; Alcantara et al., 2013).

Among the main properties of this compound, xylitol is non-cariogenic and has anti-cariogenic power (Makinen et al., 2000; Bressanello et al., 2016). It is non-cariogenic because the microorganisms normally found in the oral cavity are not able to metabolize it, which prevents them from producing acids that attack tooth enamel and cause lesions that produce caries. It is anti-cariogenic, promoting the remineralization of initial lesions. An additional explanation for its beneficial dental effects is the reduction in the amount of *Streptococcus mutans*, the main pathogenic agent responsible for caries, in saliva, obtained through the regular use of chewing gum containing this polyalcohol (Autio et al., 2002; Makinen et al., 2000; Alanen, Holti, and Pienihakkinen, 2000; Vernachio, Vezina, and Mitchell, 2007).

Currently, xylitol is mainly produced by a chemical process that requires extensive operations of ion exchange and chromatographic fractionation to obtain high-purity xylose. The hydrogenation step takes place in discontinuous reactors at high pressures (50 atm) and temperatures in the range of 80-140 ºC, using a nickel-based catalyst. After removing the catalyst by filtration and ion exchange, the hydrogenated solution containing xylitol is concentrated and undergoes chromatographic fractionation using cationic resins. Xylitol crystallizes when pure (Mikkola et al, 2000; Pérez-Bibbins, et al., 2016). Thus, the use of this method has disadvantages, which make the final product more expensive, especially the use of high pressures and temperatures, in addition to the need for intensive purification steps both in the "upstream" and in the "downstream" (Parajó, Domínguez and Domínguez, 1998).

The biotechnological route has the potential to reduce costs since it allows operation under milder temperature and pressure conditions and does not require extensive substrate purification steps before its transformation into xylitol (Barrio et al., 2017; Pérez et al., 2019). However, the concentrations, yields, and production rates obtained in the fermentation process using lignocellulosic hydrolysates are still bottlenecks for large-scale processes, although they can be circumvented by selecting the correct fermentation system, operation mode, and cultivation conditions (Pereira Jr. et al., 2008; Liu et al., 2020).

The concomitant production of ethanol and xylitol has been evaluated in recent studies. For instance, Cortivo et al. (2018) used a recombinant strain of the yeast *Saccharomyces cerevisiae* to produce ethanol and xylitol. The authors used oat and soy hull hydrolysates for this purpose. When hydrolysates containing similar concentrations of glucose and xylose were used, the maximum consumption of xylose was approximately 35% under anaerobic conditions. However, hydrolysates composed mainly of xylose resulted in the consumption of 73% of xylose, and ethanol was formed with yield of 0.33 g/g. In bioreactor cultures under oxygen limitation, xylose consumption was approximately 65%, and the main product was xylitol, reaching a final concentration of 8.17 g/L.

1.1.3 Biological steps of the process: enzymatic hydrolysis and fermentation

The enzymatic hydrolysis step usually takes place right after the pre-treatment of the lignocellulosic biomass. Cellulose hydrolysis requires a group of enzymes that act synergistically, resulting in a glucose-rich medium. Commonly, commercial enzyme preparations of cellulases also contain xylanases, which can release pentoses from hemicellulose, if this fraction is present in the pre-treated material (Canilha et al., 2012).

Enzymatic hydrolysis is an enzyme-catalyzed reaction that uses water to break one molecule into two other molecules. One of the catalyzed reaction products receives a HO^- group, while the other reaction product receives a hydrogen cation (Pereira Jr. et al., 2008).

Cellulases are composed of an enzymatic complex in which there are enzymes that act synergistically and are subdivided into three classes: endo-1,4-β-D-glucanases or endoglucanases, which break the glycosidic bonds inside the cellulose chains, creating new terminals; exo-1,4-β-D-glucanases or cellobiohydrolases, responsible for the action on the terminals, leading to cellobiose; and 1,4-β-D-glucosidases, which hydrolyze cellobiose to glucose (Arantes, and Saddler, 2010; Odega et al., 2010). Cellulases are produced by fungi and bacteria and can be classified according to their place of action in the cellulosic substrate (De Castro et al., 2010).

Consequently, the cellulosic insoluble substrates are converted into soluble sugars by the action of these enzymes (Cabella et al., 1981). It is known that when the enzymes act together, there is a better yield than the sum of the individual yields, with at least three forms of synergy between cellulases being known (Lynd et al., 2002). The first is known as EnG-ExG synergy, in which the action of endoglucanases occurs in amorphous regions, leading to the release of reducing and non-reducing terminals, on which type I and type II cellobiohydrolases (CBHs) will act, respectively. Another synergy is ExG-ExG, in which CBHs I and CBHs II act simultaneously on reducing and non-reducing terminals, releasing cellobiose. The last one is the ExG-BG and EnG-BG synergy, in which cellobiohydrolases act by releasing cellobiose and oligosaccharides, which are the substrate of β-glucosidase, which then releases glucose.

Endoglucanases work by cleaving the glucose molecule (as shown in Figure 1.5) internally at random positions. They are also called CMCases, with the systematic name being 1,4-β-D-glucan-4-glucanohydrolase, according to the IUBMB - International Union of Biochemistry and Molecular Biology (De Castro et al., 2010). These enzymes mainly catalyze the hydrolysis of amorphous cellulose and release molecules of small molar mass that have both reducing and non-reducing terminals, where exoglucanases can connect (Ahmed et al., 2018; Ostergaard and Olsen, 2011; Zhang and Zhang, 2020). Zhao and Zhao (2012), citing other authors, report that most endoglucanases have an active site with the shape of a slit, which allows the enzyme to bind and cleave the cellulose chain, releasing glucose, soluble cellodextrins or insoluble fragments of cellulose. There are also endoglucanases that have the ability to hydrolyze crystalline cellulose and generate products such as cellobiose and cellodextrins.

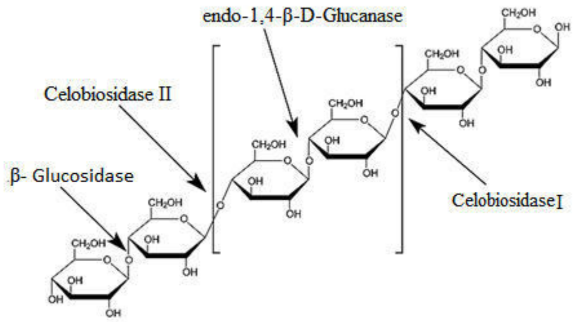

Figure 1.5. Points of action of enzymes in the structure of glucose. Adapted of Fatma et al. (2018)

Exoglucanases act on the ends of microcrystalline cellulose molecules and are classified into two groups: glucanohydrolases and cellobiohydrolases. The first group, named 1,4-β-D-glucan-glucan-hydrolases, is not widely reported in the literature but can release glucose directly from the polymeric chain (De Castro et al., 2010; Chen and Khanna, 2018). Cellobiohydrolases, or 1,4-β-D-glucan-cellobiohydrolases, can be divided into two types: enzyme type I (CBH I), which hydrolyzes reducing terminals (R), and type II (CBH II), which hydrolyzes non-reducing terminals (NR) (Ostergaard and Olsen, 2010).

β-glucosidases are enzymes that promote the hydrolysis of cellobiose and soluble oligosaccharides. These enzymes can be inhibited by-products, similar to cellobiohydrolases (De Castro et al., 2010; Choudhary, et al., 2016). Another group of enzymes that has attracted attention is the lytic polysaccharide monooxygenases (LPMOs), which are oxidizing enzymes. LPMOs have the ability to oxidize polysaccharides, thereby assisting hydrolases in the hydrolysis of cellulose (Figueiredo et al., 2018).

The xylanases catalyze the hydrolysis of xylan present in the biomass. Depending on the pre-treatment method used, hemicellulose may remain in the solid material obtained along with cellulose, and in this case, it can be hydrolyzed together with the latter (Arantes, and Saddler., 2010) using enzymes such as xylanases in formulations or acting together with enzymatic cellulase cocktails. Since the xylans present in hemicellulose are heteropolysaccharides, cleaving them into monomeric sugars requires a combination of enzymes such as endo and exo-xylanases, mannanases, beta-xylosidase, alpha-glucuronidase, and alpha-arabinofuranosidase. Hydrolysis can occur randomly, releasing linear and branched heteroxylo-oligosaccharides (Biely, 1985; Antunes et al., 2015). Among the main classes of hemicellulose-degrading enzymes, 1-4 beta D-xylanase is the main constituent of the xylanolytic system. This enzyme degrades the polysaccharide beta-1-4-xylan into xylose (Canilha et al., 2010; Rodrigues et al., 2012).

Various methods of enzymatic hydrolysis can be found in the literature. Some works have employed both low and high solids loading (Sahara et al., 2017), with the latter being the most promising option for obtaining hydrolysates with high sugar concentrations and, consequently, a high product concentration during fermentation.

Ramos (2019) suggests that fixed bed reactors are suitable for enzymatic hydrolysis due to their high efficiency, low cost, ease of installation, operation, scale-up, control, and reduced shear force. However, this system also presents some problems such as possible bed obstruction, the emergence of preferential paths, and inefficient heat and mass flows (Cardona et al., 2010; Carneiro et al., 2020). Terán-Hilares et al. (2016) used a packed bed column reactor for enzymatic hydrolysis of sugarcane bagasse that was pretreated by HC-assisted process. In this study, the authors achieved approximately 30 g/L of glucose and 10 g/L of xylose, using 13.2% of the initial load of solids in the reactor and a hydrolysis time of 24 hours. This resulted in a reported 50% yield of enzymatic cellulose hydrolysis. In another work, Dong et al. (2020) used a packed-bed reactor with an initial charge of 12% solids and achieved 40 g/L of glucose during enzymatic hydrolysis of rice straw that was pre-treated by an alkaline process. They also reported a yield of cellulose hydrolysis of 55% for pretreatment with high alkali concentration.

After the enzymatic hydrolysis step, fermentation takes place through the metabolic activity of microorganisms (Patinvoh and Taherzadeh, 2019). Bioethanol is produced by the fermentation of sugars by various yeasts, with *Saccharomyces cerevisiae* being the most commonly used strain. This conventional strain can withstand high osmotic pressure, tolerate low pH values (up to 4), and has an optimal temperature of around 30°C, resulting in high yields in ethanol production (Limayem and Ricke, 2012).

Regardless of the raw material, the fermentation of hydrolyzed sugars follows the same basic principle. In the case of lignocellulosic biorefineries, the sugars present in the medium resulting from biomass hydrolysis are hexoses and pentoses. Hexoses are easily assimilated and fermentable for microorganisms used in alcohol plants (Mesa et al., 2010). However, microorganisms such as *S. cerevisiae* do not have metabolic pathways to carry out the metabolization of pentoses, except those that are genetically modified for this purpose (Zhang et al., 2010).

Nevertheless, several microorganisms are capable of natively metabolizing pentoses, such as *Scheffersomyces stipitis* and *Candida shehatae*, each having unique characteristics and different fermentation parameters (Balat, 2011; Gírio et al., 2010).

In hemicellulose hydrolysates of sugarcane bagasse, xylose is the most abundant monomeric sugar (Rodrigues et al., 2015). For its metabolization, xylose is typically converted to the compound xylulose via direct isomerization in most bacteria. However, in yeast and filamentous fungi, this conversion takes place in stages based on redox reactions, where the enzyme xylose reductase (XR) converts xylose into xylitol. Xylitol is then converted into xylulose under the action of the enzyme xylitol dehydrogenase (XD) (Figure 1.6).

After xylitol is converted to xylulose, metabolism continues with a phosphorylation reaction catalyzed by the enzyme xylulokinase. This enzyme converts xylulose to xylulose 5-P, which is then metabolized to intermediates of the glycolytic pathway such as glyceraldehyde 3-P and fructose 6-P via the phosphopentose pathway, as shown in Figure 1.6. Both compounds are converted into pyruvate via the glycolytic pathway. Under conditions of oxygen restriction, pyruvate is converted to ethanol through two sequential reactions: decarboxylation by the enzyme pyruvate decarboxylase, which converts pyruvate to acetaldehyde, and reduction to ethanol by the enzyme alcohol dehydrogenase (Toivari et al., 2001; Jeffries and Jin, 2004; Jeffries et al., 2006). Under conditions of aerobiosis, pyruvate follows the Krebs cycle and the electron transport chain, generating energy and favoring the production of biomass (Rodrigues et al., 2006).

Under oxygen-restricted conditions, some yeasts may also accumulate xylitol, mainly in yeasts where XR is NADPH-dependent (Jeffries, 2006). In such cases, there will be less availability of NAD+ for the XD enzyme, resulting in xylitol accumulation, which can be excreted and accumulated in the medium (Jeffries and Lin, 2004). In some yeasts, the first reaction may occur in the presence of cofactors NADPH or NADH under oxygen-restricted conditions, resulting in little accumulation of xylitol and following the metabolic flow for conversion to ethanol (Hahn-Hãgerdal et al., 1994).

Regarding the production of 2G ethanol and other bioproducts, various process configurations can be found in the literature for the fermentation and enzymatic hydrolysis steps, considering the possibility of carrying out the fermentation separately or simultaneously with the hydrolysis of hexoses and pentoses. Four main types of simultaneous saccharification and fermentation (SSF), simultaneous saccharification and co-fermentation (SSCF), and consolidated bioprocessing (CBP) (Van Zyl et al., 1988).

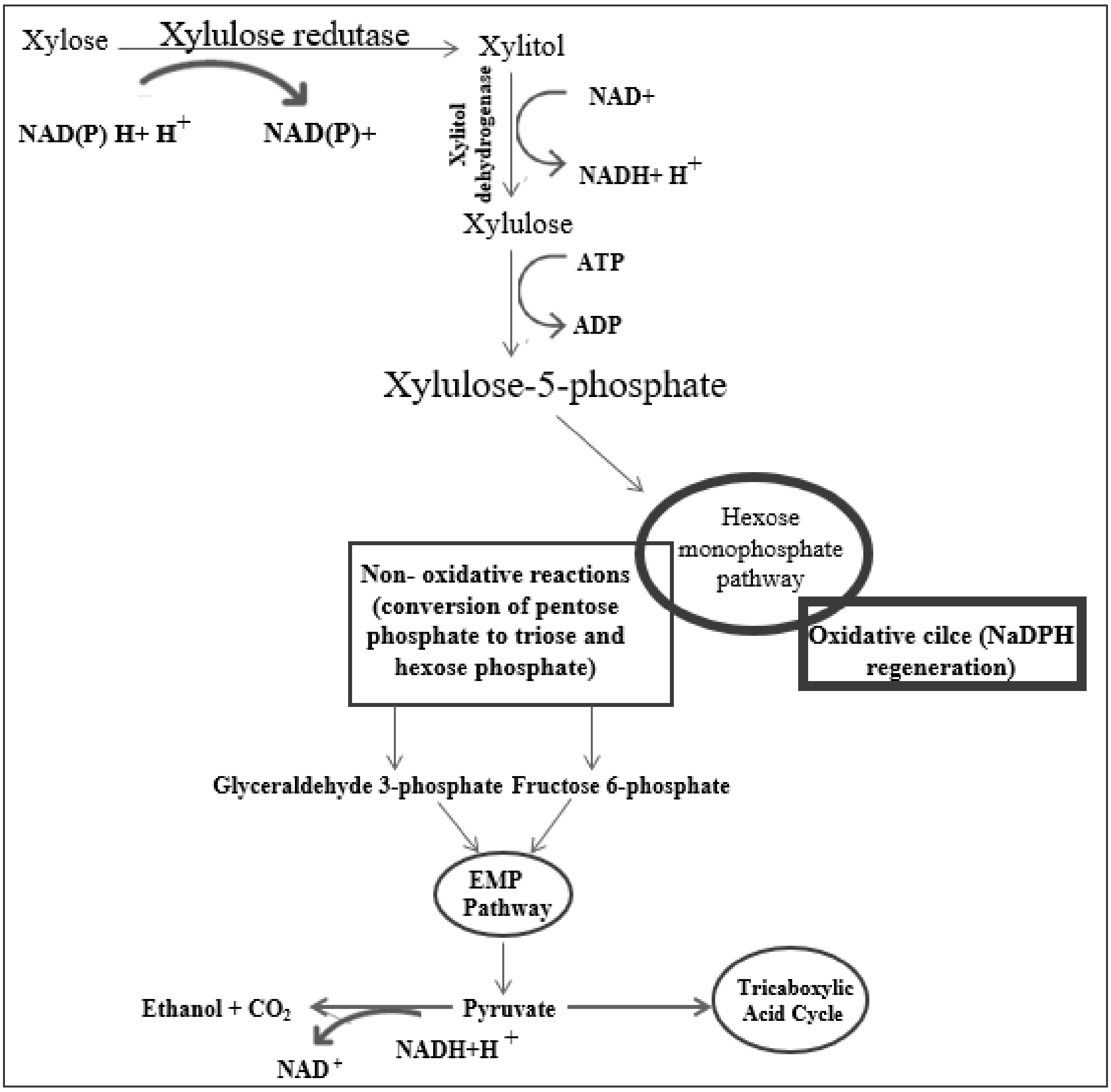

Figure 1.6. Metabolic pathway of pentose assimilation in yeast Adapted of Rodrigues et al. (2006).

The most common configuration is SHF, in which the lignocellulosic material undergoes pre-treatment and the resulting glucose-rich solution is then transferred to another tank where fermentation occurs. SSCF and SSF are process configurations in which enzymatic hydrolysis and fermentation occur simultaneously, usually in the same reactor. The difference between the two is that the SSCF process involves the reactor. The difference between the two is that the SSCF process involves the simultaneous co-fermentation of hexoses and pentoses, while the SSF process involves only glucose fermentation (Van Zyl et al., 2011).

The use of SSF/SSCF processes allows for rapid consumption of the sugars released from hydrolysis by microorganisms, minimizing the inhibition of cellulolytic enzymes by hydrolysis products (Van Zyl et al., 2011; Ask et al., 2012). In fact, the inhibition of enzymatic hydrolysis by cellobiose and glucose has been reported, and the conversion of glucose to ethanol in the same process prevents the accumulation of these sugars, favoring the enzymatic reaction. SSF/SSCF has advantages over separate hydrolysis and fermentation processes, including shorter processing time, minimized process complexities, and reduced capital investment (Chandel et al., 2014; Antunes et al., 2019). One disadvantage of these configurations is that the conditions for enzymatic hydrolysis and fermentation are generally the same, which may not be optimal for both processes. In the present project, an alternative is included in which interconnected column reactors allow for simultaneous conduction of the biological steps while maintaining the optimized conditions of each process (Téran Hilares et al., 2017).

Consolidated bioprocesses involve the simultaneous production of ethanol and enzymatic hydrolysis performed by microorganisms or microbial consortia, which are also capable of producing the enzymes required to break down cellulose molecules in the hydrolysis step. Therefore, these microorganisms can hydrolyze sugars and metabolize them, producing ethanol (Rodrigues et al., 2006). The complexity of this process and the difficulty in finding suitable microorganisms or microbial consortia are limitations of this approach (Lin et al., 2020).

1.2 Objectives

The general objective was to improve the viability of sugarcane bagasse biorefineries by employing innovative approaches to crucial process stages, including hydrodynamic cavitation-assisted oxidative processes as an alternative to biomass pre-treatment, and both sequential and simultaneous hydrolysis and fermentation systems.

Following, the specific objectives:

- Evaluate and optimize pre-treatment conditions using ozone and peroxide oxidative process systems assisted by hydrodynamic cavitation at pH 7.0, and optimize ozone input and peroxide concentration.
- Evaluate and optimize pre-treatment at different pH levels (4-7) with an oxidation process assisted by hydrodynamic cavitation, incorporating the action of ozone.
- Evaluate the oxidative pre-treatment, assisted by hydrodynamic cavitation, in an acidic environment with the presence of iron sulfate catalyst and ozone.
- Evaluate the potential of hydrodynamic cavitation as a strategy to improve the enzymatic hydrolysis of sugarcane bagasse pretreated by hydrodynamic cavitation-assisted process.
- Determine the conditions for high production of ethanol and xylitol in sequential fermentations, with *Saccharomyces cerevisiae* IR2 producing ethanol in a medium based on the hydrolysate of hydrodynamic cavitation pre-treated sugarcane bagasse carbohydrate fractions, and *Candida tropicalis* UFMGBX12 producing xylitol from xylose present in vinasse obtained after removal of ethanol by distillation.

- Evaluate the SSCF process and fed-batch SSCF system for ethanol production from hydrodynamic cavitation pre-treated material using interconnected column reactors and *Scheffersomyces parashehatae* UFMG - HM 52.2.

1.3 Thesis overview

In this work, new possibilities were discussed for using hydrodynamic cavitation in different stages of biorefineries, as well as new ways to produce ethanol and xylitol from sugarcane bagasse. This thesis focused on key bioprocessing steps, including pretreatment with oxidants, enzymatic hydrolysis, and new fermentation strategies. In addition to this first introductory chapter, which provides an overview of the entire work, this thesis is divided into six other chapters.

Chapter II provides a detailed discussion of the history and main uses of cavitation, while Chapter III provides a review about different pretreatments using HC and oxidants in recent years.

In Chapter IV, a new alternative was studied involving HC-assisted process using oxidants agents to pretreat sugarcane bagasse at a neutral pH, followed by a sequential fermentation process for ethanol production with *S. cerevisae* and xylitol production from the vinasse of the first fermentation using *C. tropicalis*. Chapter V reported the study of HC-assisted pretreatment in acid medium with oxidant agents, besides the addition of iron sulfate in optimized conditions. Also, ethanol was produced in an SSCF fermentation with *S. parahetahae* in two different ways: batch SSCF and fed-batch SSCF.

Chapter VI reported the HC potential to assist the enzymatic hydrolysis step, showing the process resulted in increase in hydrolysis efficiency without great decreasing in the enzyme activity. This process was compared with the UC-assisted hydrolysis system.

Finally, Chapter VII covers the future works and general conclusions of this thesis.

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CHAPTER II

Recent Advancements and Prospects of Using Cavitation-assisted Pretreatment of Lignocellulosic Biomass for Production of Biofuels

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ABSTRACT

Last decades, the cavitation phenomenon has been evaluated for different industrial applications. More recently, cavitation has been used for biomass pretreatment, a fundamental step in biorefineries, required to modify the lignocellulosic biomass structure and composition, favoring subsequent enzymatic hydrolysis of the present carbohydrates. The use of cavitation for this application has been resulting in advantages as high efficiency and low process time. However, to be used in biorefineries, an adequate addressing of cavitation-assisted pretreatment strategies is fundamental, aiming to profitable yield and productivity of the processes at industrial scale. In the developing biorefineries, different alternatives of cavitation pretreatments and operation modes have been considered and systematically evaluated, mainly taking into account particularities as time of pretreatment, temperature and solid:liquid ratio. Ultrasonic and hydrodynamic cavitation systems have been used for the pretreatment of lignocellulosic biomass under different conditions. Ultrasound has been more studied, and a number of different lignocellulosic materials have been pretreated under diverse ultrasound frequencies combined with other pretreatment technologies. Hydrodynamic cavitation studies for this application are more recent, presenting advantages as easier scale up. In hydrodynamic cavitation studies, orifice plate-based reactors are the most commonly reported in the literature. Considering cavitation-assisted pretreatment is a promising alternative for biorefineries, this chapter discusses the main advances in this research area. The main cavitation reactors and the different approaches are also discussed, including the authors' investigations presenting specific advances for hydrodynamic cavitation pretreatment. An overview of different approaches for cavitation pretreatments was presented, focusing on current techniques, advancements, and future perspectives.

Keywords: Hydrodynamic Cavitation; Ultrasound cavitation, Optical cavitation, Biomass pretreatment, biorefinery

2.1 Introduction

Lignocellulosic biomass is considered an abundant and cheap renewable source available to produce biofuels and other interesting compounds. It is world production ranges up to 1.8 x10^{11} tons per year (Haq et al., 2021), representing a rich reservoir of carbon and energy (Elumalai et al., 2018). Lignocellulosic materials include agricultural and forest residues and by-products (E.g. sugarcane bagasse and straw, corn cob, rice straw, wheat straw, etc.) and are widely available and still underutilized raw materials in obtaining compounds of interest. In Brazil, for example, sugarcane bagasse corresponds to an abundant material obtained after the extraction of sugarcane juice in sugarcane plants. Although most of this is used in the industries themselves for the production of steam and electricity, there is a surplus whose search for alternative uses is highly desirable (Dias et al., 2009; Dantas et al., 2013; Gonçalves et al., 2015).

The use of biomass has been considered in the context of biorefineries, which are industrial processing plants that, by using biomass as an input, have its processes and equipment highly integrated, producing a range of products with higher added value, such as fuels, energy and chemicals. Research efforts to make biorefineries viable are motivated by their inherent association with sustainability. Actually, based on the current search for the transition of the economy and culture from a system based on fossil fuels and carbon sources to a sustainable bioeconomy, biorefineries are in the context of policies and efforts of society to minimize effluents, use of renewable sources, reduction of greenhouse gas (GHG) emissions and other pollutants, among other environmental aspects always linked to the search for economic viability and social responsibility (Hendriks & Zeeman et al., 2009, Eskander, & Fankhauser, 2020).

In the developing lignocellulosic biorefineries, three main initial stages have been used, including pretreatment, enzymatic hydrolysis, and fermentation for the production of biofuel and chemicals (Clark, & Deswarte 2015, Ferreira et al., 2017; Yamakawa et al., 2018). Biofuels have corresponded to the main products of currently studied biorefineries, considering the global necessity of changing the current petroleum-based fuels by renewable options, due to environmental concerns as GHG emissions (Baer et al., 2000; Herzog et al., 2000; Montzka, Dlugokencky, & Butler, 2011). Lignocellulosic biomass is, for example, a source of sugars which can be converted in ethanol, in this case, called "second generation ethanol" (Dias et al., 2011; Aditiya et al., 2016; Santos et al., 2016).

The great interest in lignocellulosic biomass is due to its composition, which includes cellulose (30 - 60%), hemicellulose (20 - 40%), and lignin (15 - 35%) (Dahadha et al., 2017; Preethi et al., 2021). Holocellulose (cellulose + hemicellulose) of the lignocellulosic biomass can be converted into fermentable sugars such as glucose and xylose by the action of several enzymes (Nawaz et al., 2022; Estrella-Gonzales et al., 2019;). However, due to the high crystallinity of the cellulose and hemicellulose-lignin matrix coating, this raw material is recognized as a highly recalcitrant (Mankar et al., 2021; Bhatia et al., 2021; Baruah et al., 2021).

Many methods have been developed to fragment the lignocellulosic material into simple compounds. Currently, studies have been directed to an enzymatic hydrolysis of cellulose to obtain a glucose-enriched hydrolysate (Hendriks, & Zeeman et al., 2009; Xiros & Olsson et al., 2014). In this step, C5 sugars as xylose can be also obtained (Hanchar et al., 2007), as the commercially available cellulases preparation also have other enzymes, such as xylanases.

To directly proceed to enzymatic hydrolysis of carbohydrates from biomass is not feasible, considering the recalcitrance of the material impairs the enzyme access to the substrate. Thus, developing biorefineries have included a previous step, called pretreatment, which modifies the biomass structure, increasing the enzymatic hydrolysis yield (Hendriks & Zeeman et al., 2009; Kumar et al., 2020; Prado et al., 2022).

Pretreatments are commonly classified into four large groups, physical/mechanical, chemical, physicochemical, and biological (Kumar et al., 2020; Shirkavand, et. al., 2016 and Mankar et al., 2021), as shown in Figure 2.1. Indeed, a number of different pretreatment approaches have been investigated, such as steam explosion, ammonia fiber explosion supercritical CO_2, liquid hot water, acid hydrolysis, alkaline hydrolysis, oxidative delignification, steam explosion, organo solv, ionic liquids, and biological (ex. using fungus) (Zheng et al., 2014; Arenas Cárdenas et al., 2017)

Physical/mechanical pretreatments are pointed as expensive, and commonly used as a supporting approach in lignocellulosic biomass particle reduction, altering the crystallinity of the cellulose and increasing the surface area (Park et al., 2010; Kumari & Singh, 2018). They include equipment for grinding, milling or chipping the material, usually requiring high quantity of energy to be effective.

Chemical pretreatments are used with different reagents, as acid and alkaline compounds, organic solvents, and ionic liquids (Norrrahim et. al., 2021; Oke et. al., 2016). They are reported as efficient methods, due to high hydrolysis yield, however presenting disadvantages, including low efficiency and high cost (Hou et al., 2017; Xu et al., 2020).

On the other hand, physicochemical pretreatments have been reported as highly efficient to increase the enzymatic digestibility of the biomass (Li et al., 2013; Jacquet et al., 2012), by many alternatives such as steam explosion, ammonia fiber explosion and ultrasound or hydrodynamic cavitation, each one with their own advantages and disadvantages (Brodeur et al., 2011).

Biological pretreatments, as those ones using wood degrading fungi, are reported as interesting alternatives, but have important drawbacks as the long time required in the process besides low yields (Shirkavand, et al., 2016).

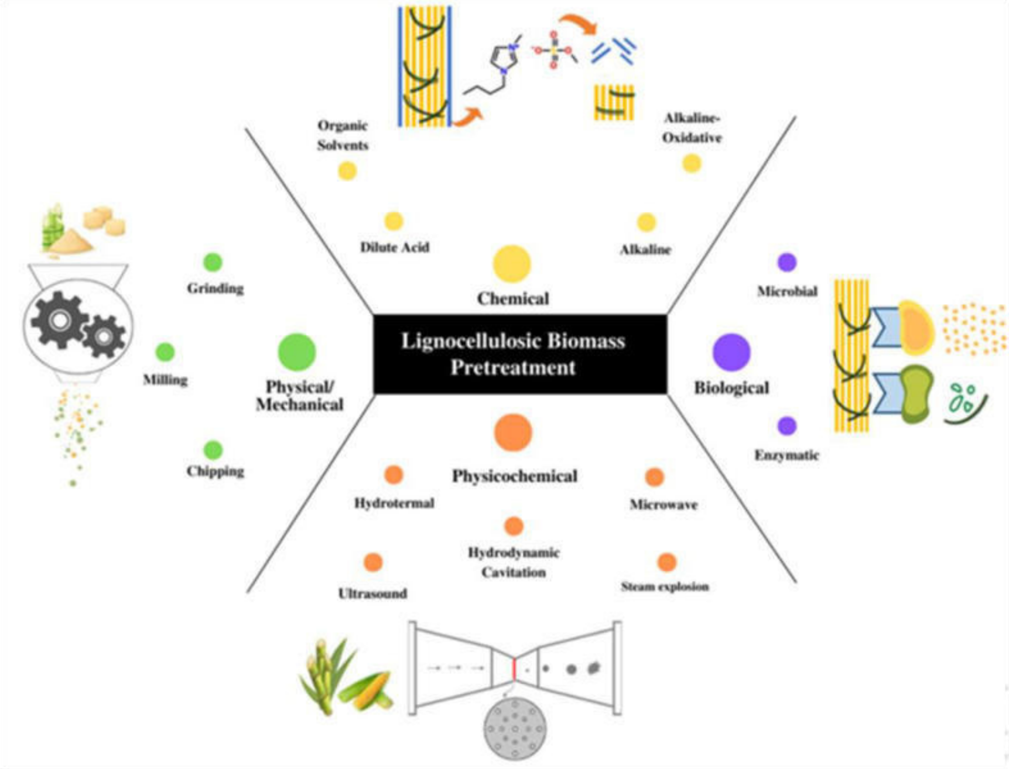

Figure 2.1. Classification of lignocellulosic biomass pretreatments (adapted from Shirkavand, et al. (2016), and Mankar et al. (2021))

Actually, although with many reported options, pretreatments for lignocellulosic biomass still outstanding as one of the significant bottlenecks for biorefineries viability. As stated by Hernández-Beltrán et al. (2019), the main limitations of the pretreatments are the energy and cost, operation, inhibitory compounds formation, as well as process yield, which directly affect the production of new molecules. Thus, the known available options still face difficulties for current biorefineries to achieve energy balance and economic viability (Vu et al., 2020; Venturini et. al., 2020; Lopes et. al., 2022).

