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ENGINEERING SCHOOL OF LORENA

RULY TERÁN HILARES

Hydrodynamic cavitation as a new approach for sugarcane bagasse pretreatment aiming to second generation ethanol production

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Advisor: PhD. Júlio César dos Santos

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Thesis dedicated at my family

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“Life is like riding a bicycle. To keep your balance, you must keep moving….”

(Albert Einstein)

ABSTRACT

TERÁN HILARES, R. **Hydrodynamic cavitation as a new approach for sugarcane bagasse pretreatment aiming to second generation ethanol production** 2017. 192 p. Thesis (Doctoral Science) – Engineering School of Lorena, University of Sao Paulo, Lorena, 2017.

Renewable energy sources have been proposed as a viable option to mitigate the consumption and the dependence of fossil fuels. Among the available alternatives, lignocellulosic biomass has shown great potential for bioenergy generation, and biofuels as ethanol can be obtained by fermentation from sugars present in cellulosic and hemicellulosic fractions of biomass. However, for the efficient release of fermentable sugars during the enzymatic hydrolysis step, a pretreatment process is required to modify the material in its structure and composition. In this context, hydrodynamic cavitation (HC) was proposed in this work as a new and promising alternative for pretreatment of sugarcane bagasse. Firstly, the variables NaOH concentration, solid/liquid (S/L) ratio and HC process time were optimized in HC assisted pretreatment. In optimized conditions (0.48 mol/L of NaOH, 4.27% of S/L ratio and 44.48 min), high lignin removal (60.4%) and enzymatic digestibility of cellulose fraction (97.2%) were obtained. Based in those results, new variables (inlet pressure, temperature, alkali concentration) were included for evaluation in a second stage of the study aiming to reduce the HC pretreatment time. In this case, temperature and alkali concentration showed more significance on lignin removal and hydrolysis yield of carbohydrate fraction in pretreated biomass. No significant difference in pretreatment efficiency was observed in 20 and 30 min of process time in the best conditions (70 °C, 3 bar of inlet pressure and 0.3 mol/L of NaOH). The dimensionless cavitation number influence also was evaluated in two levels (0.017 and 0.048), resulting higher efficiency using low cavitation number which was obtained using orifice plate with 16 holes (1 mm of diameter). Using the last optimized conditions and lower temperature (60 °C instead 70 °C) in order to avoid the foam formation when black liquor is reused, other alkalis ($Ca(OH)_2$, Na_2CO_3, KOH) were evaluated in combination with HC and compared to the use of NaOH. High enzymatic conversions of carbohydrate fraction were observed in biomass pretreated using KOH-HC and NaOH-HC; additionally, NaOH black liquor was reused in 10 sequential batches. The pretreated biomass using fresh and reused black liquor were mixed and used for simultaneous saccharification and fermentation process (SSF) in interconnected column reactors, resulting in 62.33% of hydrolysis of total carbohydrate fractions and 17.26 g/L of ethanol production (0.48 g of ethanol/g of glucose and xylose consumed). Finally, the addition of oxidant agent (H_2O_2) in the alkali HC-process was optimized. In selected conditions (0.29 mol/L of NaOH, 0.78 % v/v of H_2O_2 and 9.8 min), 95,43% and 81.34% of enzymatic hydrolysis yield of cellulose and hemicellulose fraction were achieved respectively, using 5% of solid loading (S/L) in the hydrolysis process. When packed bed flow-through column reactor using 20% of S/L was used, 74.7% cellulose hydrolysis yield was reached. Sugars present in hydrolysate were also fermented into ethanol in bubble column reactor resulting in a yield value of 0.49 g/g and 0.68 g/L.h of productivity. By analyzing the results as a whole, HC was shown as a promising technology to accelerate the pretreatment time under mild conditions, showing advantages as simplicity of system and possibility to application in industrial scale.

Keywords: Lignocellulosic biomass; Sugarcane bagasse; Second generation ethanol, Hydrodynamic cavitation; Column reactors

RESUMO

TERÁN HILARES, R. **Cavitação hidrodinâmica como uma nova abordagem para o pré-tratamento do bagaço de cana-de-açúcar visando à produção de etanol de segunda geração**. 2017. 192 p. Tese (Doutorado em Ciências) – Escola de Engenharia de Lorena, Universidade de São Paulo, Lorena, 2017.

O uso de fontes de energia renováveis tem sido proposto como uma alternativa viável para reduzir o consumo e a dependência de combustíveis fósseis. Entre as alternativas disponíveis, a biomassa lignocelulósica apresenta grande potencial para geração de bioenergia, sendo que biocombustíveis como o etanol podem ser obtidos por fermentação a partir de açúcares presentes em suas frações celulósicas e hemicelulósicas. No entanto, para a liberação eficiente de açúcares fermentáveis na etapa de hidrólise enzimática, é necessário um processo prévio de pré-tratamento para modificar a estrutura e composição do material. Neste contexto, no presente trabalho a cavitação hidrodinâmica (CH) foi proposta como uma nova e promissora alternativa para o pré-tratamento do bagaço de cana-de-açúcar. Em uma primeira etapa, as variáveis concentração de NaOH, relação sólido/líquido (S/L) e tempo de processo foram otimizadas no pré-tratamento assistido por CH. Em condições otimizadas (0,48 mol/L de NaOH, 4,27% de relação S/L e 44,48 min), elevados valores de remoção de lignina (60,4%) e digestibilidade enzimática da fração de celulose (97,2%) foram obtidos. Com base nesses resultados, novas variáveis (pressão à montante, temperatura e concentração de álcali) foram incluídas para avaliação em uma segunda etapa do estudo com o objetivo de reduzir o tempo de pré-tratamento com CH. Neste caso, a temperatura e a concentração de álcalis foram as mais importantes na remoção de lignina e influenciaram na hidrólise das frações carboidrato da biomassa pré-tratada. Não houve diferença significativa na eficiência do pré-tratamento em 20 e 30 minutos de tempo de processo nas melhores condições (70 ° C, 3 bar de pressão a montante e 0,3 mol/L de NaOH). A influência do adimensional "número de cavitação" também foi avaliada em dois níveis (0,017 e 0,048), resultando em maior eficiência usando o número de cavitação mais baixo, que foi obtido usando placa de orifício com 16 furos (1 mm de diâmetro). Usando estas condições otimizadas e menor temperatura (60 ° C ao invés de 70 ° C) para evitar a formação de espuma quando o licor negro é reutilizado, outros álcalis (Ca $(OH)_2$, Na_2CO_3, KOH) foram avaliados em combinação com CH e comparados com o uso de NaOH. Conversões enzimáticas elevadas das frações carboidrato foram observadas em material pré-tratado utilizando KOH-CH e NaOH-CH; além disso, o licor negro de NaOH foi reutilizado em 10 bateladas sequenciais. As biomassas pré-tratadas com licor negro reutilizado e fresco foram misturadas e utilizadas em processo de sacarificação e fermentação simultâneas (SSF) em reatores de coluna interligados, resultando em 62,33% de hidrólise das frações carboidrato e 17,26 g/L de produção de etanol (0,48 g de etanol/g de glicose e xilose consumidos). Finalmente, a adição de agente oxidante (H_2O_2) no processo alcalino-CH foi otimizado. Nas condições selecionadas (0,29 mol/L de NaOH, 0,78% v/v de H_2O_2 e 9,8 min), 95,43% e 81,34% de rendimento de hidrólise enzimática das frações de celulose e hemicelulose, respectivamente, foram obtidos utilizando 5% de carregamento de sólidos (S/L) no processo de hidrólise. Quando foi utilizado reator de coluna de leito fixo com 20% de S/L, atingiu-se 74,7% de rendimento de hidrólise de celulose. Os açúcares presentes no hidrolisado também foram fermentados em etanol em um reator de coluna de bolhas, resultando em um valor de rendimento de 0,49 g/g e 0,68 g/L.h de produtividade. Analisando-se os resultados de uma forma global, demonstrou-se que a CH é uma tecnologia promissora para acelerar o tempo de pré-tratamento em condições amenas, mostrando vantagens como simplicidade do sistema e possibilidade de aplicação em escala industrial.

Palavras chaves: Biomassa lignocelulósica; Etanol de segunda geração; Cavitação hidrodinâmica; Reatores de coluna

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LIST OF ABBREVIATIONS

HC	Hydrodynamic cavitation
SCB	Sugarcane bagasse
PBFTCR	Packed bed flow-through column reactor
SSF	Simultaneous Saccharification and Fermentation
SHF	Separate Hydrolysis and Fermentation
CBP	Consolidated Bioprocess
vvm	Volume of air per volume of medium per minute
Cv	Adimensional cavitation number

SUMMARY

CHAPTER I

Introduction, thesis overview and objectives

1.1 Introduction and thesis overview

Sugarcane bagasse (SCB) is a massively produced bio-waste from the sugar-alcohol industry. Typically, it still has more than 60-70% of carbohydrates in its composition in the form of holocelluloses (collectively cellulose and hemicellulose) and remaining 30%-40% as a non-carbohydrate fraction, mainly lignin, besides extractives and ash (PANDEY et al., 2000; TERÁN-HILARES et al., 2016). Currently, about 70-90% of SCB is burned to electricity generation (~150 kWh/t cane) inside the industry and the remaining is stockpiled (KHATIWADA et al., 2016). SCB is being heavily cultivated in Brazil specifically, within last year, amounting even up to about 657 million tons of resulting in the generation of 184 tons of bagasse (50% moisture content) (CONAB, 2017).

This enormous availability of SCB in Brazil makes it an attractive choice to be employed for the sustainable production of biofuels and commodity chemicals and thus to contribute for the bio-economy of Brazil. Biofuels, specifically lignocellulosic ethanol, is produced by the fermentation of SCB hydrolysates has received increasing attention due to its technical suitability to substitute geological fuels (VAN EIJCK; BATIDZIRAI; FAAIJ, 2014). This sustainable fuel typically known as “second generation ethanol” is considered to solve environmental problems by way of reducing greenhouse gas emissions and fossil fuel dependence simultaneously (VAN EIJCK; BATIDZIRAI; FAAIJ, 2014; ISIKGOR; BECER, 2015).

To fully exploit the potential use of SCB for bioprocesses, its carbohydrate fractions must be extracted by way of overcoming its well-known recalcitrance. In this regard, biomass pretreatment and subsequent enzymatic hydrolysis has been envisaged as one promising approach for biorefineries. This pretreatment step is required to turn substrate more accessible to the enzymes by either decreasing crystallinity, increasing surface area, partially dissolving hemicelluloses, enhancing the porosity, modifying or dissolving the lignin etc. Currently, this pretreatment is a major bottleneck for the commercialization of bio-based refineries. Therefore, the development of efficient pretreatment technologies is of an absolute need (AGBOR et al., 2011). There have been a fair deal of pretreatment studies mentioned in the literature; however, they still suffer from such limitations viz, extensive consumption of chemical, needing for harsh thermo-mechanical treatments, complexity of

chemical system, less efficiency and high energy consumption (RABEMANOLONTSOA; SAKA, 2016).

Recently, hydrodynamic cavitation (HC) has been reported as one promising strategy with unique advantages compared to other methods such as less chemicals consumption, low temperatures and short process time (KIM et al., 2015; NAKASHIMA et al., 2016). Moreover, HC process was demonstrated as improving the pretreatment efficiency due to lignin removal and increase in the porosity of the material, resulting in higher enzymatic digestibility and thus favoring biological conversion process for bioethanol production or others biomolecules (E-PIC s.r.l, 2016; TERAN HILARES et al., 2016).

As a new and promising strategy, HC-pretreatment needs to be elaborated specifically considering the study of the influent process variables and a systematic optimization of the pretreatment conditions. Moreover, HC-based systems established on different systems configurations are crucial for their process integration in a biorefinery. Among from these such possibilities, simultaneous saccharification and fermentation process (SSF) stands out as one such potential option.

This strategy is advantageous to avoid the main problems associated with the inhibition of enzymes action by end-product (cellobiose and glucose); additionally, the reduction in the number of vessels enhaces the process economics (LIU et al., 2014). However, for it to be fully beneficial for bio-refineries a rigorous and optimized enzymatic hydrolysis and fermentation is vital. Indeed, these two steps have different optimum temperatures and the commonly used SSF approach includes the use of one-pot strategy with a temperature intermediate between the optimum ones (KIM et al., 2015). A new approach can be the use of interconnected reactors with immobilized cells for fermentation, allowing the use of optimized temperature for enzymatic hydrolysis and fermentation process in each reactor, besides the use of high solid loading avoiding thus the inhibition of enzyme activity.

Therefore, this thesis is focused on the development of new approaches related to HC pretreatment and subsequent SSF steps. Besides this introductory chapter, as an overview of the whole work, this thesis has been divided in seven other chapters. Chapter II covers a discussion about composition, processing and limitation of using lignocellulosic biomass in bioprocesses. In the next chapter III, the application of HC has been extensively discussed regarding its impact on pretreatment strategies. In

subsequent chapter IV, results about the effects of variables as concentration of alkali, solid loading and process time in HC-pretreatment performance has been mentioned, specifically considering the system without temperature control. Experiments corresponding to this chapter were carried out in the Environmental BioTechnology and BioEnergy Laboratory of Korea Advanced Institute of Science and Technology, (KAIST) South Korea. In chapter V, the evaluation of the effects of variables as temperature, inlet pressure and alkali concentration in the HC-pretreatment process has been discussed. Chapter VI shows the potential use of HC in combination with other alkalis and the black liquor reuse for additional successive pretreatment processes, besides the use of the pretreated biomass for ethanol production in SSF process using immobilized cells and interconnected reactors. The chapter VII, the evaluation of the addition of a potential oxidant (hydrogen peroxide) for NaOH-HC-pretreatment process has been thoroughly discussed. The final chapter VIII corresponds to the future works and conclusion of this these.

1.2 Objectives

The main objectives of this research work were the following:

— Evaluation of the potential of HC technology for lignocellulosic biomass pretreatment, considering the important parameters and the perspectives for future developments;

— Evaluation and optimization of the main influent variables in HC-assisted sodium hydroxide pretreatment without temperature control (alkaline concentration, solid loading and time of reaction), aiming to improve the enzymatic hydrolysis of cellulosic fraction of pretreated sugarcane bagasse;

— Evaluation and optimization of the main influent variables in HC-assisted sodium hydroxide pretreatment (alkaline concentration, inlet pressure and temperature), aiming to improve enzymatic hydrolysis of cellulosic fraction of pretreated sugarcane bagasse;

— Evaluation of different chemical (NaOH, KOH, Na_2CO_3 ,$Ca(OH)_2$) and reuse of black liquor for pretreatment of sugarcane bagasse in HC assisted process using previously optimized conditions;

— Evaluation of the feasibility of bioethanol production in SSF process with immobilized cells of *Scheffersomyces stipitis* NRRL-Y7124 using alkaline-HC pretreated SCB as raw material at high solids loading in interconnected column reactors;

— Evaluation and optimization of the main influent variables in sodium hydrogen peroxide (NaOH-H_2O_2) pretreatment assisted by HC (NaOH and H_2O_2 concentration, and time), aiming to improve enzymatic hydrolysis of cellulosic fraction of pretreated sugarcane bagasse;

— Evaluation of enzymatic hydrolysis of NaOH/H_2O_2/HC pretreated SCB in high solid-content column reactor (20%);

— Evaluation of the fermentability of hydrolysate obtained from NaOH/H_2O_2/HC pretreated SCB to produce ethanol in SHF process using column reactor and free cells of the yeast *Scheffersomyces stipitis* NRRL-Y7124.

1.3 References

AGBOR, V. B. et al. Biomass pretreatment: Fundamentals toward application. **Biotechnology Advances**, v. 29, p. 675–685, 2011.

CONAB – COMPANHIA NACIONAL DE ABASTECIMENTO. Acompanhamento da safra brasileira: cana de açúcar, V. 3 - SAFRA 2016/17 N.4 - Quarto levantamento, 2017. Available in: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/17_04_19_11_27_36_boletim_cana_portugues_-_4o_lev_-_16-17.pdf. Accessed in: 26 Jul. 2017.

E-PIC S.R.L.. Biomass pretreatment (BIOGAS), 2016. Available in: http://www.epic-srl.com/en/cavitationtechnologies/ biomass-pretreatment-biogas. Accessed in: 8 Apr. 2017.

ISIKGOR, F.H.; BECER, C. R. Lignocellulosic biomass: a sustainable platform for the production of bio-based chemicals and polymers**. Polymer Chemistry**, v. 6, p. 4497-4559, 2015.

KHATIWADA, D. et al. Optimization ethanol and bioelectricity production in sugarcane biorefineries in Brazil. **Renewable Energy**, v. 85, p. 371-386, 2016.

KIM, I. et al. Hydrodynamic cavitation as a novel pretreatment approach for bioethanol production from reed. **Bioresource Technology**, v. 192, p. 335–339, 2015.

LIU, Z. H. et al. Simultaneous saccharification and fermentation of steam-exploded corn stover at high glucan loading and high temperature. **Biotechnology for Biofuels**, v.7, p. 167-172, 2014.

NAKASHIMA, K. et al. Hydrodynamic cavitation reactor for efficient pretreatment of lignocellulosic biomass. **Industrial Engineering Chemistry Reseasrch**, v. 55, p. 1866–1871, 2016.

PANDEY, A. et al. Biotechnological potential of agro-industrial residues. I: sugarcane bagasse. **Bioresource Technology**, v. 74, p. 69-80, 2000.

RABEMANOLONTSOA, H.; SAKA, S. Various pretreatments of lignocellulosics. **Bioresource Technology**, v. 199, p. 83–91, 2016.

TERÁN-HILARES, R. et al. Successive pretreatment and enzymatic saccharification of sugarcane bagasse in a packed bed flow-through column reactor aiming to support biorefineries. **Bioresource Technology**, v. 203, p. 42–49, 2016.

VAN EIJCK, J.; BATIDZIRAI, B.; FAAIJ, A. Current and future economic performance of first and second generation biofuels in developing countries. **Applied Energy,** v. 135, p.115-141, 2014.

CHAPTER II

Lignocellulosic biomass as raw material to produce ethanol and others bio-based products

Abstract

Agro-industrial and forest residues are potential feedstocks to produce ethanol and chemicals in a sustained way. The carbohydrate fractions, present within their native conformation, correspond to cellulose and hemicellulose which represent about 60-70% of their dried weight. These carbohydrates can be hydrolyzed by different ways to generate monomeric sugars for fermentation processes, with the enzymatic hydrolysis route as the most commonly adapted option. However, for an efficient biological hydrolysis of cellulose, a previous pretreatment process is necessary to disrupt the recalcitrant structure of biomass. In this way, several pretreatment technologies have been proposed such as chemical, physical, physicochemical and biological methods. However, currently this step has been considered as a persistent bottleneck in biorefineries prior to their commercialization. Other current challenges besides pretreatment are related to enzymatic hydrolysis process specifically employing high solids loading (>10%), use of an efficient reactor for hydrolysis and development of efficient configuration for biological conversion of biomass into bio-chemicals. Considering the technological bottlenecks in pretreatment and non-favorable economic situation, Brazilian 2G ethanol industrial companies have not yet reached their installed capacities. An option for future investments could be a biorefinery with a process intensification for the production of polymers, biosurfactants, lactic acid and other interesting molecules beyond ethanol. In this context, this chapter deals with the potential of lignocellulosic biomass (mainly SCB) as raw material to produce ethanol and other bio-based products. Moreover, brief descriptions of the process steps and their commonly used configurations have also been mentioned.

Keyword:

Lignocellulosic biomass, Sugarcane bagasse, 2G Ethanol, Pretreatment, Hydrolysis, Fermentation, Biorefinery

2.1 Introduction

Considering the current environmental problems associated with the extensive use of fossil fuels and petroleum based products, different environmental policies have been proposed. For example, the European Commission in climate and energy package sets three key targets as their 2020 vision: 20% cut in greenhouse gas emission, 20% of EU energy from renewables and 20% improvement in energy efficiency (EUROPEAN COMMISSION, 2009). Also, other global agreements to strengthen the global response to climate change by the reduction of greenhouse gases emission were fixed in the 21-Annual Conference of Parties (COP21) and Paris climate agreement (UNITED NATIONS, 2015), reaffirming the goal of limiting global temperature increase to below 2 degrees Celsius.

Biomass is an alternative renewable feedstock for the energy production maintained in a sustained way. According to National Renewable Energy Laboratory (2017), wood is still the largest biomass energy resource today but other sources of biomass can also be used. Among from them, agro-industrial residues and their by-products are available around the world and include a variety of lignocellulosic materials, such as corn straw and stover, rice straw and husk, wheat straw and sugarcane bagasse (SCB). SCB is relatively abundant and massively available feedstock in Brazil due to the well-developed sugar-ethanol industry (PEREIRA et al., 2015).

Due to its higher content of carbohydrate fractions, SCB can be used for second-generation ethanol and others commodity molecules production (SINDHU et al., 2016). However, the inherited structural complexity and close association of carbohydrates with lignin in them, they show recalcitrance, turning difficult the biological steps commonly used in biorefineries (enzymatic hydrolysis of carbohydrate fractions and fermentation of released monomeric sugars) (CRIMES et al., 2017). This fact turns necessary a prior step named pretreatment that can be performed by a number of different ways to turn macromolecular carbohydrates (mainly cellulose) more accessible to the enzymes (SIQUEIRA et al., 2017). Despite of the different pretreatment methods available in the scientific literature, this step is considered a persistent bottleneck in lignocellulosic biomass conversion. Thus, development of an efficient and low cost pretreatment process is one of the main challenges in conversion of biomass.