As a new alternative, in the last years, cavitation has been proposed as an emerging physicochemical pretreatment. Ultrasound cavitation (UC) has been applied to different biomasses, resulting in high efficiency in the subsequent enzymatic hydrolysis step (Bussemaker et al., 2013, Perrone et al., 2016). The advantages of this technique include low process times, the use of mild conditions, and the possibility of higher performance combining with different options of pretreatment (Gogate et al., 2006; Prado et al., 2022; Bimestre et al., 2022). However, there are some drawbacks in UC-assisted pretreatment, mainly related to the difficulties of scale up (Mason et al., 2014; Mahamuni, & Adewuyi, 2010; Pandit et al., 1996) that still need to be overtaking. Thus, more recently, hydrodynamic cavitation (HC)- assisted pretreatment has been studied, with energy efficiency higher than the obtained with UC techniques, and possibility of scale up (Malani et al., 2021; Bimestre et al., 2022).

Thus, this chapter includes an explanation of the cavitation phenomenon and its relevance as an emerging pretreatment of biomass. Firstly, a brief historical overview about the development of knowledge about cavitation is presented, followed by showing some general aspects of this phenomenon. Cavitation systems are shown as promising alternatives for addressing the limitations in the pretreatment step in biorefineries. Hydrodynamic cavitation and ultrasonic cavitation are described, highlighting the main advances in the application of these technologies in different uses reported in the last years, besides discussing some future perspectives about this theme.

2.2 Phenomenon of cavitation

2.2.1 Brief historical overview about knowledge about cavitation phenomenon

Cavitation is a phenomenon in which occurs the formation, growth, and implosion of vapor bubbles in a liquid (Shah et al., 1999; Bai et al., 2020). Regarding to development of research work, Figure 2.2 shows a brief historical timeline from the first observations of the cavitation phenomenon until the present day, as well as its applications.

In the 1754, Euler, in Berlin, observed by the first time that this phenomenon occurs in water wheels, modifying their performance (Dmitry, Gerasibrov, and Yurin, 2022). The effects of cavity within a fluid were also considered in 1859 by Besant. Subsequently, in 1873, a study was developed by Osborne Reynolds when investigating the over-speeding of propellers. The first recorded account of cavitation effects on a ship was in 1893 by Reynolds and Parsons, when verifying that this phenomenon occurs when performing speed tests on British warships. In this case, failures in the propulsion system were observed due to the appearance of severe damage to the propellers of the vessels. This damage was suggested as caused by bubble formation, vibration and high noise in the region of the propellers and water turbines (Carlton, 2018; Yusvika et al., 2020). In 1894 cavitation was observed as occurring in a circulating channel. Based on the various observations for that time, the phenomenon was later named cavitation by Froude in 1895 (Carlton, 2018; Leighton et al., 2012; Biryukov et al., 2022).

In 1917, the English physicist and mathematician Lord Rayleigh, in an attempt to explain the characteristic noise generated in tests on ship propellers, proposed a mathematical model to explain the collapse of cavities in liquids, achieving prediction of the generation of high temperatures and pressures at the event site during the collapse one that formed bubbles and studied the phenomenon with dissolved gases in the flow liquid (Suslick, 1990; Llewelyn et al., 2014).

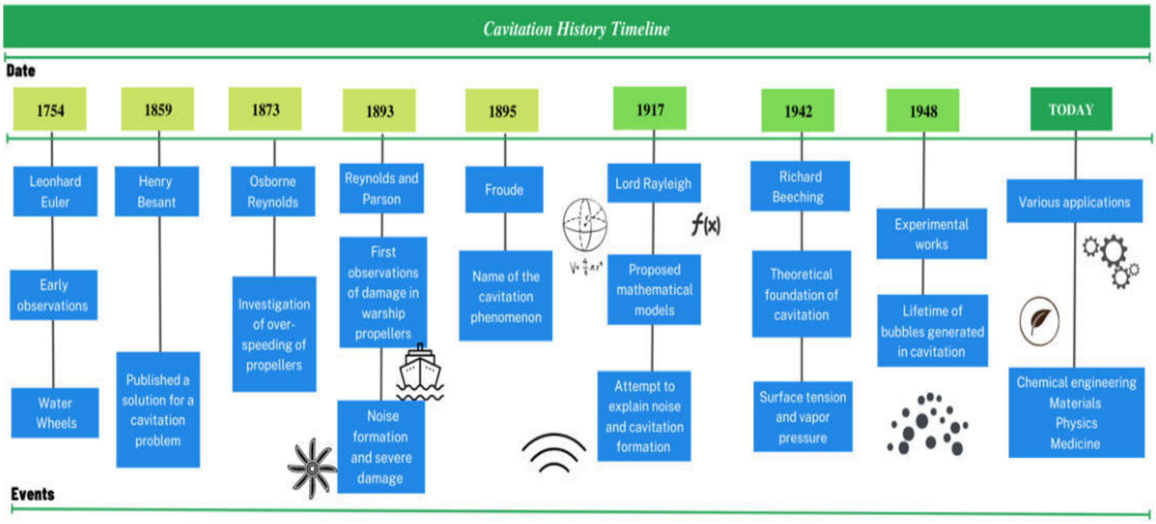

Figure 2.2. Brief historical overview of the advances in cavitation phenomenon studies (adapted from Llewelyn et al. (2014), and Carlton (2018))

Rayleigh called the puzzling phenomenon that causes wear on water turbines and ship propellers as common an incomprehensible spherical void formed suddenly and very quickly. Later, in 1942, the English researcher, physicist, and engineer Richard Beeching added to Rayleigh's studies the effects of surface tension and vapor pressure in the bubble in his theoretical studies. In 1948, some more experimental works were conducted to understand this phenomenon by using high-speed cinematography to propose the lifetime of a bubble formed by cavitation, which confirmed high radial velocities and acceleration subsequently after the formation and collapse of the bubble. The cavitation phenomenon was reported by Burriele in 1951 (Noltingk & Neppiras, 1950; Llewelyn et al., 2014).

Due to the potential application of cavitation, the number of different research has been increasing over the last three decades, mainly in the areas of chemical engineering, materials, biological and physics, as well as medicine. Currently, different cavitation strategies have been employed in the pretreatment of lignocellulosic biomasses, wastewater treatment, as well as in the food and biomedical industries (Kooiman et al., 2020; Prado et al., 2022; Wang et al., 2022; Askarniya et al., 2022).

2.2.2 Cavitation: general aspects

The cavitation phenomenon can be obtained in different ways. The literature reports different methods to produce cavitation, among sonic, hydrodynamic, laser-induced (optical), and particle cavitation (Gogate et al., 2006; Santos et. al., 2009). In all cases, the same phenomenon of pressure drop occurs, originating the “hot spots”, differentiating them by the way in which this phenomenon is originated in the medium (Bimestre et al., 2022; Hilares et al., 2019; Prado et. al., 2022)

Optical cavitation is produced by high-intensity laser light, with the photons breaking the liquid continuity. Particle cavitation is produced by a beam of elementary particles, for example, a beam of neutrons breaking up a liquid, as in the case of the bubble chamber (Dos Santos, 2009; Padilla Martinez et al., 2014). The both, optical and particle cavitation, can be studied for different applications. Laser-induced cavitation, for example, has been used for cleaning of surfaces, peening, and wastewater treatment (Song et al., 2004; Gu et al., 2020; Padilha-Martinez et al., 2014). However, for biomass pretreatment, the research work has been focused last years in sonic cavitation and hydrodynamic cavitation (Bimestre et al., 2022; Prado et. al., 2022).

Sonic cavitation, also called acoustic cavitation, is generated by the incidence of sound waves with high frequency (16 kHz to 10 MHz) in a liquid medium (Pinjari & Pandita, 2010; Capári et al., 2016).

In sonic cavitation, the formed microbubbles perform different movements, depending on the frequency and intensity of the applied sound waves, that may present stable oscillatory movements (periodic movement in equilibrium) for several cycles or even transient movements, grouping in a single phase the stages of formation and growth (Moholkar, Sable & Pandit, 2000; Capári et al., 2016; Liu et al., 2019). Regarding to cavitation in the systems, there are two main features of bubble dynamics to be considered: (1) the maximum bubble size and (2) the distance traveled by the bubble before the collapse, i.e., bubble life. Indeed, the maximum bubble size defines the cavitation intensity. Bubbles grow at low pressure or high temperature. Larger bubbles implode with a higher intensity and can cause greater effects on a substance than smaller bubbles. For example, a large number of imploding bubbles can alter the structure of different biomass (Madison et al., 2017; Terán Hilares et al., 2018; Bimestre et al., 2022).

A stable and inertial cavitation phenomenon can be generated. Stable cavitation is characterized by the formation and oscillation of bubbles around an equilibrium radius at low ultrasonic intensity, while the inertial cavitation is characterized by the emergence, expansion, and collapse of bubbles, releasing large amounts of energy, and occurring during a single acoustic compression cycle. Due to the high-energy release in inertial cavitation, this phenomenon is desirable for biomass pretreatment (Iskalieva et al., 2012; Sidana, A., & Yadav, 2021).

Furthermore, the effects of sonic cavitation are varied, from erosion, sonoluminescence and catalyst in chemical reactions, mainly promoted by the generation of high localized temperatures and pressure (up to 5000 ºC and 500 atm) during bubble collapse (Suslick et al., 1990; Wu et al., 2013). This effect of ultrasound inducing or accelerating chemical reactions is already well established, and being effectively reported in literature (Moholkar, Sable & Pandit, 2000; Iskalieva et al., 2012; Kim et al, 2015, Nakashima et al., 2016).

The physical (mechanical) effects promoted by the cavitation are important, and include high shock waves capable of breaking crystallinity structures and promoting the appearance of microholes, with a generation of high levels of shear caused by the strong jets generated in the process (Saharan, Brave & Pandita, 2011; Sawant, et al. 2008). Chemical effects include the generation of some oxidant chemical species as the hydroxyl radical (Moholkar, Sable & Pandit, 2000).

The main limitation of sonic cavitation is the hard scale up from laboratory to the industrial scale, since the effects are limited to regions closely to the surface of the sonicator, being feasible in small operation volumes. In addition, there are other limitations such as the lack of energy dissipation uniformity under optimal conditions and the severe erosion (wear) of the sonicator surface, especially when operated at high power required in industrial scale reactors (Moholkar, Sable & Pandit, 2000). Furthermore, the hydrodynamic cavitation has been shown to be more efficient for large-scale operations than acoustic cavitation in various others chemical processes such as hydrolysis of fatty oils, polymerization and depolymerization of aqueous solutions (Gogate et al., 2006).

Hydrodynamic cavitation (HC), as well as other cavitation processes, is characterized by the formation, growth, and implosion of (microscopic) bubbles in a liquid medium, but, in this case, the process is generated by the passage of liquid through a specifically imposed region where the local pressure is reduced to values below the vapor pressure of the liquid, such as occurs in Venturi tubes and orifice plates. After this region, the pressure in increased again, resulting in the collapse of the bubbles, thus generating the so-called “hot spots”, promoting physical and chemical transformations. (Bradve, et al. 2014; Saharan, Brave & Pandita, 2011). Indeed, this phenomenon includes dissolved gases in the flowing liquid. High pressure shock waves are propagated from implosion centers, inducing cyclic stresses on solid surfaces, and exposed surfaces that can result in structural vibration, noise, and surface wear (Cioncolini et al., 2016; Vieira et al., 2016).

Hydrodynamic cavitation is more efficient than acoustic cavitation for many applications due to its capacity to oxide organic substances allied to low-cost operation, easier scalability, and high-power efficiency (Raut-Jadhav et al., 2016; Bimestre et al., 2022). In these hydrodynamic cavitation systems, however, concerns must be driven to the design of the pretreatment reactor to prevent clogging. In the work of Terán-Hilares et. al., (2017), e.g., the biomass is retained in a cavitation compartment and prevents system clogging.

Hydrodynamic cavitation is a phenomenon with a possibility for application in different areas. Applications reported in the literature include wastewater treatment. Indeed, the literature has shown hydrodynamic cavitation systems to accelerate the degradation of specific compounds such as commercial pesticide methomyl, 4-chloro-2-aminophenol for microbial disinfection of water (Badve et al., 2015; Barik and Gogate, 2016, Raut-Jadhav et al., 2016).

Other works used hydrodynamic cavitation for cells disruption and oil extraction from microalgae, besides intensification of biodiesel production (Maddikeri et al., 2014, Ghayal D, Pandit AB, Ratho, 2013, Chuah et al., 2016). This technology has also being used in more recent research for food application, as a work performed to obtain desirable properties in tomatoes juices (Terán Hilares et al., 2019b).

HC process was used by Save, Pandit, and Joshi (1994) in the process of disruption of cells, resulting in almost one order of magnitude more energy efficiency compared to established techniques such as ultrasonication and mixer-blender methods. In other work, Pandit and Joshi (1993) used the cavitation for improved oil extraction. In that work, the hydrolysis of castor oil and kendi oil was aided by Venturi reactor and an ultrasound system in order to evaluate the cavitation effect, obtaining better performance in oil extraction in the HC system. In 2014, Maddikeri, Gogate, and Pandit (2014) used hydrodynamic cavitation for the synthesis of biodiesel by the interesterification of waste cooking oil. Those authors used the Venturi tube reactor for this synthesis and the result showed a maximum yield of 90%.

Among the advantages of hydrodynamic cavitation, the mild operating conditions of temperature and pressure, short process times, and simplified construction system are features that outstand this technique as the pretreatment of biomass (Terán-Hilares et al., 2018).

2.3 Pretreatment assisted by cavitation systems

The development of sustainable and economical technologies for the pretreatment of biomass have been the focus of recent research. Following, some details of application of different cavitation systems for biomass pretreatment are presented and discussed.

2.3.1 Lignocellulosic biomass pretreatment using Ultrasonic Cavitation

Ultrasonic cavitation has been reported as an innovative biomass pretreatment technique. Compared to other pretreatment methods, UC has advantages such as a short time process and mild conditions. Indeed, acoustic cavitation pretreatment contributes to more exposed surfaces and increases the performance of enzymatic hydrolysis (Halow et al., 2006; Shchukin et al., 2011; Devadasu et al., 2020).

Under ultrasonic irradiation, the growth of bubbles in a liquid can occur through the different mechanisms: with high-intensity low-frequency ultrasound (20 kHz) cavitation nuclei may grow rapidly through inertia effects, while with low-intensity high-frequency ultrasound (> 50 kHz) a cavity may also grow, but slowly, in a process called rectified diffusion.

The cavities' surface area is slightly greater during expansion than compression. The cavity also can reach a critical size where it can no longer efficiently absorb energy from the sound field to sustain itself. Thus, this process can generate microbubbles and implode too. In this process, microbubbles had 10-100 μ m diameter when observed at the beginning of implosion. High pressure can modify the microbubble's structures; when the pressure is reduced, cavity clusters collapses (Shchukin et al., 2011).

In 2011, a UC system was used for lignocellulosic biomass pretreatment, with a report about the use of the steam explosion/CO_2 for corn stover pretreatment. These systems caused changes in the biomass structure that increased the enzymatic hydrolysis celluloses yield. This pretreatment increased 15% the hydrolysis yield in comparison with *in natura* biomass (Tian et al., 2011).

For sugarcane bagasse, one pretreatment with an ultrasonic bath was reported in 2016 using a alkaline an medium, resulting in a glucan hydrolysis yield of 94%, and the removal of 70% of hemicellulose (Liu et al., 2016). In the same year, Li et al. (2016) used UC for extracting cellulose nanocrystals from wood flour by a two-step process that comprised ethanol and peroxide solvothermal pretreatment, and an ultrasonic disintegration process. Indeed, it is common to find UC combined with alkalis or other chemicals to increase pretreatment performance. In that work, characterization results showed that 97% of the total lignin and 70% of the hemicellulose could be removed in a single ultrasound pretreatment step. Additional treatment with alkaline hydrogen peroxide removed the residual lignin and hemicellulose, and resulted in high-purity cellulose.

In another example, Perrone et al., (2016) used ultrasonic irradiation by using a frequency of 22 kHz and power of 50 W with ozonation (32 mg/ min) and alkali (0.1 mol/L) for delignification of 20 g of sugar cane bagasse during 65 min, verifying that this combined process resulted in high glucose enzymatic yield (95%) and 27 mg/g of lignin removal.

Xiong et al. (2017) used the Fenton process (peroxide and iron) combined with ultrasound cavitation for the pretreatment of rice straw. That study resulted in an efficient method for 70 % lignin removal from lignocellulosic biomass, enhancing the production of fermentable sugars in the enzymatic step in the optimized condition with 1.6 M of peroxide and 0.4 M of iron, during 3 h in 25 ºC with 22kW of ultrasound. Indeed, Bagheri et al. (2015) used an ultrasound cavitation system with metal-catalyzed (iron or TiO_2) and peroxide, for the pretreatment of sugarcane bagasse. In the optimal conditions experiment, the delignification of SCB achieved 78% with metal-catalyzed TiO_2, adding peroxide (only 1%), and using 60 min sonication time, at 75◦C. The ultrasound system used 50% wave amplitude and 70% ultrasound duty cycle. The pretreatment yielded 12.94 g/L, 14.86 g/L of xylose and glucose, respectively, after hydrolysis of pretreated sugarcane bagasse.

In a work of 2020, Devadasu et al. (2020) verified that the isolation of lignin from sawdust biomass using the ultrasound-assisted cavitation approach was 2 to 3 times more effective compared to a conventional delignification method.

There are also interesting studies about ultrasound cavitation-assisted pretreatment of biomass for biogas production. E.g., Capari et al. (2016) studied UC pretreatment of corn lignocellulosic material for biogas production. The authors compared ultrasound cavitation pretreatment with steam explosion methodology. The ultrasonic cavitation, in the optimized experiment, used a time of 10 min, ultrasonic power of 750W, ultrasound frequency of 20 kHz, and 25ºC of temperature. In comparison, the steam explosion did not use mild conditions, with a process performed at 150ºC temperature for 30 min. The results proved the efficiency of UC pretreatment, which, using mild conditions, resulted in 40 % more production in the anaerobic process.

In one more recent work, Tsai et al. (2018) pretreated corn stalks with a dual-frequency ultrasound technique for biogas production. The results showed that the cavitation of dual-frequency (57 kHz, 20kHz, at 100W) ultrasound was more intense than single frequency (20kHz- 100W), verifying that the biogas production was 59 % higher in the dual frequency system (Tsai et al., 2018). Additionally, Zhengben et al., (2018) studied the eucalyptus decomposition using the UC-alkali process. In that study, authors showed that UC system pretreatment with 0.1 mol/L of NaOH (single frequency 28 kHz-300W, during 1.5 hours) also presented more gas production in less temperature (308ºC) than thermal decomposition (400ºC).

In another recent work about biogas production, Zielinski et al. (2019) used ultrasonic cavitation in the pretreatment of wheat straw by a system with a power of 400 W, and a frequency of 24 kHz (54 min). The authors observed that UC pretreatment increased 25 % of the biogas aerobic production, by compared with steam explosion pretreatment (160ºC and 45 min).

Examples of literature work dealing with ultrasound cavitation pretreatment for different biomasses conducted in different devices are also presented in Table 3.1

An interesting innovation for UC biomass pretreatment includes a combination with laser cavitation, that can be optimized by the combination with peroxide (H_2O_2). For example, Al-Momani et al. (2022) developed a new method of pretreatment of residual algae biomass for the production of bioethanol and biopolymer through the application of UC laser-hydrogen peroxide-Fe-nanoparticle pretreatment (Mv-H_2O_2-Fe-Pt). The authors reported that the pretreatment yielded the release of 0.988 g of total sugars per gram of biomass, presenting a higher value compared to acid (0.58 g/g) and enzymatic (0.52 g/g) pretreatment.

An important drawback is the energy cost of ultrasound cavitation pretreatments is high, impairing the economic viability of the process. Thus, there are options of hydrodynamic cavitation reactors that can be an interesting alternative, as following discussed.

Table 3.1. Examples of literature work dealing with ultrasound cavitation pretreatment for different biomass

Biomass	**Pretreatment conditions**	**Main results**	**References**
Sugarcane bagasse	UC reactor with 0.4 mol/L of Na_2CO_3 and 0.6mol/L of H_2O_2, 4% solid/liquid ratio, 30ºC, 0.25mm of particle size, during 60 mim in UC with 50kHz	Maximal reducing sugars 275 g/Kg, 71.2 % of lignin removal	Nakashima et. al., 2016
	UC reactor with 1L, 20-50kHz, 100W during 30 min at 80ºC	53.17% of glucan hydrolysis yield	Yu et al., 2018
	5-HMF and the ultrasound system were used for pretreatment of sugarcane bagasse in a UC-reactor added with acid ionic liquid. The UC system used 120W power, 20 Hz-50 Hz frequency, woring at 140ºC, for 30 min	42.62 %, 28.63 %, and 19.01% of glucan, hemicellulose, and lignin in pretreated biomass, respectively; 65.72 % of glucan hydrolysis yield in the subsequent enzymatic step	Li et al., 2020
Grass clipping	Alkaline process using 0.75% of $Ca(OH)_2$ for total time of 30 min and ultrasound process with 25 kHz frequency and 60-650W in 75ºC	Maximal reducing sugars 414 mg/g; 23.0 % of lignin removal	Wang et al., 2017
Wheat straw	UC reactor with 1L, 20-50kHz, 100W during 30 min in 80ºC	48 % of glucan hydrolysis yield	Yu et al., 2018
	Ultrasonic bath (8L), 0.2 mol/L Na_2CO_3, 1mol/L H_2O_2, power of 150W, frequency 22 Hz-40 Hz, total time of 70 min	The composition of pretreated biomass was: 36.3 %, 31.4 %, and 9.2 % of glucan, hemicellulose, and lignin in pretreated biomass, respectively, with more than 87 % of lignin removal	Devadasu et al., 2020

2.3.2 Lignocellulosic biomass pretreatment using Hydrodynamic Cavitation

Hydrodynamic cavitation (HC) has emerged as one of the most potential technologies for lignocellulosic biomass pretreatment. In this way, the work of Kim et al., (2015) was a pioneer regarding to the use of this technology to increase the enzymatic digestibility of biomass in a biorefinery context. In that work, the authors also evaluated the use of hydrodynamic cavitation generated by one orifice plate, together with alkali (NaOH) for reed pretreatment in order to increase enzymatic accessibility in the next step. Since then, the topic has been studied for possible new pretreatment combinations, using different devices to generate cavitation, and different process conditions (Hilares et al., 2017; Hilares et. al., 2020; Nakashima et al., 2016).

It is interesting to note that, since the beginning of studies with hydrodynamic cavitation-assisted biomass pretreatment, it has constantly been used in many works together with alkalis to create a synergism between the chemical compound and the physical chemical phenomenon. This synergism allows for achieving more effective results when comparing the processes separately. For example, in the work of Nalawade, (2020), cavitation by itself was able to remove 9.1% of lignin removal, but, when using alkali-UC, a removal of 63.9% was observed. Besides alkalis, the use of other chemicals such as oxidizing agents has also been reported too.

It is possible to notice an increase in the number of works published on the subject from 2016. For example, Téran-Hilares et al. (2016), presented an optimized pretreatment condition of sugarcane bagasse by using Hydrodynamic cavitation generated by an orifice plate and NaOH, achieving 60.4 % lignin removal and 52.1 % glucan content in the biomass pretreated, as well as 97.2 % cellulose digestion after 48 hours of subsequent enzymatic hydrolysis. From this work, the authors stated that the used hydrodynamic cavitation-assisted pretreatment resulted in a low concentration of inhibitors (0.1 g/L of furfural, 0.39 g/L of acetic acid, and 0.06 g/L of hydroxy methyl furfural), an interesting result for subsequent microbiological steps. In the same year, Nakashima et al., (2016) also used a Venturi tube to create hydrodynamic cavitation for biomass pretreatment, comparing the method with the use of ultrasonic cavitation. Corn stover was the biomass and, in some experiments, cavitation was carried out together with oxidizing agent sodium percarbonate. According to the authors, when an oxidizing agent is not used, it is expected to obtain lignin in its macromolecular fraction; however, its use promotes an efficient removal of this macromolecule. Regarding the Venturi tube, they stated that the smallest of the evaluated diameters (1.4 mm and 1.8 mm) presented the best result for sugar release during enzymatic hydrolysis. Finally, the authors identified that hydrodynamic cavitation) presented better results when compared to ultrasonic cavitation in energetic terms (2.24 x 10^{-5} $g_{glucose}$ per Joule and 0.11 x 10^{-5} $g_{glucose}$ per Joule, respectively).

Patil et al., (2016) evaluated a third way to create the hydrodynamic cavitation phenomenon for biomass pretreatment, using a rotational system. The authors evaluated rotor speed, biomass loading, and pretreatment time for biogas production from wheat straw. Untreated biomass presented the worst results (31.8 mL of methane), followed by the one that was submitted to cavitation (77.9 mL) and, finally, the biomass submitted to cavitation together with oxidizing agent showed the best result (172.3 mL).

In 2017, Madison et al. (2017), performed studies with hydrodynamic and ultrasonic cavitation for the pretreatment of sugarcane bagasse. The authors also concluded the same as Nakashima et al., (2016) regarding the energy profile of the process, but stated that cavitation would not be interesting in a biorefinery context due to high energy cost. On the other hand, this statement was contested by other authors who observed that the conditions assessed by Madison et al., (2017) would not be optimized, and the economic analysis carried out did not consider the entire process, since biorefineries produce electricity from the burning of biomass (Prado et al., 2022).

In another work with sugarcane bagasse, Hilares et. al., (2017) performed a parametric study to determine optimal conditions of cavitation pretreatment generated by orifice plates. The authors also evaluated the inlet pressure in the cavitation zone, temperature, NaOH concentration, and the number of orifices in the plate. They determined that the optimal conditions were be 3 bar, 70ºC, 0.3 M of NaOH, and 16 orifices – reaching 93.05% and 94.45% of cellulose and hemicellulose hydrolysis yield, respectively. The authors stated that 20 minutes of a process provided the highest cellulose hydrolysis yield. Regarding the plate design, those with more holes leaded to worse results due to the increase in the cavitation number, which leaded to a less intense phenomenon.

In other investigation, Hilares et. al., (2017b) evaluated the use of different alkalis (NaOH, KOH, Na_2CO_3, and $Ca(OH)_2$ associated with hydrodynamic cavitation to pretreated sugarcane bagasse, also evaluating, the reuse of the black liquor in successive batches. The authors determined that NaOH presented the better results, and the reuse of black liquor in five times in successive pretreatments was possible without a loss in performance.

Indeed, by using the energy generated by cavitation to enhance the efficiency of chemical reagents, this pretreatment approach proves to be interesting and promisor, since it results in the removal of lignin, silica and hemicellulose (partially), besides the increase of cellulose digestibility due to the raise in porosity, crystallinity and surface area

(Bimestre et al., 2022). Allied with this, the reuse of remaining black liquor reduces chemical reagents requirements and effluent treatment costs.

In 2018, aimed to unravel the effect of other agents in the HC process applied to biomass pretreatment, Terán Hilares et. al., (2018) evaluated the use of hydrogen peroxide (0.78% v/v). Pretreatment was capable of achieving 95.4 % of cellulose hydrolysis – a result superior to that obtained by Hilares et. al., (2017), and in a shorter time. However, the use of such agents brings a concern since there is a need to neutralize the recovered biomass before sending it to the enzymatic hydrolysis step, which demands high amounts of water and can make the process more expensive and/or increase process time (Prado et al., 2022)

Thangacelu et. al., (2018) studied the use of hydrodynamic cavitation (orifice plate) associated with enzymatic treatment (laccase) to delignify and pretreat corn cob to produce ethanol. Enzyme loading (3-10 U per gram), solids in the reactor (2.5-5%), and reaction time (5-60 minutes) were studied. Optimized conditions were 6.5 U per gram of biomass, lowest solid content and the longest reaction time – reaching 47.4% of lignin removal. Researchers stated that a cavitation is an interesting approach because it presents a profitable energy balance and easy scale-up. However, a productive bottleneck would be the low solid content in the reactor, which still requires further studies.

So far, analyzing the articles published from 2015 to 2018, it is interesting to note the increase in interest in the subject and how the progression in knowledge has come in a sharp way. While in 2015 only one article on the subject was published, in 2018 it was already possible to see the use of cavitation with different biomasses, different devices for its generation, and its combination with other methods for improve your efficiency. Furthermore, it is also interesting to notice the optimization of process parameters, such as the NaOH content (0.48 M to 0.3M) and reaction time (44.48 minutes to 9.95 minutes) – thus making the process more and attractive.

Hilares et. al., (2019) analyzed the optimized condition from Terán-Hilares et. al., (2018) in new process configurations for HC pretreatment of sugarcane bagasse: sequential batches, semi-continuous and continuous systems. Results obtained for sequential batches were 42 g of glucose/ 100g of biomass, 32 to 35 g of glucose/ 100g of biomass for semi-continuous (7.5 minutes for average a residence time), and, for the continuous system, results reached 38 to 46 g of glucose/ 100g of biomass with 7.5 minutes as average residence time, and 32-38 g of glucose/ 100g of biomass with 3.75 minutes as average residence time. Results demonstrated the feasibility of semi-continuous and continuous systems for hydrodynamic cavitation biomass pretreatment. Certainly, semi-continuous continuous systems are interesting for industrial applications in biorefineries as they reduce dead time, energy and operational costs in general as well as in most cases leads to higher productivity (Bimestre et al., 2022, Prado et al., 2022).

In 2020, Hilares et. al., (2020) evaluated how the number of orifices and their diameters influence a continuous cavitation operation. Regarding the mechanical parameters, the authors corroborated the work of Hilares et. al., (2018) which stated that the higher the cavitation number, the less intense – therefore less effective – is the process. Regarding the operation mode, researchers reaffirm the plausibility and effectiveness of the continuous operation.

The advancements in studies in hydrodynamic cavitation-assisted pretreatment applied to biorefineries have been continued. In 2020, Bimestre et al., (2020) applied one mathematical modeling for hydrodynamic cavitation-assisted pretreatment optimization – a common practice approach in the field of process engineering. Those authors used a mathematical model to optimize the hydrodynamic cavitation generated by a Venturi tube in relation to pressure ratio, diameter and length of the throat zone. Based on the model, authors optimized the process conditions regarding NaOH loading, solid/liquid ratio and reaction time. In optimized conditions (4.90% of NaOH, 2.03% of solid/liquid ratio and 58.33 minutes of reaction time), 56.01 % of lignin removal was verified.

The innovative work of Prado et al., (2023) described a new hydrodynamic cavitation-assisted pretreatment associated with an advanced oxidative (ozone flowrate of 10 mg/min and 0.61% of hydrogen peroxide) aimed to obtain bioproducts in sequential fermentations. Pretreatment showed 83% and 78% of glucan and xylan hydrolysis yield, respectively. Subsequently, authors used the obtained hydrolysate to produce two products by sequential fermentations: ethanol was produced by *Saccharomyces cerevisiae*, and after distillation of this alcohol, xylitol was produced from vinasse by *Candida tropicalis*. Ethanol and xylitol yield of 0.41 g per gram and 0.55 g per gram were reached, respectively, verifying volumetric productivities of 8.33 g $L^{-1}h^{-1}$ and 0.64 g $L^{-1}h^{-1}$, respectively. This strategy outstands the process performed in mild conditions of pretreatment as well as the possibility to obtain high ethanol production and a high value-added product, i.e. xylitol.

Hydrodynamic cavitation has been currently evaluated for biomass pretreatment aiming at different bioproducts. For example, in the work carried out by Nalawade et. al., (2020), different pretreatments (alkaline, acid, sequential acid-alkaline, sequential alkaline-acid and cavitation with alkaline) were studied to obtain L-lactic acid from sugarcane bagasse. The cavitation phenomenon was generated using equipment based on Vortex, verifying best conditions with a result 20 % higher compared to other evaluated conditions.

In the 2019, in the study conducted by Zielinski et. al., (2019), the use of cavitation for biomass pretreatment of *Sida hermaphrodita* silage to generate biogas was assessed, considering a small facility. The facility was monitored for 330 days and the effects of hydrodynamic and ultrasonic cavitation on methane production were analyzed. Ultrasonic cavitation showed higher biogas production (460 L CH_4/ kg volatile solids) compared to hydrodynamic (430 L CH_4/ kg volatile solids); however, after the energy balance, hydrodynamic cavitation was more interesting since it produced 61 kWh/d, compared to 52 kWh/d for ultrasonic and 56 kWh/d for control. In this way, authors confirmed Nakashima et al., (2016) and Madison et al., (2017), that observed hydrodynamic cavitation presents a more interesting energy profile.

In 2022, Thangavelu et. al., (2022) studied the use of hydrodynamic cavitation (orifice plate) with lime to pretreat corn cob to produce biogas. Parameters such as lime and biomass loading, and reaction time, were analyzed. Under optimized condition (0.1 g g-1 of lime, 5% of biomass, and 60 minutes of reaction), 38.1% of lignin removal and 22.6% increase in cellulose content were obtained – pointing to the efficiency of the pretreatment. In other work, Nagarajan et. al., (2017) evaluated the use of hydrodynamic cavitation (generated by vortex) to increase biogas production from sugarcane bagasse. Authors determined that when using cavitation, a negative energy balance is changed to a positive one, as the production of biogas is increased by up to 113% - making the process advantageous and attractive for investment, corroborating the works of Patil et. al., (2016), Zielinski et. al., (2019) and Thangavelu et. al., (2022).

From the timeline of published scientific manuscripts about hydrodynamic cavitation between the years 2015 and 2023, it is noted how the subject has been attracting more and more remarkable attention from scientists. The Cavitation technique is starting to show encouraging results for biomass pretreatment, showing its versatility in the production of bioproducts (biofuel, biogas, and lactic acid, for example) and in the processing of different lignocellulosic biomasses. In addition to proving to be a plausible process to combine with other methods to improve its effectiveness, it can be easily scaled up.

2.3.2.1 Hydrodynamic cavitation reactors and the different devices used to generate the phenomenon

Different of other pretreatment methods, HC-assisted techniques are not well adapted to be studied in usual lab systems, as Erlenmeyer flasks. Thus, even in bench studies, the design and evaluation of different kinds of reactors are required, favoring future studies in pilot or industrial systems. Together with the evaluation of different reactors designs, studies of operation modes as batch, semicontinuous, and continuous have been performed (refs), showing the flexibility of the technology.

Hydrodynamic cavitation for biomass pretreatment has been generated using an orifice plate, rotation reactor, or Venturi tube (Makino et al., 1983; Chedeville et al., 2007; Wu et al., 2012). The adequate choice of hydrodynamic cavitation device is fundamental, allowing a controlled process, and providing optimal pretreatment conditions. Each reactor is designed and used to meet the required conditions through a different process. Among the main parameters, the cavitation device options show an important role since they are directly related to the cavitation phenomenon (Prado et al., 2022; Bimestre et al., 2022).

Reactors based on an orifice plate or Venturi tube are non-rotational hydrodynamic cavitation reactors (NRHCRs) and are usually composed of a feed tank, a pump, a cavitation zone and control/measurement instrument. Flow constrictors are the most used devices, showing advantages, e. g., cheap orifice plates and simple and robust equipment with Venturi tubes, which present low energy utilization, being capable to process high flow rates (Bimestre et al., 2022; Hilares et al., 2017).

Figure 2.3 shows a schematic representation of a non-rotational hydrodynamic cavitation reactor and its variations, designed through mechanical constrictions.

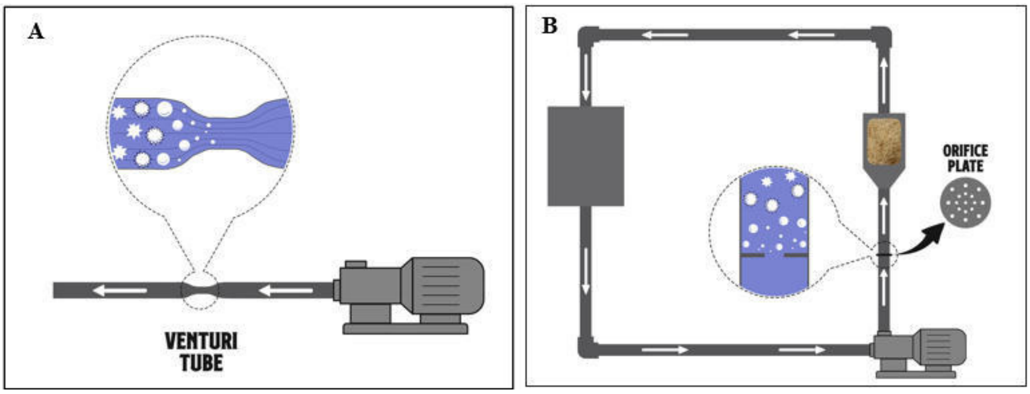

Figure 2.3. Schematic representation of non-rotational hydrodynamic cavitation reactors. A) Hydrodynamic Cavitation Venturi tube reactor B) Hydrodynamic cavitation orifice plate reactor

The dimension of the Venture tube is defined using Venturi theory. The calculation of a venturi tube consists of determining the ratio between the diameter of the central section and the diameter of the pipe, based on a desired differential pressure. Bernoulli's equation is used to describe the behavior of fluids in motion inside a tube; it is used to calculate the desired differential pressure which is evidenced in the hydrodynamic cavitation reactor (Al-Bahadly et al., 2011; Nakashima et al., 2016).

Orifice plate cavitation reactors, illustrated in Figure 2.3.B, have been the most common device used for different biomasses such as sugarcane bagasse, corn stove, reed, and straw rice, applied for further enzymatic saccharification and sugars obtaining

(Bimestre et al., 2020; Prado et al., 2022). This configuration is very simple e has been reported using different conditions of temperature, inlet pressure in the plates, number of holes in the plate, and using different chemical reagents (Gogate et al., 2006; Gogate and Pandit, 2005). Although simple batch is the most frequently applied operation mode, repeated batch, and continuous processes are also reported in the literature (Terán Hilares et.al., 2019; Hilares et al., 2020)

According to Gogate et al. (2006), if a liquid is flowing through an orifice, the reduction of the transversal section (change in diameter) of the flow increases the velocity, witnessing the pressure. During flow expansion, close to the orifice, the flowing fluid tends to fill the tube and, before reaching the inner part, it creates turbulences and vortices outside the narrowing of the fluid streamlines. Due to this turbulence and the head loss generated by the vortices, a permanent loss of pressure is unavoidable and full pressure regeneration will not occur. However, the static pressure in the fluid flow through the orifice is less than the inlet flow pressure in the system. For a certain velocity, the pressure may actually drop below the vapor pressure of the pumped liquid, causing part of the fluid to vaporize (Gogate and Pandit, 2005). In addition, with the reduction of pressure in the system, gases are released that are active even without being cavitated as a liquid. These gas bubbles also oscillate and then swell, and can generate radicals after passing through pulses of pressure and temperature (Gogate et al., 2006).

In the work of Gogate & Pandit (2005), different diameters of orifices were studied in orifice plate device in the hydrodynamic cavitation system, and the authors presented recommendations on their use, as following. The optimization of the constraint diameter used to generate cavities must be done according to the application. Larger diameters with a small number of cavities are recommended for applications that require non-intense cavitation, while smaller diameters with a larger number of holes must be used when improving the intensity of cavitation. The selection of the flow per orifice setting must be made in such a way as to control the desired cavitation intensity.

Advanced rotational hydrodynamic cavitation reactors (RHCRs, Figure 2.4) have been also shown as a highly effective device. RHCRs are usually composed of a front cover, rotor, side cover, and rear cover. To artificially induce HC, a number of cone cylinder-shaped orifices can be manufactured on the surfaces of the rotor, front cover, and rear cover in a single equally spaced radial row. The RHCRs systems usually include an electrical motor with a rotational speed controlled by a frequency inverter (Sun et al., 2021, Zheng et al., 2022).

Another type of reactor that can be found is with a high-speed homogenizer, which, according to Gogate et al. (2006), consists of a vane rotor and a stator, which are made of stainless steel. The vane wheel is driven by a variable voltage motor (the allowable limit for the homogenizer is 30V or 3.5A, resulting in a maximum speed of 16000 rpm). The distance between the rotor and the stator can be changed using different geometric configurations and this distance together with the operational parameters define the cavitation zone. Cavitation conditions are generated after the liquid passes through the rotor-stator assembly, according to the principle similar to the orifice plate assembly. As the rotor speed increases, the velocity generated by the liquid increases beyond a certain velocity, defined as the critical velocity of the onset of cavitation. Cavities are formed due to the local pressure drops below the vapor pressure of the medium.

RHCRs have been used for water decontamination processes. Sun et al., (2021) studied a rotational hydrodynamic cavitation system, which was used for water disinfection. The system was compared with thermo-system water disinfection, and the hydrodynamic cavitation reactor had 20 percent more efficiency. The RHCR achieved a disinfection rate of 100% for *Escherichia coli* with an expense of \$2.72/m^3 at the optimal condition (Sun et al., 2021). However, this reactor has not been reported in the literature for sugarcane bagasse pretreatment.

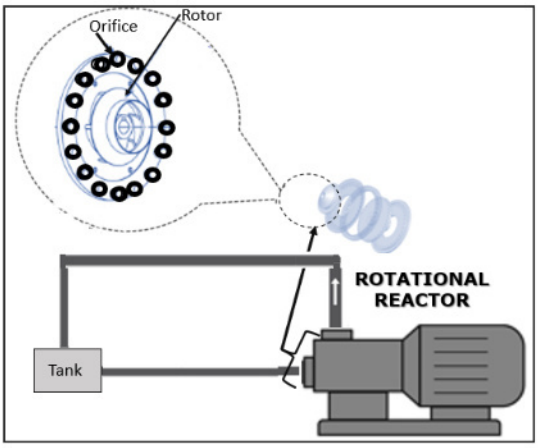

Figure 2.4. Schematic representation of rotational hydrodynamic cavitation reactor

2.4 Optical cavitation: laser system

Besides hydrodynamic and ultrasonic cavitation, there are a few research works using laser-induced cavitation.

Optical cavitation has been studied for different applications, as medical ones. It is discussed in literature, for example, with respect to cavitation erosion after the use laser system in one surgery, collateral damage in laser surgery, or the cavitation-mediated enhancement of pulsed laser in tissue (Zhong et al., 2020; Juhasz et al., 1996). For the last application, the real impact occurs when a laser had been generated, and consequence there is formation of liquid jets away from and towards the boundary with tissue (Zhong et al., 2020; Brujan et al., 2001). The damage interaction of a laser-induced cavitation bubble with an elastic boundary could depend on the distance between bubble and tissue.

For medical applications, the maximum liquid jet velocity could be 960 m s^{-1}. Such high-velocity jets penetrate the elastic boundary even through a water layer of 0.35 mm thickness, and this phenomenon must be controlled during the surgery (Brujan et al., 2001; Vogel et al., 2020).

The laser beam applied under a liquid induces changes in the properties of this fluid, which results in the formation of bubbles located close to the laser focus points. This type of energy deposition-induced cavitation has the advantage of being easily controllable and manageable compared to other cavitation methods. When the laser beam hits a fluid, the laser energy is absorbed by the electrons of the atoms present in the fluid, generating a plasma. This plasma reaches high temperatures (about 1700 °C) and causes the fluid to vaporize, which consequently increases the system pressure. This environment with high temperature and pressure can cause the pyrolysis of the solute present in the liquid, resulting in its degradation (Tong et al., 2023).

In some cases, as in the degradation of methylene blue, the increase in temperature due to laser emission causes pyrolysis of this compound. In addition, laser cavitation induces chemical and physical modifications of the fluid, such as high-speed water jets that stimulate a shear force capable of breaking the hydrogen bonds of water molecules, forming free radicals ·OH and ·H.

In the case of the application of laser cavitation for pretreatment of plant biomass, CO_2 laser-based was reported in the literature (Philipp et al., 1998; Tian et al., 2016). This laser located in the infrared region is considered one of the main highest-powered continuous waves (Tian et al., 2011; Tian et al., 2012). Also, another type of laser pretreatment can be performed in combination with hydrogen peroxide (AlMomani et al., 2022).

The wavelength (nm) and energy (mJ) of the laser, as well as the number of irradiations, are the most important parameters in laser cavitation. It is important to consider that starting wavelength of the laser beam since chemical compounds absorb different amounts of energy depending on wavelengths. Tong et al. (2023) proposed the degradation of methylene blue dye by laser cavitation using 532 nm wavelength.

In laser cavitation, both the bubble and the formed shock wave are directly influenced by the pulsed laser energy (uJ), i.e., the variation in energetic excitation that causes effects on the cavitation intensity (Agrez et al., 2023).

2.5. Conclusion, and Future Perspectives

The viability of biorefineries requires studies seeking for a maximum performance and reducing the costs of the main steps involved in the process. The main and most persistent bottleneck in biorefineries is the pretreatment step, and the development of innovative alternatives is highly desirable. The use of processes assisted by cavitation has potential, making it possible to obtain high yields in the enzymatic hydrolysis of the biomass pretreated using this technology.

In this chapter, the cavitation phenomenon and its relevance as an emerging pretreatment of biomass were presented. Hydrodynamic cavitation (HC) and ultrasonic cavitation (UC) were described, highlighting the main advances in the application of these technologies reported in the last years. HC and UC are promising technologies for the intensification of different pretreatments for lignocellulosic materials under mild conditions such as short process time, and low temperatures. Specifically, Hydrodynamic cavitation pretreatment results in a high percentage of increase in the enzymatic digestibility, with a higher energy efficiency and easier scale up compared to Ultrasonic Cavitation. Hydrodynamic Cavitation processes have been studied using different reactors such as those ones based on orifice plates, Venturi tube, and rotational cavitation.

Cavitation pretreatment associated with the use of other methods, as mild chemical pretreatment, has been shown to be more efficient in increasing the enzymatic digestibility of the material compared to the use of cavitation only.
Analyzing the published scientific manuscripts last years, it is interesting to remark the increase in interest on the subject and how the progression in knowledge had come in a sharp way. The process of the pretreatment assisted by cavitation has potential to contribute to the techno-economic viability of the process, eventually increasing the profitability of a biorefinery (Gevari et al., 2020; Gholami et al., 2021). However, in this sense, an evaluation of technological alternatives for the cavitation method is still necessary.

Real problems that are constantly observed in all biorefineries and that contribute to making the pretreatment process complicated include operational issues involving the mechanical section; in the case of sugarcane bagasse, the high-water absorption of the material results in a viscous suspension with fiber and silica (Bimestre et al., 2020). Application of hydrodynamic cavitation in combination with extruder continuous process, for example, for intensification of biomass pretreatment processes is a novel and potentially suitable approach for implementation in lignocellulosic biorefinery (Byun et al., 2020; Peeters et al., 2013). Developing new mechanical devices to assist in the biomass transport process can contribute to a more efficient pretreatment for biorefineries.

In the last years, other approaches, to be considered includes studies with more intensive use of modeling and simulation tools, which could indicate new alternatives, also giving the potential of the technique for obtaining high-added value products for biorefineries. The use of computational simulation and economic analysis tools allows the experimental results can be considered in relation to their effects on the biorefinery in a global way, enabling the analysis of its viability and guiding new experimental developments. Thus, future evaluation of cavitation-assisted pretreatments in techno-economic studies is required. In this way, studies in higher scale reactors are required, and the already shown potential of continuous operation mode should be considered.

Certainly, cavitation systems, particularly hydrodynamic cavitation systems, stand out in further studies for biomass pretreatment, presenting process feasibility and versatility applied to bioproducts manufacturing (biofuels, high-added value products, biogas, cellulosic pulp, and others).

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CHAPTER III

A review on recent developments in hydrodynamic cavitation and advanced oxidative processes for pretreatment of lignocellulosic materials

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ABSTRACT

Environmental problems due to utilization of fossil-derived materials for energy and chemical generation has prompted the use of renewable alternative sources, such as lignocellulose biomass (LB). Indeed, the production of biomolecules and biofuels from LB is among the most important current research topics aiming to development a sustainable bioeconomy. Yet, the industrial use of LB is limited by the recalcitrance of biomass, which impairs the hydrolysis of the carbohydrate fractions. Hydrodynamic cavitation (HC) and Advanced Oxidative Processes (AOPs) has been proposed as innovative pretreatment strategies aiming to reduce process time and chemical inputs. Therefore, the underlying mechanisms, procedural strategies, influence on biomass structure, and research gaps were critically discussed in this review. The performed discussion can contribute to future developments, giving a wide overview of the main involved aspects.

Keywords: Hydrodynamic cavitation, Advanced Oxidative Processes, biomass pretreatment, biorefinery

3.1 Introduction

Environmental concerns are currently experienced worldwide by clear signs of climate change such as melting of polar ice caps, natural disasters, fluctuations in rainfall frequencies as well as excessive greenhouse gases emissions (IPCC, 2021; Chaturvedi and Verma, 2013; Tsegaye et al., 2019; Dragone et al, 2020; Das et al., 2021).

Therefore, in the latest decades, concerns regarding economic, environmental, and energy security aspects have motivated the development of sustainable processes by using renewable sources. For example, the second-generation ethanol produced from lignocellulosic biomass, combined with other bioproducts, stands out as a promising environmentally friendly process (Bhatia et al., 2021; Kumar et al., 2020).

As a matter of fact, the use of plant-derived biomass is highlighted, as it represents around half of all world´s biomass availability. In this regard, approximately 13 billion tons of lignocellulosic biomass is generated annually. Lignocelulose biomass accounts for enable of the energy potential of biofuel and represents a rich source of sugars. Studies have been intensified for the use of this biomass along with bagasse for the production of cellulosic ethanol and others bioproducts such as xylitol and biosurfactants. Development of this technological path will allow for taking full advantage of biomass, increasing ethanol production without expanding the area cultivated. However, in order for this technology to be viable certain challenges must be overcome, including the establishment of appropriate conditions of pretreatment and hydrolysis of these materials for release of fermentable sugars. (Baruah et al., 2018; IPCC, 2021). Amongst, sugarcane bagasse, wheat straw, rice straw, corn cobs, fruit residues, and other agricultural and forest residues comprise important biomass for using as raw material in industrial processes (Philippini et al., 2020; Haldar, and Purkait, 2021). Yet, a few hurdles in their usage still remain to be solved in order for the process to become largely feasible in biorefineries. Indeed, lignocellulosic materials are composed by a recalcitrant structure which hinder enzymatic digestibility of carbohydrates in hydrolysis step, resulting in lower sugar yield in biorefineries (Gao et al., 2014; Du et al., 2020; Wang and Lee, 2021; Haldar & Purkait, 2021).

Currently, studies about the industrial use of carbohydrate fractions of lignocellulosics in biorefineries have been directed towards some fundamental initial steps: pretreatment, enzymatic hydrolysis and fermentation. Enzymatic hydrolysis of cellulose requires a previous pretreatment, usually chemical or physico-chemical, although some biological alternatives have been also evaluated (Chiaramonti et al., 2012; Baruah et al., 2018; Antunes et al., 2019). Pretreatments aim to disrupt or modify the biomass structure, facilitating the subsequent processes of saccharification and fermentation. Overall, pretreatments can be considered as the main limiting process stage, representing more than 30% of the total manufacturing cost (Vanneste et al., 2017; Haldar, and Purkait, 2021).

Taking this into account, a handful of well-developed pretreatments have been used as the basis for many processes in biorefineries (Hirani et al., 2018; Ho et al., 2019; Woiciechowski et al., 2020; Girón-Navarro et al., 2021). Among the most used pretreatment techniques, the alkali, dilute acid, and steam explosion are still costly and energy-intensive, in addition, to exhibit technical obstacles such as corrosion and formation of inhibitory compounds for the following biological stages of the process. The bottleneck in the biorefinery's production process is the pre-treatment and enzymatic hydrolysis stages, and the pre-treatment encounters barriers to increasing industrial scale and a low-cost process that can be carried out continuous (Lee et al., 2021; Mankar, Pandey and Pant, 2021).

To meet the market demand for more sustainable solutions, the incessant search to reduce costs and attempt the scale-up of production has allowed the development of new technologies, which could potentially solve many of the bottlenecks encountered in the biomass conversion techniques (high energy consumption, high cost, and low productivity). Within this context, alternatives such as the use of Advanced Oxidative Processes (AOP) and Hydrodynamic Cavitation (HC) have emerged (Kim et al., 2015; Andersen et al., 2019). AOPs are techniques in which radicals are used for degrading recalcitrant materials in mild conditions. Hence, they could also offer the possibility to lessen the cost of pretreatment and increase the digestibility of lignocellulosic biomass (Xie et al., 2017; Ho et al., 2019). HC is a technology under development that has been enhanced for the pretreatment of lignocellulosic biomass due to its advantages, such as lower processing time and chemical inputs, the versatility of operation modes, and its scaling-up potential (Kim et al., 2015; Nakashima et al., 2016; Mabee et al., 2021).

Thus, this review provides a survey on AOPs and HC as emerging tools in the pretreatment of plant-derived biomasses, presenting their fundamental concepts, recent advancements, limitations, and promising procedural strategies.

3.2.Lignocellulosic biomass

Lignocellulosic biomass is one of the most abundant raw materials in the world. Due to its carbohydrate-rich composition, these biomasses can be used in fermentative processes to obtain different bioproducts (Das et al., 2021; Sun et al., 2016; Ubando, Felix, and Chen, 2020). Lignocellulosic consist mainly of lignin, cellulose, and hemicellulose fractions, which are interconnected, constituting a recalcitrant structure. Lignin, for example, is present as a coat, overlapping hemicellulose and cellulose, representing a barrier to protect the biomass, avoiding its degradation by microbes or chemicals (Tsegaye et al., 2019; Das et al., 2021). Among present carbohydrates, cellulose is particularly hard to hydrolyze, considering the hydrogen bonds formed among its molecules and the presence of crystalline regions, besides the close association of this fraction with the other components of biomass (Wu et al., 2019).

Aiming to increase the yield of enzymatic hydrolysis step performed to obtain fermentable sugars, it is quite attractive to utilize pretreatment methods that combine high efficiency, low energy requirement, and less chemical inputs in order to enable their implementation in larger scales with none or minimal environmental impacts (Wyman et al., 2005; Agbor et al., 2011; Dragone et al., 2020).

Pretreatment methods can be classified according to their type and applied technology: chemical, physical, physicochemical, and biological, in addition to their combination (Silverstein et al., 2007; Agbor et al. 2011; Sun et al., 2016). Although chemical pretreatment methods can have economical and environmental drawbacks, they are most commonly reported in literature (Ubando, Felix and Chen, 2020). E.g. dilute acid pretreatment conditions had been studied at temperatures between 120 to 180°C (Alvira et al., 2010; Huang et al., 2019). Under the harsh conditions of pretreatment, biomass fractions could be converted into fermentative inhibitors such as furans. They are a family of polymers, which are used as raw material for chemical solvents, furans are formed as a result of the chemical processes used in the pre-treatment step. These furan-derived compounds (e.g., furfural, phenols and dicarboxylic furanic acids) have acts as inhibitors of microorganisms, damaging cell walls and membranes, inhibiting cell growth, reducing enzymatic activities and consequently the production of ethanol, these compounds (Zhang et al., 2010, Olea et al., 2012). On the other hand, alkaline pretreatments had been studied under a wide range of conditions, with temperatures ranging from ambient to 150°C (Woiciechowski et al., 2020). Moreover, physical methods such as extruder combined with chemical compounds have also been evaluated (Ubando, Felix, and Chen, 2020). Actually, many researchers are developing new alternatives for overcoming the chemical disadvantages such as the use of high temperatures, including advanced oxidative pretreatments. Subsequently, after pretreatment operations, the obtained solid material rich in cellulose can be submitted to the biological steps of enzymatic hydrolysis and fermentation (Travani et al., 2016). In hydrolysis, a broth rich in glucose (and, depending on the pretreatment option, other sugars as xylose) is produced, which can be transformed into ethanol by the action of specific yeasts, followed by the distillation of alcohol (Adsul et al., 2011; Gao et al., 2014).

The enzymatic hydrolysis of the cellulose fraction occurs through the action of cellulases. Cellulases comprise a complex set up of enzymes, mainly composed of three kinds of hydrolase enzymes with different specificities. Endo-1,4-β-D-glucanases randomly hydrolyze internal β-1,4-glucosidic bonds, while cellobiohydrolases I and II (CBH) convert cellulose into cellodextrins. Besides, 1,4-β-D-glucosidases hydrolyze cellobiose and cellodextrins to glucose (Jørgensen et al., 2007; Wyman, 2013; Bornscheuer et al., 2014). These enzymes act synergically (Leite et al., 2003), and can be aided by other ones, as LPMOs (Lytic polysaccharide monooxygenases) are a new class of microbial copper enzymes involved in the degradation of recalcitrant polysaccharides that they are a new solution in this step (Mussatto et al., 2019). Moreover, commercially available celluloses preparations include a number of enzymes to enhance the cellulose hydrolysis yield, acting in other biomass fractions remaining in the pretreated material, as xylanases, pectinases, and others (Hu, Arantes, and Sanddler, 2011; Motta et al., 2013).

Hemicellulosic fraction can be also converted into monosaccharides by acidic or enzymatic hydrolysis (Zhang et al., 2018; Yamakawa et al., 2020). Depending on the chosen pretreatment alternative and conditions, hemicellulose fraction will remain in the pretreated material, and thus can be hydrolyzed together with cellulose in enzymatic hydrolysis step. Raw materials as sugarcane bagasse or corn stover are rich in xylans, and xylanase enzymes, obtained from bacteria and fungi, can hydrolyze the β (-1,4)-D-xylopyranosyl bond, releasing xylose and xylooligosaccharides monomers (Knob; Carmona, 2010). Among the xylanases, depolymerization is rendered by endo-β-1,4-xylanases, which hydrolyze the xylan backbone oligosaccharide, β-xylosidases which hydrolyze short-chain xylooligosaccharides (Rao et al., 2006; dos Santos et al., 2011; Silva-Fernandes et al., 2017; Maffei et al., 2019). After the hydrolysis step, the sugars released from the polysaccharides will be used in the fermentation process to produce different bioproducts.

One of the main challenges of the hydrolysis step is to development efficient hydrolysis process by using low enzyme loading and high solid content in the process. However, high solid loading leads to high lignin content in the system, producing the unproductive adsorption of cellulases into its surface, impairing their action on cellulose. Indeed, lignin can hamper the accessibility of enzymes, reducing the hydrolysis yield and the generation of the final product (Adsul et al., 2011; Paudel et al., 2017; Das et al., 2021). Furthermore, other issues are the crystallinity degree of the cellulose (Converse et al., 1988; Jeya et al., 2012; Paudel et al., 2017; Das et al., 2021).

Alternative pretreatment methods have drawn researchers' attention, such as the AOPs (Advanced oxidation processes), that stand as an interesting and promising approach due to their potential in lignin removal as well as their eco-friendly status.

Another interesting alternative is the use of hydrodynamic cavitation (HC)-assisted processes. HC is a physicochemical process, and, among other effects in biomass, can generate oxidative chemical species (Yang et al., 2018; Terán-Hilares et al., 2019; Wu et al., 2019). Briefly, these types of pretreatment use hydroxyl radicals with high oxidative potential upon recalcitrant organic molecules structures, such as lignin. They can be boosted by UV light radiation and their application for the treatment of lignocellulosic materials showed promising results (Zhu et al., 2020; Teran-Hilares et al., 2020). Following, the oxidative pretreatments, including their general aspects, current challenges, and characteristics, are discussed aiming to address potential solutions for modern biorefineries. The oxidative processes have been included as an advantageous pre-treatment because it results in a pre-treated mass fraction with a low content of lignin and hemicellulose and, with mild operating conditions. Many studies have sought to optimize this technology (Chen et al., 2012; Mendes et al., 2014).

3.3 Types of the advanced oxidative process

Amongst the available pretreatments in the literature, AOPs are an interesting and promising option. The AOPs bring up the possibility of reducing the load of chemical reagents used in the process, hence, lessening the overall expenditures. In this case, the utilization of low-cost oxidizing agents such as ozone is beneficial, once these agents favor the formation of free radicals that remove lignin and increase the effectiveness of the pretreatment (Ravindran; Jaiswal, 2016; Rodriguez et al., 2017).

The AOPs enable the cleavage organic structures, transforming them into smaller molecules (Liu et al., 2019). It is based on an outstanding ability to generate hydroxyl radicals, which oxidize compounds and react 100 - 1000 fold faster than alternative oxidants such as O_3 (Polizzi, 2007). In these processes, there are several ways to generate the HO$^{\cdot}$ radical (Dutra et al., 2018; Davaritouchaee et al., 2019). Depending on the radical-forming conditions, these oxidative reagents tend to exhibit different oxidation potentials ranging from 1.36 to 2.80 V (Dutra et al., 2018; Davaritouchaee et al., 2019).

Advanced oxidative processes can be carried out by reagents such as H_2O_2 or O_3, and the efficiency of the processes can be increased with the use of metallic catalysts (Fe^{+2}, Cr^{+2}). Radicals can react with different organic structures (Girón-Navarro et al., 2021), having the necessary energy input to oxidize any recalcitrant organic component (Malato et al., 2002; Polizzi, 2007; Yang et al., 2018).

AOPs are classified in non-catalytic processes (e.g. ozonization and peroxide oxidation process); and catalytic systems (e.g. electrochemical process, Fenton, and photo-Fenton) (Krishnan et al., 2010; Xie et al., 2017; Zhao et al., 2021). Ozonization, electrochemical process, Fenton and UV light can be highlighted as potential methods for the emerging pretreatments of lignocellulosic material (Figure 3.1).

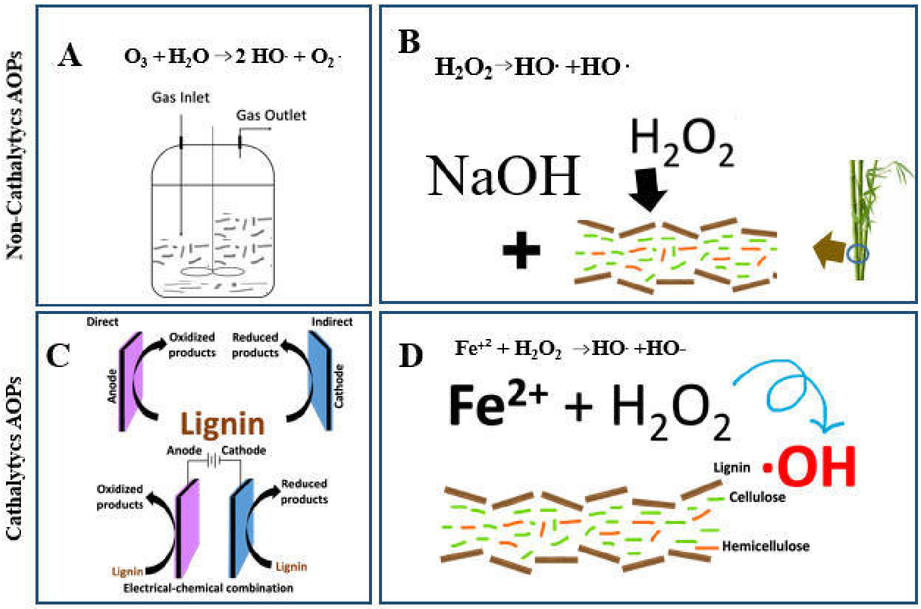

Figure 3.1. Different oxidative process types. (A) Advanced oxidative process with ozone, (B) Advanced oxidative process with peroxide and alkali, (C) Electrochemical process, (D) Fenton process.

3.3.1 Non-catalytically advanced oxidative process

3.3.1.1 Ozonolysis advanced oxidative process

An ozone decomposition reaction is complex and can be greatly influenced by experimental factors and the concentration of different species (Yang et al., 2018). The main responsible for the decomposition of ozone is the anion (HO^- or $HO\cdot$), which is generated in an aqueous medium. The anion reacts quickly with several organic compounds (Peixoto et al., 2018; Ho et al., 2019). Indeed, the reaction between ozone and the hydroxyl ion gives rise to the formation of the hydroperoxyl radical $HO_2^{\bullet}$, hydroperoxyl radical superoxide $O_2^{\bullet}$, and the hydroperoxyl radical in acid-base equilibrium.

Likewise, substances could be converted into superoxide radicals $O_2^{\bullet\,-}$ and/or $HO_2^{\bullet}$ which act as chain reaction promoters (Yang et al., 2018; Girón-Navarro et al. 2021). Organic molecules (R) can also act as promoters. Ozone has an oxidation potential of 2.07 V, which makes it suitable for plant cell wall degradation. Henceforth, this process has been studied for some decades to increase lignin removal from biomass and enzyme yield (Bhange et al., 2015; Ben'ko and Lunin, 2020). Lignin removal and ozone consumption are dependent on different parameters such as reactor design, moisture content, particle size, pH, reaction time, ozone/airflow, and ozone concentration (Travani et al., 2016).

Nevertheless, two parameters must be primarily considered for this process:

- Moisture content of the sample which directly affects the speed of the reaction between biomass and ozone. The moisture is the most important ozonization process parameter. It has an important role in aiding mass-transfer between the reactant species as well as enhancing the effectiveness of radical formation. Most researchers agree that the factor moisture could count with different interactions closely with the biomass, for example water could change interactions with ozone and with the substrate, eliciting the degradation reactions (Souza-Correa et al., 2013; Travani et al., 2016; Liu et al., 2019).