Fermentable sugars can be obtained from cellulosic fraction of SCB by an enzymatic hydrolysis of pretreated material, resulting in hydrolysate rich in glucose (TERÁN-HILARES et al., 2016). Regarding hemicellulose, it can be hydrolyzed during pretreatment, when e.g. dilute acid or steam explosion are the chosen technology (JIANG et al., 2015) or can remain in the pretreated material and be hydrolyzed during enzymatic step by using cellulases and hemicellulases commercially available enzyme blends (ZHANG; XU; ZHOU, 2014), in both cases resulting in a xylose-enriched hydrolysate.

After hydrolysis, monomeric sugars can be used for ethanol and other chemicals production by fermentation (KAWAGUCHI et al., 2016; TERÁN HILARES et al., 2017a). These two biological steps (enzymatic hydrolysis and fermentation) can be carried out employing different configurations. Traditionally, they are performed in separate processes in sequence named as separated hydrolysis and fermentation (SHF). However, in this case, cellulase activities can be inhibited by end-products (glucose and cellobiose), retarding the reaction kinetics (KAWAGUCHI et al., 2016). In order to avoid inhibition problems by hydrolysis end-product in SHF, SSF/SSCF processes (Simultaneous hydrolysis and fermentation or co-fermentation) have been proposed as potential alternatives (KAWAGUCHI et al., 2016; LOACES; SCHEIN; NOYA, 2017). There is also other interesting configuration, called consolidated bioprocess (CBP), which corresponds to an attempt to integrate enzyme production to enzymatic hydrolysis and fermentation steps (KAWAGUCHI et al., 2016).

Therefore, in this chapter the related topics are briefly discussed to show the potential of lignocellulosic biomass (mainly SCB) as raw material to produce ethanol and other bio-based products. Firstly, a discussion about biomass composition is presented, followed by a description of the process steps and their configurations commonly used for processing lignocelluloses. Finally, a literature review about ethanol and alternative products obtained from biomass has been presented.

2.2 Lignocellulosic biomass: composition and availability

Lignocellulosic biomass includes the forest and agro-industrial residues and their by-products. This material is mainly comprised of carbohydrate fractions of cellulose and hemicellulose covered by lignin (Table 2.1), besides other minor

components as ash and extractives. Carbohydrate fractions correspond to more than 60% of dry mass. These fractions are closely linked and covered by lignin, turning the material more rigid, impermeable and resistant against any chemical and biological attack (ANWAR; GULFRAZ; IRSHAD, 2014; SINDHU et al., 2016). Lignocellulosics such as sugarcane bagasse, rice straw, corn stover and wood are the usual feedstocks used in bioprocesses considering their large availability in different countries around the world (TYE et al., 2016).

Table 2.1 – Samples of composition of different lignocellulosic biomasses reported in literature

	Dry weight composition (%)			
Lignocellulosics	Cellulose	Hemicellulose	Lignin	References
Sugarcane bagasse	40	26.1	24.1	Terán-Hilares et al. (2016)
Sugarcane straw	34.8	23	24.1	Rocha et al. (2017)
Corn stover	37.28	17.57	8.2	Wang et al. (2017)
Rice straw	35.3	18.5	16.9	Ahmed et al. (2017)
Wheat straw	31.6	28.1	20.1	Salapa et al. (2017)
Eucalyptus wood	49.9	20.3	27.4	Carvalho, Queiroz and Colodette (2016)

Source: Personal archive

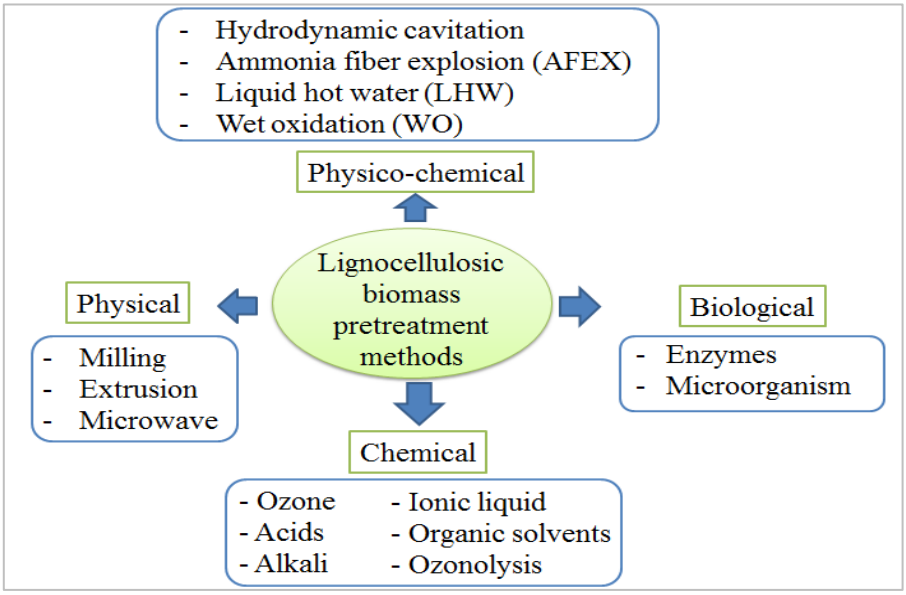
In Brazil, the most abundant biomass feedstock corresponds to sugarcane bagasse, which is a low-cost biomass obtained after sugarcane milling and juice extraction. During 2016/2017 crop, about 657 million of tons of sugarcane were produced (CONAB, 2017). Considering the proportion of 280 kg of bagasse per ton of sugarcane (50% moisture content) (RABELO et al., 2011), about 184 millions of tons of bagasse were produced in this season. Currently, most of this biomass is burned to electricity generation inside the sugar-alcohol industry; however, even so a great amount of SCB is wasted. The large availability of bagasse prompts its application for the production of fuels and chemicals by fermentation. However, for this purpose, some previous processes are required, as discussed in the following section.

2.3 Processing of lignocelluloses for the industrial production of fuels and chemicals

2.3.1 Pretreatment

In order to modify the recalcitrant structure of lignocellulosic biomass to improve the subsequent hydrolysis step, different pretreatment methods have been reported in the scientific literature. Even considering the different available alternatives (Figure 2.1), this step is still considered as a persistent bottleneck in lignocellulosic biorefineries.

Figure 2.1 – Classification of lignocellulosic biomass pretreatment methods



Source: Based in the work of Mosier et al. (2005), Mood et al. (2013) and Chen et al. (2017).

Each pretreatment method presents a specific mechanism on the biomass; however, overall changes correspond to the modification in the structure (decreasing the crystallinity, reducing the degree of polymerization and increasing the porosity) or changing in the composition (hemicellulose or lignin removal), turning the pretreated material more suitable for enzymatic attack. Below are some of the main chemical and physico-chemical pretreatment methods such as alkaline pretreatment, acid pretreatment, steam explosion and ammonia fiber explosion (AFEX) discussed briefly.

Steam explosion (SE) is a physio-chemical pretreatment method. It is one of the oldest and more effective method that facilitate enzymatic hydrolysis and is currently the most commonly investigated to develop the commercial biorefineries (RAVINDRAN; JAISWAL, 2016). In this process, the biomass is submitted to high pressure (15-48 bar) and temperature (200-250 °C) for a few times (0.5 – 5 min); after this, the biomass is rapidly decompressed. That abrupt decompression results in the breakdown of the lignin-carbohydrate complex (PIELHOP et al., 2016). The main disadvantages of this method include incomplete disruption of the lignin-carbohydrate matrix which leads to the condensation and precipitation of soluble lignin components on the carbohydrate fraction turning the biomass less digestible, besides generation of inhibitory compounds due to harsh process conditions (SUN; CHENG, 2002; AGBOR et al., 2011).

Ammonia fiber/freeze explosion (AFEX) process is another physio-chemical method. This process require high pressure (10-50 bar) and lower temperatures (<100 °C) than SE process (AGBOR et al., 2011). Under such conditions, hemicellulose and lignin are partially removal and the cellulose crystallinity is reduced. The pretreated biomass presents high enzymatic digestibility yield and there is no formation of inhibitors, allowing the use of hydrolysate in fermentation process without any previous detoxification treatment (CHEN et al., 2017). Some disadvantages of this method correspond to high cost of ammonia and ineffectiveness when the biomass presents high lignin content in its composition (18-30%) (AGBOR et al., 2011).

Other pretreatment options correspond to chemical methods using acids (inorganics and organics), alkalis, ozone, organosolv or ionic liquid. Acid pretreatment can be carried out using concentrated acid or dilute acid solutions. Concentrated acid is performed using concentration of acids higher than 30% and temperatures about 100 °C or less. In those conditions carbohydrates are hydrolyzed into monosaccharides without necessity of a subsequent enzymatic hydrolysis (CHEN et al., 2017). However, although they are powerful agents for cellulose and hemicellulose hydrolysis, concentrated acids are toxic, corrosive and hazardous and require reactors that are resistant to corrosion. Besides, their use results in high operational and maintenance costs which offer difficulties in the commercial scale application (AGBOR et al., 2011; RAVINDRAN; JAISWAL, 2016). Considering these drawbacks, dilute acid pretreatment using concentration of acids lower than 4

wt.% was proposed as a previous pretreatment to enzymatic hydrolysis. In dilute acid pretreatment, the hemicellulose is hydrolyzed into monomeric sugars and turns cellulosic fraction more exposed to cellulases. However, for an efficient pretreatment, high temperatures (140-240 °C) and pressures (> 10 bar) are necessary. In those conditions, fermentation inhibitory products such as HMF, furfural, acetic acid and formic acid are co-produced (JÖNSSON; MARTÍN, 2016).

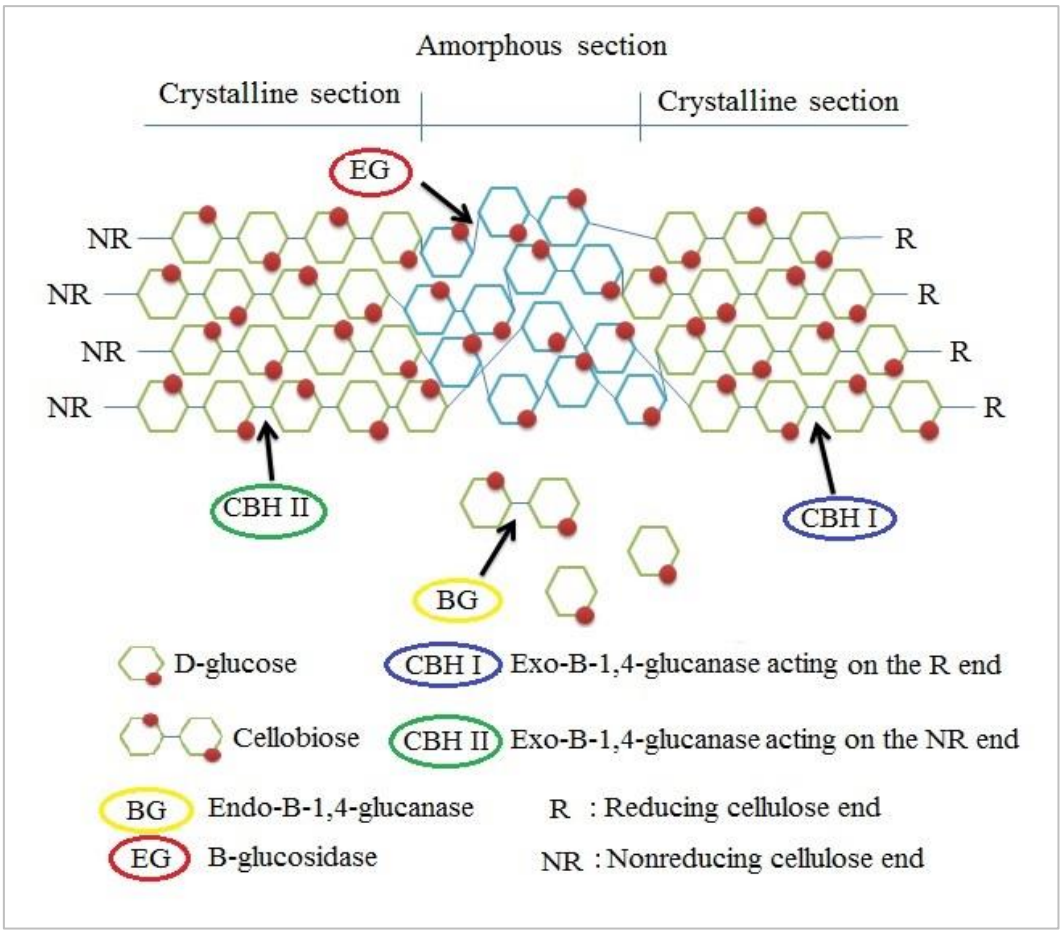
Considering the limitations associated with chemical pretreatment specifically with acids, alkaline methods have been proposed as appropriate alternatives, mainly due to less severity required during the process. Alkalis such as NaOH, KOH, $Ca(OH)_2$ and ammonium hydroxide have been commonly used for pretreatment (PHITSUWAN; SAKKA; RATANAKHANOKCHAI, 2016; TERÁN-HILARES et al., 2016). Main effects of alkaline pretreatments correspond to the lignin removal, besides partial removal of acetyl groups and uronic acids substitutions on hemicelluloses (KIM; LEE; KIM, 2016; RAVINDRAN; JAISWAL, 2016). After alkali pretreatment, the biomasses also present reduction in degree of polymerization and increase of the internal surface area due to swelling of cellulose (RAVINDRAN; JAISWAL, 2016). However, this method still offers some disadvantages which correspond to the necessity of high concentration of alkali when the process is carried out under low temperature, long process time, resulting in high cost of processing with the alkalis.

In this regard, different combined technologies have been proposed to improve the pretreatment efficiency using low concentration of alkalis and even so some reported works correspond to vacuum-assisted alkaline pretreatment (LV et al., 2017), ultrasound (WANG et al., 2017) and hydrodynamic cavitation (KIM et al., 2015; NAKASHIMA et al., 2016; MADISON et al., 2017). Ultrasound technology and hydrodynamic cavitation in alkaline medium enhance the enzymatic hydrolysis efficiency, allowing to reduce chemical consumption in the process. Actions of cavitation include chemical and mechanical effects that favor changes in both the biomass structure and composition (WANG et al., 2017). However, in the case of ultrasound technology, it presents some drawbacks as high energy consumption, complexity to application in large scale and high cost of system. Considering its disadvantages, hydrodynamic cavitation technology was proposed as alternative to ultrasound in pretreatment process and was studied extensively in this thesis.

2.3.2 Hydrolysis of carbohydrate fractions

After pretreatment process, the carbohydrate fractions, mainly cellulose, present in pretreated biomass is enzymatically hydrolyzed. For complete hydrolysis of cellulose fractions into monomers, a synergetic action of an enzymatic complex is usually necessary. Hydrolases are the main biocatalysts used in this way, including endoglucanases (EGs) (E.C. 3.2.1.4), exoglucanases/cellobiohydrolases (CBHs) (E.C. 3.2.1.91), and β-glucosidases/cellobiases (BGs) (E.C. 3.2.1.21) (VAN DYK; PLETSCHKE, 2012; EZEILO et al., 2017). A representative model of enzyme attack on cellulose is shown in Figure 2.2. As shown, EGs randomly attack the cellulose and create new chain ends; CBHs catalyze the hydrolysis of chain ends into cellobiose, and BGs break cellobiose into glucose (HURON et al., 2016).

Figure 2.2 – Schematic representation of the enzymatic hydrolysis of cellulose by cellulases



Source: Adapted from Ezeilo et al. (2017).

In the last years, the interest in a group of oxidases that contribute with degradation of cellulose has increased. This group corresponds to enzymes with auxiliary activity 9 (AA9/formerly GH61) and 10 (AA10/formerly CBM 33), the lytic polysaccharide monooxygenases (LPMO) (AACHMANN et al. 2012; CORRÊA; SANTOS; PEREIRA, 2015), that provide new ends for cellulases recognition and action in cellulose.

Current commercial cellulase preparations are usually a blend of different enzymes, including also other enzyme groups, as xylanases and pectinases, to help the hydrolytic process (MALGAS et al., 2017).

The efficiency of carbohydrate fraction hydrolysis is influenced by several factors such as cellulase activity, solid loading, temperature, pH, presence of surfactants, configuration of reaction vessel and characteristics of biomass (SUN; CHENG, 2002; TERÁN–HILARES et al., 2016). About this topic, there are good reviews in literature, as the articles published by Sun and Cheng (2002), Modenbach and Nokes (2013) and Khare, Pandey e Larroche (2015).

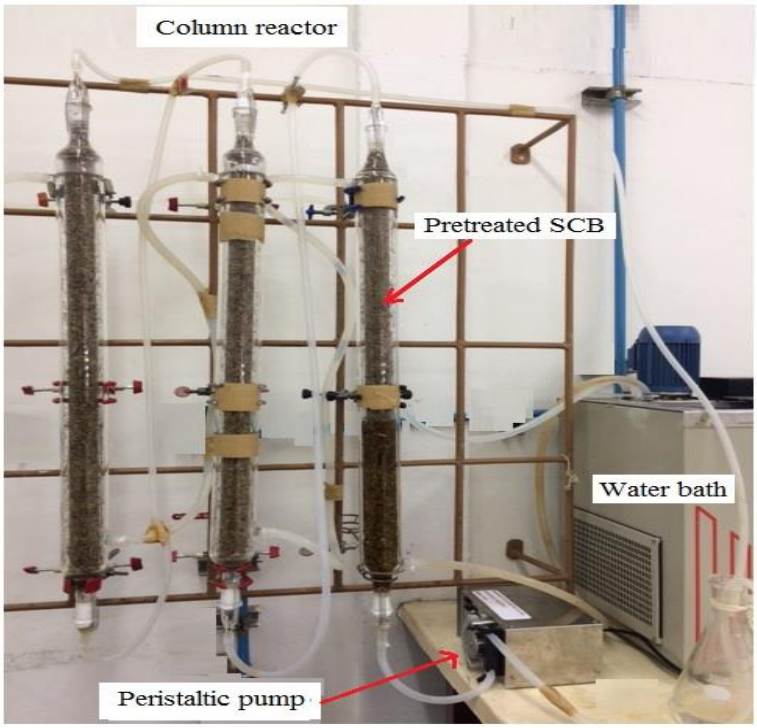
Among the different variables that influence enzymatic hydrolysis, high solid loading in the reactor is fundamental in order to obtain high sugar concentrations for fermentation process; however, one of the major challenges when high solid loading is used corresponds to the limited water availability in the reactor (MODENBACH; NOKES, 2013; TERÁN-HILARES et al., 2016). The availability of water in the medium is fundamental for catalyst transport, reducing its viscosity and solubilizing the released sugars to avoid end-products inhibition (ROCHE; DIBBLE; STICKEL, 2009).

Enzymatic hydrolysis using high solid loading in Erlenmeyer flasks and specified reactors was previously reported by Roche, Dibble e Stickel (2009), Silva et al. (2016), Terán-Hilares et al. (2016) and Du et al. (2017). For example, in report of Roche, Dibble and Stickel (2009), roller bottle reactors (RBRs) were used for enzymatic hydrolysis of corn stover pretreated with dilute acid in steam explosion process. In that work, 15, 20 and 30% of solid loading were hydrolyzed using GC220 (Genencor-Danisco, NY, USA) enzyme at 20 mg protein/g cellulose. Higher conversion yield (~90%) was observed using 15% of solids, that value decreased to ~84% and 70% of yield when solid loading was increased to 20 and 30%, respectively. Additionally, using 20% of solids with 30 mg of enzyme GC220/g of substrate, 65% and 85% of cellulose conversion yield were achieved in 2 and 7 days,

respectively. In the work of Du et al. (2017) was also reported enzymatic hydrolysis of corn stover at high solid loading. In that study, about 76%, 72% and 60% of cellulose conversion yield were achieved using 5%, 10% and 20% of solid loading, respectively.

The hydrolysis of cellulose fraction at 20% of solid loading of hydrothermally treated SCB was reported by Silva et al. (2016). In that work, about 75% and 70% of conversion yields were achieved in 72h of hydrolysis using CTH2 (blend of Cellic CTec2 and Cellic HTec2) and CT2 (Cellic CTec2) , respectively, at enzyme loading of 20 FPU/g of glucan. In the work of Terán-Hilares et al. (2016) a hydrolysis of SCB pretreated in alkaline condition (0.3 mol/L, 70 °C and during 4h) at high solid loading (13.2%) was reported in a packed bed flow-through column reactor (PBFTCR). In that work, 49% and 57% of cellulose and hemicellulose fractions were hydrolyzed, respectively, after 48h at 20 FPU/g of dry SCB. This kind of reactor was also evaluated in the Biopolymers, Bioreactors and Process Simulation Laboratory (LBBSIM) of Engineering School of Lorena-University of São Paulo (EEL-USP) using three columns in series (Figure 2.3).

Figure 2.3 – Packed bed flow-through column reactor used for enzymatic hydrolysis at high solid loading



Source: Personal archive

A problem when high solid loading is used for enzymatic hydrolysis is also related to unproductive adsorption of cellulases in residual lignin in pretreated biomass, resulting in an increased enzyme dosage to produce high hydrolysis yield (LIN et al., 2017). One alternative to overcome this drawback without increasing in enzyme loading corresponds to addition of surfactants. Actually, this strategy can increase the conversion of cellulose into soluble sugars, as demonstrated in studies of Castanon and Wilke (1981), Eriksson, Börjesson and Tjerneld (2002), Kristensen et al. (2007) and Lin et al. (2017).