- Ozone concentration, due to its correlation with its consumption rate and the removal of lignin (Travani et al., 2013; Ben'ko & Lunin, 2020).

Pretreatment of different biomasses has been studied involving the ozonolysis process, such as Eurasian aspen, scots pine, wheat straw, rice straw, sugarcane bagasse, cotton stalks, corn stover, eucalyptus, among others. Thereof, It has been observed that ozonolysis increases the saccharification yield of biomass up to 80 % (Travani et al., 2013; Kádár et al., 2015; Perrone et al., 2016; Andersen et al., 2019). For example, Travani et al., (2013) worked with sugarcane bagasse in a fixed bed reactor and therein, different moisture levels were examined. The best result was achieved at 80% (w/w) moisture level which rendered a 46% of glucan hydrolysis yield in the saccharification step, whereas lignin removal was up to 60%. Lower the moisture content, more inhibitory compounds such as furans are formed and have been detected in the downstream liquor after the pretreatment. The action of ozone alters the structure of lignin, thus, several degradation byproducts are encountered within, including a wide range of aromatic and polyaromatic compounds. These compounds are further converted into carboxylic acids which are also identified in the pretreatment liquor (Schultz-Jensen et al., 2011; Travani et al., 2016).

In another work, in a multi-layer reactor, Kaldar et al (2015) studied the enzymatic hydrolysis of wheat straw pretreated with plasma-assisted ozone oxidation. In that research, the pretreated biomass had 49% of glucan hydrolysis yield. The pretreatment liquor was analyzed with ATR-FTIR and Scanning Electron Microscopy (SEM) which indicated the degradation of aromatic structures. The compounds presented in the pretreatment liquor were discriminated as 4-hydroxybenzoic acid, vanillin, vanillic acid, coumaric acid, ferulic acid and 2-methoxyacetophenone.

In a more recent report, Perone et al. (2016) have undertaken studies to develop alternative pretreatment of sugarcane bagasse (SCB) with ozone at a rate of 38 mg/min, supplemented with 0.1mol/L NaOH for 60 min. With the assistance of ultrasonic cavitation (16 - 22 kHh, 55W), this pretreatment resulted in 85% yield of glucan hydrolysis and more than 50% removal of lignin. Subsequently, FTIR analysis of lignin showed alterations in its aromatic structure which could be most likely associated with the ozone effects. The functional groups of lignin were highly altered. Indeed, these structures are potential targets for the ozone attack in the oxidative pretreatment.

The ozonolysis process is carried out under mild conditions, with ambient temperature and pressure, without producing saccharification and fermentation inhibitors. This oxidizing agent can be used with the addition of other chemical components in order to increase the efficiency of the pretreatment, such as the addition of peroxide. Xie et al. (2017) used wood biomass for ethanol production and compared the effects of oxidative and non-oxidative pretreatment approaches. The former used a combination of biological followed by an alkaline with peroxide pretreatment. The latter was conducted solely by the mediation of biological action. Maximal sugar yield was reached after the oxidative pretreatment, comprising a value of 348.8 mg of sugar/g dry biomass. Besides, lignin removal was 2-fold higher in the oxidative (42% lignin removal) strategy with respect to its counterpart (19% lignin removal). Subsequently, ethanol titer was about 40% higher with the oxidative-pretreated biomass-derived medium was applied in the fermentation.

Different authors confirmed the desestructuracion of lignin (Mottiar et al., 2016; Travani et al., 2016). In this alignment, the pretreatment liquor of coniferous wood after ozonation have been studied by Mottiar et al. (2016). In that study, structural analysis exhibited the occurrence of glyoxylic, oxalic, formic, and acetic acids as the main byproducts in this liquor. In fact, the structure of lignin was definitely modified with the action of ozone. The FTIR analysis showed that aromatic peaks decreased after the ozonation pretreatment, and the XRD (X-ray diffraction) analyses elucidated that crystallinity of its structure increased 5 % after the pretreatment.

3.3.1.2 Peroxide and UV light oxidative process

Hydrogen peroxide is a reagent used to promote advanced oxidative processes, mainly in alkaline conditions. It dissociates into the HOO^- ion in aqueous solution and is responsible for the oxidation of carbonyl and ethylene groups, in addition to being an initiator for the formation of other radicals such as hydroxyl ($^{\cdot}OH$) and superoxide anion ($^{-}O_2$) (Perroni et al., 2016; Ho et al. 2019; Lee et al., 2020). These radicals promote the oxidation of lignin and depolymerization of biomass (Kumar et al. 2020). The use of alkaline hydrogen peroxide (AHP) has been considered viable in the pretreatment of lignocellulosic material (Ho et al. 2019; Anderson et al., 2019). The main disadvantages of these processes are loads of peroxide, NaOH, and water (Bittencourt et al. 2019; Krishnan et al. 2010). The result of these reactions is the formation of radical products that react with oxygen (aqueous and aerated medium), starting a cascade of degradative reactions (Safarzadeh-Amiri; Bolton and Cater, 1997; Yang et al., 2018).

Bittencourt et al. (2019) studied AHP with 5 M of NaOH, 55ºC, 12 h, and H_2O_2 with a loading 0.3g-3.0g. The formation of chemically modified lignin may be observed by the ratio of insoluble lignin (IL)/soluble lignin (SL) in the pretreated SCB. It was evident once the ratio IL/SL increased roughly 3 % after AHP pretreatment. Moreover, Ayeni and Daramola (2017) showed that AHP removed 78% of the lignin from biomass in an oxidative pretreatment (with 1% v/v H_2O_2, 120°C, 0.14 MPa). In that study they used two alkalis (CaOH and NaOH with 0.1g/g at 0.2g/g biomass) and examined the performance of lignin removal. The results showed more than 4-fold increase in cellulose content in pretreated biomass. The FTIR (Fourier transform infrared spectroscopy) analysis showed that C=C band decreased after the pretreatment according to the presence of $HO^{\cdot}$ radicals and XRD analysis determined 6% increase of the biomass crystallinity.

3.3.2 Catalytic advanced oxidative process

The ozonolysis, photocatalysis, wet oxidation, electrochemical process, Fenton, and photo-Fenton reaction are just some of the vast catalytically oxidative processes. They have been used in the depolymerization of lignocellulosic biomass in bioenergy production. (Travani, et al., 2016; Den et al., 2018; Lee et al., 2020; M'Arimi et al., 2020).

3.3.2.1 Electrochemical process

The electrochemical process is a metal-mediated catalytic process that can contribute to the removal of the recalcitrant structures of lignin as well as aid the alteration of its aromatic structure (Du et al., 2020). Amongst several techniques, the anodic electro-oxidation, also known as direct electro-oxidation, using Ni, Pb/PbO_2, Ti/SnO_2, Sb_2O_3, RuO_2-IrO_2/Ti, Pt, Ni/NiOOH, and graphite as catalysts, are some of the most applied strategies for lignin removal (Panigrahi, and Dubey et al., 2019; Di Fidio et al., 2021). In short, biomass modification can be performed from the cathode, reducing lignin, or from an oxidoreduction reaction using an anode and a cathode (Zhu et al., 2014; Den et al., 2018).

Electrochemical pretreatments are considered to be low cost, reagent-free, and environmentally friendly, and can be carried out under hard or mild reaction conditions (Stiefel et al., 2016; Marino et al., 2016; Wijaya et al., 2020; Garedew et al., 2021). In this way electrochemical lignin conversion strategies, including electro-oxidation and hybrid electro-oxidation are efficient methods for lignin depolymerization and even for lignin fractionation, retrieving the biomass black liquor (Zhang et al., 2016; Chen et al., 2019; Du et al., 2020).

The use of this technique has not been reported for biomass pretreatment aiming at the retrieval of sugars for biorefineries. However, the literature supports the potential of electrochemical pretreatments for biorefineries, considering their effect on lignin. For instance, Stiefel et al. (2016) studied electro-oxidative cleavage of lignin-Kraft conducted in an ion-exchange membrane-assisted reactor separating anolyte and catholyte at high temperatures (170ºC). The reaction compartment was filled with sodium hydroxide solution. The electrodes used for the electron-oxidation were platinum and nickel with a surface area of 25 cm².

Analogously, Di Marino et al. (2018) have also worked with lignin-Kraft. Instead, their process used electrodes of graphite and nickel in a similar membrane-assisted reactor with a surface area of more than 21 cm²; this work evaluated different times (20-420min) of reaction. Both aforementioned studies found monomeric phenolic compounds such as vanillin and acetovanillone, carboxylic acid, and phenanthrene-carboxylic acid as byproducts of the delignification processes. Thus, the use of the examined techniques could be an interesting alternative for the simultaneous pretreatment of biomass and the generation of lignin-derived compounds.

In another report, Guy et al. (2016) also worked with lignin-kraft and used electron-oxidation with NaOH in membrane-assisted reactors. However, this study was performed under milder reaction conditions, such as weaker bases and lower temperatures (100°C). The authors observed formation of either unsubstituted or substituted methoxyphenols.

Thus, the potential of those techniques is seemingly high for biorefineries and more studies about this theme should be conducted in the future to understand more about this process (Marino et al., 2016; Wijaya et al., 2020; Garedew et al., 2021).

3.3.2.1.1 Fenton advanced oxidative process

Fenton is another type of catalytic AOP which combines hydrogen peroxide with iron ions (or other metals such as copper and titanium) (Yang et al., 2018). The presence of metal ions accelerates the production of free radicals such as the hydroxyl ($HO^{\bullet}$) (Rao et al., 2006; Kato et al., 2014). Furthermore, the Fenton reaction has been shown to have advantages, as it can be performed at moderate temperatures and can work with relatively high solids loading (Jung et al., 2015). Michalska et al. (2012) worked with rice straw and evaluated three different iron oxide options (30-75 mg), with the best result being 71 mg of iron oxide per gram of biomass (almost 30 % delignification). They used 1.0 mol/L of peroxide for 60 min and 1kg of biomass. More than 71 mg promoted metal coagulation.

Choosing an optimal concentration of iron and H_2O_2 in Fenton processes is important from a practical and economic viewpoint. Bhange et al. (2015), for example, worked with garden biomass using the Fenton process (500 ppm iron and 1000-10000 ppm peroxidase, 60ºC), investigating different times (60-180 min). The best result showed an increase in H_2O_2 concentration in Fenton's reagent significantly increased the rates of lignin degradation (20%), and the yield of hydrolysis glucan was more than 70%. The results also indicated that Fenton pretreatment increased the degradation of the lignin aromatic structure and enhanced sugars yield at 60ºC and 180 min.

In another work by Bagheri et al. (2015), the delignification of SCB achieved 78% with metal-catalyzed (iron or TiO_2) ultrasound-assisted peroxide at the optimal conditions of 1% H_2O_2, 4 g SCB load, 60 min sonication time, 2:100 M ratio of metal salt, under 75

°C. The ultrasound used 50% wave amplitude and 70% ultrasound duty cycle. The pretreated SCB yielded a titer of 12.94 g/L, 14.86 g/L of xylose, glucose, and arabinose, respectively. The pretreatment liquor presented the formation of carbonyl radicals, which increasing this due to the presence of TiO_2. The analysis of FTIR showed the decrease of the weaker band (C=O) after the pretreatment in the biomass. SEM demonstrated that crystallinity increased 20% more in the pretreated bagasse that confirmed the removal of hemicellulose and lignin.

Another work by Wu et al. (2019) conducted the pretreatment of rice straw. In that study 0.3 kg of biomass, 0.02 mol/L Fe^{+2} and 1.5 mol/L H_2O_2 reacted for 2 hours, ranging the pH 6-7. Under optimal pH (6.0) condition a 572 g/Kg of sugar yield was achieved after enzymatic hydrolysis step. The untreated and pretreated rice straws were submitted to SEM analysis which indicated that the former presented a regular and intact morphology with a smooth and compact surface, whereas the latter showed deformations within the surface. These results suggested that pH might induce the change in the lignin surface of pretreated rice straw, reducing non-specific interactions between lignin and cellulose. It was found that reduced pH decreased the negative charge on the lignin surface, decreasing its hydrophilicity and assisted the oxidative degradation, reducing the recalcitrance of the substrate.

3.3.2.1.2 Photo-Fenton advanced oxidative process

The presence of light radiation is another booster factor in the formation of radicals and, by combining hydrogen peroxide, solid semiconductors (e.g. ZnO, Fe_2O_3, TiO_2, ZnS) as catalysts, we have the so-called Photo-Fenton pretreatment (Giannakis et al., 2019; Melo, 2009). In this photocatalytic process, degradation reactions occur in the interface between the bulk solution and the solid. The advantage of this process is related to faster degradation of recalcitrant organic materials with a low input cost when compared to other already mentioned oxidative processes.

Actually, the photo-Fenton process has great potential for degradation of organic molecules. Exposito et al. (2018) evaluated the degradation process of the organic structure of carbamazepine. This process used 150 mL/L of peroxide with 2.5 mg/L of iron, pH 5.0, for 5 min with or without UV -light (50W, 220nm). The action of Photo-Fenton with UV light promoted 80% of degradation of this recalcitrant structure, compared to a biological traditional treatment which promoted only 33% of degradation.

Photo-Fenton has not been evaluated for pretreatment of lignocellulosic, but, considering the good results obtained for oxidation of organic molecules, there is a great potential to be used in biorefineries. This potential is also reinforced considering similar processes, which, although not using photo-Fenton, have been employing metal catalyst and UV for biomass pretreatment. E.g. the study by Yang et al. (2018) compared oxidative pretreatments with and without the presence of ultraviolet light for the pretreatment of sisal waste. Pretreatment was performed using UV light (500W, 200 nm), varying the peroxide (0.02-0.8 g/g biomass) and pH (8-12). The process was conducted for 6 h under 60ºC. Promising results were reported in pH 12 with 0.8g/g peroxide, regarding a glucan hydrolysis yield of 91.6% in the saccharification step. Markedly, the presence of light enabled an overall cost reduction of 20% and removal of 71% of the lignin. Besides, XRD analysis detected an increase in the degree of crystallinity and a significant modification in its structure after the UV light-assisted peroxide pretreatment.

In a more recent work, Zhu et al. (2020) evaluated removal of lignin in wheat straw. In that work, the authors studied the effects of NiO concentration in the presence of peroxide and with different wavelengths of UV light (350-780 nm). Overall, in that study, the wavelength of 780 nm removed 65.73% of lignin in the presence of 3 % of metal in the pretreatment. The UV light unsettle the intricate assembly of biomass and release the highly recalcitrant lignin. The pretreatment increased the wheat straw yield glucan hydrolysis by 86% after pretreatment.

3.3.3 Other combinations of advanced oxidative process for pretreatment of lignocellulose biomass

In the literature, there are more combinations with the different advanced oxidative processes that had been used for pretreatment by biomass. E.g. Yu et al. (2014) studied AOPs with the combination of ammonia and peroxide for the pretreatment of Miscanthus biomass. In this work, temperature (60-150°C), peroxide loading (1-5% H_2O_2), and reaction time (1-6 hours) were examined, using a fixed ammonia concentration at 30 % (m/v). The optimal conditions were found using 5% H_2O_2, under 130°C within 4 hours reaction time, whereby 89% of lignin was removed. Subsequently, at the enzymatic hydrolysis step, 90% and 73% of glucan and xylan were converted into monomeric sugars, respectively. The XRD of pretreated biomass indicated that this method had a more accentuated effect in the lignin and hemicellulose amorphous regions, whereas the degree of crystallinity considerably increased. In addition, the ATIR analysis showed a reduction in the aromatic band intensity, which indicates the removal of lignin-like structures.

In another approach, Xiong et al. (2017) studied Fenton-assisted ultrasonic cavitation (UC) for the pretreatment of rice straw. By applying a solution of 1.6 M of H_2O_2, 0.4 M Fe^{+2} under 25°C during 3 hours reaction, they examined the effect of different UC potency, ranging from 200-600W with a fixed frequency of 22 KHz. Under the optimized conditions obtained with 600W, lignin removal achieved 70%. The characterization showed that Fenton's reagent was more efficient in changing the specific surface area than ultrasound alone, this surface was with less mechanical structure than the surface of untreated biomass. Nevertheless, the combination of Fenton's reagent and UC resulted in an improvement of 93% of sugar yield after the enzymatic hydrolysis in comparison to both strategies separately conducted. Thus, the combined ultrasound cavitation and Fenton's reagent process was been proved a reliable and effective method.

3.4 Hydrodynamic Cavitation as a promising alternative

The development of new technologies for the pretreatment of lignocellulosic biomass is a current research challenge. In this way, hydrodynamic cavitation (HC) is another emerging alternative for addressing the limitations in the pretreatment step, which has as one of its main effects the generation of oxidative species in the medium, as following discussed.

3.4.1 Hydrodynamic Cavitation: main Concepts

Cavitation is a phenomenon in which vapor/gas bubbles are formed in bulk liquid, followed by their coalesce and collapse (Madison et al., 2017). Actually, cavitation is an extremely complex process dictated by several mechanisms stepwise. Indeed, a sequence of phenomena are observed, including nucleation, growth, and the collapse of bubble cavities. Likewise, heat and mass transfer occur simultaneously between the bulk liquid and the bubble-aggregates as well as cavitation noise that leads to sonoluminescence. All these features build up together a background of more than one century of empirical observations. Even so, comprehensive experimental studies are still conducted to investigate the dynamic behavior of cavitation bubbles (Nakashima et. al., 2016; Devadasu et. al., 2020).

Table 3.1 summarizes illustrative examples of alternative combinations of AOPs for biomass pretreatment.

Table 3.1. Another combinations of advanced oxidative process for biomass pretreatment

Pretreatment Condictions	Lignocellulosic Biomass	Main Results	Reference
Two processes: **1.** NH_4OH 30%, in different temperatures, 90-150ºC; 1:10 solid/liquid ratio for different times 1-6h, followed by a pretreatment with NH_4OH solution plus H_2O_2 in the same step (1-5% of H_2O_2) for 4h in 130ºC; **2.** NH_4OH and H_2O_2 in two-step separately; NH_4OH 30% for 2h at 130ºC, followed by H_2O_2 (1%-5%) for 2h 130ºC 1:6 solid/liquid.	Miscanthus	Glucan and xylan hydrolysis yield of 90% and 73%, respectively	Yu et al. (2014)
Alkaline process at 83 ºC, 2% (w/w) NaOH, with liquid-to-solid ratio of 13:1 (mL/g) for 6h, and then added with 3% (v/v) H_2O_2 at 50ºC for 2h.	Corn straw	Maximal reducing sugars 348.77mg/g of biomass; Lignin removal 50%	Wang et al.(2015)
Fenton-assisted UC 1.6 M of H_2O_2, 0.4 M Fe^{+2} 25˚C 3 h, UC potency, ranging from 200-600W with a fixed frequency of 22 KHz. Under the optimized conditions obtained with 600W.	Rice straw	Maximal reducing sugars 428.77mg/g of biomass; Lignin removal 70%	Xiong et al.(2017)
Two processes: Firstly NaOH 100mg/g biomass, 24h, 30ºC, 10% S/L, 25g biomass; Following Cu-AHP 1mM Cu, H_2O_2 50mg/g biomass, NaOH 10mg/g biomass, 14h, 120ºC, 25g biomass.	Zip-lignin	Glucan and xylan hydrolysis yield of 71% and 67%, respectively	Bhalla et al. (2018)
1% (w/v) NaOH, with liquid-to-solid ratio of 4:1 (mL/g), and then added with 4% (w/v) H_2O_2 for 55ºC for 6h	Grass waste	Glucan and xylan hydrolysis yield of 83.5%; Lignin removal 73%	Yan et al. (2020)

In this phenomenon occurs the formation of microbubbles due to fluid acceleration and the pressure drop below the vapor pressure. Lauterborn and Bolle (1975) measured this maximum jet velocity (120 m/s). High-pressure regimes produce small perforations or erosion on solid surfaces, because of the emission of shock waves and the formation of a high-speed micro-jet that is directed towards the solid boundary upon the collapse of the bubble. This erosion could help in the degradation of recalcitrant materials. This physical phenomenon, which tends to increase over time, causes the failure of rotors, hydraulic pumps, which makes cavitation an unwanted phenomenon in industrial processes, but beneficial when used in a targeted way as for pretreatment of biomass (Badave et al., 2014; Devadasu et al., 2020; Terán-Hilares et al., 2020).

The collapse of cavities or microbubbles generated in ultrasonic and hydrodynamic cavitation produces localized zones (“hot spots”) with transient temperatures around 10,000K and pressures of up to 1000 atm, resulting in physical-chemical amendments throughout the cavitation zone (Figure 3.2) (Saharan et al., 2011; Terán-Hilares et al., 2020). Under these conditions, water molecules are dissociated, generating highly oxidizing radicals ($HO^{\bullet}$, $^{\bullet}O_2$, $HOO^{\bullet}$) (Badve et al., 2014; Liu et al., 2016; Habashi et al., 2016; Terán-Hilares at al., 2019).

According to the mode of formation, cavitation can be classified into two categories: energy deposition-induced cavitation and pressure drop-induced cavitation (Badave et al., 2014; Devadasu et al., 2020).

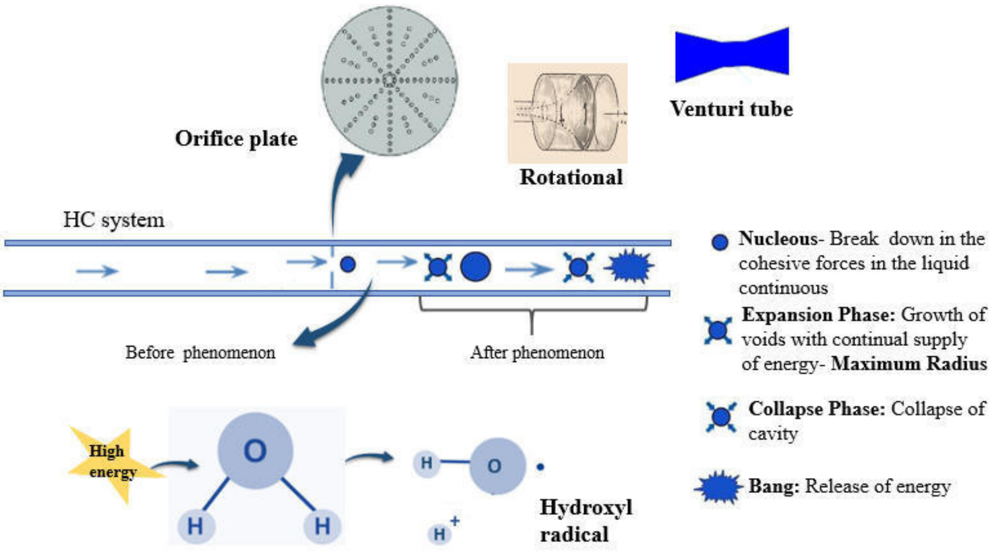

Figure 3.2. Collapse of the bubble in hydrodynamic cavitation process

Energy deposition-induced cavitation includes particle as laser-induced cavitation. This process induces the change of liquid properties and generates the cavities. Pressure drop-induced cavitation mainly consists of ultrasonic cavitation and hydraulic cavitation, whereby the former employs ultrasonic pressure load and the latter develops a special flow pattern. These processes cause changes in the liquid bulk pressure, leading to the development of cavitation (Kim et al., 2015; Liu et al., 2016). It has been reported that ultrasonic cavitation has a higher temperature and pressure of collapse than hydraulic cavitation. Although the HC process corresponds to the same phenomenon generated in the ultrasonic systems, the pressure drop in the fluid flow is mediated by devices such as orifice plate, Venturi pipe, and valves rather than the frequency of sound waves, (Kim et al., 2015; Terán-Hilares et al., 2019).

3.4.2 Hydrodynamic cavitation applied to lignocellulosic biomass pretreatment

HC effects can be useful for the process of biomass pretreatment. The generation of shock waves and microcurrents allow the degradation of nearby organic molecules, also disrupting the lignocellulosic fibers (Saharan et al., 2013; Devadasu et al., 2020). Furthermore, since it is conducted in mild conditions, such as short-time processes, the construction of a simplified system, depicted in Figure 3.3, enhances lignin removal from biomass (Terán-Hilares et al., 2017).

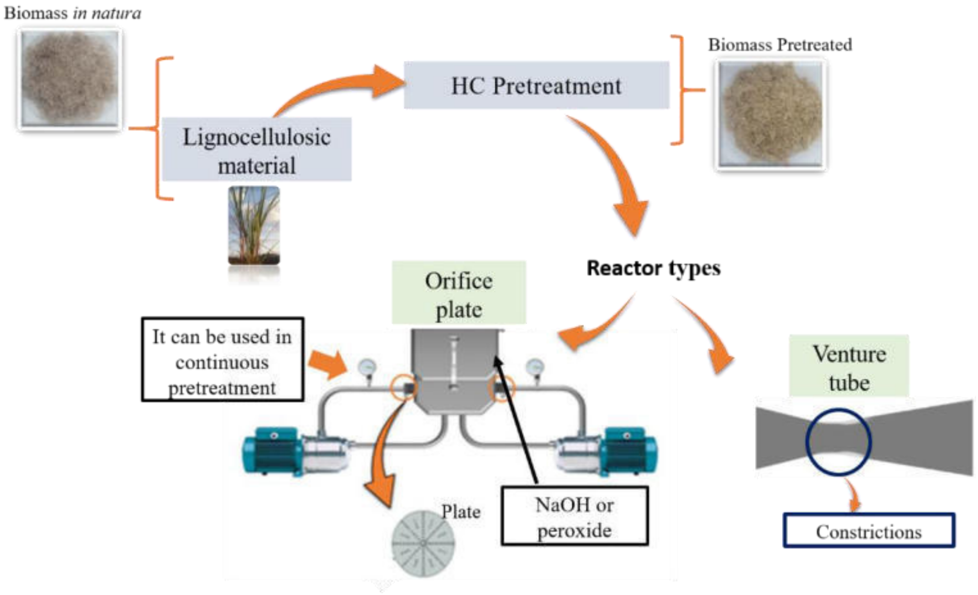

Figure 3.3. Schematic representation of hydrodynamic cavitation reactor for pretreatment of lignocellulosic biomass

This method promotes the subsequent hydrolysis of carbohydrates in a shorter time, lower price, with fewer fermentation-inhibitors formation (Habashi et al., 2016). Indeed, when HC is operated in a combination with alkali pretreatment, it achieves greater yields in the subsequent enzymatic hydrolysis (Kim et al., 2015; Nakashima et al., 2016).

In Table 3.2, recent findings in cavitation pretreatments and the advantages and disadvantages of UC (ultrasonic cavitation) and HC are shown. Within this regard, it is noteworthy to observe that time is a key parameter in those pretreatment methods (e.g., UC and HC). As described before, it is shown many advantages in the use of the HC process, such as shorter periods of time (e.g., 10 minutes process time) are described in literature when compared to UC (70 minutes). For instance, Liu et al. (2016) reported a 94% glucan hydrolysis yield in hydrolysis step after the pretreatment process in a 3-hour process of pretreatment applying UC. However, Terán-Hilares et al. (2017b) found 96% of glucan hydrolysis yield in a shorter process time of pretreatment (10 minutes).

Several alternatives have been recently evaluated, and innovative pretreatment methods assisted by hydrodynamic cavitation have been performed. HC is a versatile pretreatment method that can be modified to have other beneficial characteristics. Regarding its combination with chemical reagents, for example, Kim et al. (2015) studied an HC-assisted alkali pretreatment of reed, and, under optimized process conditions (3% NaOH, 11.8% solid loading, and 41.1min) observed lignin removal of 53.4%, with maximal reducing sugars of 326.3g/kg of biomass after 72h of enzymatic hydrolysis.

Teran-Hilares et al., (2016) worked with SCB in HC-assisted alkali method with 0.48 mol/L NaOH, for 44 min (60ºC). They observed 97% of glucan hydrolysis yield in 24 h. In another work, by a consecutive optimization approach, the authors were gradually decreasing pretreatment time and alkali loading, primarily reduced to 20 min and 0.3 mol/L, respectively (Teran-Hilares et al., 2017b). Ultimately, by reducing the amount of NaOH (0.29 M) and adding H_2O_2 at 0.78 % v/v, it was possible to decrease pretreatment time to 10 min (Teran-Hilares et al., 2018)

Table 3.2. Examples of literature works dealing with cavitation-assisted pretreatment of lignocellulosic biomass

Cavitation type	Condictions	Biomass	Main results	Advantages/ Disadvantages	Reference
Ultrasonic Cavitation	Ultrasonic bath (8L), 0.5 mol/L Na_2CO_3, 1mol/L H_2O_2 power of 150W, frequency 22 HZ-40 HZ, **Total time**:70 min	Raw saw	Lignin removal 87%; the obtained lignin was also analyzed regarding to the presence of peaks at wavelength range of 875–817, 1123–1110, and at 1599 cm^{-1}	**Advantages:** Removes hemicelluloses and lignin; increase accessible surface area **Disadvantages:** High energy consumption (J/g of sugar); difficulty of application on larger scale; the high costs; chemical agent help is requested, for example NaOH; high time	Devadasu et. al., 2020
	Bach reactor with 0.4 mol/L of Na_2CO_3 plus 0.6mol/L of H_2O_2, 4% solid/liquid ratio, 30ºC, 0.25mm of particle size, Venturi tube (length, 40mm; internal diameter 1.8 mm; throat diameter, 3.6mm). **Total time**: 60 min.	Corn Stover	Maximal reducing sugars 275 g/Kg of biomass; Lignin removal was verified by FTIR analysis by reduction in the band 1745 and 1606cm^{-1}, assigned to the side chain and aromatic ring of lignin.		Nakashima et. al., 2016
	Firstly, 32mg/O_3 for 1h, followed by alkaline process using 0.1 mol/L of NaOH for 2 h and ultrasound process for 5 min with 50KHz+ 70kHz. But followed experiments with other combinations (30kHz+ 50kHz, 40kHz + 60kHz, and 50kHz+70kHz) **Total time**: 2h and 5 min	Sugarcane Bagasse	Glucan hydrolysis yield 94%; maximal reducing sugars 240 g/kg of biomass; Hemicellulose removal 70%		Liu et al., 2016
Hydrodynamic Cavitation	3% of NaOH, 11.8% of S/L ratio, 77ºC, 0.5MPa of inlet pressure, orifice plates: 27 holes of 1mm of diameter. **Total time**: 41.1min	Reed	Glucan hydrolysis yield 85% ; maximal reducing sugars 326g/Kg of biomass; Lignin removal 53.4	**Advantages** : Remove hemicelulose and lignin; increase accessible surface area, possible use of this pretreatment in semi-continuous or continuous process; short time; Liquor reuse. **Disadvantages**: Chemical agent help is requested, for example adding NaOH in the process; studies are required for high solid loading	Kim et al., 2015
	0.4mol/L of Na_2CO_3 plus 0.6 mol/L of H_2O_2, 4% of S/L ratio, 60min, 30ºC, 0.25mm of particle size, Venturi tube (length, 40mm; internal diameter 1.7 mm; throat diameter 3.7 mm) **Total time:** 40 min	Corn stover	Maximal reducing sugars 275 g/kg of biomass		Madison et al., 2017
	Batch process, 3 bar of inlet pressure and 0.29 mol/L of NaOH, 0.78 % (v/v) of H_2O_2, 60ºC. **Total time:** 9.95 min	Sugarcane Bagasse	Glucan hydrolysis yield of 95.4%; Hemicellulosic removal 21 %; Lignin removal 45.6%		Terán-Hilares et al., 2017
	3 bar of inlet pressure and 0.3 mol/L of NaOH, 0.6 % (v/v) of H_2O_2, of process, liquor reuse. **Total time**: 10 min	Sugarcane Bagasse	Glucan hydrolysis yield of 96%; maximal reducing sugars 781g/kg of biomass; Lignin removal 27.2%		Terán-Hilares et al., 2018
	3 bar of inlet pressure and 0.3 mol/L of NaOH, 0.78 % (v/v) of H_2O_2, and 10 min in 60ºC, liquor reuse. **Total time**: 10min	Sugarcane Bagasse	Glucan hydrolysis yield of 86%; maximal reducing sugars 565g/kg of biomass; Hemicellulosic removal 53%; Lignin removal 63.3%		Terán-Hilares et al., 2019
	3 bar of inlet pressure and 0.3 mol/L of NaOH, 0.78 % (v/v) of H_2O_2, and 7.5 min in 60ºC (time residence in reactor), 15 L pretreatment 1.5 % S/L biomass, continuous process	Sugarcane Bagasse	Glucan hydrolysis yield of 80%; maximal reducing sugars 652g/kg of biomass; Hemicellulosic removal 54.47%; Lignin removal 54.4%		Terán-Hilares et al., 2020
	Venturi tube reactor with 1.5 mm diameter in the cavitation region, with 300 kPa pressure, addition of NaOH (4.90%), 2.03% solid with the process of 58.33% (solid/liquid)	Sugarcane Bagasse	Glucan hydrolysis yield of 78%; Lignin removal 56.01%; It had a solids recovery of 66% with a solid composition of 58.46% of cellulose, 14.34% of hemicellulose, and 14.03% of lignin.		Bimestre et al., 2020

Different operation modes have also been evaluated. Terán-Hilares et al. (2019) evaluated the reuse of the alkaline liquor in the HC-assisted process. In this sense, the alkaline pretreatment solution was retrieved in each subsequent pretreatment batch; and only the solid fraction of sugarcane after pretreatment was collected. In that work, the percentage of solids recovery ranged from 63.5% to 84.3%, on the same severity of the pretreatment (Terán-Hilares et al., 2019).

In other work, HC-system in semi-continuous operation mode was described by Terán-Hilares et al. (2019). That study used an alkaline hydrogen peroxide solution which was continuously recirculated for 10 min using a load of 25 g of dry SCB under 60°C. After the pretreatment, the liquid fraction was retrieved and reused in another new batch process in the same conditions.

Continuous processes are another alternative that deserves to be evaluated for pretreatment in biorefineries, considering its advantages as higher productivity. Continuous processes have been reported using a double screw extruder with an acid solution (Choi et al., 2012; Vandenbossche et al., 2016; Yin et al., 2018), ionic liquids (da Silva et al., 2013), and sodium hydroxide (Han et al., 2013), in addition to the acid-catalyzed steam explosion (Chen et al., 2019). Regarding HC, recently Terán-Hilares et al. (2020) developed a continuous process. In that work, a suspension containing 1% of sugarcane bagasse in an alkaline solution was fed into the HC-reactor at 200 mL/min flow rate. Enzymatic hydrolysis of pretreated biomass yielded 34 g of glucose/100g of biomass, and 18 g of xylose/100g of biomass, compared to 93.2 g of glucose/kg of biomass and 27.1 g of xylose /kg of biomass obtained for the untreated sugarcane bagasse (Terán-Hilares et al., 2020).

Another important aspect to pay attention to is HC pretreatment has been generating a liquor with high pH which has not been investigated until now. Considering mainly lignin removal has been observed (Nakashima et al., 2016), it would be expected to find this macromolecular fraction in the generated liquor. However, as HC has been used with oxidizing reagents as hydrogen peroxide (Terán-Hilares et al., 2019), or as it can generate oxidizing free radicals by itself, it is probable the lignin was oxidized or even degraded, and the liquor can have derived compounds as 4-hydroxybenzoic acid, vanillin, vanillic acid, coumaric acid, ferulic acid and 2-methoxyacetophenone (Kaldar et al., 2015; Travani et al., 2016).

3.5 Research gaps, economic aspects, and future perspectives

HC and advanced oxidative processes are new technologies applied in the pretreatment of biomass, and currently they present some limitations that must be addressed in the future. Thus, some authors recommend parallel surveys of these processes and different biomass characterization studies to understand the behavior of delignification (Liu et al., 2016). Furthermore, further studies should potentially focus on reducing costs and time of the processes.

Indeed, several previously described oxidative methods have been reported as promising and efficient methods for lignocellulosic biomass pretreatment, but at lab scale. In those methods, the generated radical species degrade lignin selectively and augment the subsequent saccharification; however, information about techno-economic analysis (TEA) of oxidative pretreatment methods is scarce. Such TEA analysis evaluates both the economic and technological aspects of the process, helping in the understanding about the total costs associated with ethanol and/or other bioproducts, equipment, chemicals, feedstock costs, and even energy requirements (Baral, Arita & Bradley, 2017).

Additionally, process bottlenecks that might hinder industrial-scale feasibility can also be identified (Bhalla et al., 2018).

Although TEA is a way to summarize important information within the process, its hurdles lie in data assessment. Once it is obtained, reliably interpretation whether the process is cost-effective or still needs improvement can be performed. For instance, steam explosion, dilute sulfuric acid, ammonia fiber explosion, and biological pretreatment are methods with sugar-production estimated costs ($/kg) around 0.43, 0.42, 0.65, and 1.41, respectively (Baral, Arita & Bradley 2017).

An interesting oxidative process is ozonolysis combined with ultrasound, resulting in an efficient method for lignin removal from biomass and to enhance the production of fermentable sugars in the enzymatic step (Perrone et al., 2016; Perrone et al., 2021). However, the lack of TEA for that specific process impairs it to be evaluated in comparison to other methods. Therefore, a TEA for the ozonolysis process and its combination with alternatives as ultrasound or hydrodynamic cavitation (HC) is required to compare the cost to other current methods.

Bhalla et al. (2018) compared two different alkali pretreatments for zip-lignin: a method with ethanol under 30°C and 3 M NaOH and a method using copper-catalyzed H_2O_2-assisted alkali pretreatment under 120°C, 1 M NaOH, H_2O_2 (100 mg/g biomass) with a load of 0.03g Cu. By that, the authors reported the generation of inhibitors such as 2,2′-bipyridine as well as a decrease in the saccharification yield when using H_2O_2 and Cu-AHP. In addition, regarding capital, feedstock, and operating costs, the alkali ethanol-assisted pretreatment resulted in lower values compared to 120ºC Cu-AHP. Nevertheless, Yuan et al. (2019), studied a similar process and observed an increase in the temperature of the alkaline process up to 120°C could potentially reduce the minimum fuel selling price (MFSP) by more than \$1.00 per gallon of biofuel, compared to pretreatment at the first condition of Bhalla et al. (2018) at 30 °C, which require high chemical inputs.

As previously shown, recent studies report the HC-assisted process as an efficient and promising pretreatment, with several advantages, e.g., operation in continuous mode, simple configuration of the system, low energy consumption, and the possibility of scaling (Fardinpoor et al., 2021; Terán-Hilares et al., 2020; Tsalagkas et al., 2021). The low energy consumption in HC in pretreatment process was previously reported by Kim et al. (2016); in that work, 75% of less energy consumption by kg-biomass pretreated was observed compared to ultrasound. On the other hand, Madison et al. (2017) affirm that the energy cost of hydrodynamic cavitation process is too expensive to be economical; assuming electricity costs \$0.08/kWh, the energy cost was \$16.70/kg sugar. However, those authors evaluated non optimized processes assisted buy HC. In the first experiment, the authors pretreated SCB for 2h using only HC and water at 10-20ºC, in pH 7 using Venturi tube (length 44 mm, and 1.7 mm internal diameter) as cavitation device. The second system evaluated by those authors corresponded to an HC-assisted NaOH pretreatment (orifice plate) for reed during 41min using 3% NaOH, 11% solid:liquid ratio of biomass at 77ºC. Another situation studied by those authors corresponded to HC (Venturi tube, the same as the first experiment) added with 0.4 mol/L Na_2CO_3, 0.6mol/L H_2O_2 during 60 min, in 60ºC for corn stover. The economic analysis was based only on the calculated cost of energy consumption during the process and the cost with chemicals. Thus, Madison et al. (2017) did not used optimized conditions or other advancements further reported for HC-assisted pretreatment (Madison et al., 2016; Terán-Hilares et al., 2017b). Moreover, economic analysis of a biorefinery is a more complex task. Actually, the economic evaluation of a biorefinery must consider the process at a whole, including computer-added simulation tools and taking into account the electricity production obtained from the biomass itself (Vasconcelos et al., 2020). In this way, new works should be performed in the future to take a real economic potential of HC-assisted methods.

Another aspect to be considered is all pretreatment methods produce wastewater; therefore, the development of strategies for the recovery of chemicals is a current challenge. For example, in hydrodynamic cavitation assisted alkaline or oxidative processes, a high volume of the alkaline solution has been used and is necessary to recirculate it by pumping the solution through pipes and valves to induce cavitation. This can suggest coupling other technologies such as membranes to recover sodium hydroxide, as well as to valorize lignin-hemicellulose fractions or derivatives from the wastewater.

HC and oxidative processes can also have disadvantages related with the use of different chemical species and the biomass recovery process. It is well known that the use of hydrogen peroxide as an oxidizing reagent harbors advantages due to its availability and easy use (Terán-Hilares et al., 2019; Bimestre et al., 2020). However, its use has been performed combined with more reagents and, when it is used with NaOH, neutralization of the recovered biomass is necessary. Hence, it is necessary to use large volumes of water to achieve the required pH in biomass for sequential processes, such as enzymatic hydrolysis or fermentation (Dutra et al., 2018).

3.6 Conclusion

AOPs and HC are promising technology for the intensification of different pretreatment under mild conditions (as the short time required and low concentration of chemicals) for lignocellulosic materials. Besides, the HC pretreatment results in high percentage of increase in the enzymatic digestibility and can be operated in continuous mode. AOPs assisted by the HC could establish an efficient pretreatment procedure, contributing to the viability of lignocellulosic biorefineries. Yet, further studies are still required to better understand the biomass modification after these oxidation processes, including knowledge about the composition of generated liquors, besides designing appropriate devices for a high-performance process.

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CHAPTER IV

Hydrodynamic cavitation-assisted oxidative pretreatment and sequential production of ethanol and xylitol as innovative approaches for sugarcane bagasse biorefineries

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ABSTRACT

In the present work, a new alternative of hydrodynamic cavitation-assisted pretreatment associated with an advanced oxidative process was proposed, together with a new approach to obtain bioproducts in sequential fermentations. In a first step, the sugarcane bagasse was pretreated in experiments conducted according to a 2^2 face centered statistical design to evaluate the influence of the variables O_3 flow and percentage of H_2O_2. The pretreated material was submitted to enzymatic hydrolysis in a fixed bed reactor, obtaining 120g/L of sugars (adding together glucose, xylose and arabinose). Enzymatic hydrolysate of sugarcane bagasse (SCB) was fermented to ethanol by *Saccharomyces cerevisiae* IR2. After distillation of this alcohol, the obtained vinasse was used to produce xylitol by *Candida tropicalis*. Influent variables in pretreatment were evaluated by performing experiments according to a statistical design, and, at optimum conditions (ozone flowrate of 10 mg/ min, and H_2O_2 concentration of 0.61%), about 84% and 78% of glucan and xylan hydrolysis yield were obtained in enzymatic hydrolysis, respectively. In the sequential fermentations for ethanol and xylitol production, yield values of 0.41 g/g and 0.55 g/g, respectively, were obtained, with corresponding volumetric productivities of 8.33 g/Lh and 0.64 g/Lh, respectively. The proposed strategy was shown as a promising approach for biorefineries, considering the mild conditions of pretreatment and the possibility of high ethanol production using S. cerevisiae in a fermentation process similar to that one already available in sucro-alcoholic sector, followed by xylitol production in vinasse-based medium.

Keywords: Second generation biorefinery; Sequential fermentation strategy; Biomass pretreatment; Sugarcane bagasse

4. 1 Introduction

The world has been motivated by environmental concerns to develop research about alternative fuels from renewable sources, aiming to mitigate Greenhouse gas emissions and to reduce the dependence of fossil-based products. Among the available options, second-generation ethanol stands out, with other interesting bioproducts that can be concomitantly produced in biorefineries, creating new and sustainable processes using biomass as raw material (Philippini et al., 2020; Kumar et al., 2020; Dragone et al., 2020; Bittencourt et al., 2021).

Actually, in Brazil, sugarcane bagasse (SCB) is an abundant raw material, obtained as by-product in sugar and alcohol industrial sector. The country is responsible for the production of 30 billion liters of ethanol per year, corresponding to an annual generation of more than 100 million of tons of sugarcane bagasse (USDA, 2022).

A limitation for the use of lignocellulosic biomass in biorefineries is its high recalcitrance, which hinders enzymatic digestibility of polysaccharides (mainly cellulose), and, consequently, the release of fermentable sugars to obtain bioproducts (Baruah et al., 2018). Thus, pretreatment is a critical stage for the use of lignocellulosic biomass and can represents almost 30% of the whole process cost (Kim et al., 2015).

Currently emerging pretreatments such as Hydrodynamic Cavitation (HC) have been evaluated, with promising results to overcome the negative points of conventional pretreatments (Prado et al., 2022; Fardinpoor et al., 2021). HC has important advantages, such as low processing time, low reagents requirement, the versatility of operation modes, and easy scale-up (Nakashima et al., 2016; Thangavelu et al. 2022; Bimestre et al., 2022).

Hydrodynamic cavitation has been a strategy to enhance the efficiency of lignocellulosic biomass pretreatment. The HC modifies the lignocellulosic structure due to the high energy released in "hotspots" of cavitation, which can help to generate oxidative radicals to aid the breaking of lignin (Fardinpoor et al., 2021). HC also results in increase in the surface area and in the porosity of biomass (Fardinpoor et al., 2021; Bimestre et al., 2022; Hilares et al., 2017). However, until now there are a relatively small number of papers published about this topic, as reported in recent review articles (Fardinpoor et al., 2021; Hilares et al., 2017). Thus, some challenges are still to be overcome, aiming to reducing even more the quantity and cost of reagents used together with HC, favoring the viability of the technique.

There are previous works of our research group reported in the literature about HC-assisted pretreatment of biomass, using processes in an alkaline medium (Hilares et al., 2019), or in an alkaline medium containing H_2O_2 (Wu et al., 2016). Hilares et al. (2020), for example, reported alkaline HC-assisted pretreatment of sugarcane bagasse, obtaining 54 g of total reducing sugars released per 100 g of pretreated biomass, by using 0.3 mol/L of NaOH and 0.78% of H_2O_2 for 10 min in the pretreatment performed in HC-reactor with an orifice plate with 16 holes of 0.65 mm each one. However, new studies should be performed aiming to reduce the quantity of reagents necessary in the process, and in this way, advanced oxidative processes (AOPs) could be an interesting and non-previously reported alternative for HC-assisted pretreatment of biomass.

Actually, as pointed in a recent review of Prado et. al. (2022), AOPs can generate radicals to degrade recalcitrant materials, with benefits such as a low-cost pretreatment in mild conditions, increasing the digestibility of lignocellulosic biomass, and reducing process time and chemical inputs (Prado et al., 2022). HC association with AOPs using reagents as O_3 and H_2O_2, can favor the formation of free radicals that remove lignin, complementing the quantity of radicals such as HO_2^- and $O_2^\cdot$ (Eq. 1 and Eq. 2) that are already formed on-site by HC , increasing the effectiveness of the pretreatment (Noyori et al., 2003). Thus, HC/AOP combined processes could be interesting, resulting in high lignin removal by oxidation, due to the synergy between the two techniques, which produce high quantity of oxidant hydroxyl radicals (Bimestre et al., 2022; Antunes et al., 2014).

$H_2O_2 \leftrightarrow HO_2^- + H^+$ Eq.1

$O_3 + HO^-_2 \rightarrow HO^\cdot + O_2^{-\cdot} + O_2$ Eq.2

Besides to consider the pretreatment approach, all fractions of biomass should be taken into account to obtain interesting products, aiming at favoring the utilization of lignocellulosic as raw material in biorefineries (Dulie et al., 2021). Regarding to carbohydrate fractions, cellulose can be hydrolyzed to obtain glucose, a monomeric sugar directly fermentable by the *S. cerevisiae*. On the other hand, hemicellulose can represent until one-third of biomass, and its hydrolysis results in a xylose-enriched mixture (Queiroz et al., 2022). Xylose can be also used to produce ethanol by genetically modified microorganisms or even by naturally-xylose-fermenting yeasts (Dulie et al., 2021), but other value-added compounds could be produced and result in a more profitable biorefinery. Among options, xylitol is a versatile, and valuable biomolecule, with important applications in chemical, pharmaceutical, and food sectors. Although currently produced mainly by a chemical process, the xylitol production has been evaluated through bioprocesses and this route has advantages such as the use of comparatively milder conditions, no requirement of extensive xylose purification steps, with low cost of product (Latif et al., 2011).

Simultaneous ethanol and xylitol production has been studied (Latif et al., 2001) but this alternative could present low productivity for both products, with non-optimized conditions for each one. Latif and Rajoka. (2001), for example, reported simultaneous ethanol and xylitol production using co-culture of two different yeasts (*Scheffersomyces stipitis and Candida tropicalis*), obtaining 20g/L (Yp/s was 0.36) of ethanol in 72h, besides 8 g/ L of xylitol (Yp/s of 0.23).

An interesting approach to produce high concentrations of ethanol and xylitol with high productivities would be the sequential production of these compounds, which could occur in a process similar to that one already used for first generation ethanol. The production of this fuel in Brazilian industry is usually performed by the fermentation of sugarcane juice or molasses, carried out by the yeast *Saccharomyces cerevisiae* (Siles et al., 2011). This yeast is the most traditionally used in industrial process, considering its advantages as be resistant to high ethanol concentrations, and production of ethanol from medium with high concentration of sugars in relatively short time (about 8h-12h) (Siles et al., 2011). For first generation ethanol from sugarcane, this process is consolidated, involving milling, fermentation, and distillation. Thus, considering the above-mentioned advantages of sugars fermentation with *S. cerevisiae*, a similar process could be advantageous to produce ethanol in glucose-enriched hydrolysates obtained from cellulose of the sugarcane bagasse. Also, in sucro-alcohol Brazilian industry, a great quantity of vinasse is produced in alcohol distillation step. In the country, for each ton of sugarcane, 70 liters of alcohol and 600 to 1000 liters of vinasse are produced (Aparecida et al., 2013; Ramos et al., 2021). If a hydrolysate from cellulose and hemicellulose fractions of sugarcane bagasse was used in alcohol production by *S. cerevisiae*, the xylose is expected to remain in fermented broth, thus remaining in the vinasse obtained in distillation of ethanol. Then, the cultivation of a xylitol producing microorganism in vinasse-based medium would be an interesting approach to integrate 2G ethanol and xylitol production.

This work evaluated innovative approaches for pretreatment of SCB and for ethanol and xylitol production. The pretreatment was performed by combining HC/AOP in a medium added with ozone and H_2O_2. It resulted in biomass enriched in cellulose and hemicellulose, which was hydrolyzed in a column bioreactor (Wu et al., 2016). As the resulting hydrolysate had a mixture of sugars, mainly glucose and xylose, an approach of a sequential production of ethanol and xylitol was proposed and evaluated. *S. cerevisiae* was used to produce ethanol from glucose with high productivity, and, after ethanol distillation, the produced vinasse containing xylose was used for xylitol production by *C. tropicalis.* The flocculent *S. cerevisiae* IR2 strain was used aiming to favor its reuse in the process (Ramos et al., 2021).

4.2 Material and Methods

4.2.1 Raw material and chemicals

Ipiranga Agroindustrial LTDA (Descalvado, São Paulo, Brazil) donated the Sugarcane bagasse (SCB). This raw material was dried under sunlight until 10% of moisture, and it was milled until particle size between 0.60 mm and 1.18 mm, which was used for the study.

Commercial cellulases preparation CELLIC CTEC-2 (with 110 FPU/mL) was purchased from Sigma-Aldrich Brasil Ltda. (Cotia, SP, Brazil). Peptone (part number 91079-38-8) and yeast extract (part number Y1625), and other chemicals (all of analytical grade) were also purchased from Sigma-Aldrich Brasil Ltda.

4.2.2 Hydrodynamic cavitation-assisted oxidative pretreatment

Pretreatment was performed in a HC system, which was described elsewhere (Hilares et al., 2017). An oxidative pretreatment system was adapted to the cavitation reactor, The Ozonator (Ozone & Life, Waterone, Kansas City- KN, USA) was linked by a pipe to feed O_3 to the HC reactor. As shown, the system is composed mainly of a HC-reactor (cavitation zone), in which biomass is placed inside a screen. Liquid medium is recirculated by a 11032.5W centrifuge pump (Grundfos CM 5-3, Bombas Grundfos do Brasil Ltda., São Bernardo do Campo, SP Brazil), generating the cavitation in a 16 holes-orifice plate. A recirculation tank is also present to warrant an enough volume of fluid to be suctioned by the pump.

A 2^2 central composite face-centered design, with triplicate at the center point (Table 4.1), was used for the pretreatment runs. The results were analyzed aided by Design-expert software v. 13 (Stat-Ease, Inc., Minneapolis, US) and Statistica for Windows (StatSoft, Inc. V.5 Tulsa, OK, USA). The evaluated variables were ozone flow (from 2 to 10 mg/min) and concentration of H_2O_2 in the medium (0 to 1%) in pH 7.0. The studied range for ozone flow was based on the limits of capacity of available system, and the range for H_2O_2 was determined to be lower than the values used in a previous work of HC-pretreatment without AOP (Wu et al., 2016).

Response variables were the composition of the pretreated material and the hydrolysis yield of the carbohydrate fractions of the material, which was evaluated in an enzymatic hydrolysis step carried out in Erlenmeyer flasks according to section 2.3.

All pretreatment runs were carried out with 35 g of bagasse loaded in the system, which was also charged with 3 L of a H_2O_2 solution, in concentrations according to statistical design. The solution was recirculated in the system with a flow of 5 m^3/h, and ozone was fed continuously into the reactor cavitation, with air flow in the ozonator (Ozone & slamp; Life, model O&Samp:L 3,0RM) of 0.2 m^3/ min and the ozone flow adjusted according to statistical design (total gas flowrate from 12 to 76 mL/min, corresponding to an ozone flow of 2 to 10 mg/min). The pretreatment time was 10 min, 60ºC, with a pressure of 3 atm upstream of the orifice plates. After this step, the pretreated bagasse was separated from the liquid medium by filtration and washed with distilled water. Subsequently, the components (cellulose, hemicellulose, and lignin) of pretreated bagasse were quantified.

At optimized conditions, which was indicated by an empiric mathematical model composed after statistical analysis, experiments were performed in triplicate to confirm the prediction of the model. Also, control experiments were performed under those conditions: a. Without orifice plates in the cavitation system (control without HC), using the same recirculation flow in the pump; b. Without ozone (only with air inlet from ozonation system) nor H_2O_2 in the cavitation system (only HC). Additionally, a control was carried out with the enzymatic hydrolysis of untreated biomass performed according to section 4.2.3.

4.2.3 Enzymatic Hydrolysis in Erlenmeyer flasks to evaluate the pretreatment

This step, using the pretreated SCB, was carried out in Erlenmeyer flasks (125mL) with 5% of solid loading in a citrate buffer solution (50 mM) with pH 4.8. The enzymatic hydrolysis was performed employing Cellic® CTec2 (Novozymes Latin America Ltda., Brazil), a commercial cellulases enzyme blend, with a loading of 20 FPU g^{-1} of dry pretreated SCB. Reaction was performed at 50°C and 200 rpm for 24h. Concentrations of hexoses and pentoses released in hydrolysis were analyzed by high performance liquid chromatography (HPLC). Hydrolysis yields were calculated as Equations 3 and 4 (Lu et al., 2012).

$$Glucan\ hydrolysis\ yield(\%) = \frac{(G*0.9*V)}{(CC*M)}*100\% \qquad \text{Eq. 3}$$

$$Xylan\ hydrolysis\ yield(\%) = \frac{(Xyl*0.88*V)}{(CC*M)}*100\% \qquad \text{Eq. 4}$$

Where:

G = concentration of glucose g /L released from glucan in enzymatic process

Xyl = concentration of xylose g/ L released from xylan in enzymatic process

V = total volume of reactional medium

CC = percentage of glucan or xylan in raw SCB

M = mass of SCB used in hydrolysis enzymatic

4.2.4 Enzymatic hydrolysis in column reactor

Column enzymatic hydrolysis process was performed as described by (Ramos et al., 2021), with 21% of solid loading (40 g of SCB, total liquid 190 mL). The reactor consisted of one column with 30 mm of internal diameter. Liquid medium was composed by a citrate buffer solution (50 mM) with pH 4.8, and Cellic® CTec2 (Novozymes Latin America Ltda., Brazil), using 20 FPU /g of dried pretreated biomass. Following, hydrolysate was vacuum concentrated at 50°C in a Rotary Evaporator. Then, concentrated hydrolysate was used in sequential fermentation experiments.

4.2.5 Sequential production of ethanol and xylitol by *Saccharomyces cerevisiae* IR2 and *Candida tropicalis* UFMGX12.

4.2.5.1 Microorganism, inoculum preparation and cells immobilization

S. cerevisiae IR2 and *C. tropicalis* UFMGX12 were obtained from stock cultures available in the Sustainable Bioproducts Laboratory (LBIOS) at the Engineering School of Lorena–University of Sao Paulo, Brazil. Inoculum was prepared in 250 mL Erlenmeyer flasks containing 100 mL of medium, the cultivation carried out in a BIO CB SSB rotary shaker (ERETZBIO, São Paulo, Brazil), for 24 hours, 150 rpm, at 30ºC.

For the yeast *S. cerevisiae* IR2, the medium was composed of 50 g/L of glucose, 10 g/L of bacteriological peptone and 10 g/L of yeast extract. For *C. tropicalis* yeast, the medium was composed of 60 g/L xylose, 20 g/L yeast extract, 2 g/L NH_4SO_4, 0.1 g/L $CaCl_2.2H_2O$. For both fermentations, with initial pH adjusted to 5.5.

Subsequently, both cells were recovered by centrifugation at 5000g for 10 min, washed and suspended in water (distilled) to obtain a suspension with high cell density, which was used as an inoculum in order to obtain 20 g/L and 1 g/L of initial cell concentration in the fermentation process performed with *S. cerevisiae* and *C. tropicalis*, respectively.

4.2.5.2 Ethanol and xylitol sequential production

The sequential production was performed using two processes, the first fermentation aimed ethanol production by *S. cerevisiae* in a medium formulated with concentrated hydrolysate (containing 150 g/L of glucose, 60 g/ L of xylose), supplemented with 10 g/ L of peptone, and 10 g/ L of yeast extract (conditions according to Ramos et. al. (2021). The process was performed in a rotary shaker in 125mL Erlenmeyer flasks loaded with 75mL of medium, with agitation of 150 rpm, at 30°C for 24h. After the process, cells were separated from medium by decantation, followed by centrifugation at 5000g for 10 min.

Then, ethanol was removed from the fermented broth by a vacuum rotary evaporator at 55°C for 6h. The complete removal of ethanol was verified by analysis of samples by HPLC. Afterwards, the resulting liquid mixture (vinasse) containing xylose was fermented by *C. tropicalis* to obtain xylitol. This second fermentation was performed in a rotary shaker in 50 mL flasks loaded with 25mL of medium containing: vinasse (60 g/L of xylose), supplemented with 20 g /L of yeast extract, 2 g/ L NH_4SO_4 and 0,1 g/L $CaCl_2.2H_2O$, according to De Arruda et al. (2017). Medium was adjusted to initial pH of 5.5 with aqueous solution of NaOH 0.1 mol/L, and fermentations were performed with agitation of 150 rpm, at 30°C for 48h. For both fermentations, periodic samples were withdrawn from the medium for analysis of sugars and products.

For fermentation process, yield values (Yp/s) were calculated considering the ratio between the concentrations of obtained product and consumed substrate. Efficiency values were calculated as the ratio between the obtained and the theoretical values of Yp/s, which are 0.51 g/g for ethanol and 0.917 g/g for xylitol (De Arruda et al., 2017). Productivity values (Qp) were calculated by dividing the concentration of obtained product by the time of fermentation.

4.2.6 Analytical methods

4.2.6.1 Humidity

The sugarcane bagasse moisture was determined by using an infrared balance Mark M163 (BEL Engineering, Piracicaba-SP).

4.2.6.2 Compositional characterization of bagasse

The components of *in natura* and pretreated bagasse were quantified in cellulose, hemicellulose, lignin, ashes, and extractives, following the methodology of Mesquita, Ferraz, and Aguiar [36]. Solid recovery was calculated by the ratio between the dry mass of the material after pretreatment and before pretreatment (Eq.5).

$$\%Solid\ rec. = \left[\frac{m\ SCB\ pret.}{msCBstart}\right] \times 100 \quad \text{Eq. 5}$$

Where:

Solid rec = Solid recovery, in percent (%)

m SCB pret. = dry mass of the material after pretreatment (g)

m SCB start = dry mass of the material before pretreatment (g)

4.2.6.3 Analysis of sugars, acetic acid, ethanol, and xylitol

These compounds were analyzed by HPLC on Agilent Technology 1200 series chromatograph (Agillent Technologies 1200, New York, USA). Before analysis, liquid phase was filtered in Sep Pak C18 filters and analyzed in chromatograph, according to Antunes et. al. (2014).

4.2.6.4 Biomass concentration

For the experiments using *S. cerevisiae*, after cultivation, all the medium volume was centrifuged at 3000 g for 20 min, cells were washed two times with distilled water, and the biomass concentration was measured considering the dry mass obtained after drying cells in an oven at 105ºC.

For experiments using *C. tropicalis*, periodic samples were removed from medium, and the yeast concentration was measured by turbidimetry (Antunes et al., 2014).

4.2.6.5 Structural analysis of sugarcane bagasse by Fourier Transform Infrared Spectroscopy (FTIR)

The Fourier Transform Infrared Spectroscopy (FTIR) was performed in an equipment Shimadzu IR Prestige-21(Shimadzu, Kyoto, Japan). Samples were previously prepared in potassium bromide (KBr) pellets pressed at 50 kN. Spectra were collected in transmittance mode, with 64 scans, 4 cm^{-1} resolution and a spectral range from 4500 to 400 cm^{-1}. The samples analyzed were SCB *in natura*, and SCB pretreated with HC-assisted oxidative process in the optimized conditions.

4.2.6.6 Structural analysis of sugarcane bagasse

Different structures in SCB surfaces were observed using LEO44Oi Electron Microscopy/Oxford (Cambridge, England), as described by Antunes et al., (2014). Crystallinity index (CrI) in raw and pretreated SCB was determined by X-ray diffraction (XRD), according to Segal et al., (1959). The crystallinity was scanned over the range of 2θ = 5-50° and the crystallinity index (CrI) was calculated using Eq.6 .

$$CrI(\%) = \left[\frac{(I\ crystalline - Iamorphous)}{Icrystalline}\right] \times 100 \quad \text{Eq. 6}$$

Where:

I crystalline= Intensity at 22.3°

I amorphous = Intensity at 16.1°

4.2.6.7 Chemical oxygen demand (COD), Biologic oxygen demand (BOD) and Total Organic Carbon (TOC) analysis

Vinasse obtained from ethanol production and the fermented broth obtained after xylitol production (after cells removal by centrifugation at 5000 g for 10 min) were characterized regarding to COD, BOD and TOC.

The methodologies used for COD and BOD determination were adapted from Standard Methods (APHA, 2004).

For COD determination, samples were previously diluted 10 times and NaOH (0.1 mol/L) was used to adjust the pH to 7.0. BOD5 assay was performed at 50°C for 5 days. It was measured by the difference in demand of oxygen before and after the incubation period, with deionized water previously saturated with compressed air. Dissolved oxygen was measured in samples using HANNA INSTRUMENTS®Modelo (New York, USA).

TOC determinations were performed in a total organic carbon analyzer (Shimadzu, TOC-VCPH model, Kyoto, Japan). Two calibration curves were constructed: the first for low carbon content, ranging from 0 to 100 mg/L, and the second for high content, ranging from 100 to 1000 mg/L of carbon. All samples were previously diluted up to 10 times and pH was corrected (between 2 and 3), in order to ensure total acid digestion prior to combustion. The equipment used was programmed to perform two measurements for the samples, and the analytical control was performed as a function of coefficient of variation (CV), aiming to a value less than 3% (otherwise, another measurement was performed).

4.3 Results and Discussion

4.3.1 HC-assisted AOP Pretreatment effect on enzymatic hydrolysis of SCB

SCB was pretreated under different conditions using AOP assisted by HC process, and the result of pretreatment was evaluated in biomass composition and in enzymatic hydrolysis of its cellulose and hemicellulose fractions.

Untreated biomass had 40% of glucan, 26% of hemicellulose (24% of xylan, 1% of arabinan, and 1% of acetyl), 25% of lignin, besides and 1% of ash and 3% extractives. Table 4.1 presents the results of the statistical design carried out for evaluating the effects of the variables O_3 flow (2 to 10 mg/min) and H_2O_2 concentration (0% to 1%) on the yields of enzymatic hydrolysis of glucan and of xylan, and on biomass composition and removal of its compositional fractions.