In the study of Eriksson, Börjesson and Tjerneld (2002), for example, different non-ionic species such as octylphenol(ethyleneglycol)$_{9,6}$ ether (Triton X-100) and poly(oxyethylene)$_{20}$ sorbitan-monolaurate (Tween 20) and charged as dodecyltrimethylammonium bromide (DoTAB) and Sodium dodecylsulphate (SDS) surfactants were added in the medium during enzymatic hydrolysis of cellulose of steam-pretreated spruce. In that work, authors concluded that addition of surfactant improved the conversion of cellulose by the reduction of the unproductive enzyme adsorption to the lignin part of the substrate. This is due to hydrophobic interaction of surfactant with lignin on the lignocellulose surface, which releases unspecifically bounded enzyme. Additionally, a reduction from 90% to 80% of enzyme adsorption on the biomass was observed after addition of surfactant. In another work, a mix of sodium dodecyl sulfate and cetyltrimethylammonium bromide (SDS- CTAB) at molar ratio of 0.001–0.1 mM was evaluated during enzymatic hydrolysis of corn stover (LIN et al., 2017). In that work, 80% of hydrolysis yield was observed using 30 FPU/g glucan of enzyme loading in process without supplementation with SDS-CTAB and 12.5 FPU/g glucan of enzyme loading in the presence of SDS-CTAB. Additional information about the use of surfactants can be found in the review paper published by Mondenbach and Nokes (2013).

2.3.3 Fermentation into ethanol

Sugars mainly derived as glucose, cellobiose, xylose and arabinose present in hydrolysates can be converted into ethanol, polymers, organic acids and other bio-products by fermentation process using different microorganism and using different process (batch, fed-batch and continuous).

The glucose present in hydrolysate is usually fermented by *Saccharomyces cerevisiae* into ethanol (KARAPATSIA et al., 2016; MISHRA et al., 2016). However, due to presence of xylan and other pentose-based polysaccharides in raw material, monomeric pentoses are also available as substrate and thus yeasts that can metabolize them are required. In this way, different microorganisms have been evaluated. For example, *Scheffersomyces stipitis* was used for co-fermentation of glucose and xylose present in enzymatic hydrolysate of sugarcane bagasse into ethanol, as reported by Terán-Hilares et al. (2016). In that work, 23.4 g/L of ethanol (yield of 0.4 g/g) was achieved, with complete consumption of glucose, cellobiose and xylose in the hydrolysate. In another work, *S. stipitis* and *Spathaspora passalidarum* were used to convert glucose and xylose present in SCB enzymatic hydrolysate into ethanol in fed-batch process (NAKANISHI et al., 2017). In that work, 18.52 g/L of ethanol (yield of 0.32 g/g) and 0.36 g/L.h were achieved using *S. stipitis* in a sequential fed batch cell recycle process. In the same configuration of fermentation process, 23.3 g/L of ethanol (0.46 g/g) and 0.81 g/L.h of productivity were achieved using *S. passalidarum*. For both cases, the microorganism showed preferential consumption of glucose instead of xylose, a phenomenon known as diauxic shift (repression of D-xylose reductase (XR) and xylitol dehydrogenase (XD) enzymes involved in xylose metabolism by the presence of glucose), as reported by different authors (GUTIÉRREZ-RIVERA et al., 2011; SANTOS et al., 2015).

The hemicellulosic fraction is usually obtained by acid hydrolysis, and the hydrolysate present high concentration of xylose in its composition. This sugar can be converted into ethanol by several microorganisms as *S. stipitis*, *S. shehatae* and others. Dussán et al. (2016), e.g. evaluated the influence of different variables (aeration, agitation rate and initial pH) on ethanol production from SCB hemicellulosic hydrolysate by *S. shehatae* UFMG HM 52.2 in batch process. In that work, the maximum ethanol production was 18 g/L (yield of 0.44 g/g and productivity of 0.25 g/L.h), achieved in the following condition: 0.1 vvm, 100 rpm and initial pH of 6.50.

Continuous co-production of ethanol and xylitol from rice straw hydrolysate in a membrane bioreactor using co-culture of *Saccharomyces cerevisiae* NCIM 3090 and *Candida tropicalis* NCIM 3119 was recently evaluated by Zahed et al. (2016). In that study, in the batch co-culture system, the ethanol and xylitol productions were 33.4 g/L (0.44 g/g yield) and 25.1 g/L (0.55 g/g yield), respectively. However, in

continuous co-culture process, 55 g/L and 31 g/L of ethanol and xylitol were achieved at the dilution rate of 0.03 L per hour, respectively. Thus, continuous process is an interesting strategy for bioproducts production from lignocellulosic hydrolysates. Additionally, co-culture and co-fermentation strategies can be also an interesting alternative that requires deeper studies.

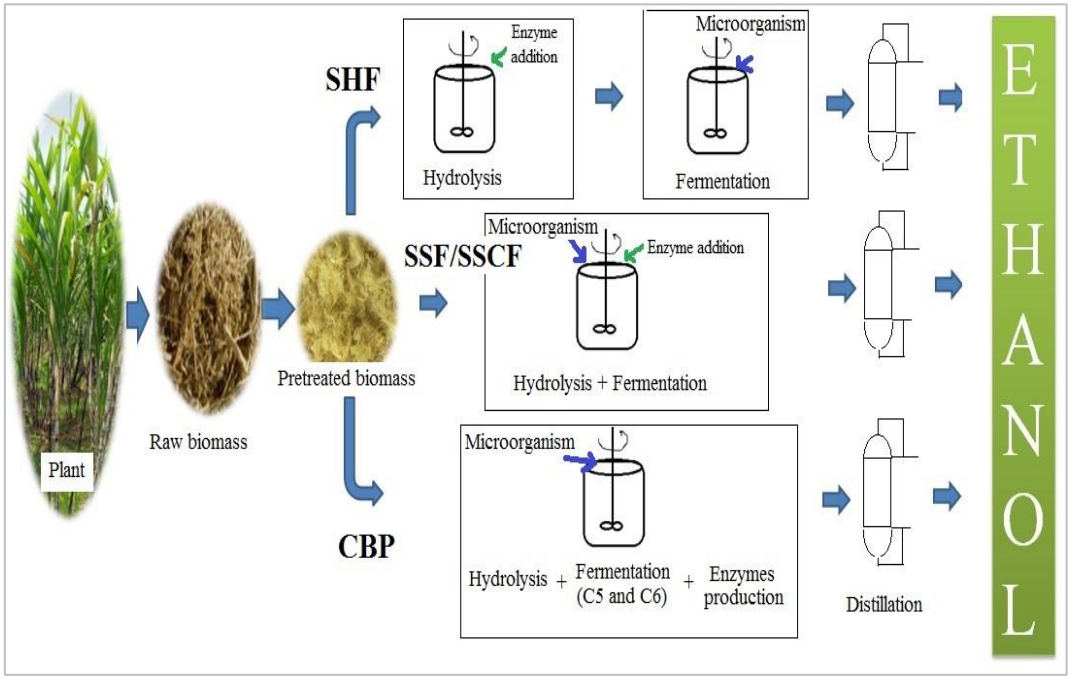
Other strategy to co-fermentation of pentose and glucose into ethanol is the use of genetically engineered microorganisms, including *S. cerevisiae*. This alternative can be exemplified by the recent work of Lee et al. (2017). In that work, the *S. cerevisiae* was modified by the introduction of xylose metabolic pathway. By using lignocellulosic hydrolysate containing initial glucose (~105 g/L) and xylose (~ 35 g/L), 55 g/L of ethanol was produced, and also the simultaneous consumption of both sugars was observed

2.4 Process configuration alternatives for biotechnological processes in biorefinery

In this section, different alternative process configurations for biorefineries have been proposed and discussed thereof. The main alternatives that have been studied for ethanol production are schematically shown in Figure 2.4.

Enzymatic hydrolysis and fermentation are usually carried out in a process configuration known as Separate Hydrolysis and Fermentation (SHF). In this system, hydrolysis of carbohydrate fraction and fermentation are carried out separately, and each process is performed in a sequence, commonly in different bioreactors. The main advantages of this process correspond to facility of working under optimized conditions of temperature and pH for each biological step (PAULOVA et al., 2015). However, the main drawback of this process corresponds to enzyme activity inhibition problems due to high glucose and cellobiose concentration in the hydrolysate. Moreover, higher capital cost requirements due to the necessity of different vessels is another drawback of this particular configuration (DAHNUM et al., 2015).

Figure 2.4 – Schematic representation of different configurations for ethanol production from biomass.



Source: Personal archive

As alternative, Simultaneous Saccharification and Fermentation (SSF) process has been proposed. In that process, the released sugars are simultaneously converted into ethanol as they are hydrolyzed from the material. When pentoses are also fermented, the process is named Simultaneous Saccharification and co-Fermentation (SSCF). The main advantages of SSF/SSCF compared to SHF corresponds to reduction in number of required vessels, less process time and cost, and non-accumulation of inhibitory products of hydrolysis (WINGREN; GALBE; ZACCHI, 2008; LOACES; SCHEIN; NOYA, 2017). However, SSF/SSCF presents also a disadvantage which corresponds to development of the process in non-optimized temperature and pH condition when the process is carried out in the same vessel (DAHNUM et al., 2015). An alternative corresponds to the use of interconnected reactors for SSF/SSCF process keeping the optimal temperatures for each step. For example, in the study of Viola et al. (2013), a two-chamber reactor separated by a membrane (acrylic tissue with 10 μm pores) was used to improve enzymatic hydrolysis and fermentation of lignocellulosic materials. In that work, using the proposed system, an increase in 20% in ethanol yield was reached compared to the value obtained using only one vessel.

Consolidated bioprocess (CBP) is another option, which integrates all biological events required for biomass conversion, and it corresponds to simultaneous performing of enzyme production, hydrolysis of carbohydrate fraction and fermentation of resulting sugars (C5 and C6) (SINGH et al., 2017). Some microorganism mainly bacteria (*Clostridium* and *Caldicellulosiruptor*) were proposed as excellent CBP candidate due to its remarkable cellulose hydrolysis ability and conversion into ethanol (AKINOSHO et al., 2014). A CBP process for ethanol production was reported by Singh et al. (2017). In that work, the raw and pretreated rice straw was used in CBP process in order to produce ethanol and other soluble metabolic end-products by newly isolated strain *C. thermocellum* DBT-IOCC19. At the end of fermentation (96 h), 2.31 mM lactate, 9.05 mM acetate, and 14.15 mM ethanol were produced. Additionally, the bacterium displayed 95.6% and 82.74% degradation of avicel cellulose at 5 g/L and 10 g/L in 96 h of fermentation, respectively. By using 10 g/L of avicel, 35 mM ethanol, 18.15 mM acetate, and 4.88 mM lactate were produced.

2.5 Second generation bioethanol production: Current scenario in Brazil

Currently, there are two plants for 2G ethanol production (GranBio and Raizen) in commercial scale in Brazil. According to Ministry of Mines and Energy of Brazil (MME, 2015), the national projection for cellulosic ethanol production in Brazil for 2024 is up to 429 million L, considering the production of seven installed and in construction plants.

The company RAIZEN started operation in 2014, with initial investment of R$ 237 million in research, development and structure. The nominal capacity projected by the company was 40 million of liter of 2G-ethanol production by year (RAIZEN, 2014). According to Energy and Mining State Secretariat of São Paulo (2017), the projected production of second generation ethanol during 2017/18 will be about 16 million of liter, value lower than 50% of nominal capacity of the company. That value is higher than obtained in 2016/17 which was near to 8 million of liters.

The Bioflex 1, the industrial unit of GranBio, also started the production of second generation ethanol from straw and sugarcane bagasse in 2014. This company was constructed to produce 82 million of liter per year. However, in 2015, the

company produced only 4 million of liters and in 2017 was projected to achieve 50% of real capacity (NOVACANA, 2016).

In both these cases, the technological bottlenecks are the transport of bagasse to reactors and the pretreatment process. Besides, other problems are also associated to current economic situation of Brazil. The National Bank for Economic and Social Development (BNDES) of Brazil is responsible for 40 to 50% of financing of Brazilian sucro-energetic industry. In the case of GranBio Company, BNDES has 15% of the business with a contribution of $190 million (NOVACANA, 2017a). However, the presence of BNDES in sucro-energetic sector has been falling year after year with successive negative records. Now, with the data for the first half of 2017 released, the situation has worsened further with reduction in 26% compared to period of 2016 (NOVACANA, 2017b).

One option to turn more favorable the economic viability of 2G ethanol production is the production of this alcohol in the context of biorefineries with a larger pool of interesting bioproducts obtained from biomass (FITZPATRICK et al., 2010). In the next section, some possibilities of products of industrial interest with potential to compose a biorefinery framework are discussed.

2.6 Production of sustainable chemicals from lignocellulosic biomass

Besides ethanol, the sugars released after hydrolysis process (mainly glucose and xylose when SCB is used as raw material) can be also used for production of different bio-based molecules, including polymers, xylitol, pigments, organic acids and others (SINDHU et al. 2016; TERÁN HILARES et al., 2017). In this section, some examples of bioproducts as polymers, biosurfactants and lactic acids, obtained from different lignocelluloses have been discussed.

2.6.1 Polymers

Polymers mainly derived from petroleum resources present properties of non-biocompatibility and non-biodegradability, limiting thus their application in different areas ranging from medicine, food additives, biosensors and others (MOGOŞANU; GRUMEZESCU, 2014). In recent years, alternative biopolymers have got attention, mainly due to the aspects of biocompatibility and biodegradable; these properties

turn possible their applications as medical material, packaging, cosmetics, food additives and others (RAJ; MUMJITHA, 2015). Biopolymers can be obtained from different sources such as animal (e.g. chitosan and collagen), plants (e.g. starch and gums) and microorganisms (e.g. polyhydroxyalkanoates (PHAs), xanthan and pullulan) or can be chemically produced from monomers obtained from biological sources (e.g. poly(lactic acid)) (KOUTINAS et al., 2014). Microbial production way is an attractive alternative mainly due to non-dependence of environmental conditions, presenting higher yield of production and production using different lignocellulosic feedstock as a carbon sources (VIJAYENDRA; SHAMALA, 2013).

Polyhydroxyalkanoates (PHAs) correspond to microbial biopolymers which are synthetized by diverse bacteria under nutrient unbalanced conditions using different substrates as sugars (glucose and sucrose), alkanes and fatty acids (RAMYA et al., 2017). The homopolymer poly-3-hydroxybutyrate (P3HB) and the copolymer poly-3-hydroxybutyrate-co-3-hydroxyvalerate (P3HB-co-3HV) are the most extensively studied PHA compounds (SILVA et al., 2004; GONZÁLEZ-GARCÍA et al., 2011). Considering the expensive cost of carbon sources (e.g. pure carbohydrates) usually used for PHA and PH3B production, inexpensive raw materials have been proposed, including agro-industrial and forest residues and by-products, such as sugarcane bagasse, wheat straw, rice straw, sweetgrass and others (CESÁRIO et al., 2014). In the work of Cesário et al. (2014), P3HB was produced by *Burkholderia sacchari* DSM 17165 using commercial sugars (glucose and xylose) and enzymatic hydrolysate of wheat straw. In that work, using commercial sugars, 0.7 g P3HB/g cell dry weight (CDW) with a yield of polymer on sugars ($Y_{P/S}$) of 0.18 g/g were achieved; moreover, using wheat straw hydrolysate, 0.6 g P3HB/g CDW and 0.19 g/g of yield were achieved.

Other interesting biopolymer corresponds to pullulan produced by *Aureobasidium pullulans*, a black-yeast-like fungus. It consists of maltotriose repeating units further connected by α-(1→6) linkages. This particular linkage confers it a considerable solubility in water compared to other polysaccharides (Wu et al., 2016). However, *A. pullulans* strains are also associated with the formation of a pigment (melanin) actually unwanted in pullulan production process, requiring additional decolorization steps, thus making it an expensive choice (WU et al., 2009; RAVELLA et al., 2010). Another key aspect in pullulan production corresponds to the necessity of replacement the expensive carbon sources (liquefied starch, sucrose

and pure carbohydrates) by low cost ones. Several alternative carbon sources rice hull hydrolysate (WANG et al., 2014) and SCB hemicellulosic hydrolysate (CHEN et al., 2014), have been used to produce this biopolymer.

In a recently study reported by Terán Hilares et al. (2017), different strategies were evaluated in order to simplify the pullulan production process using low cost carbon source. In that study, light-emitting diodes (LEDs) of different wavelengths were used to assist the fermentation process aiming to produce low-melanin containing pullulan by the wild strain of *A. pullulans* LB83 with different carbon sources. Under white light using glucose-based medium, 11.75 g/L of pullulan with high melanin content (45.70 UA_{540nm}/g of pullulan) was obtained. The process was improved by assisting the fermentation by blue LED light, resulting in 15.77 g/L of pullulan with reduced content of melanin (4.46 UA_{540nm}/g of pullulan). By using sugarcane bagasse (SCB) hydrolysate as carbon source, similar concentration of pullulan (about 20 g/L) was achieved using white and blue LED lights, with lower melanin contents in last option.

2.6.2 Biosurfactants

Biosurfactants (BSs) naturally produced by microorganisms present advantages compared to synthetic surfactants derived from petroleum or oleo-chemical sources, mainly due to low or non-toxicity, biodegradability, biocompatibility, comparable surface activity than synthetic, and several other applications (LEE et al., 2008; SAMAD et al., 2016).

BSs can be extracted from natural sources or obtained by chemical modification of them (called “first generation biosurfactants”). However, microbial BS’s (the so-called “second generation biosurfactants”) have attracted great attention in research works (MADSEN et al., 2015).

Microbial BSs are classified according to their chemical structure and include glycolipids, lipopeptides and lipoproteins, phospholipids and fatty acids, polymeric surfactants, and particulate surfactants (DESAI; BANAT, 1997).

Among BSs, Sophorolipids (SLs) can be considered the most promising to be produced from lignocellulosic hydrolysates. Actually, although there are other important BSs classes, as rhamnolipids, SLs are advantageous because they can be produced by non-pathogenic yeast strains as *Candida apicola*, *Rhodotorula*

bogoriensis, *Wickerhamiella domericqiae* and others (VAN BOGAERT; ZHANG; SOETAERT, 2011). SLs are composed by two components: a sophorose head (a dimeric sugar residue) and a hydroxylated fatty acid (ROELANTS et al., 2015).

Some current challenges in SLs production correspond to the use of low cost substrate and the optimization of fermentation parameters. Different cheaper carbon sources can be used for SLs production; however, glucose and oleic acid are the most common substrates. In this way, considering the availability and low cost of lignocellulosic biomass, different studies were reported using cellulosic hydrolysates for this purpose (MOLDES et al., 2007; SAMAD et al., 2016).

In the study of Moldes et al. (2007), detoxified hemicellulosic hydrolysate of corn cobs was used for intracellular biosurfactant production by *Lactobacillus pentosus*. In that work, 4.7 g/L of intracellular biosurfactant, 0.53 g of intracellular biosurfactant per g of biomass ($Y_{BS/BM}$) and 0.53 g of intracellular biosurfactant per g of sugar consumed ($Y_{BS/S}$), were achieved. Corn stover was also used as a substrate for SLs production by *Candida bombicola*, as reported by Samad et al. (2016). In that study, initial experiment was carried out in 250-mL Erlenmeyer flask with 50 mL of medium. About 11.6 g/L of SL (yield of 0.12 g of SL per g of carbon source) was produced in 14 days, using as carbon source the enzymatic hydrolysate of corn stover (65 g/L of initial total sugars) supplemented with 10 g/L of soybean oil. Additionally, sophorolipid was produced in 3-L of bioreactor using same hydrolysate and supplemented with yellow grease (10 g/L of initial concentration). In that condition, 52.1 g/L (0.35 g/g of sugar plus yellow grease) was achieved.

In another report, Liu et al. (2016) related the use of enzymatic hydrolysate from rice straw pretreated via SO_3 micro-thermal explosion for SLs production by *Wickerhamiella domercqiae* var. *sophorolipid* CGMCC 1576. In that work, using 60 g/L of glucose as hydrophilic carbon source and 60 ml/L of hydrophobic carbon source, 53.7 g/L of SLs were produced after 168h of fermentation at 30 °C of process temperature.

2.6.3 Lactic acid

Lactic acid (2-hydroxypropanoic acid, CH_3–CH(OH)–COOH) is a natural organic acid with several applications in food industry, polymers, pharmaceutical, cosmetic, and chemical industries, which is produced by different strains of

Lactobacillus. It can be produced in two optical isomers: L-(+)-lactic acid produced by *L. casei, L. paracasei,* and *L. rhamnosus*; D-(-)-lactic acid produced by *L. delbrueckii, L. coryniformis*, *L. jensenii*, and *L. vitulinus*; DL-lactic acid produced by *L. pentosus, L. plantarum, L. brevis, L. sake,* and *L. acidophilus* (MACK, 2004; ABDEL-RAHMAN; SONOMOTO, 2016). L-Lactic acid is used for the synthesis of poly L-lactic acid (PLLA), a biodegradable and thermostable polymer with several applications in orthopedic fixation, packaging and dental applications; on the other hand, D-Lactic acid is used for the production of poly D-lactic acid (PDLA) (JOHN; NAMPOOTHIRI; PANDEY, 2007).

Lactic acid can be produced by chemical synthesis or microbial fermentation. Some advantages of the microbial way correspond to utilization of renewable carbohydrate biomass, low temperature of production, and the production of high optical purity lactic acid by selecting an appropriate strain (KUO et al., 2015; ABDEL-RAHMAN; SONOMOTO, 2016). For fermentation process, different carbon sources have been used; for example, in the work of Kuo et al. (2015), nondetoxified wood hydrolysate was employed for optically pure L-lactic acid production by using a newly isolated and D-lactate dehydrogenase gene-deficient *L. paracasei* strain. In that work, 99 g/L of L-lactic acid with respective yield of 0.96 g/g and productivity of 2.25 g/L.h, was produced in 120h of fermentation.

In the report of Adsul, Varma and Gokhale (2007), enzymatic hydrolysate of cellulosic fraction of SCB was used for production of L-(+)-lactic acid by *L. delbrueckii* mutant Uc-3 in a SSF process. In that study, 67 g/L of lactic acid, with respective productivity of 0.93 g/L.h and yield of 0.83 g/g, was produced from 80 g/L of cellulose. Additionally, the conversion of cellobiose into lactic acid was observed in a homo-fermentative way. Oonkhanond et al. (2017) also reported the use of SCB hydrolysate for lactic acid production by *L. casei* in a 3-L bioreactor. Those authors observed production of 21.3 g/L after 120 h with a productivity of 0.63 g/L h. To summarize, the production of lactic acid from agro-industrial residues is an attractive alternative, which can contribute to the viability of biorefineries as one possible option.

2.7 Conclusion

The carbohydrate fractions present in SCB (a massive Brazilian biowaste) can be potentially deployed for the bioproduction of second generation ethanol and useful biochemicals. The structural organization of SCB along with presence of lignin makes it more recalcitrant, reducing the biological conversion of carbohydrates. Therefore, the pretreatment step has been considered as one persistent bottleneck that still requires the development of efficient methods. The carbohydrate fraction in pretreated biomass can be enzymatically hydrolyzed; however, high solid loading in the reactor is desirable to obtaining high sugars concentration. Also, different alternatives have proposed in order to increase the hydrolysis efficiency as addition of surfactant and use of specific reactors. Released sugars can be fermented into several metabolites as 2G ethanol with different process configuration (SHF/SSF/SSCF/CBP) in order to achieve high fermentation yield. In Brazil, 2G ethanol from straw and sugarcane bagasse has been produced in commercial scale; however, due to persistent technological bottlenecks and non-favorable economic situation of Brazil, companies are not achieving the installed capacity. An option to advocate economic viability of future biorefineries is the production of different commodity green materials viz, polymers, biosurfactants, lactic acid etc. instead of merely producing bioethanol.

2.8 References

AACHMANN F. L. et al. NMR structure of a lytic polysaccharide monooxygenase provides insight into copper binding, protein dynamics, and substrate interactions. **Proceedings of the National Academy of Science**, v. 109, p. 18779–18784, 2012.

ABDEL-RAHMAN, M. A.; SONOMOTO, K. Opportunities to overcome the current limitations and challenges for efficient microbial production of optically pure lactic acid. **Journal of Biotechnology,** v. 236, p.176-192, 2016.

ADSUL, M. G.; VARMA, A. J.; GOKHALE, D. V. Lactic acid production from waste sugarcane bagasse derived cellulose. **Green Chemistry,** v. 9, p.58-62, 2007.

AGBOR, V. B. et al. Biomass pretreatment: Fundamentals toward application. **Biotechnology Advances,** v. 29, p.675-685, 2011.

AHMED, M. A. et al. Optimization of twin gear-based pretreatment of rice straw for bioethanol production. **Energy Conversion and Management,** v. 141, p.120-125, 2017.

AKINOSHO, H. et al. The emergence of *Clostridium thermocellum* as a high utility candidate for consolidated bioprocessing applications. **Frontiers in Chemistry,** v. 2, p.66-72, 2014.

ANWAR, Z.; GULFRAZ, M.; IRSHAD, M. Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: A brief review. **Journal of Radiation Research and Applied Sciences,** v. 7, p.163-173, 2014.

CARVALHO, D. M.; QUEIROZ, J. H.; COLODETTE, J. L. Assessment of alkaline pretreatment for the production of bioethanol from eucalyptus, sugarcane bagasse and sugarcane straw. **Industrial Crops and Products,** v. 94, p.932-941, 2016.

CASTANON, M.; WILKE, C. R. Effect of the surfactant tween 80 on enzymatic hydrolysis of newspaper. **Biotechnology and Bioengineering**, v. 23, p. 1365-1372, 1981.

CESÁRIO, M. T. et al. Enhanced bioproduction of poly-3-hydroxybutyrate from wheat straw lignocellulosic hydrolysates. **New Biotechnology,** v. 31, p.104-113, 2014.

CHEN, H. et al. A review on the pretreatment of lignocellulose for high-value chemicals. **Fuel Processing Technology**, v. 160, p. 196–206, 2017.

CHEN, Y. et al. Production of pullulan from xylose and hemicellulose hydrolysate by *Aureobasidium pullulans* AY82 with pH control and DL-dithiothreitol addition. **Biotechnology And Bioprocess Engineering,** v. 19, p.282-288, 2014.

CONAB – COMPANHIA NACIONAL DE ABASTECIMENTO. Acompanhamento da safra brasileira: cana de açúcar, V. 3 - SAFRA 2016/17 N.4 - Quarto levantamento, 2017. Available in: http://www.conab.gov.br/OlalaCMS/uploads/arquivos/17_04_19_11_27_36_boletim_cana_portugues_-_4o_lev_-_16-17.pdf. Accessed in: 26 Jul. 2017.

CORRÊA, T. L. R.; SANTOS, L. V.; PEREIRA, G. A. G. AA9 and AA10: from enigmatic to essential enzymes. **Applied Microbiology and Biotechnology,** v. 100, p.9-16, 2015.

CRIMES, J. et al. Assessment of pre-treatment technologies for bioethanol production from sugarcane bagasse considering economics and environmental impact. **Asia-pacific Journal of Chemical Engineering**, v. 12, p.212–229, 2017.

DAHNUM, D. et al. Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch. **Energy Procedia,** v. 68, p.107-116, 2015.

DESAI, J. D.; BANAT, I. M. Microbial production of surfactants and their commercial potential. **Microbiology and Molecular Biology Reviews**, v. 61, p. 47–64, 1997.

DU, J. et al. Identifying and overcoming the effect of mass transfer limitation on decreased yield in enzymatic hydrolysis of lignocellulose at high solid concentrations. **Bioresource Technology,** v. 229, p.88-95, 2017.

DU, J. et al. Identifying and overcoming the effect of mass transfer limitation on decreased yield in enzymatic hydrolysis of lignocellulose at high solid concentrations. **Bioresource Technology,** v. 229, p.88-95, 2017.

DUSSÁN, K. J. et al. Evaluation of oxygen availability on ethanol production from sugarcane bagasse hydrolysate in a batch bioreactor using two strains of xylose-fermenting yeast. **Renewable Energy,** v. 87, p.703-710, 2016.

ERIKSSON, T.; BÖRJESSON, J.; TJERNELD, F. Mechanism of surfactant effect in enzymatic hydrolysis of lignocellulose. **Enzyme and Microbial Technology,** v. 31, p.353-364, 2002.

EUROPEAN COMMISSION. 2020 climate & energy package. the 2020 package is a set of binding legislation to ensure the eu meets its climate and energy targets for the year 2020, 2009. available in: https://ec.europa.eu/clima/policies/strategies/2020_pt#tab-0-1. accessed in: 10 apr. 2017.

EZEILO, U. R. et al. Enzymatic breakdown of lignocellulosic biomass: the role of glycosyl hydrolases and lytic polysaccharide monooxygenases. **Biotechnology & Biotechnological Equipment**, v. 31, p. 647-662, 2017.

FITZPATRICK, M. et al. A biorefinery processing perspective: Treatment of lignocellulosic materials for the production of value-added products. **Bioresource Technology,** v. 101, p.8915-8922, 2010.

GONZÁLEZ-GARCÍA, Y. et al. Polyhydroxybutyrate production by *Saccharophagus degradans* using raw starch as carbon source. **Engineering in Life Sciences,** v. 11, p.59-64, 2011.

GUTIÉRREZ-RIVERA, B. et al. Conversion efficiency of glucose/xylose mixtures for ethanol production using Saccharomyces cerevisiae ITV01 and Pichia stipitis NRRL Y-7124. **Journal of Chemical Technology & Biotechnology,** v. 87, p.263-270, 2011.

HURON, M. et al. Mechanistic modeling of enzymatic hydrolysis of cellulose integrating substrate morphology and cocktail composition. **Biotechnology and Bioengineering,** v. 113, p.1011-1023, 2015.

JIANG, L. et al. Obtaining fermentable sugars by dilute acid hydrolysis of hemicellulose and fast pyrolysis of cellulose. **Bioresource Technology**, v. 182, p. 364-367, 2015.

JOHN, R. P.; NAMPOOTHIRI, K. M.; PANDEY, A. Fermentative production of lactic acid from biomass: an overview on process developments and future perspectives. **Applied Microbiology and Biotechnology, v.**74, p.524-534, 2007.

JÖNSSON, L. J.; MARTÍN, C. Pretreatment of lignocellulose: Formation of inhibitory by-products and strategies for minimizing their effects. **Bioresource Technology,** v. 199, p.103-112, 2016.

KARAPATSIA, A. et al. Fed-batch Saccharomyces cerevisiae fermentation of hydrolysate sugars: A dynamic model-based approach for high yield ethanol production. **Biomass and Bioenergy,** v. 90, p.32-41, 2016.

KAWAGUCHI, H. et al. Bioprocessing of bio-based chemicals produced from lignocellulosic feedstocks. **Current Opinion in Biotechnology**, v. 42, p. 30-39, 2016.

KHARE, S. K; PANDEY, A.; LARROCHE, C. Current perspectives in enzymatic saccharification of lignocellulosic biomass. **Biochemical Engineering Journal,** v. 102, p.38-44, 2015.

KIM, I. et al. Hydrodynamic cavitation as a novel pretreatment approach for bioethanol production from reed. **Bioresource Technology**, v. 192, p. 335–339, 2015.

KIM, J. S.; LEE, Y. Y.; KIM, T. H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. **Bioresource Technology,** v. 199, p.42-48, 2016.

KOUTINAS, A. A. et al. Valorization of industrial waste and by-product streams via fermentation for the production of chemicals and biopolymers. **Chemical Society Reviews,** v. 43, p.2587-2598, 2014.

KRISTENSEN, J. B. et al. Use of surface active additives in enzymatic hydrolysis of wheat straw lignocellulose. **Enzyme And Microbial Technology,** v. 40, p.888-895, 2007.

KUO, Y. et al. Production of optically pure L-lactic acid from lignocellulosic hydrolysate by using a newly isolated and d -lactate dehydrogenase gene-deficient *Lactobacillus paracasei* strain. **Bioresource Technology,** v. 198, p.651-657, 2015.

LEE, S-C. et al. Characterization of new biosurfactant produced by *Klebsiella* sp. Y6-1 isolated from waste soybean oil. **Bioresource Technology,** v. 99, p.2288-2292, 2008.

LEE, Y. et al. Bioethanol production from cellulosic hydrolysates by engineered industrial *Saccharomyces cerevisiae*. **Bioresource Technology,** v. 228, p.355-361, 2017

LIN, X. et al. Effect of cationic surfactant cetyltrimethylammonium bromide on the enzymatic hydrolysis of cellulose. **Cellulose,** v. 24, p.61-68, 2016.

LIU, X. et al. Sophorolipids production from rice straw via SO3 micro-thermal explosion by *Wickerhamiella domercqiae* var. *sophorolipid* CGMCC 1576. **Amb Express,** v. 6, p.60-67, 2016.

LOACES, I.; SCHEIN, S.; NOYA, F. Ethanol production by *Escherichia coli* from Arundo donax biomass under SSF, SHF or CBP process configurations and in situ production of a multifunctional glucanase and xylanase. **Bioresource Technology,** v. 224, p.307-313, 2017.

LV, X. et al. Vacuum-assisted alkaline pretreatment as an innovative approach for enhancing fermentable sugar yield and decreasing inhibitor production of sugarcane bagasse. **Bioresource Technology,** v. 239, p.402-411, 2017.

MACK, D. R. D(-)-Lactic acid producing probiotics, D(-)-Lactic acidosis and infants. **Canadian Journal of Gastroenterology,** v. 18, p.671-675, 2004.

MADISON, M. J. Mechanical pretreatment of biomass – Part I: Acoustic and hydrodynamic cavitation. **Biomass Bioenergy**, v. 98, p. 135–141, 2017.

MADSEN, J. K. et al. The anionic biosurfactant rhamnolipid does not denature industrial enzymes. **Frontiers in Microbiology,** v. 6, p.292-310, 2015.

MALGAS, S. et al. Time dependence of enzyme synergism during the degradation of model and natural lignocellulosic substrates. **Enzyme and Microbial Technology,** v. 103, p. 1–11, 2017.

MISHRA, A. et al. Lignocellulosic ethanol production employing immobilized *Saccharomyces cerevisiae* in packed bed reactor. **Renewable Energy,** v. 98, p.57-63, 2016.

MME - MINISTÉRIO DE MINAS E ENERGIA - BRASIL / Secretaria de Planejamento e Desenvolvimento Energético. 2015. Plano decenal de expansão de energia 2024. http://www.epe.gov.br/PDEE/Relat%C3%B3rio%20Final%20do%20PDE%202024.pdf.

MODENBACH, A.A.; NOKES, S.E. Enzymatic hydrolysis of biomass at high-solids loadings – a review. **Biomass Bioenergy**, v. 56, p. 526–544, 2013.

MOGOȘANU, G. D.; GRUMEZESCU, A. M. Natural and synthetic polymers for wounds and burns dressing. **International Journal of Pharmaceutics,** v. 463, p.127-136, 2014.

MOLDES, A. B. et al. Evaluation of Biosurfactant Production from Various Agricultural Residues by *Lactobacillus pentosus*. **Journal of Agricultural and Food Chemistry,** v. 55, p.4481-4486, 2007.

MOOD, S. H. et al. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. **Renewable and Sustainable Energy Reviews**, v. 27, p. 77–93, 2013.

MOSIER, N. et al. Features of promising technologies for pretreatment of lignocellulosic biomass. **Bioresource Technology**, v. 96, p. 673–686, 2005.

NAKANISHI, S. C. et al. Fermentation strategy for second generation ethanol production from sugarcane bagasse hydrolyzate by *Spathaspora passalidarum* and *Scheffersomyces stipitis*. **Biotechnology and Bioengineering**. 2017. doi:10.1002/bit.26357.

NAKASHIMA, K. et al. Hydrodynamic cavitation reactor for efficient pretreatment of lignocellulosic biomass. **Industrial Engineering Chemistry Research**, v. 55, p. 1866–1871, 2016.

NOVACANA. Granbio paralisa temporariamente sua usina de etanol celulósico, 2016. Available in: https://www.novacana.com/n/etanol/2-geracao-celulose/granbio-paralisa-usina-etanol-celulosico-050416/. Accessed in: 1 Aug. 2017.

NOVACANA. GranBio admite atraso em usina, mas espera etanol 2G competitivo em 2019, 2017a. Available in: https://www.novacana.com/n/etanol/2-geracao-celulose/granbio-atraso-usina-etanol-2g-competitivo-2019-010617/. Accessed in: 21 Apr. 2017.

NOVACANA. BNDES limita investimentos nas usinas de etanol e alcança novo recorde negative, 2017b. Available in: https://www.novacana.com/n/industria/investimento/bndes-limita-investimentos-usinas-etanol-recorde-negativo-030817/?kmi=teranhilares@gmail.com&utm_source=Etanol&utm_campaign=09a5f5a48e-Fonte_secou_BNDES_2017_08_03&utm_medium=email&utm_term=0_9fda3940f1-09a5f5a48e-71260893. Accessed in: 23 Apr. 2017.

NREL – NATIONAL RENEWABLE ENERGY LABORATORY. Biomass Energy Basics, 2017. Available in: https://www.nrel.gov/workingwithus/re-biomass.html. Accessed in: 12 Feb. 2017.

OONKHANOND, B. et al. Lactic acid production from sugarcane bagasse by an integrated system of lignocellulose fractionation, saccharification, fermentation, and ex-situ nanofiltration. **Journal of Environmental Chemical Engineering,** v. 5, p.2533-2541, 2017.

PAULOVA, L. et al. Lignocellulosic ethanol: Technology design and its impact on process efficiency. **Biotechnology Advances,** v. 33, p.1091-1107, 2015.

PEREIRA, S. C. et al. 2G ethanol from the whole sugarcane lignocellulosic biomass. **Biotechnology for Biofuels**, v. 8, p. 44, 2014.

PHITSUWAN, P.; SAKKA, K.; RATANAKHANOKCHAI, K. Structural changes and enzymatic response of Napier grass (*Pennisetum purpureum*) stem induced by alkaline pretreatment. **Bioresource Technology,** v. 218, p.247-256, 2016.

PIELHOP, T. et al. Steam explosion pretreatment of softwood: the effect of the explosive decompression on enzymatic digestibility. **Biotechnology For Biofuels,** v. 9, p.152-160, 2016.

RABELO, S. C. et al. Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. **Bioresource Technology**, v. 102, p. 7887-7895, 2011.

RAIZEN. Tecnologia em energia renovável: etanol de segunda geração, 2014. Available in: http://www.raizen.com.br/energia-do-futuro-tecnologia-em-energia-renovavel/etanol-de-segunda-geracao. Accessed in: 12 Feb. 2017.

RAJ, V.; MUMJITHA, M. S. Fabrication of biopolymers reinforced TNT/HA coatings on Ti: Evaluation of its corrosion resistance and biocompatibility. **Electrochimica Acta,** v. 153, p.1-11, 2015.

RAMYA, R. et al. Standardization of biopolymer production from seaweed associative bacteria. **International Journal of Biological Macromolecules,** v. 102, p.550-564, 2017.

RAVELLA, S. R. et al. Extracellular polysaccharide (EPS) production by a novel strain of yeast-like fungus Aureobasidium pullulans. **Carbohydrate Polymers,** v. 82, p.728-732, 2010.

RAVINDRAN, R.; JAISWAL, A. K. A comprehensive review on pre-treatment strategy for lignocellulosic food industry waste: Challenges and opportunities. **Bioresource Technology**, v. 199, p. 92–102, 2016.

ROCHA, M. S. R. S. et al. A kinetic model for hydrothermal pretreatment of sugarcane straw. **Bioresource Technology,** v. 228, p.176-185, 2017.

ROCHE, C. M; DIBBLE, C. J; STICKEL, J. J. Laboratory-scale method for enzymatic saccharification of lignocellulosic biomass at high-solids loadings. **Biotechnology for Biofuels,** v. 2, p.28-34, 2009.

ROELANTS, S. L. K. W. et al. Towards the industrialization of new biosurfactants: Biotechnological opportunities for the lactone esterase gene from *Starmerella bombicola*. **Biotechnology and Bioengineering,** v. 113, p.550-559, 2015.

SALAPA, I. et al. Organosolv pretreatment of wheat straw for efficient ethanol production using various solvents. **Biomass and Bioenergy**, v. 100, p. 10-16, 2017.

SAMAD, A. et al. Sweet sorghum bagasse and corn stover serving as substrates for producing sophorolipids. **Journal of Industrial Microbiology & Biotechnology,** v. 44, p.353-362, 2016.

SANTOS, S. C. et al. Fermentation of xylose and glucose mixture in intensified reactors by *Scheffersomyces stipitis* to produce ethanol. **International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering**, v. 9, p. 539-544, 2015.

SECRETARIAT OF ENERGY AND MINING OF THE SAO PAULO'S STATE GOVERNMENT. Etanol 2G: produção da Raízen avança, mas deve ficar abaixo de 50% da capacidade, 2017. Available in: http://www.energia.sp.gov.br/2017/04/etanol-2g-producao-da-raizen-avanca-mas-deve-ficar-abaixo-de-50-da-capacidade/. Accessed in: 1 Aug. 2017.

SILVA, A. S. et al. High-solids content enzymatic hydrolysis of hydrothermally pretreated sugarcane bagasse using a laboratory-made enzyme blend and commercial preparations. **Process Biochemistry,** v. 51, p.1561-1567, 2016.

SILVA, L. F. et al. Poly-3-hydroxybutyrate (P3HB) production by bacteria from xylose, glucose and sugarcane bagasse hydrolysate. **Journal of Industrial Microbiology & Biotechnology,** v. 31, p.245-254, 2004.

SINDHU, R. et al. Bioconversion of sugarcane crop residue for value added products – An overview. **Renewable Energy**, v. 98, p. 203-215, 2016.

SINGH, N. et al. Cellulosic ethanol production via consolidated bioprocessing by a novel thermophilic anaerobic bacterium isolated from a Himalayan hot spring. **Biotechnology for Biofuels**, v. 10, p. 73, 2017.

SIQUEIRA, G. et al. Limitation of cellulose accessibility and unproductive binding of cellulases by pretreated sugarcane bagasse lignin. **Biotechnology For Biofuels,** v. 10, p.176-180, 2017.

SUN, Y.; CHENG, J. Hydrolysis of lignocellulosic materials for ethanol production: a review. **Bioresource Technology**, v. 83, p. 1–11, 2002.

TERÁN HILARES, R. et al. Low-melanin containing pullulan production from sugarcane bagasse hydrolysate by *Aureobasidium pullulans* in fermentations assisted by light-emitting diode. **Bioresource Technology**, v. 230, p. 76–81, 2017.

TERÁN-HILARES, R. et al. Successive pretreatment and enzymatic saccharification of sugarcane bagasse in a packed bed flow-through column reactor aiming to support biorefineries. **Bioresource Technology**, v. 203, p. 42–49, 2016.

TYE, Y. Y. et al. The world availability of non-wood lignocellulosic biomass for the production of cellulosic ethanol and potential pretreatments for the enhancement of enzymatic saccharification. **Renewable and Sustainable Energy Reviews**, v. 60, p. 155–172, 2016.

UNITE NATIONS – Framework Convention on Climate Change. Summary of the Paris Agreement, 2015. Available in: http://bigpicture.unfccc.int/#content-the-paris-agreemen. Accessed in: 23 Jun. 2017.

VAN BOGAERT, I. N. A.; ZHANG, J.; SOETAERT, W. Microbial synthesis of sophorolipids. **Process Biochemistry,** v. 46, p.821-833, 2011.

VAN DYK, J. S.; PLETSCHKE, B. I. A review of lignocellulose bioconversion using enzymatic hydrolysis and synergistic cooperation between enzymes—Factors affecting enzymes, conversion and synergy. **Biotechnology Advances,** v. 30, p.1458-1480, 2012.