As shown in Table 4.1, the pretreatment resulted in modification on the SCB composition, rendering it as a material enriched in cellulose and with lower content of lignin. Indeed, the lignin was the main fraction removed by the pretreatment (removal of 42-56%), followed by xylan (28-35%), while glucan removal was lower than18%.

Indeed, the hydrodynamic cavitation effect on the pretreatment of lignocellulosic biomass has been reported in literature (Prado et al., 2022; Bimestre et al., 2022; Hilares et al., 2020), and the chemical and physical action of cavitation provides benefits such as mild conditions for oxidative degradation, and reduction of lignin fraction of biomass under short reaction period (Hilares et al., 2017). The HC phenomenon results in high energy released into the medium, which contributes for generation of oxidative radicals (Prado et al., 2022; Hilares et al., 2020). Besides, the addition of oxidizing agents (ozone and H_2O_2) further contributes to process efficiency due to the high oxidative power (2.08V, 1.78V of ozone and H_2O_2, respectively). HC and oxidizing agents synergically act on lignocellulosic material, breaking down lignin molecules (Prado et al., 2022). HC effect is also associated with high-speed microjets action and shockwave generation due to violent cavity collapse. This improves cellulose and hemicellulose enzymatic conversion into fermentable sugars, once the specific surface area grows and total pore and micropore volumes increase (Thangavelu et al., 2022; Hilares et al., 2017).

As observed in Table 4, the compositional modification resulted in different values of glucan and xylan hydrolysis. The highest yield values (84% for glucan and 78% for xylan) were observed using ozone (O_3) flow of 10 mg/ min and 1% of H_2O_2 in the medium.

The obtained data were statistically analysed, and empiric models (Eq. 3 and 4) were composed to explain the experimental behaviour of the variables glucan and xylan hydrolysis yield. ANOVA was performed for the models, checking they were significant (*p-value*<0.05), although with significant lack of fit (*p-value*<0.05) for both responses. Also, an F test was verified. Indeed, to perform the F test, the variance was calculated for each variable. After obtaining the variations, the F statistic was calculated by dividing the largest variation by the smallest variation. Both models were reduced by removing non-significant terms (except if required for model hierarchy), and R^2 values were 95% and 83% for the models of glucan and xylan hydrolysis yield, respectively. Even with significant lack of fit, the models were used to trace response surfaces and for process optimization, considering their significance at 90% confidence level and, mainly for glucan hydrolysis yield, the high value of R^2, and F test presented the value greater than the tabulated. As shown, those models were able to predict results at optimized conditions.

Table 4.1 Composition and removal of sugarcane bagasse pretreated by HC-assisted advanced oxidative process technology according to a 2² face-centered design performed to evaluate the influence the influence of important variables in the process. Results of bagasse composition, removal of biomass components and hydrolysis yield were presented as average±standard deviation of the analytical results.

Run	Conditions (values in parentheses)		Solids Recovery (%)	Bagasse Composition			Removal			Hydrolysis Yield	
	Ozone (2 - 10 mg/min)	H_2O_2/ (0 - 1 %)		Glucan (%)	Xylan (%)	Lignin (%)	Glucan (%)	Xylan (%)	Lignin (%)	Glucan (%)	Xylan (%)
1	2 (-1)	0 (-1)	71.3	54.08	24.08	17.34	3.601	28.46	50.54	30.31	28.21
2	10 (+1)	0 (-1)	70.0	55.0	24.01	19.01	3.75	29.97	46.77	58.60	57.95
3	2 (-1)	1 (+1)	71.0	54.82	24.82	19.31	2.67	26.57	45.15	50.80	36.45
4	10 (+1)	1 (+1)	67.0	59.32	23.32	19.82	0.64	34.90	46.88	83.84	78.02
5	2(-1)	0.5 (0)	72.0	45.76	22.76	18.31	17.6	31.72	47.25	54.90	58.92
6	10 (+1)	0.5 (0)	64.0	57.06	27.06	17.47	8.70	27.84	55.27	81.87	66.60
7	6 (0)	0 (-1)	72.0	52.7	22.7	15.29	5.14	31.90	55.96	39.24	32.38
8	6 (0)	1 (+1)	73.0	51.40	21.40	19.79	6.19	34.91	42.18	61.63	46.46
9	6 (0)	0.5 (0)	72.0	52.7	23.7	17.76	5.14	28.90	48.85	79.26	72.09
10	6 (0)	0.5 (0)	72.0	52.40	23.40	17.71	5.68	29.80	48.99	78.76	68.23
11	6 (0)	0.5 (0)	72.0	52.40	23.40	17.15	5.68	29.80	50.60	77.09	69.98

Thus, based on the composed models (Equations 7 and 8), the response surfaces were traced (Figure 4.1). The enzymatic hydrolysis of the carbohydrate fractions of the sugarcane bagasse pretreated by the AOP process assisted by hydrodynamic cavitation were linearly dependent on the input flow of ozone in the cavitation reactor, with higher hydrolysis yield obtained using greater ozone flow. Regarding H_2O_2 concentration, the dependence of glucan and xylan hydrolysis yield was quadratic.

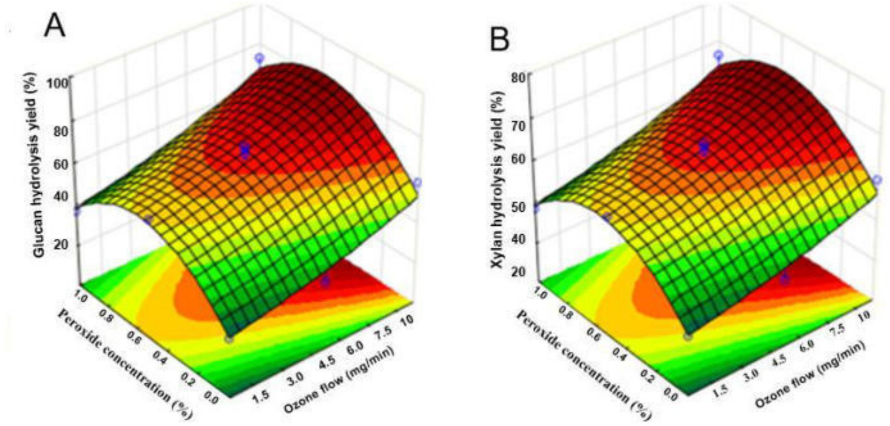

Figure 4.1. Response surface for the yield of enzymatic hydrolysis of glucan (A) and for the yield of enzymatic hydrolysis of xylan (B), as a function of H_2O_2 concentration and ozone flow used in HC-assisted oxidative pretreatment of sugarcane bagasse

Glucan hydrolysis yield (%) = $10.72 + 4.67A + 103.93B - 81.22B^2$ $R^2 = 95\%$ Eq. 7

Xylan hydrolysis yield (%) = $11.21 + 4.15A + 96.47B - 82.34B^2$ $R^2 = 83\%$ Eq. 8

Where: A is ozone flow; B is H_2O_2 concentration

The models were then used to optimize the response variables. The maximization was performed using the Design-expert software-specific tool. The optimized conditions were obtained according to the numerical tool of the Design Expert software, in order to maximize both the hydrolysis of glucan and xylan. The conditions obtained were: 10 mg/min of ozone and 0.61 % of H_2O_2.

Experiments were performed in triplicate under these conditions to confirm the models and the yield of glucan hydrolysis predicted by the model, of (90.6± 6.9) % (average value ± 95% confidence interval), was confirmed by the experimental result obtained, (84.1 ± 4.1) % (average value ± standard deviation). The yield of xylan hydrolysis predicted by the model, (80.9 ± 12.2) % (average value ± 95% confidence level), was also confirmed by the experimental result obtained (72.0 ± 1.5) % (average value ± standard deviation).

Control experiments were also performed in triplicate under the optimized conditions, as section 4.2.2. The first control used ozone inlet (10 mg/min), and H_2O_2 (0.61%) in the HC-reactor at the same recirculation flow, but without an HC-generator device (orifice plates). Thus, in this case, only the effects oxidizing agents were present. The second control considered only the HC effect (without oxidizing agents). As shown in Figure 4.2, the beneficial effect of using a combined AOP-HC system was shown, considering the enzymatic hydrolysis yields of glucan and xylan were lower than 50% and 40%, respectively, in the control experiments. Anyway, in all cases, the pretreated biomass resulted in hydrolysis yields higher than those ones observed for untreated biomass, which corresponded to 18.27±0.18 % for glucan, and 8.53±0.24 % for xylan (results were presented as average ± standard deviation).

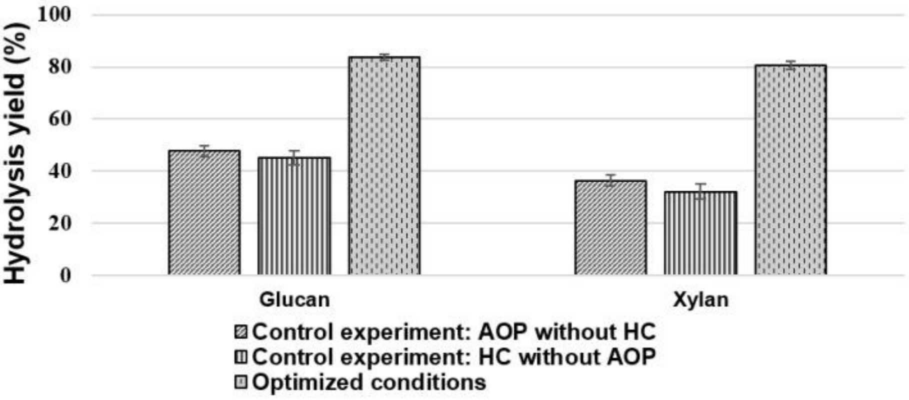

Figure 4.2. Enzymatic hydrolysis yield obtained using sugarcane bagasse pretreated by AOP-HC-assisted process at optimized conditions (10 mg/min ozone inlet, and 0.61 % H_2O_2), and obtained in control experiments performed under similar conditions, but without HC generation device (AOP without HC) and without addition of ozone nor H_2O_2 (HC without AOP). Results are average of triplicates and are shown as average ± standard deviation (error bars)

In a previous work, Teran Hilares et al. (2020) used 0.3 mol/L of NaOH and 0.78% of H_2O_2 for alkaline HC-assisted pretreatment of sugarcane bagasse, reporting a hydrolysis yield of 89 % of glucan and 78 % of xylan in biomass pretreated for 10 min. Although those results were slightly superior to the reported in the present manuscript, it is important to note we used lower quantity of H_2O_2 (0.61%) and no NaOH. Instead, ozone was used, which has been reported as a low-cost reagent (Rekhate and Srivastava, 2020).

4.3.1.1. Changes in biomass due to the pretreatment: FTIR, XRD, and SEM

In order to verify changes in biomass along pretreatment, FTIR, X-ray diffraction (XRD) and Scanning electron microscopy (SEM) were performed for *in natura* and for biomass pretreated under optimized conditions.

The samples of *in natura* and pretreated SCB presented some similar bands when analyzed by FTIR (Figure 4.3). As also observed by Cai et al. (2017) and Yan et al.

(2020), the FTIR spectrum showed bands at 3337-3432 cm^{-1} due to -OH symmetrical and asymmetrical vibration, at 2946-2895 cm^{-1} due to groups CH_2 and CH_3, and at 1031 cm^{-1} due to C–O, C═C and C–C═O elongations. They are assigned to the three fractions of biomass and are present in the spectra of both analyzed samples. In pretreated material, it was observed an accentuation in the peak 1746 cm^{-1}, referring to the free ester carboxyl of the hemicellulose fractions, the accentuation of the -OH band in the plane at 1375-1444 cm^{-1}, referring to cellulose and hemicellulose, and the disappearance of 1258 cm^{-1} peak, referring to ═O–C–O–C stretching vibrations of lignin. Also, in pretreated material occurred an accentuation of the 900 cm^{-1} peaks, referring to glycosidic bonds, in 1643-1512 cm^{-1} peak, referring to aromatic structure modification, and in 845 cm^{-1} peak, which demonstrated the C-H structure after aromatic deformation. Those phenomenona can be related to the effects of oxidative pre-treatment on the SCB, since removal of lignin explain the accentuation of characteristic peaks of glycosidic and alteration on aromatic structure fractions, also explaining the disappearance of peaks of lignin fraction.

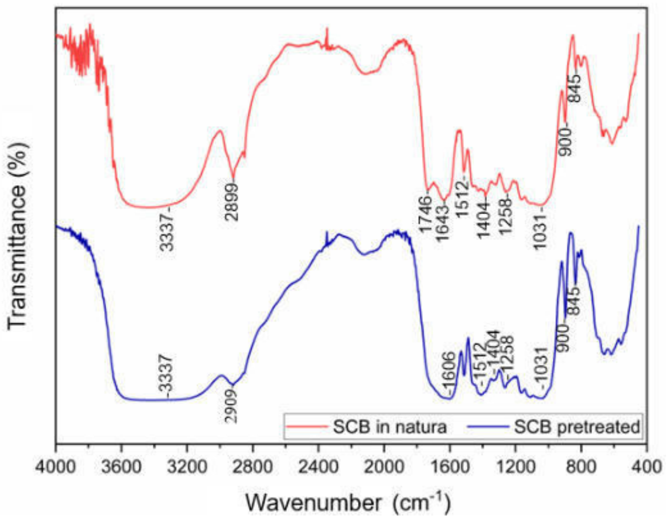

Figure 4.3. FTIR spectrum of sugarcane bagasse *in natura* and pretreated by AOP-HC-assisted process under optimized conditions (10 mg/min ozone inlet, and 0.61 % H_2O_2)

From the X-ray analysis, *in natura* SCB showed CrI of 39%, while pretreated SCB with HC-assisted oxidative process under optimized conditions presented CrI of 57%. Indeed, the sugar's fractions and lignin content of the biomass modify the CrI of lignocellulosic material (Yan et al., 2020). In the present work, the CrI of the pretreated material was higher compared to *in natura* SCB. Amorphous compounds, as lignin and hemicellulose, help decreasing the crystallinity of the material. Depending on the effect of the pretreatment, these compounds can be removed, increasing the total crystallinity of the material. However, since part of the present cellulose is a crystalline polymer, the pretreatments usually reduce this specific polysaccharide's CrI, while the material's CrI is magnified (Dulie et al., 2021).

Subsequently, the raw and pretreated sugarcane bagasse were looked over by scanning electron microscopy (SEM), in order to study the changes provoked by the pretreatment in the morphology of the structures (Figure 4.4). The images of raw sugarcane bagasse revealed the fiber surface is more homogeneous compared to the pretreated material. The structure of raw SCB is mainly covered by lignin and extractives, which is characteristic of *in natura* agricultural residues. Images of pretreated sugarcane bagasse morphology confirmed the disruption of the material due to a high removal of lignin and extractives (Dulie et al., 2021).

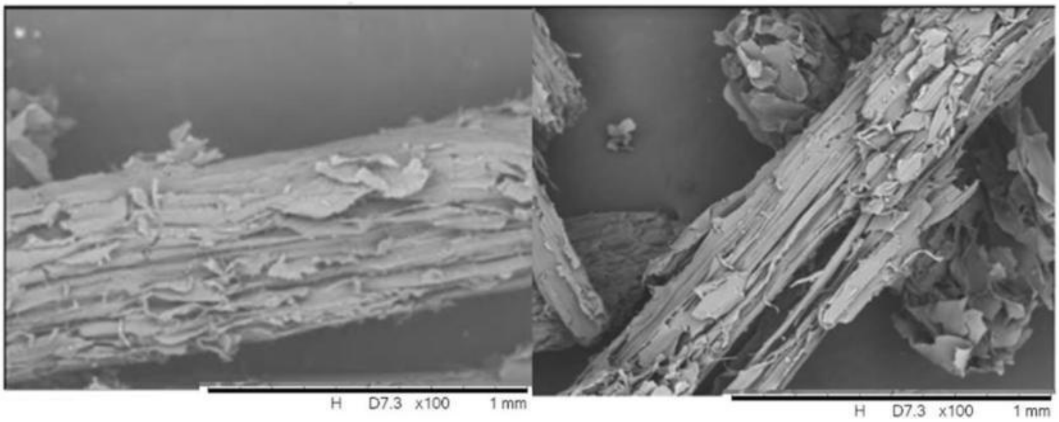

Figure 4.4. SEM images of sugarcane bagasse *in natura* and pretreated by AOP-HC-assisted process under optimized conditions (10 mg/min ozone inlet, and 0.61 % H_2O_2): *in natura* (left) and pretreated (right)

4.3.2 Sequential Fermentation production of ethanol and xylitol

Enzymatic hydrolysis was carried out in column reactor using high solid loading (21%). This is an interesting approach to obtain high sugars concentration in the hydrolysate, as previously reported (Wu et al., 2020; Ramos et al., 2021). After 48h of process, a hydrolysate with 60 g/L of glucose and 30 g/L of xylose was obtained. After this step, the hydrolysate was vacuum concentrated until reach 120 g/L and 60 g/L of glucose and xylose, respectively, aimed to the further fermentation assays.

The ethanol production process was performed with an initial cell concentration of 20 g/L, an approach usual in Brazilian sucro-alcohol industry (Ramos et al., 2021), adequate to reduce the fermentation time, thus increasing the productivity.

As shown in Figure 4.5A, after 6 hours of process, the glucose consumption had achieved 90%, and ethanol concentration attained about 50g/L, corresponding to a Yp/s of 0.41 g/g (fermentation efficiency of 82.1%) and Qp of 8.33 g/Lh. This value of Yp/s was similar to the reported by Ramos et. al. (2021). Those authors used the same yeast strain, *Saccharomyces cerevisiae* IR2, with cultivation in a bubble column bioreactor using medium based on hydrolysate enzymatically obtained from sugarcane bagasse pretreated by alkaline sulfite method. However, the maximum Qp values observed by those authors (1.58 g/Lh) were lower than the obtained in the present work.

As also can be seem in Figure 4.5A, microbial cells also increased, reaching 40 g/L in 4h of fermentation. Actually, ethanol production is reported as a growth-associated product (Ramos et al., 2021) After the process, cells were separated from fermented broth by decantation, an advantageous of using the strain IR2 of *S. cerevisiae*, which is flocculent (Ramos er al., 2021; Subhedar and Gogate,2015) a characteristic of this strain that favors biorefineries due to its ease separation and reuse (Ramos et al., 2021). Even so, aiming to warranty a free cell broth, an additional centrifugation was performed before distillation of ethanol.

Subsequently, ethanol was then separated from medium by distillation in a rotary evaporator. After removal, the residual liquid mixture remaining in the system (vinasse) presented great quantity of xylose (about 60 g/L), feasible for use as carbon source in fermentation process for value-added product obtaining, i.e., xylitol production. In this case, besides xylitol production, this process is an interesting approach to reduce the high organic loading of vinasse, considered as a problem for industry with environmental concerns (Aparecida et al., 2013).

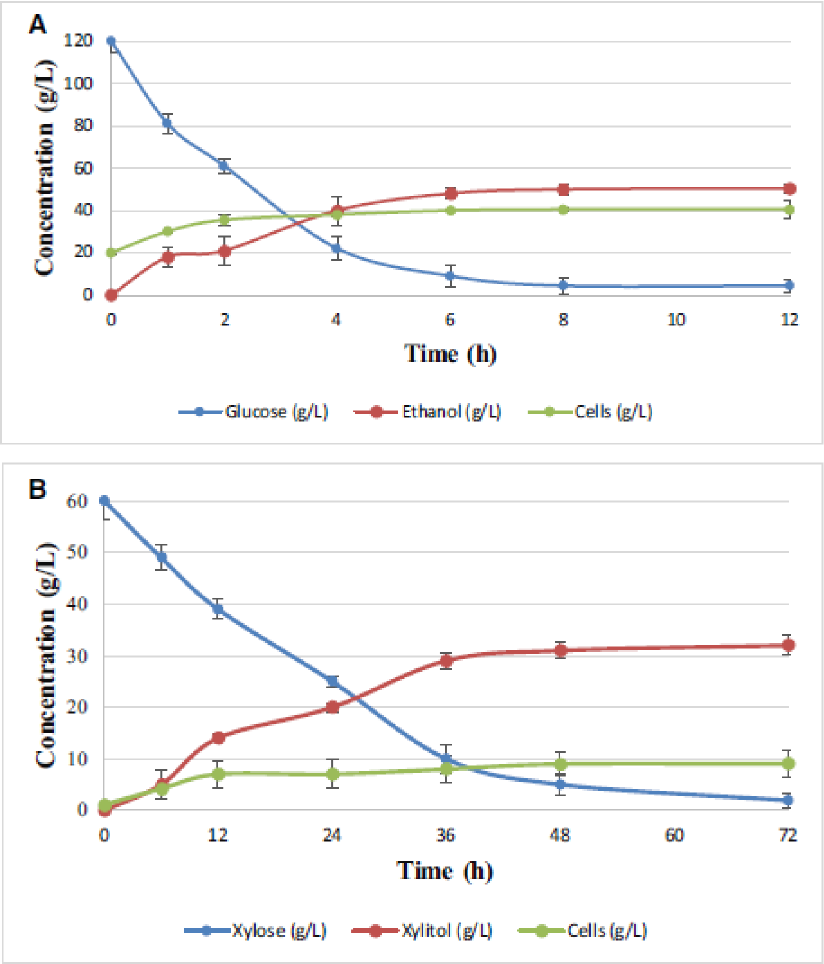

Figure 4.5. Sugar consumption and fermentation kinetic profile for (A) Ethanol with up to 82% of efficiency, and (B) xylitol production. Results are average of triplicates and are shown as average ± standard deviation (error bars)

Thus, Figure 4.5B presents xylitol production fermentation profile, showing that in 36h of the process the consumption of xylose had already achieved 84%. Furthermore, in 48h of process, maximum of 32 g/ L of xylitol was observed, corresponding to Yp/s of 0.55 g/g (60% of fermentation efficiency), and Qp of 0.64 g/ Lh.

The obtained results were similar or superior to other reported works by using different systems for xylitol production. Antunes et. al. (2021) evaluated the production of xylitol by *Candida tropicalis* UFMGBX12 in agitated Erlenmeyer flasks by using acid sugarcane bagasse hydrolysate. In that work, the fermentation was performed with a medium composed of 30g /L of xylose, 10g/L of yeast extract, 0.4g/L of magnesium sulfate and 5g/L of KH_2PO_4, and the process was conducted under 30°C and agitation of 200rpm. The authors reported production of 12g/ L of xylitol after 96h of process, showing Yp/s of 0.61 g/ g, and Qp of 0.12g/Lh. In another work, De Arruda et. al. [34] studied xylitol production by *Candida guilliermondii* FTI 20037 in 2.4L stirred tank reactor (450 rpm of agitation and 0.7 vvm of aeration) by using medium based on sugarcane bagasse hemicellulosic hydrolysate. In another work De Arruda et al., (2017) fermentation was conducted under 30°C and initial pH of 5.65g/L of xylose and supplementation with 2.0 g/L of ammonium sulfate, 0.1g/L of calcium chloride, and 10g/L of rice bran extract. The authors reported values of 0.55 g/g of Yp/s, efficiency of 60%, and Qp of 0.31g/Lh, with 35 g/L xylitol production in 84h. It is worth to mention no previous work was found reporting the production of xylitol as a sequence of ethanol production in a medium based on enzymatic hydrolysate of the cellulosic and hemicellulosic fractions of sugarcane bagasse.

As the hydrolysate used in the present work was obtained by enzymatic route, no inhibitors are expected in the medium to interfere in the evaluated fermentative processes Dos Santos et al., 2016; Bonfiglio et al., 2021; Phitsuwan et al., 2013) Besides, as the performance of the xylitol process was similar or superior compared to other reported works, the results indicate no possible metabolites of *S. cerevisiae* from ethanol fermentation remained in the vinasse in an amount enough to impair the xylitol production.

An additional consideration is related to the quality of generated wastewater. In the usual Brazilian ethanol production process, organic loading of vinasse can offer environmental concerns. Actually, there is a high volume of vinasse generated as residue of the first generation ethanol distillation. It can be used for fertigation, but its high concentration on the soil by evaporation can cause problems on subsoils (Aparecida et al., 2013). Indeed, sugarcane vinasse causes alterations in the properties of soils and biota in general (Aparecida et al.,2013; Siles et al., 2011). Taking this into account, an evaluation of organic loading of wastewater produced in the second generation sequential process proposed in the present work was performed. In this way, the values of COD, BOD5, and TOC were measured in vinasse before and after xylitol production process (cell free fermented broth). The results indicated high organic loading in the vinasse after first fermentation, but great reduction after xylitol fermentation (Table 4.2). TOC values were reduced in 91%, while COD and BOD5 values were reduced, respectively, in 93% and 97%. BOD5/COD was 0.50 in vinasse and was 0.87 in cells-free fermented broth, indicating the increase in biodegradability after xylitol production. Moreover, the fermented broth after xylitol production would have more reduction in organic loading after downstream of the product.

Table 4.2. Chemical oxygen demand (COD) and Total organic carbon (TOC) of vinasse obtained from ethanol distillation, and of the cells-free fermented broth of xylitol fermentation

Inputs	**COD (g L^{-1})**	**TOC (g L^{-1})**	**BOD (g L^{-1})**
Vinasse of First Fermentation	351.0 ± 0.50	179.0 ± 0.01	179.1 ± 0.91
Cell-free Fermented Broth	16.4± 0.05	15.64 ± 0.26	14.1 ± 1.0

4.4 Conclusion

The proposed innovative method with AOP-HC-assisted pretreatment of sugarcane bagasse was shown as promising. The influential variables in pretreatment were evaluated, and the optimized conditions corresponded to ozone flowrate of 10 mg/min and 0.61 % of H_2O_2 in the medium, with glucan and xylan hydrolysis yield values of 84% and 72%, respectively. The hydrolysate was used to produce ethanol and xylitol, with a proposal of a sequential production process using *S. cerevisiae* and *C. tropicalis.* 50 g/ L of ethanol were produced in 8h of fermentation, and an ethanol yield of 0.41 g/g was obtained. After distillation of the produced alcohol, the vinasse-based medium resulted in xylitol production of 32 g/L, corresponding to yield and productivity of 0.55 g/g and 0.64 g/Lh, respectively. The proposed strategies have potential to be used in processes similar to those ones of current first-generation ethanol industries in Brazil, with high production of ethanol by *S. cerevisiae* from glucose present in hydrolysate, followed by a high production of xylitol by *C. tropicalis* from xylose remaining in vinasses. Future works should consider economic aspects of the HC-assisted technology, also considering economic and environmental sustainability of a biorefinery using the proposed approach.

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CHAPTER V

Hydrodynamic cavitation-assisted acid pretreatment and simultaneous saccharification and co-fermentation for ethanol production from sugarcane bagasse using immobilized cells of *Sheffersomyces parashehatae*

**Chapter in preparation for submission: Prado, C. A. et al., 2023. Hydrodynamic cavitation-assisted acid pretreatment and simultaneous saccharification and co-fermentation for ethanol production from sugarcane bagasse using immobilized cells of Sheffersomyces parashehatae.*

ABSTRACT

A new alternative for hydrodynamic cavitation-assisted acid process of sugarcane bagasse (HCAAP) was proposed together with a simultaneous saccharification and co-fermentation process performed in interconnected column reactors using immobilized cells of *Sheffersomyces parashehatae*. Influent variables in HCAAP pretreatment were evaluated by statistical design, indicating that ozone flowrate of 10 mg/min of and pH 5.01 resulted in about 83% and 77% of glucan and xylan hydrolysis yield, respectively, in the further enzymatic hydrolysis process. These yield values were increased to 93% and 94% when the process was carried out adding iron sulfate to the system. In the SSCF fermentation, ethanol efficiency of 85% and volumetric productivity of 0.23g/(Lh) were obtained in batch mode operation, while in fed-batch, values of 85% and 0.39 g/(Lh) were observed, respectively. Thus, the innovative techniques evaluated were shown as promising alternatives, allowing to obtain high performance in main process of biorefineries.

Keywords: Hydrodynamic cavitation; Advanced Oxidative Processes; second generation ethanol, SSCF in interconnected columns.

5.1 Introduction

Currently, biorefineries have been highlighted as research topics, considering they include the utilization of renewable raw materials and can help the world in a transition of using fossil sources of energy and can help the world in a transition of using fossil sources of energy and carbon to a sustainable bioeconomy (Ubando et. al., 2020). Among the biomass sources, lignocellulosic materials are abundant and available worldwide, with a production that reaches up to billion tons per year (IPCC, 2021). However, the main challenge for using lignocellulosic biomass in biorefineries is its high recalcitrance, which hinders the enzymatic digestibility of polysaccharides (mainly cellulose), and hence, fermentable sugars obtaining (Gao et al., 2014; Haldar and Purkait, 2021). Thus, pretreatment is a critical stage for the use of vegetal biomass as carbon source in bioprocess, representing almost 30% of the whole process cost (Bittencourt et al., 2021; Haldar, and Purkait, 2021).

Efficient pretreatments largely reported in literature such as acid hydrolysis, alkaline pretreatment, and steam explosion can be costly and energy-intensive operations (Anderson et al., 2019; Bimestre et a., 2022). Additionally, they have presenting restrictions, for example, corrosion of equipment, and the presence of compounds that inhibit the subsequent biological stages of the process (Zheng et al., 2021; Mankar, et al., 2021). Taking this into account, interesting alternatives include emerging pretreatments such as Hydrodynamic Cavitation (HC) and the use of Advanced Oxidative Processes (AOP), that have been evaluated to overcome undesirable points of conventional pretreatments by allowing mild process conditions, and avoiding the use of high concentration of chemicals (Kim et al., 2015; Andersen et al., 2019, Prado et al., 2022).

HC pretreatment techniques present important advantages, such as low processing time, low reagents requirement the versatility of operation modes, and easy scale-up (Mabee et al., 2010; Kim et. al., 2015; Nakashima et al., 2016). However, it still a developing technology for biomass pretreatment, with just a few scientific manuscripts about this topic reported until now (Prado et al., 2022). As HC, AOP techniques are able to generate radicals that degrade recalcitrant fractions of biomass, with benefits such as a low-cost pretreatment in mild conditions, increasing the digestibility of lignocellulosic materials, as well as reducing process time and chemical inputs (Rodriguez et al., 2017; Ho et al., 2019). Thus, HC/AOP combined processes are interesting, and can result in high lignin removal by oxidation, due to the high quantity of oxidant hydroxyl radicals produced by both techniques (Mafia et al., 2014; Prado et al., 2022). HC/AOP combination were evaluated in a recent work Prado et al., (2023), which studied a pretreatment with ozone inlet and peroxide in an oxidative HC-assisted pretreatment during 10 minutes, 60ºC, and in pH of 7.0. The optimized conditions (10mg/min ozone inlet, and 0.67 % of peroxide) had 83%, and 74% of hydrolysis yield of glucan and xylan respectively.

In despite of promising results, new alternatives could be more interesting, as HC/AOP process in acid medium, which could result in different modifications in biomass, as removing more hemicellulose together with lignin, reducing the requirements of chemicals to obtain high efficiency. Additionally, as the subsequent enzymatic hydrolysis process is performed in acid medium, the material could be no washed after pretreatment, helping to obtain the pH required by cellulolytic enzymes.

All fractions of biomass should be taken into account to obtain interesting products, aiming at favoring the maximum utilization of lignocellulosic as raw material in biorefineries (Antunes et al. 2014). Regarding carbohydrate fractions, cellulose can be hydrolyzed to obtain glucose, a monomeric sugar directly fermentable by yeasts usually applied in the ethanol production industry, as *S. cerevisiae*. On the other hand, hemicellulose can represent until one-third of sugarcane bagasse (SCB) biomass, and its hydrolysis results in a xylose-enriched mixture (Dulie et al., 2021). Xylose can be also used to produce ethanol by genetically modified microorganisms or even by naturally-xylose-fermenting yeasts (Haldar et al., 2020, Jofre et al., 2021), the use of yeasts capable of efficiently assimilating both sugars, such as *Scheffersomyces stipitis* and *Scheffersomyces parashehatae*, is an interesting approach aimed to use C6 and C5 sugars from SCB hydrolysate for the production of commodities, e.g., ethanol, and high value-added products such as xylitol (Antunes et a., 2021; Farias et al., 2017). Particularly, *S. parashehatae* is a yeast that has a high tolerance for ethanol. This characteristic makes this yeast a promising option for the production of ethanol, as it allows fermentation to occur in more favorable conditions for the conversion of sugar into alcohol (Antunes et al., 2014; Faria et al., 2017).