VIJAYENDRA, S. V. N.; SHAMALA, T. R.. Film forming microbial biopolymers for commercial applications—A review. **Critical Reviews in Biotechnology,** v. 34, p.338-357, 2013.

VIOLA, E. et al. Use of a two-chamber reactor to improve enzymatic hydrolysis and fermentation of lignocellulosic materials. **Applied Energy,** v. 102, p.198-203, 2013.

WANG, D. et al. Efficient production of pullulan using rice hull hydrolysate by adaptive laboratory evolution of Aureobasidium pullulans. **Bioresource Technology,** v. 164, p.12-19, 2014.

WANG, S. et al. Ultrasound assisted alkaline pretreatment to enhance enzymatic saccharification of grass clipping. **Energy Conversion and Management**, v. 149, p. 409–415, 2017.

WINGREN, A.; GALBE, M.; ZACCHI, G. Techno-Economic Evaluation of Producing Ethanol from Softwood: Comparison of SSF and SHF and Identification of Bottlenecks. **Biotechnology Progress,** v. 19, p.1109-1117, 2008.

WU, S. et al. Production of pullulan from raw potato starch hydrolysates by a new strain of *Auerobasidium pullulans*. **International Journal of Biological Macromolecules,** v. 82, p.740-743, 2016.

WU, S. et al. Sweet potato: A novel substrate for pullulan production by *Aureobasidium pullulans*. **Carbohydrate Polymers,** v. 76, p.645-649, 2009.

ZAHED, O. et al. Continuous co-production of ethanol and xylitol from rice straw hydrolysate in a membrane bioreactor. **Folia Microbiologica,** v. 61, p.179-189, 2015.

ZHANG, Y.; XU, B.; ZHOU, W. On a novel mechanistic model for simultaneous enzymatic hydrolysis of cellulose and hemicellulose considering morphology. **Biotechnology and Bioengineering,** v. 111, p.1767-1781, 2014.

CHAPTER III

Hydrodynamic cavitation as a strategy to enhance the efficiency of lignocellulosic biomass pretreatment

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ABSTRACT

Hydrodynamic cavitation (HC) is a process technology with potential for application in different areas including environmental, food processing and biofuels production. Although HC is an undesirable phenomenon for hydraulic equipment, the net energy released during this process is enough to accelerate certain chemical reactions. The application of cavitation energy to enhance the efficiency of lignocellulosic biomass pretreatment is an interesting strategy proposed for integration in biorefineries for the production of bio-based products. Moreover, the use of HC-assisted process was demonstrated as an attractive alternative when compared to other conventional pretreatment technologies, not only due to high pretreatment efficiency resulting in high enzymatic digestibility of carbohydrate fraction, but also by its high energy efficiency, simple configuration and construction of system, besides possibility of using in large scale. This paper gives an overview about the HC technology and its potential for application on the pretreatment of lignocellulosic biomass. The parameters affecting this process and the perspectives for future developments in this area are also presented and discussed.

Keywords:

Hydrodynamic cavitation; Lignocellulosic biomass; Pretreatment; Enzymatic hydrolysis; Biorefinery; Process intensification

3.1 Introduction

Changing the current economy based on fossil fuels to a more sustainable economy based on renewable biomass has been one of the main challenges faced by the society nowadays. Such interest is mainly motivated by the concern in reducing the greenhouse gases emission into the atmosphere (MUSSATTO, 2016). Vegetable biomass in the form of forest and agricultural wastes is an abundant and attractive raw material to be used on the production of biofuels and chemicals due to its huge availability, low price, and high content of polysaccharides (SARKAR et al., 2012; TUCK et al., 2012). The development of processes for the production of biobased products from this kind of feedstock is considered crucial to ensure a sustainable low-carbon economy for the future with potential environmental, economic, and social benefits (MUSSATTO, 2016).

Lignocellulosic biomass is composed mainly by three components; cellulose, hemicellulose and lignin. Cellulose and hemicellulose are the main carbohydrate components of the biomass structure, while lignin is a polyphenolic macromolecule highly cross linked to the other components and that provides rigidity to the material cell wall (KUMAR; SINGH; SINGH, 2008). Thus, in order to recover the monomeric sugars present in the carbohydrate fractions for further use in bioprocesses, a pretreatment stage is required to open the material structure, facilitating the action of enzymes in the subsequent step of enzymatic hydrolysis (SARKAR et al., 2012).

Pretreatment modify the lignocellulosic structure by increasing the surface area and porosity of biomass; amending and/or removing the lignin fraction; partially polymerizing and removing the hemicellulosic fraction, and reducing the cellulose crystallinity (MESA et al., 2010; ZHANG et al., 2012). Numerous methods have been developed to pretreat lignocellulosic biomass, which can be classified as physical (e.g. milling, chipping, and irradiation), chemical (employing chemicals like alkalis, acids, salts, oxidizing agents and organic solvent), physicochemical (e.g. auto- hydrolysis, liquid hot water, supercritical fluids and steam explosion) or a combination of these (MUSSATTO; DRAGONE, 2016). Among these options, acid, alkaline and organosolv pretreatments have been largely studied in the last years. Acid pretreatment can be performed using either a low acid concentration at high temperature or a high acid concentration at low temperature. Of course, different

impacts on biomass structure are promoted when using dilute or concentrated acid solutions (MOOD et al., 2013; MUSSATTO; DRAGONE, 2016). Alkali pretreatment is also an option to remove lignin, acetyl groups and uronic acids from biomass, improving the cellulose accessibility for enzymatic saccharification. The most important advantages of alkaline pretreatment are the possibility to operate at lower temperatures and avoid the requirements of reactors specific for severe conditions (as usual in acid pretreatments, for example). However, there are still some drawbacks such as long residence time and neutralization of the pretreated slurry that should be addressed (LI et al., 2010; TERÁN-HILARES et al., 2016a). The organosolv process is able of breaking the internal lignin and hemicelluloses bonds, increasing the surface area and turning also the cellulose more accessible to enzymes. The main drawbacks of this method are the very low or very high boiling point of organic solvents, their flammability and volatility, and their costs (SUN; CHEN, 2008).

Although a variety of techniques has been proposed for the pretreatment of lignocellulosic biomass, the development of effective and lower-cost pretreatment methods has been strongly encouraged since this step is still one of the most expensive within the overall process of biomass conversion to biobased products (SARKAR et al., 2012). In this sense, the use of energy of hydrodynamic cavitation (HC) for biomass pretreatment is a promising alternative that is currently under evaluation for implementation in biorefineries. HC has been reported as being highly energy efficient and suitable for large scale applications (GOGATE; BHOSALE, 2013). This review deals with some specificities of HC phenomenon focusing on its possible application for biomass pretreatment and incorporation in biorefineries. A comprehensive discussion about the potential of this technology for such application, the parameters affecting this process and the perspectives for future developments in this area are also presented and discussed.

3.2 Cavitation phenomenon and its application

Cavitation phenomenon can be defined as the formation and subsequent growing and collapse of micro-bubbles due to changes of pressure at constant temperature (GOGATE; PANDIT, 2001). In the scientific literature, hydrodynamic, acoustic, optic (produced by photons of high intensity) and particle (produced by

elementary particles) cavitation are reported. However, only acoustic and HC generates desired intensity of energy suitable to accelerate physical and chemical reactions (GOGATE; KABADI, 2009). HC in particular, has shown some advantages when compared to acoustic cavitation such as easier operation, besides flexibility to vary the cavitation intensity by using different configuration in the cavitation device and operational parameters (GOGATE; PANDIT, 2001; MANCUSO et al., 2016).

Acoustic cavitation occurs when a high-amplitude ultrasonic signal (20 kHz – 100 kHz) is propagated in a liquid. This phenomenon can result in stable or inertial cavitation. Stable cavitation is characterized by the creation and oscillation of gas bubbles around an equilibrium radius that is formed in low ultrasonic intensity; while the inertial cavitation comes up when bubbles grow, expand and collapse with release of a large amount of energy that occurs during a single acoustic compression cycle (VANHILLE; CAMPOS-POZUELO, 2011). Due to high energy released during bubble collapse, inertial cavitation is considered as the main source to improve chemical and mechanical effects of ultrasonic cavitation (TANG et al., 2002). Bubble collapses lead to the generation of free radicals that accelerate chemical reactions such as for the synthesis of nano-materials, polymers and degradation of organic pollutants, etc. In addition, acoustic cavitation has promising applications in diagnostic and therapeutic areas (ASHOKKUMAR et al., 2007).

HC usually occurs in pumps, injectors and other hydraulic machines being considered undesirable for hydraulic systems. However, this net energy can be used for different industrial applications, including the use of HC reactors (WANG; ZHANG, 2009; MADDIKERI; GOGATE; PANDIT, 2014).

For design of HC reactor is necessary to consider some aspects aiming to allow an adequate process. The cavitating conditions inside the devices could be characterized using a dimensionless number, known as cavitation number (C_V), which can be defined by Eq. (3.1), where P_2 is downstream pressure (Pa), P_v corresponds to vapor pressure of water (Pa), ρ is density of solution (kg/m^3) and V_{th} is the velocity of fluid through the orifice (m/s) (KUMAR; PANDIT, 1999; DULAR et al., 2004; SARC et al., 2017).

$$C_v = 2(P_2 - P_v)/\rho * V_{th}^2 \qquad \text{Eq. (3.1)}$$

Also, the empirical correlation proposed by Gogate and Pandit (2001) among inlet pressure value (P_i, *bar*), physicochemical properties of the liquid and initial

radius of the nuclei (R_0, mm), diameter of orifice plate or Venture tube used for generation of cavities (d_0, mm) and percentage of free area of the holes (A, %), can be used to predicted the magnitude of imploding cavitation bubble pressure (Pc, *bar*) using the Eq. (3.2).

$$Pc = 7527 * A^{-2.55} * P_i^{2.46} * R_0^{-0.8} * d_0^{2.37} \quad \text{Eq. (3.2)}$$

Other parameter necessary to be considered for design of HC reactor is the cavitation yield that can be expressed as a function of the magnitude of the collapse pressure (Pc) achieved during collapse of cavities. This parameter can be calculated using the Eq. (3.3), proposed by Gogate and Pandit (2001), where K and n are dependent of each type of reaction and of the characteristic of used HC system. For example, for decomposition of potassium iodide using HC system based in orifice plate (1% of KI) with a time of operation of 60 min, an operating temperature of 30 °C and a cavitation number between 0.156– 0.87, values of $8.834x10^{-11}$ and 1.1633 for K and n, respectively, were determined.

$$\text{Cavitation yield} = K*(Pc)^n \quad \text{Eq. (3.3)}$$

The beneficial effect of HC are attributed to releases large magnitude of energy over a very small location ("*hot spots*") during collapse of cavities, resulting in very high energy densities (in the order of 10^{18} kW/m^3) due to the generation of local conditions of very high temperature (10,000 K) and pressure (1,000 atm) (Ozonek, 2012).

In this drastic condition, free radicals (OH•, O_2•, HOO•, H•) are generated by dissociation of water molecules inside the cavities due to drastic condition achieved in the hot spots, which results in either intensification of the chemical reactions or the propagation of certain reactions under mild conditions (PATELLA; REBOUD; ARCHER, 2000; GOGATE; PANDIT, 2005; RAUT-JADHAV et al., 2016).

The collapse of cavities is also usually accompanied by others secondary effects such as vibration, change in the flow hydrodynamics, noise (100 Hz to 100 kHz), thermal and light effects like luminescence (DULAR et al., 2004). The potential mechanical effect of cavitation can be attributed to microjets generated during the violent collapse of micro-bubbles, which reach high local velocities (around 100 m/s), producing impact of high pressure (>1GPa) in a very short time (approx. 1 ns) and resulting in damages on the surface of the solid materials as a consequence (PATELLA; REBOUD; ARCHER, 2000).

HC is a phenomenon with possibility for application in different areas. Initially, it was suggested by Pandit and Joshi (1993) for use on the hydrolysis of fat and oils. In that study, hydrolysis of castor oil and kerdi oil were carried out aided by HC (Venturi device) and ultrasound in order to evaluate the cavitation effect, obtaining better performance in acid value in HC-system. Later, other potential applications included wastewater treatment and biodiesel production, showing that HC (based in orifice plate device) is a simple, efficient, time saving, ecofriendly and industrially viable process (SIVAKUMAR; PANDIT, 2002; PAL et al., 2010).

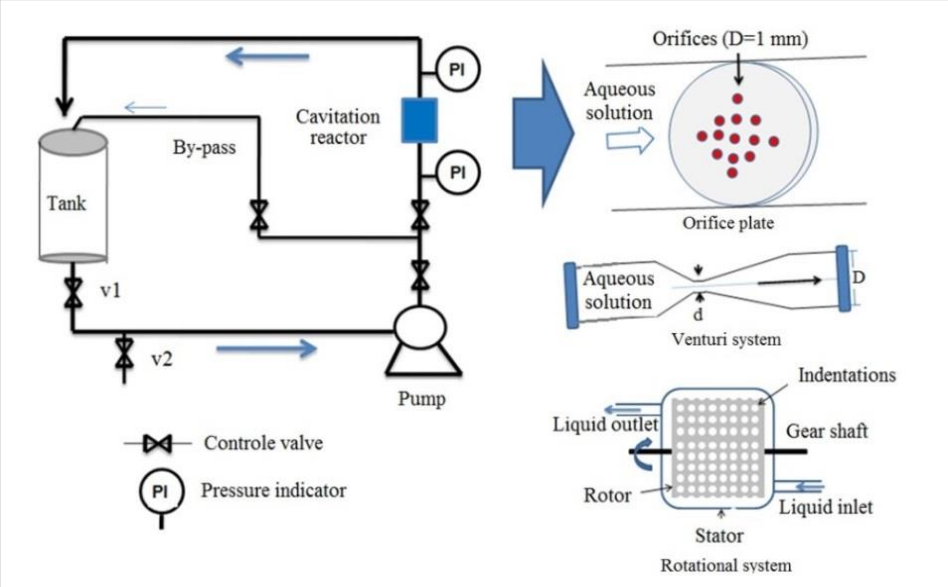
Recently, it has been proposed to accelerate the degradation of specific compounds such as 4-chloro-2-aminophenol (BARIK; GOGATE, 2016), commercial pesticide methomyl (RAUT-JADHAV et al., 2016) and azo dyes (CAI et al., 2016), for microbial disinfection of seawater (BADVE; BHAGAT; PANDIT, 2015), intensification of biodiesel production (GHAYAL; PANDIT; RATHOD, 2013; MADDIKERI; GOGATE; PANDIT, 2014; CHUAH et al., 2016), cells disruption and oil extraction from microalgae (LEE; HAN, 2015), pretreatment of lignocellulosic biomass (KIM et al., 2015; TERAN HILARES et al., 2016b; NAKASHIMA et al., 2016; MADISON et al., 2017) and for dry mill corn ethanol production (RAMIREZ-CADAVID et al., 2016). Due to the wide applications, nowadays, companies like HyCa Technologies Pvt Ltd (Mumbai, India), Cavitation Technology, inc. (Chatsworth, USA) and Arisdyne Systems® (Cleveland, USA), offer technical counseling and sale of hydrodynamic cavitation technology for different purposes.

3.3 Hydrodynamic cavitation for pretreatment of lignocellulosic biomass

Recent studies have proposed the use of HC to enhance the efficiency of lignocellulosic biomass pretreatment, which is still an expensive step in lignocellulosic biorefineries (SARKAR et al., 2012). When compared to more conventional pretreatment methods, HC-assisted process seems to be an attractive option for biomass pretreatment since it presents high energy efficiency and is of easy scalability since requires simple equipment (PANDIT, 2016).

HC can be generated in systems based on orifice plates and Venturi systems, or in rotational systems (Figure 3.1) (GOGATE; PANDIT, 2005; PATIL et al., 2016). As shown, these systems are simple and basically composed by a pump, a reservoir tank, pipes, valves and cavitation device, besides pressure and temperature indicators. This kind of equipment and instrumentation are available or can be built for use in large scale, and also a number of different design alternatives could be thought for industrial use, as coupling a number of cavitation devices to large reservoir tanks.

Figure 3.1 – Schematic diagram of different hydrodynamic cavitation systems



Source: Based in the work of Kim et al. (2015); Nakashima et al. (2016); Patil et al. (2016)

The first work reported using HC for pretreatment of lignocellulosic biomass was published in 2015, when Kim et al. (2015) reported HC generated by orifice plates as a new approach for reed (*Phragmites australis*) pretreatment aiming to produce ethanol. Since then, other works were reported using HC for pretreatment of different biomasses such as corn stover for general use in biorefineries (NAKASHIMA et al., 2016b), sugarcane bagasse for ethanol production (TERÁN HILARES et al., 2016b; MADISON et al., 2017) and milled wheat straw for biogas production (PATIL et al., 2016). All of these studies are based on orifice plates or Venturi systems, exception to the last one (PATIL et al., 2016), which used rotational equipment.

An advantage of HC-system based in orifice plate could be pointed and is associated to non-recirculation of solid through the system, avoiding clogging problem, and also to high pressure drop and consequently high cavitation intensity. Moreover, for Venture tube-based HC-system using high solid loading, special pumps (as helical) could be necessary, corresponding to more expensive equipment compared to usual centrifugal pumps. Other kind of system, a rotational device composed by a stator and rotor assembly was also used by Badve et al. (2014) for intensification of delignification of wheat straw in the paper manufacturing process. An advantage of rotational systems, as well Venturi devices, could be an ease adaptation to continuous pretreatment process.

Works dealing with lignocellulosic biomass pretreatments assisted by HC-system are summarized in Table 3.1. In the work of Kim et al. (2015), HC-assisted alkaline pretreatment of reed under optimized process conditions (3% NaOH, 11.8% solid loading and 41.1 min) resulted in high lignin removal (53.4%) and high glucose release (326.5 g/Kg of biomass) after 72h of enzymatic hydrolysis. Terán Hilares et al. (2016) also related that HC-assisted alkaline pretreatment of sugarcane bagasse under optimized conditions (1.9% NaOH, 4.27% solid loading and 44.5 min) resulted in high lignin removal (60.4%) and high yield of cellulose digestibility (97.2%) after 48h of enzymatic hydrolysis. In both reports, HC was more efficient than ultrasound-assisted alkaline or conventional alkaline pretreatment.

HC has also been found advantageous for biomass pretreatment in terms of energy efficiency. The energy requirement (3.65 MJ/Kg biomass) for HC-assisted alkaline pretreatment of reed, for example, was almost four times less than by ultrasound-assisted pretreatment (14.4 MJ/Kg biomass) (KIM et al., 2015). Pretreatment of sugarcane bagasse with HC provided 6.43×10^{-5} g glucose/J, a value higher than that obtained by using ultrasound assisted process (2.61×10^{-5} g of glucose/J) (TERÁN HILARES et al., 2016b). Nakashima et al. (2016) also reported higher efficiency for corn stover pretreatment by HC than by ultrasound assisted process (2.24×10^{-5} g glucose/J and 0.11×10^{-5} g glucose/J, respectively). However, in the work of Madison et al. (2017), 0.78×10^{-6} g glucose/J was obtained using HC-system based in Venturi tube and that value was lower than related in previous reports.

Table 3.1 – Hydrodynamic cavitation system used for pretreatment of different lignocellulosic biomass

Biomass	HC pretreatment conditions	Main changes in biomass	Enzymatic digestibility	Sugar released (g/kg of raw biomass)	Ref.
Reed	3 % of NaOH, 11.8% of S/L ratio*, 41.1 min, 77 °C, 0.5 MPa of inlet pressure, orifice plates: 27 holes of 1 mm of diameter	35-42% of lignin removal	85% of cellulose hydrolyzed in 72h	326	Kim et al. (2015)
Corn stover	0.4 mol/L of Na_2CO_3 plus 0.6 mol/L of H_2O_2, 4% of S/L ratio, 60 min, 30 °C, 0.25 mm of particle size, Venturi tube (length, 40 mm; internal diameter φ, 3.6 mm; throat diameter φ_t, 1.8 mm).	Lignin removal was verified by FTIR analysis by reduction in the band 1745 cm^{-1} and 1606 cm^{-1}, assigned to the side chain and aromatic ring of lignin	-	275	Nakashima et al. (2016)
Sugarcane bagasse	0.48 mol/L of NaOH, 4.24% of S/L ratio*, 44.48 min, 64 °C and 0.3 MPa of inlet pressure, orifice plates: 27 holes of 1 mm of diameter	60.4% of lignin removal; 52% of cellulose content in the pretreated biomass	97.2% of cellulose hydrolyzed in 48h	422	Terán Hilares et al. (2016b)
Sugarcane bagasse	Calcium hydroxide (0.1 g/g dry biomass)**, 1% of S/L ratio***, 120 min, 22 °C, Venturi tube: 15% opening, 0.572 cm cavitation throat diameter, 0.37 MPa of inlet pressure	Changes in composition were measured by indirect method (crystallinity index)	46% of cellulose hydrolyzed in 72h	325	Madison et al. (2017)

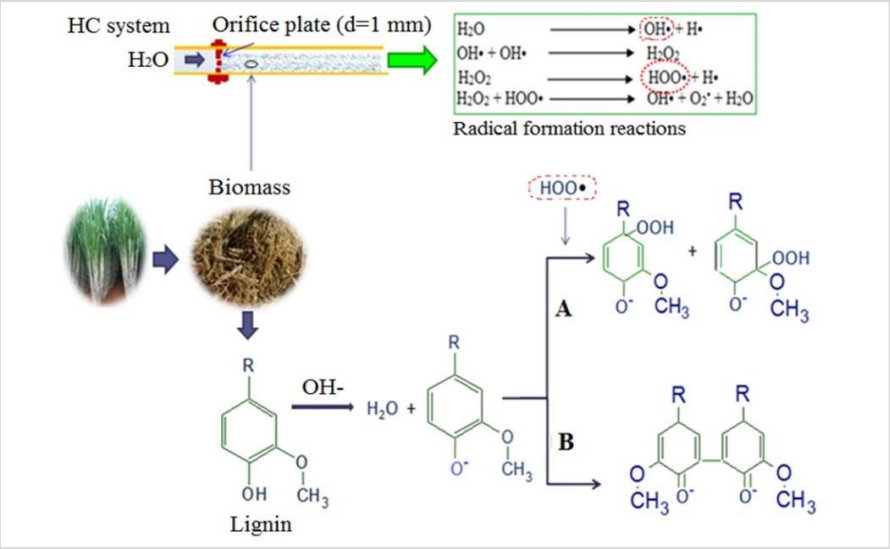
*****Solid loading was calculated considering the volume of cavitation zone (biomass was kept in cavitation zone inside of a cylindrical wire cloth). ** Pretreatment was carried out in two steps: biomass was treated using lime at 100 °C by 2h, followed by HC process. *** Solid loading was calculated based in total volume (biomass was recirculated through the system).

Source: Personal archive

The lowest value of energy efficiency reported in the study of Madison et al. (2017) could be related to a long time of process used (120 min), besides low temperature, low solid loading and low cavitation intensity of this kind of system. In that study, 0.28$/kg of glucose was calculated as the cost of energy consumption in pretreatment and this technology was considered by those authors as non-economically viable. HC pretreatment is a new technology and the available information is not enough to carry out an adequate economic evaluation, mainly considering a biorefinery context. Really, besides necessity of more optimization studies for the different HC system possibilities, in biorefineries, energy is usually be generated by using biomass itself or some of its fraction, as lignin (DIAS et al., 2009; RABELO et al., 2011), and all fractions of the lignocellulosic material can be used to obtain a number of different valuable products, besides biofuel (DIAS et al., 2012). Thus, economic viability must be evaluated considering process as a whole and simulation tools have been used in this way (DIAS et al., 2009).

The mechanism of delignification induced by HC effects on biomass is not well understood yet, but it can be attributed to physicochemical and mechanical effects of cavitation. During implosion of bubbles, the generated OH• radicals from water molecules dissociation are highly reactive and could be responsible for the oxidation of lignin molecule to some extent, as represented in the Figure 3.2.

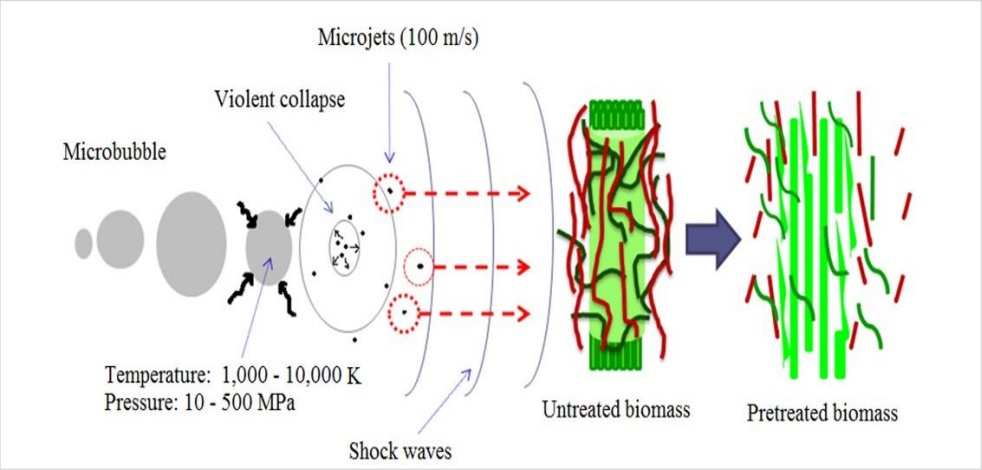
Figure 3.2 – Reaction mechanism of lignin with hydroxyl radicals (OH•) generated during hydrodynamic cavitation. A- Reaction of degradation of lignin and B- Reaction of condensation of lignin



Source: Adapted from Badve et al. (2014).

The mechanical effects of cavitation are attributed to high speed microjets and shock waves generated during asymmetric violent collapse. Those effects are responsible for the degradation by pyrolysis/molecular breakdown of organic molecules, as lignin located in the vicinity of cavities, also resulting in small perforations on the surface of the material, which can improve the accessibility of enzymes to the cellulose (BADVE et al., 2014; TERÁN HILARES et al., 2016b; PATIL et al., 2016). A schematic representation of the mechanical effect of cavitation during pretreatment of lignocellulosic biomass is shown in Figure 3.3.

Figure 3.3 – Schematic representation of mechanical effect of cavitation during pretreatment of lignocellulosic biomass



Source: Based in report of Gogate and Pandit (2005).

The generation of small perforations in the biomass structure was also reported by the company E-PIC S.r.l (2016), which related increase in the specific surface area (mm^2/g), total pore volume (mm^3/g) and total micropore volume (mm^3/g) from 1.28 to 1.92, 3.33 to 5.08, and 0.36 to 0.59, respectively, when untreated wheat straw was submitted to HC (pretreatment conditions were not reported).

Pretreatment of corn stover by hydrodynamic cavitation-assisted sodium percarbonate (HC-SP) was also reported to promote efficient lignin removal (NAKASHIMA et al., 2016). In addition, the use of sodium percarbonate, which is composed by Na_2CO_3 and H_2O_2, is very interesting because it is an environmentally friendly oxidant reagent. During HC-SP pretreatment, hydrogen peroxide (H_2O_2) is dissociated into highly reactive radicals as OH• and O_2•, which attack side chains, resulting in decomposition and removal of lignin, as a consequence. In the alkaline

medium provided by Na_2CO_3, the radical OH• generated by the collapse of microbubbles in hydrodynamic cavitation are recombined, resulting in H_2O_2 that is also dissociated into HOO•, OH• and $O_2^{\bullet}$. Therefore, the H_2O_2 generated by dissociation of SP and recombination of OH• produced by HC, increase the concentration of H_2O_2 in the medium and, consequently, improve the efficiency of pretreatment of lignocellulosic biomass (BADVE et al., 2014; TERÁN HILARES et al., 2016b; NAKASHIMA et al., 2016).

As the main purpose of pretreatment is to increase the efficiency of enzymatic hydrolysis of polysaccharides to sugars for subsequent fermentation, the generation of compounds that inhibit the microbial metabolism must be avoided (SARKAR et al., 2012; TERÁN HILARES et al., 2016b). In this sense, since HC allows obtaining high pretreatment efficiency under mild process conditions (<60°C), low generation of inhibitors compounds is expected. This is important because inhibitors can be carried with the bagasse to the enzymatic hydrolysis step of the process. Terán Hilares et al. (2016), e.g., reported some inhibitors concentration in enzymatic hydrolysate (0.1 g/L of furfural, 0.39 g/L of acetic acid and 0.06 g/L of HMF) obtained from HC pretreated sugarcane bagasse. However, more studies are necessary to better understand the release/formation of inhibitor compounds during this procedure.

3.4 Parameters affecting the cavitation intensity during biomass pretreatment

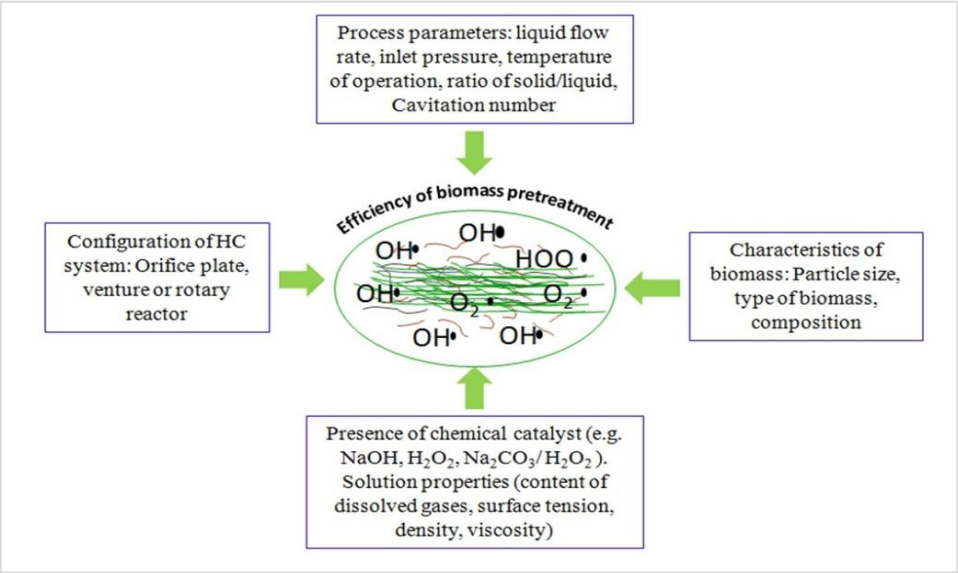
The cavitation intensity and, consequently, the efficiency of this pretreatment on biomass structure, are affected by different parameters (Figure 3.4).

Temperature of liquid and inlet pressure, for example, have been reported to affect the cavitation efficiency in different applications of HC including for degradation of herbicide alachlor, methyl parathion, imidacloprid, and 4-chloro 2-aminophenol (WANG; ZHANG, 2009; PATIL; GOGATE, 2012; BARIK; GOGATE, 2016; HABASHI et al., 2016), waste water treatment (ZUPANC et al., 2013; GOGATE; PATIL, 2015), depolymerization of aqueous guar gum (PRAJAPAT; GOGATE, 2015). In all of these studies, the cavitation efficiency was improved when the temperature of the liquid was increased from 30 °C to 45 °C. However, higher temperatures are not recommended for use in this process because

they can increase the vapor pressure of the medium, generating vaporous cavities and decreasing the intensity of cavitation during collapse, reducing then the cavitation efficiency (MISHRA; GOGATE, 2010). Moreover, the properties of the solution (density, surface tension, viscosity and content of dissolved gases) are also affected at higher temperatures, affecting also the cavitation intensity, as a consequence (OZONEK, 2012; GOGATE; PATIL, 2014; PRAJAPAT; GOGATE, 2015).

The effect of temperature during pretreatment of lignocellulosic biomass by HC has been little studied until now. Determining the optimal operating temperature for a specific system is necessary in order to achieve high cavitation efficiency and, consequently, high efficiency of pretreatment. In a recent study on the application of HC during the alkaline pretreatment of sugarcane bagasse (TERÁN HILARES et al., 2016b), the energy added by the pumping and released during collapse of micro-bubbles caused an increase in the temperature from 22 °C to 65-70 °C during 45 min of process. In that study, the lignin removal reached 60.4%, which was attributed to the physicochemical effect of cavitation (OH• radicals, microjets, shock waves) under lower temperatures and to the reaction catalyzed by sodium hydroxide under higher temperatures. However, further studies are necessary in order to evaluate the synergetic effect between temperature and cavitation intensity on both the economy and efficiency of lignocellulosic pretreatment.

Figure 3.4 – Parameters affecting the pretreatment efficiency using hydrodynamic cavitation process



Source: Adapted from Ozonek (2012).

The effect of inlet pressure on cavitation intensity has been reported for different HC applications. Enhancement of the cavitation intensity has been observed with an increase in inlet pressure due to collapse of cavities that becomes more violent, leading to a high pressure pulse and, consequently, a higher number of generated radical OH• (PATIL; GOGATE, 2012; HABASHI et al., 2016). Inlet pressure between 3-4 bar have been considered optimal for different HC applications. Above this pressure, the cavitation intensity decreases due to super cavitation phenomenon (indiscriminate growth of the bubbles), resulting in splashing and vaporization of the flow (PATIL; BOTE; GOGATE, 2014). Therefore, the reduction in the cavitation intensity leads to lower efficiency of pretreatment.

The cavitation number is another important parameter and it is strongly influenced by inlet pressure and configuration of system (number and diameter of orifice plates). Two different values of Cv were used for pretreatment of corn stover by Nakashima et al. (2016). In that study, the highest glucose release (4 g/L) during enzymatic hydrolysis was achieved using Cv of 0.29 compared to the obtained using Cv of 0.44 (3 g/L). Thus, changes in system configuration or process conditions, as an increase in the area of orifices, can increase the cavitation number with a consequent reduction in HC effect (KUMAR; PANDIT, 1999). In this way, more studies are required in order to determinate the optimal value for Cv in pretreatment process.

Chemical such as sodium hydroxide (NaOH) , lime ($Ca(OH)_2$) and sodium percarbonate ($2Na_2CO_3$ and $3H_2O_2$) have also been used for HC pretreatment of sugarcane bagasse (TERÁN HILARES et al., 2016b; MADISON et al., 2017) and corn stover (NAKASHIMA et al., 2016), respectively. In HC assisted process, the efficiency of these chemicals for biomass pretreatment is more effective due to a higher reaction rate resulting from an increase in the kinetic energy of molecules under drastic condition generated by collapse of micro-bubbles. Moreover, the dissociation of alkalis results in generation of H_2O_2 in the medium, which is dissociated into free radicals (OH•) that disrupt the lignin structure (BADVE et al., 2014).

Some characteristics of the biomass, such as kind or particle size, are also important parameters affecting the pretreatment efficiency in HC systems. Particle size reduction improves the mass transference; however, a great reduction in particle size cannot be economically justifiable (KHULLAR et al., 2013). Different particle

sizes have been reported for biomass pretreatment assisted by HC, as for example, between 1.18 and 1.70 mm for sugarcane bagasse (TERÁN HILARES et al., 2016), lower than 0.25 mm for corn stover (NAKASHIMA et al., 2016), and between 0.125 and 1.0 mm for wheat straw (PATIL et al., 2016). Depending on the size of the particle, the use of a specific pump and adequate design of diameter of hole (when orifice plates or Venturi systems are used) will be necessary in order to avoid clogging problem. In this case, other possibility is to confine the biomass in the cavitation zone, keeping this region isolated and avoiding the necessity of solid particles passing it through the orifices (TERÁN HILARES et al., 2016b).

3.5 Future perspectives: HC application for intensification of pretreatment processes

Application of HC in combination with chemical agents, for example, for intensification of biomass pretreatment processes is a novel and potentially suitable approach for implementation in lignocellulosic biorefineries (KIM et al., 2015; TERÁN HILARES et al., 2016b; NAKASHIMA et al., 2016).

When used for different applications (not including biomass pretreatment), HC uncombined with chemicals has been reported to result in low catalytic efficiency, as in this case, the effect is only due to the action of radicals generated during HC phenomenon and its mechanical effect. During the application of HC for degradation of commercial pesticide (methomyl) in aqueous solution, for example, Raut-Jadhav et al. (2016) obtained degradation of 13.9% when using HC alone and 2.86% when using hydrogen peroxide (1:30) alone. However, using a combined process (HC plus H_2O_2), 97.2% of degradation of methomyl was achieved after 60 min. The synergetic effect obtained in the combined process was attributed to the significant rate of dissociation of H_2O_2 in the presence of HC, leading to enhanced generation of OH• radicals (RAUT-JADHAVET et al., 2016).

Degradation of 4-chloro 2-aminophenol by HC-assisted process was also reported by Barik and Gogate (2016). In that study, 21.89%, 64.29% and 68.89% of degradation were achieved using in separate, HC, ozone (O_3) and UV photolysis (UV), respectively. However, in combined processes using HC+UV, HC+O_3 and HC+UV+O_3, 79.30%, 73.38% and 96.85% of degradation were achieved, respectively. The enhanced degradation of HC+UV was attributed to the increased

number of hydroxyl radicals available due to the cleavage of H_2O_2 generated in cavitation process (BARIK; GOGATE, 2016). In the case of HC+O_3, the extend of degradation could be due to the enhancement of oxidizing effect of molecular ozone in turbulence generated by HC, generation of reactive species (oxygen and hydroxyl radicals) due to decomposition under HC, and enhancement of mass transfer of ozone molecules from gas phase to the bulk solution (PATIL; GOGATE, 2012; RAUT-JADHAV et al., 2016).

The intensification of biomass pretreatment process could also be possible by combining HC with hydrogen peroxide, ozone and Fenton reaction. Hydrogen peroxide is a strong oxidant and has been used for lignocellulosic biomass pretreatment. However, its use alone may not be effective due to low reaction rates at reasonable concentration. Therefore, hydrogen peroxide has been usually applied in combination with others pretreatment methods such as acid/alkali (MUSSATTO; ROCHA; ROBERTO, 2008; COSTA et al., 2015) and microwave irradiation (SINGH et al., 2011). Nevertheless, such combined methods usually require long reaction times and, sometimes, a specific reactor design. In process assisted by HC, it would be possible to achieve high pretreatment efficiency using shorter times and lower concentration of chemicals. Further studies are also necessary to evaluate the effect of this kind of combined pretreatment on biomass enzymatic digestibility.

Combination of HC with ozone is another alternative that could be evaluated for biomass pretreatment, as ozone has been extensively used for pretreatment of different lignocellulosic materials including wheat straw (GARCÍA-CUBERO et al., 2009), sugarcane bagasse (TRAVAINI et al., 2013), and cotton stalks (SILVERSTEIN et al., 2007). In addition, ozonolysis process promotes lignin removal and considerably increases the release of fermentable carbohydrates during enzymatic hydrolysis, as a consequence (TRAVAINI et al., 2013). Fenton reaction is another option that could be evaluated in combination with HC. According to Ninomiya et al. (NINOMIYA et al., 2013), the combined use of sonocatalytic reaction (ultrasound + titanium dioxide) with the Fenton reaction enhanced the generation of hydroxyl radical, resulting in 60% of lignin degradation after 180 min, which was higher when compared to the 1.8% of lignin degradation obtained using sonocatalytic reaction only, and 49.9% lignin degradation using Fenton reaction only. Considering these results, the use HC instead of ultrasound could mean interesting alternative to improve these results. It would also be useful to evaluate, in

future studies, the use of other alkalis ($Ca(OH)_2$, Na_2CO_3 or others), cheaper than the options reported up till now, for lignocellulosic biomass pretreatment in combination with HC.

3.6 Conclusion

Hydrodynamic cavitation is a novel and promising technology for intensification of different processes under mild conditions (low temperature and atmospheric pressure). In this context, pretreatment of lignocellulosic biomass by HC-assisted process can be considered an interesting and more advantageous alternative when compared to conventional pretreatment process (like alkaline, steam explosion, acid and organosolv) or ultrasound cavitation, due to the short time required and low concentration of chemical to achieve high pretreatment efficiency, besides low energy requirements and increase in the enzymatic digestibility yield of carbohydrate fraction by increasing the porosity of the material. Controlling of the different parameters that affect the HC process could establish an efficient pretreatment process, turning it economically viable in a lignocellulosic biorefinery. In this way, further studies are still required to better understand the effects of cavitation on biomass structure and composition and to design appropriated devices for high performance process.

3.7 Reference

ANHILLE, C.; CAMPOS-POZUELO, C. Nonlinear ultrasonic standing waves: Two-dimensional simulations in bubbly liquids. **Ultrasonics Sonochemistry,** v. 18, p. 679-682, 2011.

ASHOKKUMAR, M. et al. Acoustic emission spectra from 515 KHz cavitation in aqueous solutions containing surface-active solutes. **Journal of the American Chemical Society,** v. 129, p.2250-2258, 2007.

BADVE, M. P. et al. Hydrodynamic cavitation as a novel approach for delignification of wheat straw for paper manufacturing. **Ultrasonics Sonochemistry**, v. 21, p.162-168, 2014.

BADVE, M. P.; BHAGAT, M. N.; PANDIT, A. B. Microbial disinfection of seawater using hydrodynamic cavitation. **Separation and Purification Technology**, v. 151, p.31-38, 2015.

BARIK, A. J.; GOGATE, P. R. Degradation of 4-chloro 2-aminophenol using a novel combined process based on hydrodynamic cavitation, UV photolysis and ozone. **Ultrasonics Sonochemistry**, v. 30, p.70-78, 2016.

CAI, M. et al. Sono-advanced Fenton decolorization of azo dye Orange G: Analysis of synergistic effect and mechanisms. **Ultrasonics Sonochemistry**, v. 31, p.193-200, 2016.

CHUAH, L. F. et al. Cleaner production of methyl ester using waste cooking oil derived from palm olein using a hydrodynamic cavitation reactor. **Journal of Cleaner Production**, v. 112, p.4505-4514, 2016.

COSTA, J. A. et al. Enhanced enzymatic hydrolysis and ethanol production from cashew apple bagasse pretreated with alkaline hydrogen peroxide. **Bioresource Technology**, v. 179, p.249-259, 2015.

DIAS, M. O. S. et al. Integrated versus stand-alone second generation ethanol production from sugarcane bagasse and trash. **Bioresource Technology**, v. 103, p.152-161, 2012.

DIAS, M. O. S. et al. Production of bioethanol and other bio-based materials from sugarcane bagasse: Integration to conventional bioethanol production process. **Chemical Engineering Research and Design**, v. 87, p.1206-1216, 2009.

DULAR, M. et al. Relationship between cavitation structures and cavitation damage. **Wear**, v. 257, p.1176-1184, 2004.

E-PIC S.r.l. Biomass pretreatment (BIOGAS), 2016. Available from: http://www.epic-srl.com/en/cavitation-technologies/biomass-pretreatment-biogas. Accessed in: 8 Jun. 2016.

GARCÍA-CUBERO, M. T. et al. Effect of ozonolysis pretreatment on enzymatic digestibility of wheat and rye straw. **Bioresource Technology**, v. 100, p.1608-1613, 2009.

GHAYAL, D.; PANDIT, A. B.; RATHOD, V. K. Optimization of biodiesel production in a hydrodynamic cavitation reactor using used frying oil. **Ultrasonics Sonochemistry**, v. 20, p.322-328, 2013.