For ethanol production, simultaneous saccharification and fermentation (SSF) or co-fermentation (SSCF) are interesting options, reducing problems of product inhibition in hydrolysis step (Tran et al., 2010). However, when in a same vessel, the process presents the same temperature for both processes, often low productive bioethanol production is observed in addition to long times fermentation (Haldar et al., 2020; Tran et al., 2010). In a recent study published by Hilares et al. (2020), the use of immobilized yeast cells of *S. stipitis* in calcium alginate was investigated for ethanol production. Those authors operated a SSCF system with column reactors, with one reator for enzymatic hydrolysis (loaded with biomass) and another for fermentation (loaded with immobilized cells). The columns were interconnected, making it possible to operate under the optimal temperature for hydrolysis (50°C) and for fermentation (30°C). Until now, this configuration with interconnected columns has been little studied, reported only by Hilares et al.(2017) and Hilares et al.(2020). Also, the previous works did not evaluated this SSCF process in fed-batch mode, which could increase the final product concentration by using higher loading of solid per batch.

The present work evaluated innovative approaches for the HC/AOP acid pretreatment of SCB and for ethanol production. SCB was chosen considering its high availability in countries as Brazil (Silva et al., 2016). The pretreatment was performed by combining HC/AOP in an acid medium, varying ozone inlet and pH value, also evaluating the potential of using iron sulfate as catalyst. In SSCF configuration, an interconnected columns strategy was evaluated in batch and fed-batch operation mode. Then, the present work novelties include alternatives for biorefineries which, at the best of our knowledge, were not previously reported in literature: Hydrodynamic cavitation-assisted acid pretretament process (HCAAP) and use of iron sulfate as catalyst; SSCF ethanol production in fed-batch operation mode and the use of *S. parashehatae* to produce ethanol in this system.

5.2. Material and Methods

5.2.1 Sugarcane bagasse

Ipiranga Agroindustrial LTDA (Descalvado, São Paulo, Brazil) donated the Sugarcane bagasse (SCB). This raw material was dried under sunlight until 10% of moisture and followed milled until particle size between 0.60 mm and 1.18 mm.

5.2.2 Hydrodynamic cavitation-assisted oxidative acid pretreatment

Raw sugarcane bagasse was pretreated in a hydrodynamic cavitation system described elsewhere (Hilares et al., 2017). The system is composed of a main tank, in which the biomass is retained, a centrifugal pump (1.5CV) and a recirculation tank, corresponding to 3L of total volume. Between the centrifugal pump and the main tank, there was an orifice plate, responsible for provoking the cavitation effect in the tank. The pressure in the system can be controlled by the manometer coupled to the pump, allowing adjustments with the rotation control by a variable frequency drive. To the main tank, an Ozonator Ozone & Life (Waterone, Kansas City – KN, USA) was coupled to provide O_3 to the oxidative process. Each experiment employed 35g of raw dry SCB in 3L of volume and the process was in 10 min, at 60°C. The evaluated inlet variables were ozone flow (from 0 to 10 mg/min) and pH (from 4 to 7), adjusted in the medium by adding 1 mol/L sulfuric acid aqueous solution. The studied range for ozone flow was based on the limits of the capacity of the available system.

A 2^2 face-centered central composite design with triplicate at the centerpoint was performed. Obtained results were analyzed aided by the software Design-Expert (Stat-Ease, Inc. Minneapolis, US) and Statistica (StatSoft, Inc. V.5 Tulsa, OK, USA). Empirical models and optimized conditions to maximize hydrolysis yields were defined, and experiments in these conditions were performed in triplicate to validate the models prediction. Response variables were the composition of the pretreated material and the hydrolysis yield of the carbohydrate fractions of the material, which was evaluated in an enzymatic hydrolysis step carried out in Erlenmeyer flasks according to section 5.2.3

Under optimized conditions, a test of a catalytic process was performed by adding 15 mg/L of iron sulfate, and the process was conducted by 10 to 30 minutes and compared with a control experiment without iron sulfate. The results were evaluated by performing enzymatic hydrolysis of pretreated material, carried out in Erlenmeyer flasks according to section 5.2.3.

5.2.3 Enzymatic Hydrolysis in Erlenmeyer flasks to evaluate the pretreatment performance

Enzymatic hydrolysis was carried out in Erlenmeyer flasks (125mL) with 5% of solid loading (unwashed pretreated bagasse) in a citrate buffer solution (50 mM) with pH 4.8. Cellic® CTec2 (Novozymes Latin America Ltda, Brazil), a commercial cellulases enzyme blend, was used with a loading of 20 FPU/ g of dry pretreated SCB. Reaction was performed at 50°C and 200 rpm for 24h. Concentration of hexoses and pentoses released in hydrolysis were analyzed by high performance liquid chromatography (HPLC). Hydrolysis yields were calculated as Equations 1 and 2 (Lu et al., 2012).

$$Glucan\ hydrolysis\ yield(\%) = \frac{(G*0.9*V)}{(CC*M)}*100\% \qquad \text{Eq. 1}$$

$$Xylan\ hydrolysis\ yield(\%) = \frac{(Xyl*0.88*V)}{(CC*M)}*100\% \qquad \text{Eq. 2}$$

Where:

G = concentration of glucose g/ L in hydrolysate

Xyl = concentration of xylose g/ L in hydrolysate

V = total volume of reactional medium

CC = percentage of glucan or xylan in raw SCB

M = mass of SCB used in hydrolysis enzymatic

5.2.4 Ethanol production by *Saccharomyces parashehatae* UFMG-HM 52.2 in SSCF system with interconnected columns in batch and fed-batch mode

5.2.4.1 Microorganism, inoculum preparation and cells immobilization

S. parashehatae was obtained from stock cultures available in the Sustainable Bioproducts Laboratory (LBIOS) at the Engineering School of Lorena–University of Sao Paulo, Brazil, and was kindly donated by Federal University of Minas Gerais (UFMG). The inoculum was prepared in 125 mL Erlenmeyer flasks containing 50 mL of medium, and the cultivation was carried out in a Bio rotary shaker (ERETZBIO, São Paulo, Brazil), for 48 hours, 150 rpm, at 30ºC.

For the yeast *S. parashehatae* UFMG-HM 52.2, the inoculum medium was composed of 50 g/ L of glucose, 10 g/ L of bacteriological peptone, 20 g/L of yeast extract, 30 g/ L xylose, 20 g/ L yeast extract, 2 g/ L NH_4SO_4, 0.1 g/ L $CaCl_2.2H_2O$, with initial pH adjusted to 5.5.

Subsequently, cells were recovered by centrifugation at 5000g for 10 min, washed, and suspended in water (distilled) to obtain a suspension with high cell density. Then, cells were submitted to the immobilization process, which consisted of preparing a calcium alginate cells suspension. Then, the mixture was dripped through a 3 mm diameter hose into a calcium chloride solution, which induces the formation of a calcium alginate capsule around the cells (Carvalho et al., 2004). The obtained concentration was 8 mg of cells per mg of immobilized system.

5.2.4.2 Ethanol production in the SSCF system with interconnected columns

Sugarcane bagasse, pretreated in the optimized conditions, was employed in an SSCF system composed of two reactors, connected by tubes, with a circulating liquid phase. One reactor is the column in which biomass is kept to by hydrolyzed, maintained at 50°C (optimum temperature for enzymatic hydrolysis with the designated enzyme cocktail). The second reactor is the fermentation tank, which is kept at 32°C and is homogenized by aeration. 110g of immobilized cells of *Sheffersomyces parashehatae* UFMG-HM 52.2 were added to the second reactor. Also, 190 mL of a liquid phase composed of citrate buffer (50mM, pH 4.8), Cellic CTec2 (20 FPU/g of dry PSB), and nutrients (3g/L of yeast extract, 5g/L of peptone, 0.1g/L of calcium chloride and 2g/L of ammonium sulfate) was added to the system. The air flow rate was kept at 30 mL/min and the circulation of the liquid phase between the reactors was controlled by a peristaltic pump ALITEA-XV (Bioengineering AG-Wald, Switzerland), with a 18mL/min flow. Samples were taken regularly to check sugar and ethanol concentrations.

The system was operated in batch and fed-batch mode. For batch, 16 g $_{\text{dry mass}}$ of pretreated biomass was added to the first reactor at the beginning of the process, which was performed for 72h.

For fed-batch operation, similar conditions were used, with the total solids added correspondent to 27 g of pretreated SCB, but only loading with 16 g at the beginning of the process, and 11 g added in 10h of operation, with all process during 72h. In fed-batch system, enzymatic preparation (20 FPU/ g of biomass) was added at the beginning of the reaction and also together with the SCB added in 10h of process.

For both, batch and fed-batch processes, the pretreated bagasse used was correspondent to the material obtained in HC-assisted acid pretreatment performed with addition of iron sulfate (pretreatment carried out for 30 min).

Sugars concentration was analyzed by HPLC, as described in 5.2.5.

5.2.5 Analytical methods

5.2.5.1. Humidity

The sugarcane bagasse moisture was determined by using an infrared balance Mark M163 (BEL Engineering, Piracicaba-SP, Brazil).

5.2.5.2. Compositional characterization of bagasse, analysis of sugars, acetic acid, ethanol, and xylitol

Characterization of biomass was performed according to Mesquita, Ferraz, and Aguiar (2016). Analysis of sugars (glucose, xylose, arabinose), xylitol and ethanol concentration, samples were performed by HPLC. The used equipment was a HPLC Agilent 1200 series (Agilent Technologies, Inc., USA) equipped with a Refractive index detector RID-6A and HPX-87H (300 x 7.8 mm) column (Bio-Rad, USA). Samples were analyzed in the following conditions: 45°C column temperature, 0.01N sulfuric acid as the mobile phase, 0.6mL/min flow rate and 20µL of injection volume.

5.3. Results and Discussion

5.3.1 Evaluation of influent variables in HC-assisted Acid Pretreatment of SCB

The raw biomass presented 40% glucan, 26% hemicellulose (24% xylan, 1% arabinan, and 1% acetyl), 25% lignin, 1% ash, and 3% extractives. Table 5.1 presents the results of the statistical design carried out to evaluate the effects of the variables O_3 flow (0 to 10 mg/L) and pH (4.0 to 7.0) on the glucan and xylan yield from enzymatic hydrolysis conducted with the resulting material from each studied AOP/HC acid pretreatment condition. Furthermore, the remaining biomass composition, hemicellulose, and cellulose removal are also presented for each studied condition.

As shown in Table 5.1, the pretreatment resulted in modifications to the SCB composition, rendering it as a material enriched in cellulose and with lower lignin content. Indeed, lignin was the main fraction removed by the pretreatment (46-56% removal), followed by xylan (27-50%), while glucan removal was lower than 15%.

The hydrodynamic cavitation effect on the pretreatment of lignocellulosic biomass has been reported in the literature (Kim et al., 2015; Hilares et al., 2020), which verifies that the chemical and physical action of cavitation provides benefits such as mild conditions for oxidative degradation and a reduction in the lignin fraction of biomass under a short reaction period (Hilares et al., 2019). The HC phenomenon results in high energy released into the acid medium, contributing to the generation of highly oxidative radicals (Xie et al., 2017). Furthermore, the addition of an oxidizing agent (ozone) to the acid medium further contributes to process efficiency due to its high oxidative power (2.78V for ozone). HC and oxidizing agents act synergistically on lignocellulosic material, breaking down lignin molecules and removing hemicellulose. Hemicellulose removal is significantly increased in an acid medium, more so than in other pretreatments such as alkaline pretreatments (Philippini et al., 2020; Ubando et al., 2020; Prado et al., 2022).

The HC effect is also associated with mechanical effects such as high-speed microjet action and shockwave generation due to violent cavity collapse. This improves cellulose and hemicellulose enzymatic conversion into fermentable sugars, as the specific surface area grows and the total pore and micropore volumes increase (Hilares et al., 2017; Kim et al., 2015).

As observed in Table 5.1, the compositional modification resulted in different values of glucan and xylan hydrolysis. The highest yield values (84% for glucan and 78% for xylan) were observed using an ozone (O_3) flow of 10 mg/L and a pH of 4 in the medium.

The obtained data were statistically analyzed, and empirical models (Table 5.2) were developed to explain the experimental behavior of the dependent variables, namely glucan and xylan hydrolysis yield. ANOVA (Table 5.3) was performed for the models, and it was found that both independent variables coefficients were significant for glucan and xylan hydrolysis yields ($p < 0.05$). Moreover, the coefficient correspondent to the interaction of variables was also significant for xylan hydrolysis yield. Both models were reduced by removing non-significant terms (except if required for model hierarchy), and the R^2 values were 91% and 85% for the models of glucan and xylan hydrolysis yield, respectively, taking into account their significance at a 95% confidence level. As below discussed, these models were able to predict results at optimized conditions and generate the surface responses presented in Figure 5.1.

Table 5.1. Composition and removal of fractions of sugarcane bagasse pretreated by acid HC-assisted oxidative process technology according a 2^2 face-centered central composite design performed to evaluated the influence of important variables in the process

Experiment	Conditions (codified values in parenthesis)		Solid Recovery (%)	Pretreated Sugarcane Bagasse composition (%)			Removal (%)			Hydrolysis yield (%)	
	Ozone (mg/min)	pH		Glucan	Xylan	Lignin (%)	Glucan (%)	Xylan (%)	Lignin (%)	Glucan (%)	Xylan (%)
1	0 (-1)	4 (-1)	71	50.08	20.08	25.34	11.11	40.31	30.84	49.74	28.26
2	10 (+1)	4 (-1)	63	54.01	19.01	19.82	7.23	40.12	49.61	84.04	77.93
3	0 (-1)	7 (+1)	70	52.82	24.82	19.84	7.59	27.08	46.96	48.96	36.85
4	10 (+1)	7 (+1)	70	54.04	22.32	19.91	5.56	35.83	28.77	52.77	58.95
5	0(-1)	5.5 (0)	63	56.36	21.76	21.41	11.48	42.25	52.85	54.93	58.92
6	10 (+1)	5.5 (0)	64	56.06	27.06	21.47	10.30	41.34	56.27	79.67	70.43
7	5 (0)	4 (-1)	72	52.7	21.7	20.19	5.30	37.01	44.96	67.24	32.42
8	5 (0)	7 (+1)	64	55.4	21.4	19.80	11.14	36.81	47.81	61.63	46.06
9	5 (0)	5.5 (0)	72	52.7	23.7	17.79	5.34	31.01	43.16	79.22	71.88
10	5 (0)	5.5 (0)	72	52.4	23.4	17.78	5.70	29.77	51.17	78.26	68.23
11	5 (0)	5.5 (0)	72	52.4	23.4	17.15	10.06	29.89	52.64	77.24	70.48

Table 5.2 Adjusted Models for the response variables enzymatic hydrolysis yield for glucan and xylan, according to the results obtained in experimental design. A: ozone flow (mg/min); B: pH

Response Variable	**Empiric model**
Glucan hydrolysis yield (X_1%)	$X_1 = -38.87 + 1.56 \cdot A + 42.42 \cdot B - 0.13 \cdot A \cdot B - 3.81 \cdot B^2$
Xylan hydrolysis yield (X_2%)	$X_2 = -222.46 + 0.34 \cdot A + 108.02 \cdot B - 9.74 \cdot B^2$

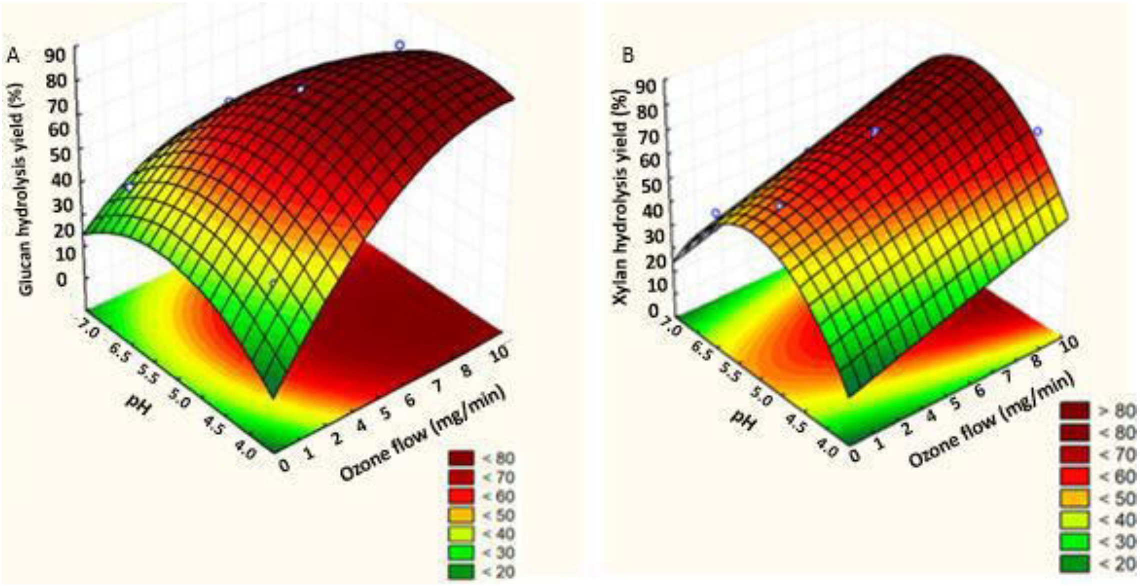

Figure 5.1. Response surface for the yield of enzymatic hydrolysis of glucan (A) and for the yield of enzymatic hydrolysis of xylan (B), as a function of peroxide concentration and ozone flow used in HC-assisted oxidative pretreatment of sugarcane bagasse

As shown in Figure 5.1 and in the empirical models in Table 5.2, the O_3 inlet had a positive effect on the hydrolysis of glucan and xylan, as observed in the empirical models that showed positive linear terms for ozone flow. Although statistical analysis suggests benefits of an additional increase in the value of this variable, the current study has already covered the maximum level feasible for the used experimental equipment (10 mg/min ozone flow). Actually, a gradual increase in hydrolysis yields with the addition of this oxidative agent is capable of inducing modifications in the structure and composition of the biomass due to its high oxidative power (2.78V) (Girón-Navarro et al., 2021; Ghanbari et al., 2020).

As also can be observed in Fig. 5.1 and Table 5.2, the enzymatic hydrolysis of the carbohydrate fraction in sugarcane bagasse acid-pretreated by hydrodynamic cavitation-assisted oxidative process is influenced at a quadratic level by the pH of the sulfuric acid solution, indicating a possible pH condition in the studied range that can maximize the enzymatic hydrolysis yield of the biomass. Indeed, lignocellulosic biomass pretreated under acid conditions undergoes structural modifications, as verified in the literature, resulting in an enhanced hydrolysis yield. However, acid conditions favor the ozone action (Barik et al., 2016), resulting in very intensive oxidative pretreatment which could generate oxidation products that sediment in the material structure, impairing the enzymatic hydrolysis (Wang et al., 2018; Lai et al., 2014).

The empirical models in Table 5.2 were used to determine an optimized condition aiming to maximize the response variables; a specific tool of the software Design-Expert was used maximization for hydrolysis yield for sugars, obtaining 10 mg/min of ozone flow rate and pH 5.10 as optimized conditions targeted to maximized values of glucan and xylan yield. The predicted results for enzymatic hydrolysis of glucan and xylan using 10 mg/min of ozone flow rate and pH 5.10, considering a 95% confidence interval, were 81.31±10.93% and 78.57±12.41% (average ± 95% confidence level interval), respectively. This pH is a good option because the hydrolysis process used a similar pH (4.8), and in this condition, no new adjustment is necessary.

Table 5.3. Analysis of variance (ANOVA) and coefficient of determination (R^2) for the fitted models for hydrolysis yield (glucan and xylan) as a function of the studied variables

Yield of enzymatic hydrolysis of glucan (%)						**Yield of enzymatic hydrolysis of xylan (%)**					
Soure	**Sum of square**	**Degrees of freedom**	**Mean square**	**F**	***P***	**Source**	**Sum of squares**	**Degrees of freedom**	**Square mean square**	**F**	***P***
Model	1904.66	5	380.92	10.31	0.0108	Model	2354.19	3	784.73	10.04	0.0063
Ozone flow (A)	810.73	1	810.73	22.57	0.0051	Ozone flow (A)	1033.86	1	1033.86	13.22	0.0083
pH (B)	235.12	1	235.12	6.80	0.0477	pH (B)	10.27	1	10.27	0.13	0.7277
AB	207.78	1	207.78	5.71	0.0618	B^2	1310.06	1	1310.06	16.76	0.0046
A^2	286.40	1	276.40	7.86	0.0371	Residual	547.30	7	78.19		
B^2	180.96	1	180.96	5.07	0.0721	Lack of fit	539.58	5	107.92	27.95	0.0349
Residual	179.92	5	35.98			Pure Error	7.72	2	3.86		
Lack of fit	179.83	3	59.94	1436.36	0.0007	Total	2901.49	10			
Pure Error	0.08	2	0.04			R^2	0.83				
R^2	0.91										

Experiments in the prediction were performed in triplicates, obtaining 83.94% ± 5.39 of yield for glucan enzymatic hydrolysis and 74.08% ± 4.86 (average ± standard deviation) of yield for xylan enzymatic hydrolysis. These results had lower variation compared to the predicted data (in the range of 95% confidence level), thus confirming the models. The obtained results also confirm the beneficial effect of the pretreatment process using oxidative processes assisted by hydrodynamic cavitation in mild acid conditions. The pretreated biomass composition was determined, obtaining 58.40% ± 3.71 of cellulose, 26.45% ± 1.68 of xylan, and 20.62% ± 6.97 of lignin.

After the optimization of the HCAAP of the lignocellulosic biomass, it was found that the hydrolysis yield had not reached the maximum already reported with this technology, such as in a study by Hilares et al. (2019), which found a glucan hydrolysis yield of 93%. Therefore, upon analyzing the pretreatment processes, an opportunity to improve this yield through oxidative processes with the presence of a catalyst was observed. The literature suggests that the use of iron as a catalytic agent aids in the degradation of organic compounds as an efficient methodology (Gholampour et al., 2016; Hu and Long, 2016). Then, it was decided to test the use of 15mg/L (Gholampour et al., 2016) of iron sulfate for HCAAP under the optimized conditions at different times, in order to increase enzymatic digestibility.

Then, the optimized conditions were used with a longer pre-treatment time and a catalyst process with the presence of iron sulfate (as shown in Figure 5.2). The values obtained for the hydrolysis yield of glucan and xylan for experiments carried out for a 10-minute time period without the presence of ferrous sulfate (80.77% ± 3.10 and 76.18 ± 2.99, respectively) are consistent with the values predicted by the experimental design models for the optimized condition (81.21 ± 10.93% and 78.97 ± 12.41%, for glucan and xylan, respectively). An improvement in the glucan hydrolysis yield is observed when the pre-treatment time is extended, reaching yields above 90% for 20 and 30 minutes without the presence of the catalyst (93.75% and 94.69%, respectively). For the xylan hydrolysis yield, the values obtained were similar to those for materials pre-treated at different times. With the presence of the catalyst $FeSO_4$, on the other hand, a glucan hydrolysis yield above 90% was obtained in just 10 minutes of pre-treatment.

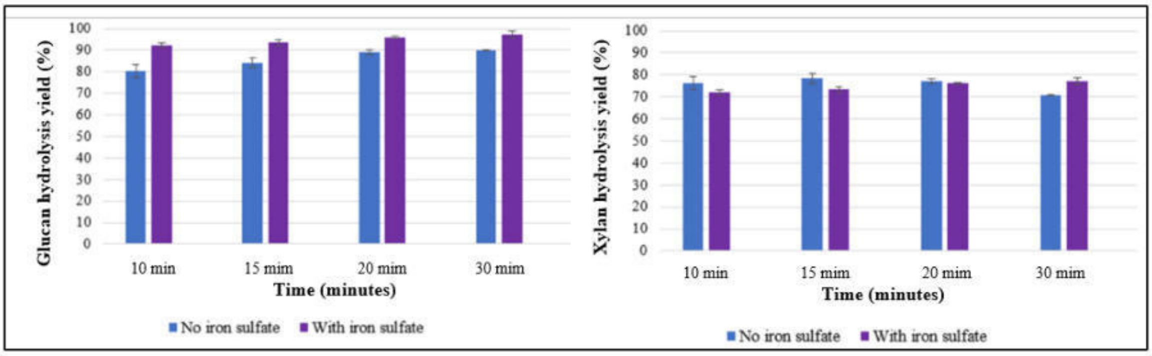

Figure 5.2. Yield of enzymatic hydrolysis of glucan (A) and of xylan (B) obtained in HC-assisted acid pretreatment with and without addition of catalyst iron sulfate

5.3.2 SSCF Fermentation production of ethanol

A two-reactor system with a circulating liquid phase was employed to evaluate the production of ethanol by *S. parashehatae* simultaneously occurring with the hydrolysis of sugarcane bagasse, which was previously pretreated for 30 min using the pretreatment with iron sulfate described in previous section. This material had the following composition: 53% of glucan; 27% of xylan; 18% lignin.

The batch experiment was performed in triplicate, and the results are reported in Figure 5.3, presenting the concentrations of substrate (xylose and glucose), cells, product (ethanol), and by-product (xylitol). The final ethanol concentration obtained after 72 hours of the process was 17 g/L, with a co-production of 4 g/L of xylitol. Then, a volumetric productivity of 0.24 g/ (L.h) was observed. Regarding to the global efficiency of the process, it was estimated in 85%, calculated as the ratio between the obtained ethanol concentration and the maximum which could be obtained if all the present glucan and the xylan were hydrolyzed, and if the ethanol fermentation had the theoretical yield of 0.511 g/g.

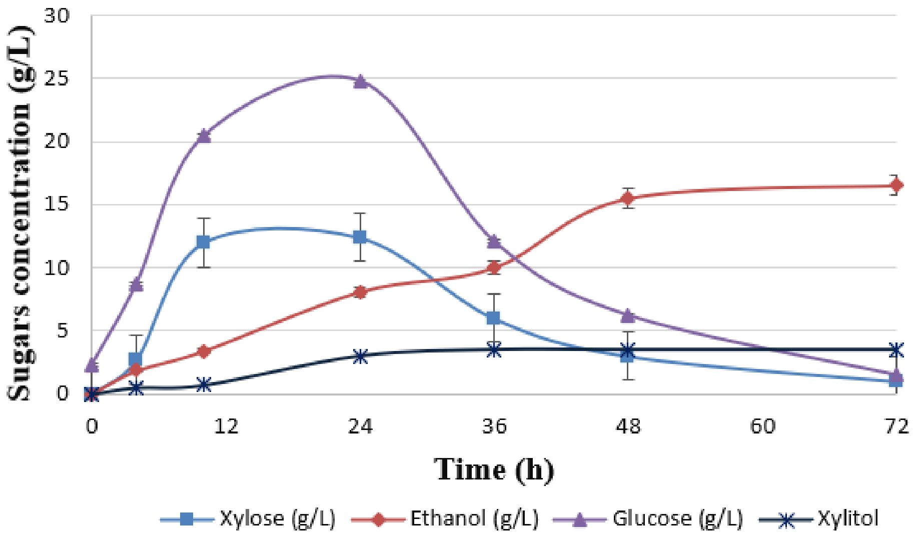

Figure 5.3. Kinetic profile of concentration of substrate, cells and products in a batch SSCF- system for the production of ethanol by *Sheffersomyces parashehatae* UFMG-HM 52.2. Results are average of triplicates and are shown as average ± standard deviation (error bars).

In the SSF configuration, hydrolysis and fermentation are carried out simultaneously, mitigating inhibition by product as the sugars are consumed by the microorganism as they are released by the hydrolysis of cellulose.

SSF process was evaluated in a similar system by Hilares et al (2017), which reported 18 g/L of ethanol using the yeast *S. stipis*, with 84 % of efficiency. In other work with similar system, Hilares et al (2020) reported 23 g/L of ethanol and 81% of efficiency, also using *S. stipis*.

As also can be observed in Figure 5.3, low concentration of xylitol was obtained. Indeed, xylitol is a by-product of ethanol-producing yeasts which are able to metabolize pentoses. This behavior is different from xylitol-producing yeasts such as *Candida tropicalis*, which has NADPH-dependent xylose reductase, presenting, under oxygen limited conditions, cofactor imbalance in the first steps of xylose metabolism, resulting in xylitol accumulation (Santos et al., 2015). Ethanol-producing yeasts have NADH-dependent xylose reductase, avoiding the cofactor imbalance, resulting in ethanol accumulation under oxygen limited conditions.

When the process was conducted in fed-batch operation mode, 28 g/L of ethanol and 5 g/L of xylitol were produced in 72 hours (Figure 5.4). This value of ethanol concentration corresponded to a volumetric productivity of 0.39 g/(L.h), and a global efficiency of 85%.

The productivity obtained in fed-batch operation was higher than the obtained in batch process, showing the promising approach of fed-batch system, which allows a higher solid loading, intensifying the process by processing more biomass in the same equipment at the same time. Actually, as the biomass is enzymatically hydrolyzed, more space is turning available in the reactor, allowing to add more pretreated material.

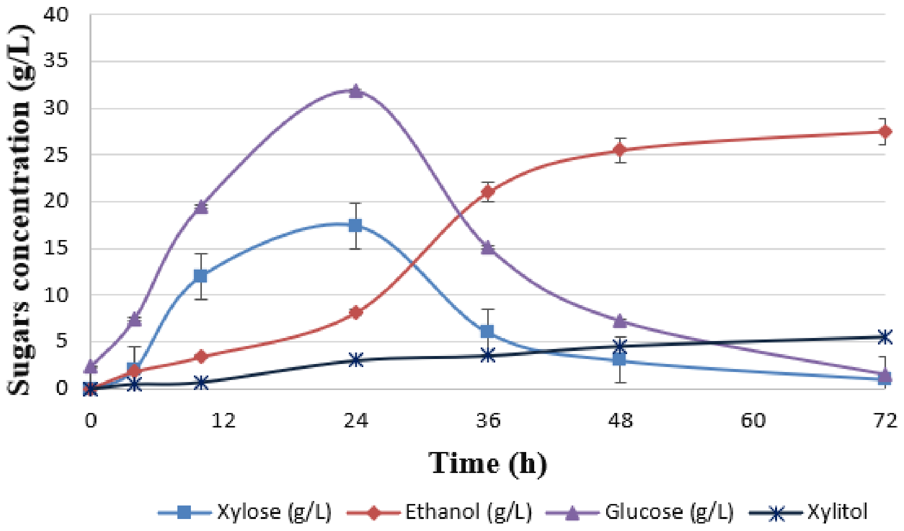

Figure 5.4. Kinetic profile of concentration of substrate, cells and products in a SSCF-fed- batch system for the production of ethanol by *Sheffersomyces parashehatae* UFMG-HM 52.2. Results are average of triplicates and are shown as average ± standard deviation (error bars)

5.4 Conclusion

The proposed innovative method which combined oxidants agents in a HCAAP pretreatment of sugarcane bagasse, has been shown to be a potential alternative for biorefineries. Influential variables in the pretreatment process were evaluated, and optimized conditions were determined, corresponding to an ozone flow rate of 10 mg/L and a pH of 5.01 in the medium. These conditions resulted in glucan and xylan hydrolysis yield values of 84% and 78%, respectively. The use of iron sulfate allowed to increase these yield values, resulting in more than 90% of glucan hydrolysis yield in 10 min of process. The resulting hydrolysate was then used to produce ethanol using a proposed SSCF production process that employed *S. parashehatae*. The fed-batch process resulted in 28 g/ L of ethanol production and a volumetric productivity of 0.39 g/(L.h) in 72 hours of fermentation, allowing to increase the solid loading in the system. By using the strategy of SSCF with interconnected columns, the enzymatic hydrolysis can be efficient even with higher solids loading, due to the mitigation of inhibition of the process by hydrolysis products, associated to keeping the optimum temperature for each biological step.

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CHAPTER VI

A proof of concept of hydrodynamic cavitation as a feasible new technology to improve the efficiency of enzymatic hydrolysis of carbohydrate fractions of sugarcane bagasse

**Chapter submitted for publication: Prado, C. A. et al., (2023c). A proof of concept of hydrodynamic cavitation as a feasible new technology to improve the efficiency of enzymatic hydrolysis of carbohydrate fractions of sugarcane bagasse., Biomass Conversion and Biorefinery.*

ABSTRACT

A pretreatment step is required to increase the enzymatic digestibility of biomass in biorefineries, and, last years, hydrodynamic cavitation (HC) has been reported as a promising alternative. In the same way, the enzymatic hydrolysis step itself could be also enhanced if assisted by HC. In the present work, a new alternative of hydrolysis included a hydrodynamic cavitation-assisted process. Thus, the effect of HC in the enzymatic hydrolysis of sugarcane bagasse was evaluated, firstly by performing an experimental design taking enzyme loading and temperature as studied variables. Conditions were optimized, and then control experiments were carried out with enzymatic hydrolysis in Erlenmeyer flasks, in system without HC, and in an ultrasound assisted- process. Also, a sequential strategy to increase the concentration of released sugars in the hydrolysate was evaluated, performing four cycles of fed-batch hydrolysis process. Under optimized conditions (18 FPU/g and 50°C), 93% and 77% of glucan and xylan enzymatic hydrolysis yield, respectively, were observed after 12 hours of process. These yields were higher than those observed for ultrasound-assisted enzymatic process, with 51% less energy consumption. A repeated fed-batch strategy was used and was shown as interesting to increase the sugars concentration in the hydrolysate, without reduction in hydrolysis yields. The potential of the proposed technique was demonstrated, and HC was proved as an interesting alternative to assist enzymatic hydrolysis.