GOGATE, P. R.; BHOSALE, G. S. Comparison of effectiveness of acoustic and hydrodynamic cavitation in combined treatment schemes for degradation of dye wastewaters. **Chemical Engineering and Processing: Process Intensification**, v. 71, p.59-69, 2013.

GOGATE, P. R.; KABADI, A. M. A review of applications of cavitation in biochemical engineering/biotechnology. **Biochemical Engineering Journal**, v. 44, p.60-72, 2009.

GOGATE, P. R.; PANDIT, A. B. A review and assessment of hydrodynamic cavitation as a technology for the future. **Ultrasonics Sonochemistry**, v. 12, p.21-27, 2005.

GOGATE, P. R.; PANDIT, A. B. Hydrodynamic cavitation reactors: a state of the art review. **Reviews in Chemical Engineering**, v. 17, p.1-85, 2001.

HABASHI, N. et al. Hydrodynamic cavitation as a novel approach for pretreatment of oily wastewater for anaerobic co-digestion with waste activated sludge. **Ultrasonics Sonochemistry**, v. 31, p.362-370, 2016.

KHULLAR, E. et al. Effect of particle size on enzymatic hydrolysis of pretreated Miscanthus. **Industrial Crops and Products**, v. 44, p.11-17, 2013.

KIM, I. et al. Hydrodynamic cavitation as a novel pretreatment approach for bioethanol production from reed. **Bioresource Technology**, v. 192, p.335-339, 2015.

KUMAR, P. S.; PANDIT, A. B. Modeling Hydrodynamic Cavitation. **Chemical Engineering & Technology**, v. 22, p.1017-1027, 1999.

KUMAR, R.; SINGH, S.; SINGH, O. V. Bioconversion of lignocellulosic biomass: biochemical and molecular perspectives. **Journal of Industrial Microbiology & Biotechnology**, v. 35, p.377-391, 2008.

LEE, I.; HAN, J-I. Simultaneous treatment (cell disruption and lipid extraction) of wet microalgae using hydrodynamic cavitation for enhancing the lipid yield. **Bioresource Technology**, v. 186, p.246-251, 2015.

LI, M. et al. Cold sodium hydroxide/urea based pretreatment of bamboo for bioethanol production: Characterization of the cellulose rich fraction. **Industrial Crops and Products**, v. 32, p.551-559, 2010.

LUO, J.; FANG, Z.; SMITH, R. L. Ultrasound-enhanced conversion of biomass to biofuels. **Progress in Energy and Combustion Science**, v. 41, p.56-93, 2014.

MADDIKERI, G. L.; GOGATE, P. R.; PANDIT, A. B. Intensified synthesis of biodiesel using hydrodynamic cavitation reactors based on the interesterification of waste cooking oil. **Fuel**, v. 137, p.285-292, 2014.

MADISON, M. J. et al. Mechanical pretreatment of biomass – Part I: Acoustic and hydrodynamic cavitation. **Biomass and Bioenergy**, v. 98, p.135-141, 2017.

MANCUSO, G. et al. Decolourization of Rhodamine B: A swirling jet-induced cavitation combined with NaOCl. **Ultrasonics Sonochemistry**, v. 32, p.18-30, 2016.

MESA, L. et al. An approach to optimization of enzymatic hydrolysis from sugarcane bagasse based on organosolv pretreatment. **Journal of Chemical Technology & Biotechnology**, v. 85, p.1092-1098, 2010.

MISHRA, K. P.; GOGATE, P. R. Intensification of degradation of Rhodamine B using hydrodynamic cavitation in the presence of additives. **Separation and Purification Technology**, v. 75, p.385-391, 2010.

MOOD, S. H. et al. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. **Renewable and Sustainable Energy Reviews**, v. 27, p.77-93, 2013.

MUSSATTO, S. I. A closer look at the developments and impact of biofuels in transport and environment; what are the next steps? **Biofuel Research Journal,** v. 3, p.331-331, 2016.

MUSSATTO, S. I.; DRAGONE, G. M. Biomass Pretreatment, Biorefineries, and Potential Products for a Bioeconomy Development. In: MUSSATTO, S. I. (ed). **Biomass Fractionation: Technologies for a Lignocellulosic Feedstock Based Biorefinery**, Massachussetts: Elsevier Inc., 2016.

MUSSATTO, S. I.; ROBERTO, I. C. Alternatives for detoxification of diluted-acid lignocellulosic hydrolyzates for use in fermentative processes: a review. **Bioresource Technology**, v. 93, p.1-10, 2004.

MUSSATTO, S. I.; ROCHA, G. J. M.; ROBERTO, I. C. Hydrogen peroxide bleaching of cellulose pulps obtained from brewer's spent grain. **Cellulose**, v. 15, p.641-649, 2008.

NAKASHIMA, K. et al. Hydrodynamic Cavitation Reactor for Efficient Pretreatment of Lignocellulosic Biomass. **Industrial & Engineering Chemistry Research**, v. 55, p.1866-1871, 2016.

NINOMIYA, K. et al. Sonocatalytic–Fenton reaction for enhanced OH radical generation and its application to lignin degradation. **Ultrasonics Sonochemistry**, v. 20, p.1092-1097, 2013

OZONEK, J. **Application of Hydrodynamic Cavitation in Environmental Engineering**. New York; 2012.

PAL, A. et al. Biodiesel production through hydrodynamic cavitation and performance testing. **Renewable Energy**, v. 35, p.619-624, 2010.

PANDIT, A. B. Hydrodynamic cavitation technology : Industrial applications, In: Ghosh, P.; Raj. B. (Eds). **The main of an engineer**. Singapore: Springer, 2016. p. 329-340.

PANDIT, A. B.; JOSHI, J. B. Hydrolysis of fatty oils: effect of cavitation. **Chemical Engineering Science,** v. 48, p.3440-3442, 1993.

PATELLA, R. F.; REBOUD, J.; ARCHER, A. Cavitation damage measurement by 3D laser profilometry. **Wear**, v. 246, p.59-67, 2000.

PATIL, P. N. et al. Intensification of biogas production using pretreatment based on hydrodynamic cavitation. **Ultrasonics Sonochemistry**, v. 30, p.79-86, 2016.

PATIL, P. N.; BOTE, S. D.; GOGATE, P. R. Degradation of imidacloprid using combined advanced oxidation processes based on hydrodynamic cavitation. **Ultrasonics Sonochemistry**, v. 21, p.1770-1777, 2014.

PATIL, P. N.; GOGATE, P. R. Degradation of methyl parathion using hydrodynamic cavitation: Effect of operating parameters and intensification using additives. **Separation and Purification Technology**, v. 95, p.172-179, 2012.

PRAJAPAT, A. L.; GOGATE, P. R.. Intensification of depolymerization of aqueous guar gum using hydrodynamic cavitation. **Chemical Engineering And Processing: Process Intensification**, v. 93, p.1-9, 2015.

RABELO, S. C. et al. Production of bioethanol, methane and heat from sugarcane bagasse in a biorefinery concept. **Bioresource Technology**, v. 102, p.7887-7895, 2011.

RAMIREZ-CADAVID, D. A. et al. Effects of hydrodynamic cavitation on dry mill corn ethanol production. **Process Biochemistry**, v. 51, p.500-508, 2016.

RAUT-JADHAV, S. et al. Intensification of degradation of methomyl (carbamate group pesticide) by using the combination of ultrasonic cavitation and process intensifying additives. **Ultrasonics Sonochemistry**, v. 31, p.135-142, 2016.

SARC, A. et al. The issue of cavitation number value in studies of water treatment by hydrodynamic cavitation. **Ultrasonics Sonochemistry**, v. 34, p.51-59, 2017.

SARKAR, N. et al. Bioethanol production from agricultural wastes: An overview. **Renewable Energy,** v. 37, p.19-27, 2012.

SILVERSTEIN, R. A. et al. A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. **Bioresource Technology**, v. 98, p.3000-3011, 2007.

SINGH, A. et al. Enhanced saccharification of rice straw and hull by microwave–alkali pretreatment and lignocellulolytic enzyme production. **Bioresource Technology**, v. 102, p.1773-1782, 2011.

SIVAKUMAR, M.; PANDIT, A. B. Wastewater treatment: a novel energy efficient hydrodynamic cavitational technique. **Ultrasonics Sonochemistry**, v. 9, p.123-131, 2002.

SUN, F.; CHEN, H. Organosolv pretreatment by crude glycerol from oleochemicals industry for enzymatic hydrolysis of wheat straw. **Bioresource Technology**, v. 99, p.5474-5479, 2008.

TANG, H. et al. An investigation of the role of cavitation in low-frequency ultrasound-mediated transdermal drug transport. **Pharmaceutical Research,** v. 19, p.1160-1169, 2002.

TERÁN-HILARES, R. et al. Successive pretreatment and enzymatic saccharification of sugarcane bagasse in a packed bed flow-through column reactor aiming to support biorefineries. **Bioresource Technology**, v. 203, p.42-49, 2016a.

TERÁN HILARES, R. et al. Hydrodynamic cavitation-assisted alkaline pretreatment as a new approach for sugarcane bagasse biorefineries. **Bioresource Technology**, v. 214, p.609-614, 2016b.

TRAVAINI, R. et al. Sugarcane bagasse ozonolysis pretreatment: Effect on enzymatic digestibility and inhibitory compound formation. **Bioresource Technology**, v. 133, p.332-339, 2013.

TUCK, C. O. et al. Valorization of Biomass: Deriving More Value from Waste. **Science,** v. 337, p. 695-699, 2012.

WANG, X.; ZHANG, Y. Degradation of alachlor in aqueous solution by using hydrodynamic cavitation. **Journal of Hazardous Materials**, v. 161, p.202-207, 2009.

ZHANG, L. et al. Effects of acid and alkali treated lignocellulosic materials on cellulase/xylanase production by *Trichoderma reesei* Rut C-30 and corresponding enzymatic hydrolysis. **Biomass and Bioenergy,** v. 37, p.16-24, 2012.

ZUPANC, M. et al. Removal of pharmaceuticals from wastewater by biological processes, hydrodynamic cavitation and UV treatment. **Ultrasonics Sonochemistry**, v. 20, p.1104-1112, 2013.

CHAPTER IV

Hydrodynamic cavitation-assisted alkaline pretreatment as a new approach for sugarcane bagasse biorefineries

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ABSTRACT

Hydrodynamic cavitation (HC) was employed in order to improve the efficiency of alkaline pretreatment of sugarcane bagasse (SCB). Response surface methodology (RSM) was used to optimize pretreatment parameters: NaOH concentration (0.1-0.5 mol/L), solid/liquid ratio (S/L, 3-10%) and HC time (15-45 min), in terms of glucan content, lignin removal and enzymatic digestibility. Under an optimal HC condition (0.48 mol/L of NaOH, 4.27% of S/L ratio and 44.48 min), 52.1% of glucan content, 60.4% of lignin removal and 97.2% of enzymatic digestibility were achieved. Moreover, enzymatic hydrolysis of the pretreated SCB resulted in a yield 82 and 30% higher than the untreated and alkaline-treated controls, respectively. HC was found to be a potent and promising approach to pretreat lignicellulosic biomass.

Keywords:

Hydrodynamic cavitation, Alkaline pretreatment, Enzymatic digestibility, Response surface methodology (RSM), Sugarcane bagasse

4.1 Introduction

Sugarcane bagasse (SCB) is an important feedstock for cellulosic ethanol production in many countries and particularly so in Brazil (CLAUSER et al., 2016). SCB, a lignocellulosic biomass, is composed of carbohydrates (cellulose and hemicelluloses), lignin, and other minor components as extractives and inorganic compounds (VALLEJOS et al., 2015).

All these components are intertwined via lignin acted as glue in an exceedingly complex way, rendering the whole material strong and recalcitrant to biological degradation (BENJAMIN; CHENG; GÖRGENS, 2013; TERÁN-HILARES et al., 2016). It is this reason that lignocellulosic has to go through pretreatment to be able to be susceptible to enzymatic hydrolysis (RAGHAVI et al., 2016; KIM; LEE; KIM, 2016). Examples of such pretreatment methods include ammonia fiber explosion, hydrothermal treatment, acid or alkaline treatment, ultrasound and microwave irradiation (KIM et al., 2013; ANGARITA et al., 2015).

Hydrodynamic cavitation (HC) is one potential option, because the HC-assisted process is known to possess advantages of high energy-efficiency and scaling-up easiness in comparison to a popular and powerful method ultrasound (US), and also to reduce the use of chemical consumption, still with high lignin removal and high glucose yield (SHARMA et al., 2008; KIM et al., 2015).

In the HC, cavitation is generated by pressure variation in a flowing liquid, which can be caused by constriction (venture nozzles, orifice plates or throttling valve). When a liquid passes through the constriction, a number of micro-bubbles (vapor cavities) are formed due to a decrease in pressure below the vapor pressure of the liquid. Subsequently, the micro-bubbles collapse due to slowed flow and pressure recovery (SHARMA et al., 2008; GONÇALVES et al., 2014). This bubble collapse is strong enough to generate localized “hot spots” with transient temperatures of about 10,000 K, and pressure of about 1000 atm, which can induce chemical and physical transformations. Besides, water molecules are dissociated into chemical products, like hydroxyl radical (OH•) that is one of the most powerful oxidants and excellent initiator of chain reactions (OZONEK, 2012; LI et al., 2015). Another important effect is the generation of shock waves during violent collapse of cavities that also are responsible for pyrolysis/molecular breakdown of organic molecules trapped inside or in the vicinity of cavities (SAHARAN et al., 2013).

The mechanical means, despite its distinctive effectiveness, has limitedly been utilized especially for the purpose of biomass pretreatment. In this work, therefore, the HC was adopted and combined with alkaline pretreatment of sugarcane bagasse. Response surface methodology (RSM) was employed as a tool to find important variables and optimal conditions.

4.2 Materials and Methods

4.2.1 Materials

Sugarcane bagasse was obtained from Usina São Francisco (Sertãozinho-SP, Brazil). The biomass was air-dried and milled in a Benedetti 270 hammer mill (Mill Benedetti Ltda, Pinhal-SP, Brazil). The milled bagasse was classified using standard Tyler sieves (10 and14 Mesh) and powders with particle sizes between 1.18 and 1.70 mm were collected, stored at room temperature in a sealed container and used for this study. SCB was comprised of 40.6% glucan, 26.3% of xylan and 24.9% of lignin on dry weight basis.

4.2.2 HC-assisted alkaline pretreatment

Pretreatment of SCB was performed in a laboratory HC-system. The HC-system consisted of a reservoir and a stainless steel cylindrical cavitation reactor which were connected to a centrifugal pump with a power of 1.5 kW. A radial form of orifice plate with 27 holes of 1 mm diameter was used in all experiments (KIM et al., 2015). Pressures of upstream and downstream were kept at 3 and 0.3 bar, respectively. In order to aid each particle in fully experiencing the cavitational effect, sugarcane bagasse was kept in a cylindrical wire cloth (40 mesh) that was placed within so-called cavitation zone in the cavitation reactor. Alkaline solution was passed through the cavitation zone continuously. Dry solid/liquid ratio (S/L) was calculated on the basis of the cavitation reactor volume of 100 mL.

For the sake of comparison, conventional alkaline and ultrasound-assisted pretreatment of SCB were also performed along with the optimal combination of HC assisted pretreatment parameters (as section 6.2.6). The US-assisted pretreatment

was carried out using a probe type ultrasonic processor (VCX 750, USA) at a power of 300W (40% amplitude) and a frequency of 20 kHz.

After pretreatment, the solid fraction was washed, dried and characterized to determinate its main components as cellulose, hemicelluloses and lignin (SLUITER et al., 2011).

4.2.3 Enzymatic hydrolysis

Enzymatic hydrolysis experiments were carried out in a 125 mL Erlenmeyer flask containing 50 mM sodium citrate buffer solution (pH=4.8) at 5% (w/v) solid loading. A novel enzyme blend Cellic C-Tec (Novozymes, Denmark) corresponding to 20 FPU/g of dry biomass was used in all experiments and saccharification was proceeded for 48 h (BAHRANI; RAEISSI; SARSHAR, 2015). Hydrolyzates were withdrawn periodically and analyzed for sugar concentration by high performance liquid chromatography (HPLC) equipped with a HPX-87H column (Bio-Rad, USA) (KIM et al., 2015).

4.2.4 Scanning electron microscopy (SEM) analysis

Surface morphology of the pretreated SCB was characterized by Scanning Electron Microscope SU5000 (Tokyo, Japan) with acceleration voltage of 10 kV and working distance of around 50 μm, and compared with the untreated SCB (CHANDEL et al., 2014).

4.2.5. X-ray diffraction

Crystallinity index of the untreated and pretreated SCB was analyzed using XRD-600 diffractometer (Shimadzu, Tokyo, Japan). The X-ray diffractometer was set at 40 kV and 30 mA. Samples were scanned over the range of 2θ = 5-50° and the crystallinity index (CrI) was determinate using Eq. (4.1) (SEGAL et al., 1959).

$$CrI\ (\%) = [(I_{Crystalline} - I_{Amorphous})/I_{Crystalline}] \times 100\% \qquad \text{Eq. (4.1)}$$

Where: $I_{crystalline}$= Intensity at 22.3° and $I_{amorphous}$= Intensity at 16.1°.

4.2.6. Experimental design

Response surface methodology (RSM) was used to optimize the HC-assisted alkaline pretreatment aiming at high glucose yield. Three independent variables, NaOH concentration (X_1), solid/liquid ratio (X_2) and reaction time (X_3), were studied. The ranges of pretreatment conditions were established as follows: NaOH concentrations of 0.1–0.5 mol/L, solid/liquid ratios of 3–10%, and reaction times of 15–45 min. A total of 15 experimental trials of the three variables were designed by Box–Behnken design using the Design-Expert software 8.0 (Stat-Ease, Inc., USA) (BOX; BEHNKEN, 1960).

4.3. Results and discussion

4.3.1. Hydrodynamic cavitation-assisted alkaline pretreatment

Solid recovery after pretreatment was found to fall between 74.2 and 86.5%, and the maximal lignin removal (54.6%) was observed when the pretreatment was done at 0.5 mol/L of NaOH, 6.5% solid/liquid ratio and 45 min of HC time (no. 8; Table 4.1).

The response variable lignin removal was adjusted by a two factor interaction (2FI) model (R^2=0.945) and the significance of each coefficient on the lignin removal was evaluated by *p*-value (lower then 0.01) and *F*-value (42.82); all this indicated a high statistical significance of the model, which is shown in the Eq. (4.2) in terms of actual values of the studied variables. In this model, only significant terms (*p*-value < 0.05) were considered.

$$Y_1 = 26.82 - 6.80X_1 - 0.77X_2 + 0.09X_3 + 1.57X_1X_3 \qquad \text{Eq. (4.2)}$$

Where: Y_1= Lignin removal (%), X_1=NaOH concentration (mol/L), X_2=S/L ratio (%) and X_3=Time (min)

Under a pretreatment condition of 0.48 mol/L of NaOH, 4.24% S/L ratio and 44.48 min of process, the maximal lignin removal predicted by the model (59.5 ± 2.69 %) was confirmed by an experimental result of 60.4%, validating the adjusted model.

Table 4.1 – Solid composition of SCB after HC-assisted alkaline pretreatment and removal components during pretreatment

Run	Experimental variables			Solid recovery (%)	Solid composition (%)			Removal of components (%)	
	NaOH (mol/L)	S/L ratio (%)	Time of process (min)		Glucan	Xylan	Lignin	Xylan	Lignin
1	0.1	3	30	86.54	41.39	25.19	20.42	17.11	29.02
2	0.5	3	30	81.09	48.58	22.25	17.17	31.40	46.91
3	0.1	10	30	86.05	47.55	24.80	21.65	18.86	25.22
4	0.5	10	30	78.81	48.30	22.50	17.20	32.58	45.57
5	0.1	6.5	15	86.50	45.71	24.49	21.80	19.45	24.28
6	0.5	6.5	15	84.49	43.59	25.19	21.22	19.08	28.01
7	0.1	6.5	45	83.45	45.68	25.01	20.29	35.62	32.02
8	0.5	6.5	45	74.21	51.35	21.28	15.35	39.95	54.61
9	0.3	3	15	85.25	44.40	24.07	19.93	21.98	32.32
10	0.3	10	15	85.24	46.33	23.78	21.94	22.93	24.91
11	0.3	3	45	77.68	46.80	23.37	16.14	30.97	50.05
12	0.3	10	45	79.32	48.12	22.12	18.54	33.29	40.94
13	0.3	6.5	30	81.72	46.74	23.93	18.33	25.64	39.98
14	0.3	6.5	30	81.57	47.85	22.97	18.18	28.76	39.46
15	0.3	6.5	30	81.82	46.91	23.29	18.80	27.54	38.22

Source: Personal archive

In all experiments, an increase in glucan fraction was observed, due to xylan and lignin removal. Glucan content in the HC-based pretreated SCB was also adjusted at 2FI model (R^2=0.911) with *p*-value lower than 0.01 and *F*-value of 18.50 and the adjusted model is shown in Eq. (4.3). The glucan content predicted by the model (51.7 ± 0.87%) under pretreatment condition of 0.48 mol/L of NaOH, 4.24% of S/L ratio and 44.48 min was confirmed by an experimental result of 52.1%, validating the established model.

$$Y_2 = 40.72 + 2.65X_1 + 1.02X_2 - 0.09X_3 - 2.30X_1X_2 + 0.65X_1X_3 \quad \text{Eq. (4.3)}$$

Where: Y_2= Glucan content (%), X_1=NaOH concentration (mol/L), X_2=S/L ratio (%) and X_3=Time (min)

The use of HC appeared to accelerate the delignification process most likely by means of both physical and chemical effect of cavitation. The collapse of micro-bubble cavities generates enormous destructive forces, which not only brings about the disintegration of biomass via high turbulence and micro-jets (KIM et al., 2015; NAGULA; PANDIT, 2016), but also in a chemical sense the dissociation of water molecules and thus the generation of radicals like $HO^{\bullet}$, $HOO^{\bullet}$ and $O_2^{\bullet}$. These radicals oxidize lignin molecules, eventually yielding low molecular weight organic products and even carbon dioxide; and this effect becomes more powerfully with an alkaline catalyst (VELMURUGAN; MUTHUKUMAR, 2012; BADVE et al., 2014).