Keywords: Hydrodynamic cavitation; Enzymatic hydrolysis; Sugarcane bagasse; Repeated fed-batch process.

6.1 Introduction

The valorization of lignocellulosic biomass into high value products is a great opportunity of development in many countries, with economic and environmental advantages. Agro-industrial residues and by-products as sugarcane bagasse, corn stover, wheat straw, and others, are largely available in many countries (Kumar et al., 2020; Bittencourt, et al., 2021). Aiming to convert efficiently these materials, efforts have been focused on the development of new alternatives of pretreatment, and new configurations of hydrolysis and fermentation. Among the bottlenecks to biomass processing, pretreatment and enzymatic hydrolysis are among the most important, this last step expensive due to drawbacks as high enzyme cost and long process time (Ogbonna et al., 2001; Hernández-Pérez et al., 2019, Dulie et al., 2021, Bathia et al., 2021).

Hydrodynamic Cavitation (HC)-assisted process has attracted the interest of research in biomass processing last years, considering studies reported in the pretreatment step (Prado et al., 2022; Bimestre et al., 2022). Cavitation results in generation of oxidative radicals, shock waves and microjets, which synergically change the composition and structure of lignocellulosic biomass (Kim et al., 2015; Hilares et al., 2017a; Bimestre et al., 2020, Thangavelu et al., 2022). HC was also used by Thangavelu et al. (2018) to assist a pretreatment of corncob in presence of enzymes (laccases), increasing significantly the lignin removal. However, at the best of our knowledge, until now there are no reports of using HC to assist the enzymatic hydrolysis step of carbohydrate fractions of a pretreated biomass, although literature indicates this possibility, considering ultrasound cavitation effect was previously reported (Subhedar and Gogate, 2015; Adewuyi & Deshmane, 2015; Singh et al., 2015), reducing the diffusion-limiting barrier and improving the enzyme/substrate binding, with an increase in reaction rate. Subhedar and Gogate (2015) reported that, under effect of ultrasound cavitation, the reducing sugars release increased in 2.4 times in the hydrolysis process. It is expected that, as occurs in biomass pretreatment, the energy efficiency per sugar generated could be better in HC process compared to ultrasound.

Therefore, the novelty of this work corresponded to evaluate, by the first time, the use of hydrodynamic cavitation to assist the enzymatic hydrolysis step of carbohydrate fractions of sugarcane bagasse. The used bagasse was previously pretreated also using an HC-assisted process. Sugarcane bagasse (SCB) was chosen as model of study considering the great availability of this biomass as by-product of sugar-alcohol industry is countries as Brazil (Lopes et al., 2018; Ramos et al., 2021; Zheng et al., 2021). The objective was to perform a proof of concept, showing the HC not resulted in deleterious effect in enzymes activity enough to impair the process, but, on the contrary, increased the enzymatic hydrolysis performance compared to systems without HC or assisted by ultrasound.

6.2. Material and Methods

6.2.1. Raw material and pretreatment

Sugarcane bagasse (SCB) sample was kindly donated by Ipiranga Agroindustrial-LTDA (Descalvado, São Paulo, Brazil). It was dried at sunlight until about 10% of humidity and classified Tyler standard sieves, with the fraction between 16 and 24 MESH used in the experiments. The moisture content of lignocellulosic biomass was determined gravimetrically using a Mark M163 infrared balance (BEL Engineering, Piracicaba-SP).

SCB was then pretreated by an HC-assisted advanced oxidative process based on the methodology described by Hilares et al. (2020), added with 0.71% hydrogen peroxide, and with an ozone inlet flow rate of 10 mg/L supplied by an Ozonator (Ozone & Life, Waterone, Kansas City- KN, EUA). The pretreatment was performed for 10 minutes at

60 °C in a batch HC reactor (Hilares et al., 2020). The raw and pretreated biomass were characterized regarding its composition (Mesquita, Ferraz, and Aguiar, 2016).

6.2.2. Hydrodynamic cavitation-assisted enzymatic hydrolysis

Enzymatic hydrolysis was performed in a hydrodynamic reactor system described elsewhere (Hilares et al., 2017b), with the cavitation generated by a plate of 16 holes, each one with 1mm of diameter, with the liquid medium recirculating in the system with a flowrate of 5 m^3/h.

Process was optimized by performing experiments according to a 2^2 face-centered composite experimental design with triplicate at the center point. For all runs, the system was loaded with 2.5 liters of 50 mM citrate buffer pH 4.8, and 30 grams of pretreated SCB.

The process was performed for 8 hours, varying the temperature (40-50°C) and enzyme loading (5-20 FPU per gram of dry biomass). The enzyme preparation used was cellulase Cellic® CTec2 (Novozymes Latin America Ltda., Brazil). The answer variables were enzymatic hydrolysis yield of glucan and of xylan (Table 1). The results obtained in the experimental design were analyzed aided by the software Design-expert (Stat-Ease, Inc., Minneapolis, US), and Statistica for Windows (StatSoft, Inc. V.5 Tulsa, OK, USA).

Under optimized conditions, a sequential strategy to increase the concentration of released sugars in the hydrolysate was evaluated, performing four cycles of fed-batch hydrolysis process. In this way, HC reactor was firstly loaded with 30 g of pretreated biomass and 2.5 L of 50 mM citrate buffer pH 4.8, and then fed with additional 10 g of pretreated biomass after 6h of process. For both times of process (0h and 6h), cellulases were also added at proportion of 18 FPU g^{-1} of fed dry biomass. After 12h, biomass was taken from the reactor, and new pretreated biomass was added, starting a new cycle under same conditions of loading of enzymes and biomass described for the first cycle (hydrolysate was kept inside the reactor). The procedure was repeated until complete four cycles of 12h each.

6.2.3 Control experiments: Erlenmeyer flasks, ultrasound-assisted process, and process in HC-system without cavitation device

Control experiments were performed under conditions optimized according to section 6.2.2.

Enzymatic hydrolysis of pretreated biomass was carried out in 125 mL Erlenmeyer flasks loaded with 0.24 g of sugarcane bagasse, 20 mL of 50 mM citrate buffer pH 4.8, and 18 FPU of cellulase Cellic® CTec2 per gram of dry biomass. Flasks were kept in a rotary shaker at 200 rpm, at 50 °C for 12 h.

Other control experiment was performed using pretreated SCB in the cavitation system, but without orifice plates to generate cavitation (control in reactor without HC).

Also, another control experiment was performed to compare the HC with the ultrasound cavitation-assisted process. Ultrasound assisted hydrolysis of pretreated SCB was performed using a 250 mL Becker in a probe ultrasonic processor (VCX 750, PLANATC-CBU100, USA) with a power of 300 W (40% amplitude) and a frequency of 20 kHz, similar to conditions used by Kim et al., (2015). This process was carried out for 12h with 1.2g of SCB, 18 FPU/g of enzyme loading in 100 mL of 5 mM buffer citrate solution (pH 4.8).

Additionally, to verify the enzyme stability under the experimental conditions, HC system was loaded with 2.5 L of 5 mM buffer citrate solution (pH 4.8) and 540 FPU of cellulases. The FPU of enzymes in the liquid medium was then monitored for 48h of operation.

6.2.4 Analytical methods

Bagasse obtained after enzymatic hydrolysis was washed and dried with abundant distilled water to a neutral pH (pH 7), and dried at room temperature for subsequent characterization in terms of glucan, xylan, and lignin, according to Mesquita, Ferraz, and Aguiar (2016). The liquid obtained in the processes was analyzed by High-Performance Liquid Chromatography (HPLC).

Concentrations of xylose, glucose, arabinose, and acetic acid were determined by HPLC in an Agillent Technologies 1200 Series chromatograph equipped with a refractive index detector and a Bio-Rad Aminex HPX-87H column (300×7.8mm), at 45°C, 0.005 mol/ L sulfuric acid as eluent in a flow of 0.6 mL/ min, and sample volume of 20 μL. Samples were previously diluted in deionized water and filtered in Sep-Pak C18 column (Waters Stevens Creek Blvd, Santa Clara, United States).

Energy consumption was measured by a power meter LCD Display Digital DDM15SD (Anself-LTD, Charleston, USA). This equipment measures the power of the equipment during the time of use, then this potential is multiplied by the total time of process.

FPU activity of cellulases was analyzed according to Goose et al. (1987).

6.3 Results and Discussion

6.3.1 Evaluation of variables that influence the performance of Hydrodynamic Cavitation-assisted enzymatic hydrolysis of pretreated SCB system

The composition of *in natura* biomass used for pretreatment was: 40% of cellulose, 26% of hemicellulose (24 % xylan, 3.6% of acetyl, and 2% arabinosil), 25% of lignin, and 6% of ash and extractives. For pretreated biomass, the composition was: 52% glucan, 26 % xylan, 18% lignin. As shown, the pretreatment resulted in lower content of lignin in pretreated biomass compared with the raw material, mainly enriching the material with cellulosic fraction, similarly to previously reported for HC-assisted pretreatment of SCB (Hilares et al., 2020; Ramos et al., 2021).

The enzymatic process was performed as described in section 6.2.2. Table 6.1 presents the matrix of the statistical design carried out, including the response variables yield of enzymatic hydrolysis of xylan and of glucan.

As observed in Table 6.1, the high enzymatic hydrolysis yields of glucan (84%) and of xylan (72%) were observed in run 4, using higher levels of the studied variables. Also, the lower yield for glucan hydrolysis (21%) was observed using lower levels of the studied variables (run 1), while, for xylan, the lower hydrolysis yield (7%) was observed using center level for enzyme loading and lower level for temperature (run 7).

Table 6.1. Results of the 2^2 face-centered design performed to evaluate the influence of important variables in the HC-assisted enzymatic hydrolysis of sugarcane bagasse pretreated by HC-assisted process

Run	Conditions (coded values in parentheses)		Hydrolysis Yield	
	Enzyme loading (FPU/g)	Temperature (ºC)	Glucan (%)	Xylan (%)
1	5 (-1)	40(-1)	21.00	19.2
2	20 (+1)	40 (-1)	81.20	60.0
3	5 (-1)	50 (+1)	70.00	30.0
4	20 (+1)	50 (+1)	83.8	71.6
5	5(-1)	45(0)	29.80	17.3
6	20 (+1)	45 (0)	64.20	56.6
7	12.5 (0)	40 (-1)	60.10	7.3
8	12.5 (0)	50 (+1)	60.20	18.6
9	12.5 (0)	45 (0)	72.10	16.5
10	12.5 (0)	45 (0)	70.00	16.0
11	12.5 (0)	45 (0)	72.00	16.2

Results were statistically analyzed, and quadratic models (Eq. 1 and 2) were composed for the answer variables. For both models, non-significant terms were removed

($p>0.05$), except if necessary for model hierarchy. ANOVA (Table 6.2) showed both fitted models were significant ($p<0.05$), although the model for glucan hydrolysis yield had a significant lack of fit ($p<0.05$). In despite of this, R^2 values were high for both models (≥97%), which then were used to compose response surfaces, as shown in Figure 6.1.

$$Y_G\,(\%) = 33.62 + 2.13A - 0.01A^2 + 0.044B \qquad \text{Eq. (1)}$$

$$Y_X\,(\%) = 21.05 + 2.14\,A - 0.010\,A^2 + 0.27\,B \qquad \text{Eq. (2)}$$

Where: Y_G and Y_X correspond to yield of enzymatic hydrolysis of glucan and xylan (%), respectively. A is enzyme loading (FPU/g), B is temperature (°C). R^2 for glucose and xylose hydrolysis yield models were 98.03 and 96.95, respectively.

As shown in Eq. 1 and 2, for both glucan and xylan hydrolysis yield, the dependence with temperature was linear, indicating higher values of this variable was better for higher yields. However, for enzyme loading, the dependence of the yields was quadratic, indicating a possible optimum value in the studied range.

As can be seem in Figure 6.1, the profile of the curves was similar for glucan and xylan hydrolysis yield, with higher values observed at 50°C and loading between 15-20 FPU g^{-1}. Indeed, the models were used to optimize both response variables, aiming at their maximization. For this, the specific tool of the Design-expert software was used. The optimized conditions were adjusted according to the numerical tool of the Design Expert software, in order to maximize the hydrolysis of glucan and xylan (with a greater degree of importance for both, represented by "+++++" in the software). Another criterion was to minimize the enzyme load (with little importance, represented by "+" in the software).

Table 6.2. Analysis of variance (ANOVA) and coefficient of determination (R^2) for the fitted models for hydrolysis yield of glucan and of xylan

Yield of enzymatic hydrolysis of glucan (%)						**Yield of enzymatic hydrolysis of xylan (%)**					
Source	**Sum of square**	**Degrees of freedom**	**Mean square**	**F**	***P***	**Source**	**Sum of squares**	**Degrees of freedom**	**Square mean square**	**F**	***P***
Model	4025.84	1	1401.95	175.71	<0.0001	Model	1780.45	1	445.11	8.75	0.0112
Enzyme Loading (A)	3683.79	1	3683.79	461.71	<0.0001	Enzyme Load(A)	269.61	1	269.61	5.3	0.0609
Temperature (B)	125.49	1	125.49	15.73	0.0054	Temperature (B)	521.17	1	521.17	10.25	0.0186
A^2	396.55	1	396.55	49.7	0.0002	A^2	471.65	1	471.65	9.27	0.0227
Residual	55.85	7	7.88			Residual	305.22	6	50.87		
Lack of fit	55.59	5	11.12	85.52	0.0116	Lack of fit	250.76	4	62.69	2.3	0.325
Pure error	0.26	2	0.13			Pure error	54.46	2	27.23		
Total	4261.69	10				Total	2085.68	10			
R^2	98.03					R^2	96.95				

The conditions obtained were: 18 enzyme FPU and 50ºC. Experiments were carried out in triplicate under these conditions to confirm the models and the glucan hydrolysis yield predicted by the model, of 85.47 ± 4.6% (mean value ± 95% confidence interval), was confirmed by the experimental result obtained, (83.30 ± 3.1) % (mean value ± standard deviation). Even with a significant lack of fit, the models were used to trace response surfaces and for process optimization, considering their significance at 95% confidence level and, mainly for glucan hydrolysis yield, the high value of R^2. As following shown, those models were able to predict results at optimized conditions. The xylan hydrolysis yield predicted by the model, (70.47 ± 5.1) % (mean value ± 95% confidence level), was also confirmed by the experimental result obtained (71.12 ± 1.52) % (mean value ± standard deviation.

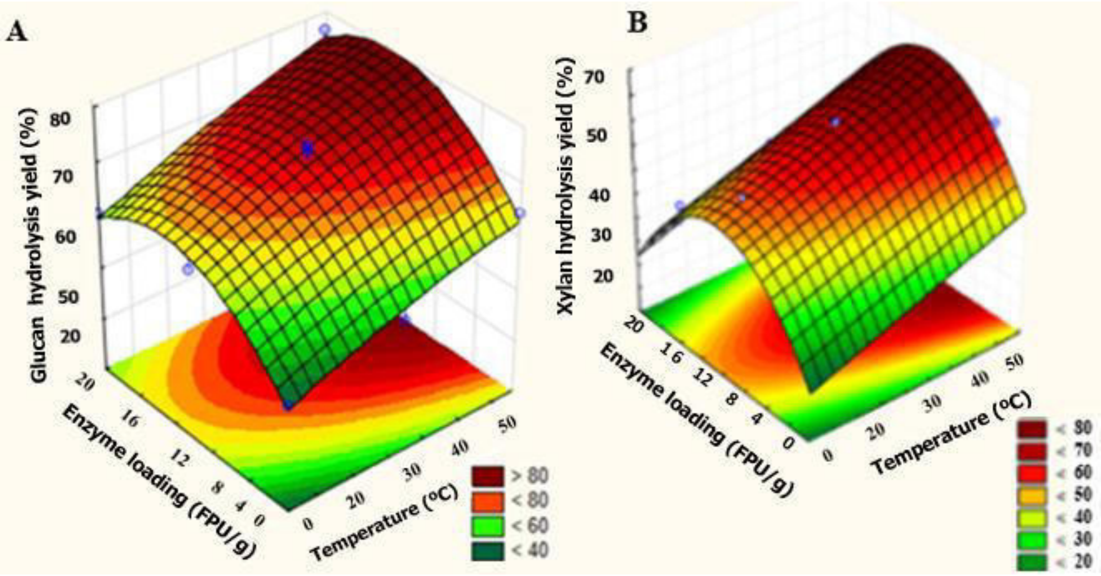

Figure 6.1. Response surface for the yield of enzymatic hydrolysis of glucan (a) and of xylan (b), as a function of enzyme loading and temperature

6.3.2 Control experiments

Under optimized conditions, experiment was also carried out for 12h, resulting in glucan and xylan hydrolysis yield of 92.90±1.24 % and 77.33±1.54 %, respectively. With this time of process, control experiments were performed in Erlenmeyer Flasks and in

HC-system without orifice plates (control without HC). The obtained results of glucan and xylan hydrolysis yield were 53.01±0.25 and 48.72±0.75, respectively, in Erlenmeyer flasks control, and 44.25±1.30 and 40.51±0.83, respectively, in the control without HC. As can be observed, the beneficial effect of HC in the enzymatic process was shown, with enzymatic yields 1.5 to 2 times higher compared to the controls.

Indeed, the enzymatic process had efficient action together cavitation process. The hydrodynamic cavitation effect on the pretreatment of lignocellulosic biomass is known, and has been reported in the literature (Kim et al., 2015; Hilares et al., 2020; Prado et al., 2023). Chemical and physical action of cavitation provides benefits such as mild conditions for oxidative degradation, and reduction of lignin fraction of biomass under short reaction period (Hilares et al., 2019). The HC phenomenon results in high energy released into the medium, which contributes to the generation of oxidative radicals (Xie et al., 2017).

The results obtained in HC-assisted system was also interesting if compared to the observed in previous work, as reported by Hilares et al. (2019). In that work, using HC-assisted alkaline pretreated sugarcane bagasse, 91% hydrolysis yield of glucan and 81 % hydrolysis yield of xylan were obtained in 24 h of enzymatic hydrolysis in Erlenmeyer flasks loaded with 5% solid/liquid ratio (50ºC, Cellic-CETEC-2, 250 rpm in shaker).

As pointed in introduction section, previous works reported beneficial effects of ultrasound (US)-assisted enzymatic hydrolysis of biomass (Kim et al., 2015, Nakashima et al., 2016). Thus, a control experiment was performed in US system, and the results, in 12h, were 86.50±0.54 and 67.54±1.04, respectively, for glucan and xylan hydrolysis yield. As shown, HC-assisted process was more effective to enhance enzymatic hydrolysis of HC-pretreated sugarcane bagasse. Also, the energy consumption was 31.75 kWh for HC-assisted enzymatic process, compared to a consumption of 65.01 kWh for

US-assisted process (Table 6.3). Actually, the higher efficiency and lower energy consumption of HC compared to US-assisted processes has already been reported for biomass pretreatment (Hilares et al., 2017b). Besides, HC systems have been reported as of easier scale-up compared to US systems (Flores et al., 2021; Bimestre et al., 2022).

Table 6.3. Control experiments: ultrasound-assisted process, and process in HC-system without cavitation device in 8 hours and 12 hours

Runs	**Conditions**		**Hydrolysis Yield (%)**		**Total energy**
	Time (h)	**Treatment**	**Glucan**	**Xylan**	**consumption (kWh)**
1	8	HC	83.34±1.54	71.63 ±1.54	20.84
2	8	UC	69.60±0.34	58.20±1.04	48.79
3	12	HC	92.90±1.24	77.33±1.54	31.75
4	12	UC	86.50±0.54	67.54±1.04	65.01

Additionally, to verify if HC-system promoted enzymes inactivation, a control experiment was carried out for 48h, adding 540 FPU of cellulases at the beginning of the process (corresponding to 18 FPU of cellulases for 30 g of bagasse), but without biomass. The relative activity was kept as 100% of the initial in 12h of the process (Figure 6.2), indicating that no enzymatic inactivation occurred in this time of process. After 36h, the relative activity was 93% of the initial, and in 48h, 73% of the initial. As shown, the system did not promote higher enzyme inactivation as an effect of HC in 48h.

In despite, of the high enzymatic hydrolysis yields, the HC-assisted process evaluated in the present work resulted in a low sugar concentration at the end of 12h of hydrolysis (6.7 g/L of glucose, and 3.8 g/L of xylose). However, this drawback is not inherent to the technique, but it was due to the cavitation system available in the laboratory. Actually, the available system has a small volume cavitation zone, designed to allow experiments with small amounts of biomass sample. Also, the volume of liquid used in the system is high due to the suction and discharge pipes required by the centrifugal pump available to obtain the required flowrate to generate cavitation. Thus, in the future, new systems will be built using the same pumps, but with larger cavitation zones, allowing the use of higher solid-liquid ratios. Anyway, using the available system, the possibility of obtaining a higher concentration of sugars in the hydrolysate was evaluated using a repeated fed-batch strategy, as following reported.

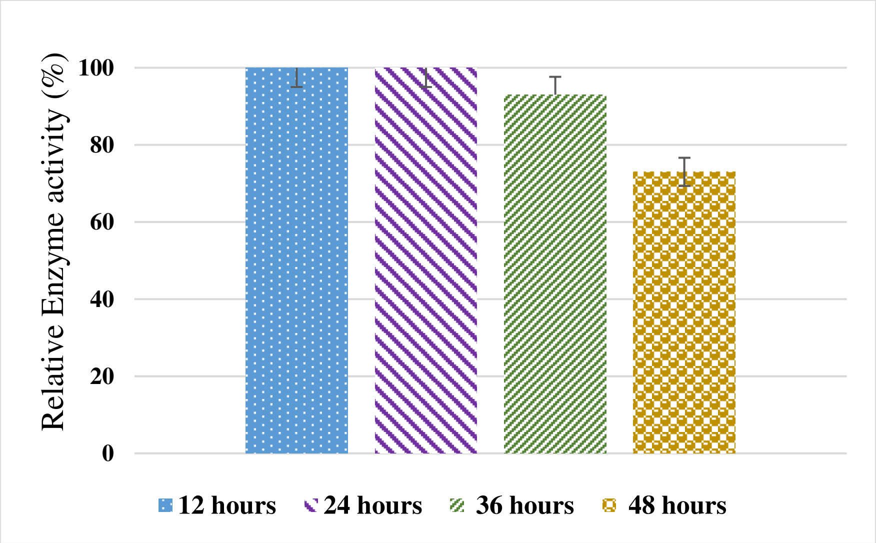

Figure 6.2. Cellulases activity measured in the liquid mixture in the HC reactor in the control experiment performed by loading the system only with enzymes and buffer

6.3.3 Repeated fed-batch mode for HC-assisted enzymatic process

The released sugars in the hydrolysis process of SCB using the fed-batch repeated process are shown in Figure 6.3. In this strategy, as part of solids are transformed in soluble compounds along the time of hydrolysis, a new loading of pretreated biomass was performed in 6h of each batch. Besides, after 12h of process, the remaining solid was removed of the cavitation system, and a new pretreated biomass loading was performed, keeping the liquid in the reactor. For each solid loading, additional enzyme (18 FPU/g of biomass) was also charged in the reactor. After the first batch of 12h (Figure 6.3), glucose and xylose concentrations were 10.1 g/L and 5.2 g/L, respectively, with hydrolysis yields similar to the previously obtained in simple batch system (93.4 and 78.6 for glucan and xylan, respectively). Thus, the beneficial effect of fed-batch strategy was demonstrated to increase the sugars concentration, without reduction in hydrolysis yields.

Terán Hilares et al. (2016) used a packed bed column reactor for enzymatic hydrolysis of sugarcane bagasse that was pretreated by CH. In this study, the authors achieved approximately 30 g/L of glucose and 10 g/L of xylose, using 13.2% of the initial load of solids in the reactor and a hydrolysis time of 24 hours. This resulted in a reported 50% yield of enzymatic cellulose hydrolysis. In another work, Tsai et al. (2020) used a packed-bed reactor with an initial charge of 12% solids and achieved 40 g/L of glucose during enzymatic hydrolysis of rice straw that was pre-treated by an alkaline process.

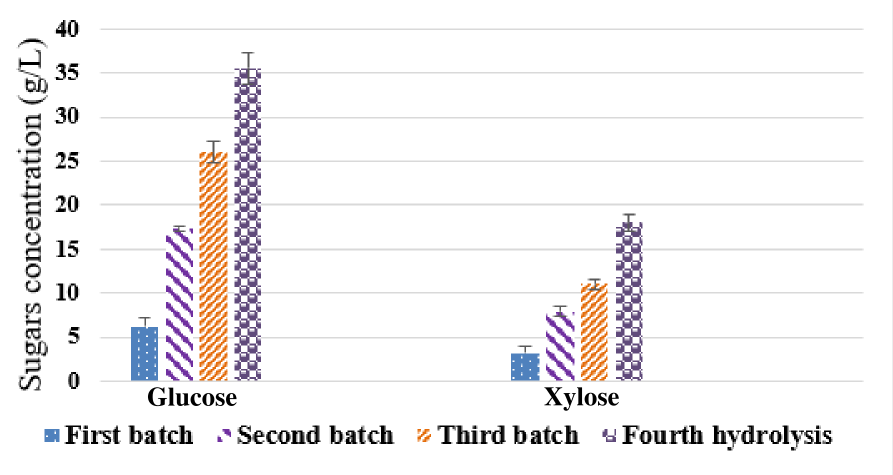

Figure 6.3. Cumulative sugars concentration obtained in hydrolysate obtained by HC-assisted enzymatic hydrolysis of HC-pretreated sugarcane bagasse in repeated fed-batch process

The beneficial effect of repeated batches was also shown. Indeed, after four repeated batches, corresponding to 48h of system operation, 36.2 g/L of glucose and 16.1 g/L were obtained in hydrolysate, corresponding to hydrolysis yields of glucan and xylan of 94% and 82%, respectively. This sequential process increased the sugar level during the enzymatic process and increased solid/liquid ratio to 4.8 percent (with 120g of SCB).

Of course, considering the initial solid loading is still low (not an inherent drawback of the technique, as previously discussed), the proposed sequential strategy could be even more interesting in future experiments using higher initial solid:liquid ratios.

6.4. Conclusion

The intended proof of concept was successful, showing the HC not resulted in reduction in enzymes activity enough to impair the process, resulting in higher enzymatic hydrolysis performance compared to systems without HC or assisted by ultrasound. Indeed, HC-assisted enzymatic hydrolysis of HC-pretreated SCB resulted in high efficiency, with about 93% and 77% of enzymatic hydrolysis yield of glucan and xylan in 12h, respectively (50ºC, 18 FPU/g). A strategy of repeated fed-batches was shown as promising to increase the sugars concentration in hydrolysate, without reduction in the hydrolysis yields. By using this strategy, sugars concentration was increased 4-6 times in the hydrolysate compared to the values obtained in simple batch mode. The efficiency of HC to assist enzymatic hydrolysis of biomass was shown, and future studies are necessary to optimize and scale up the process, paying special attention to strategies to increase the solid loading in the reactor.

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CHAPTER VII

Conclusions, and Future works

7.1 Conclusions

In the context of replacing traditional pre-treatments for biofuel production, which contributes to current sustainability measures as an energy strategy by biorefineries, the use of hydrodynamic cavitation has emerged as an innovative and promising solution. The technique offers several advantages as a pretreatment, including the ability to carry out the process under mild conditions (i.e., low temperature and in a short period).

This study aims to contribute to the generation of knowledge in the field of bioenergy by evaluating new technologies for the biorefinery of sugarcane bagasse. Specifically, the study proposes the use of hydrodynamic cavitation with oxidative processes as an innovative strategy for the pre-treatment of lignocellulosic biomass. Additionally, the study explores the use of hydrodynamic cavitation in the enzymatic hydrolysis step, which is a novel approach.

An extensive experimental work was carried out, with three different pre-treatments that combined advanced oxidative processes with hydrodynamic cavitation at neutral and acidic pH levels. The catalytic oxidative process assisted by hydrodynamic cavitation was also evaluated, using iron sulfate as a catalyst due to its abundance and affordability. Ozone was used as an oxidative source at acidic pH levels.

Different fermentation strategies were also studied, including Simultaneous Saccharification and Co-Fermentation (SSCF) with two interconnected reactors - one for the enzymatic hydrolysis step and another for fermentation and co-fermentation of ethanol production by the yeast *Sheffersomyces parashehatae*. Additionally, sequential fermentation for the production of ethanol and xylitol was studied, with fermentation first carried out with the yeast *Saccharomyces cerevisiae* IR2, which has flocculant characteristics. Following this fermentation, the yeasts were decanted, and ethanol distillation was carried out. Subsequently, the production of xylitol was performed using *Candida tropicalis* UFMG35 cultured in the obtained vinasses.

The results obtained were highly satisfactory and in the set of the obtained data the following can be highlighted:

- In the study of oxidative pretreatment assisted by HC, using a flowrate of 10 mg/min of ozone and 0.61% of peroxide, hydrolysis yields of 83% for glucan and 78% for xylan were obtained during a 10 minutes process at pH 7.0.
- Combined AOP-HC-assisted acid pretreatment of sugarcane bagasse was shown to be a potential method. Influential variables in pretreatment were evaluated, and optimized conditions were found at an ozone flowrate of 10 mg/min and a pH of 5.01, resulting in glucan and xylan hydrolysis yields of 84% and 78%, respectively, after a 10-minute process.
- Best pretreatment performance was obtained in HC-assisted acid pretreatment with ozone and iron sulfate, resulting in more than 90% of glucan hydrolysis yield in 10 min of process, reaching 95% and 92% of hydrolysis yields for glucan and xylan in 30min of pretreatment.
- In the sequential fermentations for ethanol and xylitol production, yield values of 0.41 g/g and 0.55 g/g, respectively, were obtained, with corresponding volumetric productivities of 8.33 g/(L.h) and 0.64 g/(L.h), respectively. The proposed strategy was shown to be a promising approach for biorefineries, considering its high ethanol production and effective use of vinasses to obtain xylitol.
- In the SSCF batch system, the ethanol production after 72 hours of the process was 17 g/L, with a co-production of 4 g/L of xylitol. A volumetric productivity of 0.24 g/(L.h) was observed, with 85% of global efficiency.

- Fed-batch SSCF with interconnected columns is interesting for biorefineries, with the potential to be used in ethanol production processes. In this study, the production of ethanol by S. *parashehatae* UFMGX12 from glucose and xylose present in sugarcane bagasse was 28 g/L, with a co-production of 5 g/L of xylitol in 72 hours. This process reached 85% of global efficiency, with a volumetric productivity of 0.39 g/(L.h).
- Hydrodynamic cavitation was found to be a promising solution to improve the enzymatic hydrolysis step, which is typically a bottleneck in biorefineries. While this study is still in its initial phase and requires testing at a larger scale to ensure its viability, it has the potential to be a more economical option than ultrasonic cavitation-assisted hydrolysis or traditional enzymatic hydrolysis. In the performed experiment, the sugarcane bagasse had about 93% and 77% of enzymatic hydrolysis yield of glucan and xylan, respectively, in a short period (12 hours, 50°C, 18 FPU). In was possible to carry out repeated fed-batches that were adequate to increase the sugar concentration in hydrolysate, without a reduction in the hydrolysis yields.

7.2 Suggestion of future works

- To evaluate intensification strategies of the pretreatment process, with the combination of hydrodynamic cavitation and Fenton.
- To evaluate the pretreatment process with a photo-Fenton oxidative hydrodynamic cavitation -assisted system.
- To scale up the oxidative pretreatment assisted by hydrodynamic cavitation, evaluating different strategies.
- To scale up the enzymatic hydrolysis step assisted by hydrodynamic cavitation, also evaluating different strategies.

APENDIX

List of publications: articles, book chapter and patents

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