Temperature is an important parameter in caviation, with its effect highest at a range of 30-50°C (MEROUANI et al., 2016). Temperature in this study did increase from 23°C to 64°C in 45 min. The lignin removal and changes in structure can, therefore, be attributed to both the cavitation effect bellow 50°C and above it the alkaline affect (WANG et al., 2010). A similar study of the HC-based reed pretreatment showed that a maximal lignin removal of 53% could be achieved at a condition of 5% of NaOH, 5% solid/liquid ratio and 40 min of HC operation (KIM et al., 2015).

4.3.2 Optimization of pretreatment for maximal glucose yield

Enzymatic hydrolysis of the HC-treated SCB is summarized in Table 4.2. Enzymatic digestibility was highest (91.0%) for the SCB pretreated in the run 8 (0.5 mol/L of NaOH, 6.5% of solid to liquid ratio and 45 min of HC time).

An ANOVA test was performed and the results are presented in Table 4.3. The *p*-value for the model was lower 0.001, with F-value of 128.0, indicating that the model was highly significant. In the quadratic model shown in Eq. (4.4), only significant terms with *p*-value lower than 0.05 were included. The coefficient of determination (R^2) was 0.986, implying the model could explain 98.6% variability of the response variable within the range of the studied variables. Model quality could also be verified by comparing the predicted value with the observed values, as shown in Table 4.2. The interactive effect of each twice variables on the enzymatic hydrolysis of HC-based pretreated SCB are shown in the Figure 4.1A, 4.1B and 4.1C.

Table 4.2 – Enzymatic digestibility of SCB pretreated in HC-assisted alkaline condition.

Run	Experimental variables used for pretreatment			Enzymatic digestibility after 48h	
	Concentration NaOH (mol/L)	S/L ratio (%)	Time of process (min)	Experimental (%)	Predicted (%)
1	0.1 (-1)	3 (-1)	30 (0)	37.62	39.55
2	0.5 (+1)	3 (-1)	30 (0)	76.10	75.61
3	0.1 (-1)	10 (+1)	30 (0)	32.12	32.68
4	0.5 (+1)	10 (+1)	30 (0)	67.79	68.74
5	0.1 (-1)	6.5 (0)	15 (-1)	28.19	24.98
6	0.5 (+1)	6.5 (0)	15 (-1)	53.81	51.61
7	0.1 (-1)	6.5 (0)	45 (+1)	46.52	47.25
8	0.5 (+1)	6.5 (0)	45 (+1)	90.99	92.73
9	0.3 (0)	3 (-1)	15 (-1)	46.35	51.64
10	0.3 (0)	10 (+1)	15 (-1)	44.31	44.77
11	0.3 (0)	3 (-1)	45 (+1)	86.79	83.34
12	0.3 (0)	10 (+1)	45 (+1)	75.16	76.47
13	0.3 (0)	6.5 (0)	30 (0)	64.02	64.06
14	0.3 (0)	6.5 (0)	30 (0)	66.16	64.06
15	0.3 (0)	6.5 (0)	30 (0)	65.61	64.06

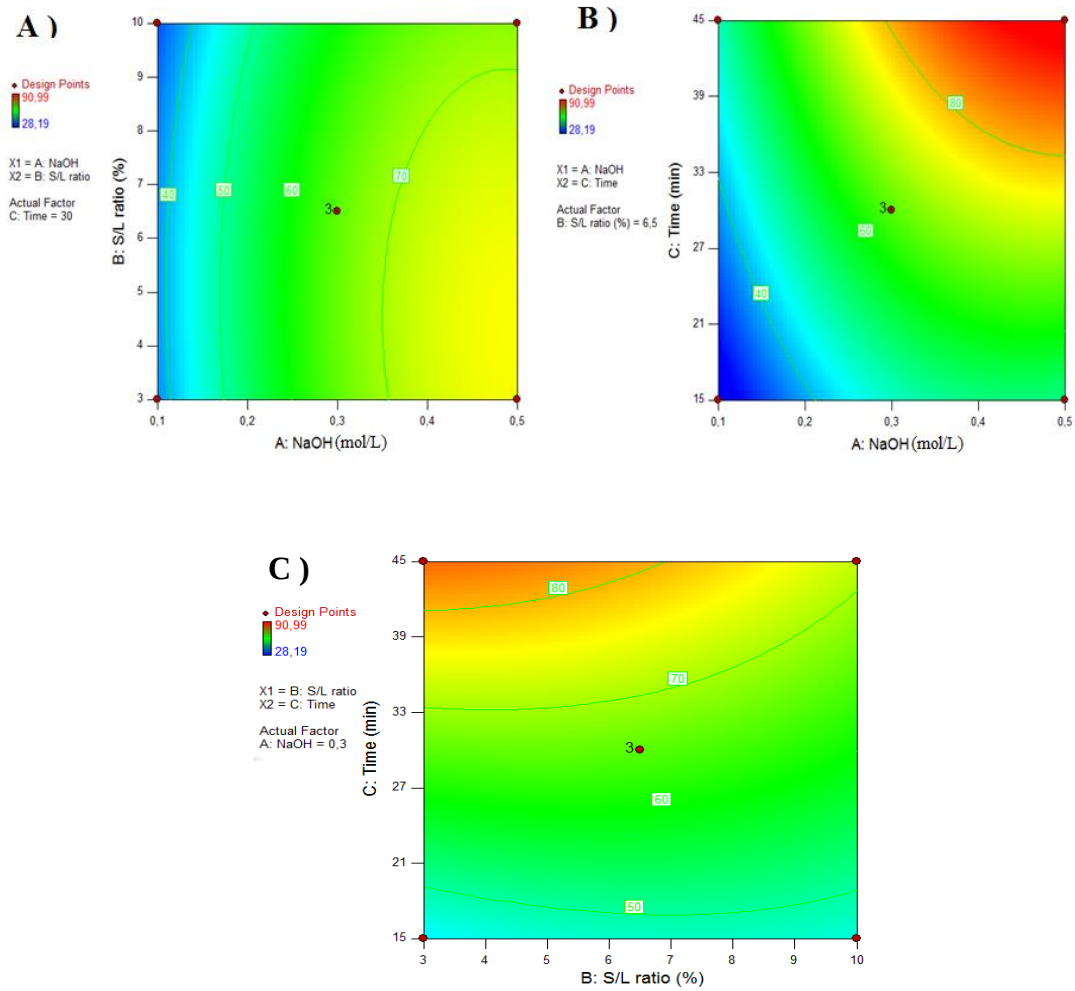
Source: Personal archive

Table 4.3 – Analysis of variance (ANOVA) for the adjusted quadratic model for enzymatic digestibility of SCB pretreated with HC

Source	Sum of Squares	Degrees of freedom	Mean Squares	F-Value	*P*-value (Prob > F)	
Model	5160.64	5	1032.13	127.98	< 0.0001	Significant
Concentration of Sodium hydroxide (X_1)	2600.65	1	2600.65	322.46	< 0.0001	
Solid/liquid ratio (X_2)	94.39	1	94.39	11.70	0.0076	
Time of process (X_3)	2009.78	1	2009.78	249.20	< 0.0001	
X_1X_3	88.83	1	88.83	11.01	0.0090	
X_1^2	366.99	1	366.99	45.50	< 0.0001	
Residual	72.59	9	8.07			
Lack of Fit	70.12	7	10.02	8.11	0.1141	Not significant
Pure Error	2.47	2	1.24			
Total	5233.22	14				
R-square:0.9861						

Source: Personal archive

Figure 4.1 – Contour plots for response enzymatic digestibility of sugarcane bagasse pretreated with HC. A- Concentration of NaOH vs. S/L ratio; B- Concentration of NaOH vs. Time; C- S/L ratio vs. Time



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Optimal pretreatment conditions were determined from the quadratic model considering the maximal enzymatic digestibility in the range of the studied variables (0.48 mol/L of NaOH, 4.24% of solid/liquid ratio and 44.48 min of HC process). Using the model, 94.9 ± 2.84% of enzymatic hydrolysis yield was predicted and this value was corroborated by the experimental value (97.2 %), indicating the model was indeed adequate.

Using the optimal pretreatment combination, an alkaline only and US-assisted alkaline (US-NaOH) pretreatments were also carried out for comparison (Table 4.4 and Figure 4.2).

Table 4.4 – Comparison of solid composition and enzymatic digestibility of SCB pretreated under different conditions

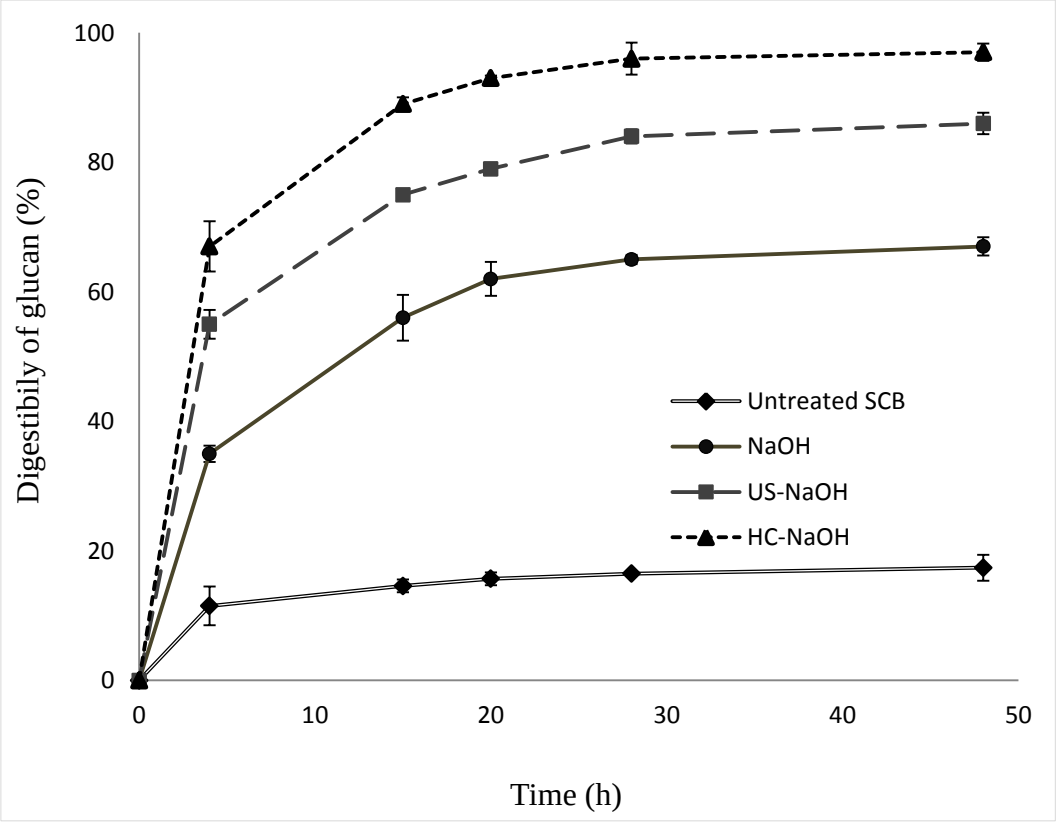
Pretreatment	Solid composition (%)			Lignin removal (%)	Enzymatic digestibility 48h (%)
	Glucan	Xylan	Lignin		
Alkali assisted by hydrodynamic cavitation	52.1 ± 1.32	23.6 ± 0.65	13.8 ± 1.32	60.4 ± 2.43	97.2 ± 1.34
Alkali assisted by ultrasound	51.8 ± 2.12	23.7 ± 1.08	14.2 ± 0.54	59.6 ± 1.65	86.5 ± 3.12
Alkali alone	48.3 ± 0.26	23.8 ± 0.64	16.8 ± 1.11	50.3 ± 1.34	67.6 ± 1.41
Untreated SCB	40.6 ± 1.21	26.0 ± 0.02	24.9 ± 0.99	-	17.4 ± 2.02

Values are means ± standard deviations of triplicate determination.

Source: Personal archive

Alkaline pretreatment alone showed a low efficiency (50.3% of lignin removal) in comparison with ultrasound assisted (59.6%) or hydrodynamic cavitation assisted (60.4%) pretreatment. This result was confirmed by susceptibility to enzyme attack, where in the untreated SCB exhibited only 17% hydrolysis of glucan in 48h of reaction, but the HC-based pretreatment yield was 97.2%. The HC was surely very effective physical disruptor as observed by loosened structure or perforation on the surface of fiber, substantially better than the US (86% of yield), which was in line with the finding with reed (KIM et al., 2015) and SCB pretreated with ultrasound (81.8% of yield) reported by Silva et al. (2016).

Figure 4.2 – Comparative of yield of digestibility of glucan during enzymatic hydrolysis of SCB pretreated under HC with untreated, alkaline alone and US-assisted alkaline process (experiments were in triplicate and results were shown as average ± standard deviation)



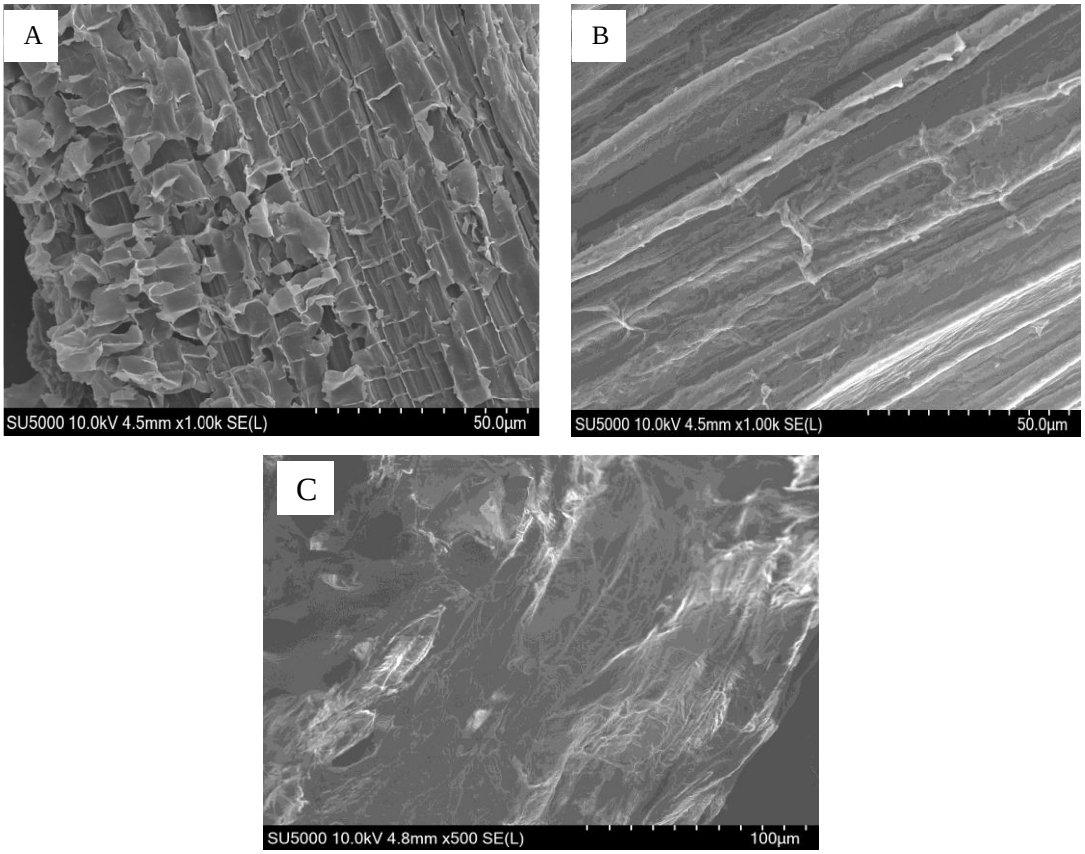
Source: Personal archive

Finally, these results of inhibitors in the enzymatic hydrolizate (0.1 g/L of furfural, 0.06 g/L of acid acetic and 0.39 g/L of HMF) indicated that the HC-assisted alkaline pretreatment of SCB did not produce to a significant level of known inhibitors like furfural, acetic acid and HMF. This is advantageous because these inhibitors could be carried with the material and impairs the subsequent fermentation process. The concentrations of such inhibitors were similar to those pretreated with an alkaline solution (0.3 mol/L of NaOH, 70°C and 4 h) (TERÁN-HILARES et al., 2016). In this study, other types of potential inhibitors like phenolic compounds were not analyzed and thus we cannot necessarily guarantee the complete absence of the inhibition of ensuing fermentation.

4.3.3 Scanning electron microscopy (SEM) analysis

The surface on the HC-treated SCB was visualized by SEM to see any noticeable changes (Figure 4.3). As expected, the treated samples displayed characteristic roughness, indicating the power and effectiveness of the HC; this altered and loosened structure was likely to have a lot to do with its enhanced enzymatic digestibility. The distinctive scars or perforations seen on the surface were attributable to localized hot spots generated by, though transient, exceedingly high temperature and pressure of the cavitation (OZONEK, 2012; BADVE et al., 2014). Microstreaming, microjet erosion and acoustic streaming were also reported to play a role. Ramadoss and Muthukumar (2016) found that when SCB was treated by TiO_2 with hydrogen peroxide that was assisted by US, perforation was created on the surface of fiber. When SCB was pretreated with an alkali alone (0.3 mol/L of NaOH, 70°C for 4h), no perforation appeared (TERÁN-HILARES et al., 2016).

Figure 4.3 – SEM analysis of SCB. A- Untreated, B, C- Pretreated in HC-system



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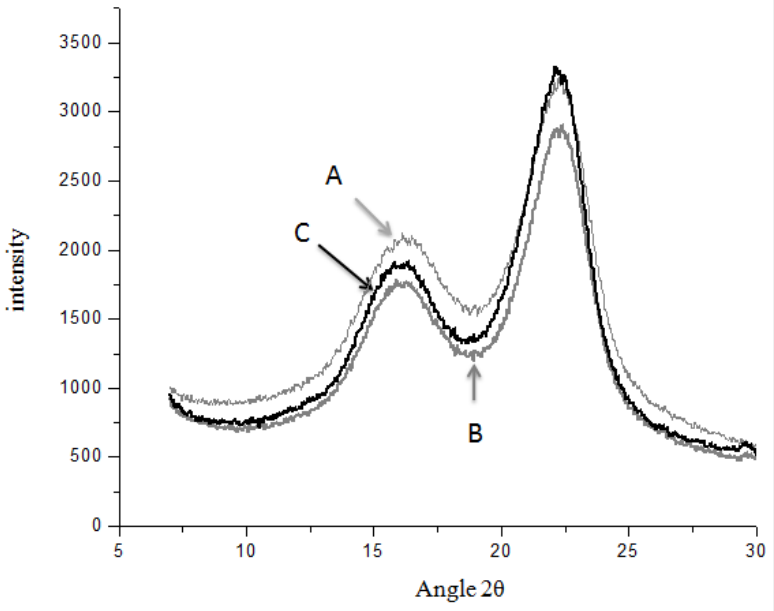
4.3.4 X-ray diffraction analysis of pretreated SCB

XRD analysis was performed to find out how much the HC-assisted alkaline treatment affected crystallinity index (CrI) of the SCB (Figure 4.4). The CrI, albeit imperfect, is an indicator of crystallinity that the lignocellulosic material possesses and it is influenced by glucan, xylan and lignin composition of the material (KUMARI; DAS, 2015).

The degree of the increase in the CrI, however, was not as high as values reported by Velmurugan and Muthukumar (2012) and Terán-Hilares et al. (2016): 28% of increase using ultrasonic-assisted alkaline pretreatment and 29.8% using only NaOH, respectively. To our delight, this somewhat counterintuitive phenomenon ended up exceedingly high enzyme digestibility (97.2%). It is likely because the cavitation affected not only the amorphous potion but also crystalline portion of cellulose fibers.

The collapse of micro-bubbles, by way of the micro-jets and localized heating, might give rise to perforation of the surface of fiber, reducing the crystallinity of cellulose and increasing the accessibility of enzymes. Besides, shock waves generated in cavitation system also are responsible for pyrolysis/molecular breakdown of organic molecules (as cellulose) in the vicinity of cavities (SAHARAN et al., 2013; KIM et al., 2015). The CrI is not an absolute and direct index of the enzyme digestibility after all.

Figure 4.4 - XRD patterns of untreated and pretreated SCB under HC and conventional alkaline. A- Untreated; B- Alkaline pretreated; C- HC-assisted alkaline pretreated.



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4.3.5 Comparison of energy efficiency of HC- and US-assisted pretreatment

The comparison of energy efficiency during SCB pretreatment under HC and US process is summarized in the Table 4.5, and it is compared with other biomass pretreatment like reed (KIM et al., 2015) and corn stover (NAKASHIMA et al., 2016). The cavitation yield by unit energy input was calculated according to Eq. (4.5) reported by Tao et al. (2016).

As a shown, HC system have high energy efficiency in comparison with US-assisted pretreatment process. A similar result was reported for other potential applications of HC, as simultaneous treatment (cell disruption and lipid extraction) of wet microalgae (LEE; HAN, 2015), and pretreatment of rubber seed (*Havea brasilensis*) oil via esterification reaction (BOKHARI et al., 2016).

Table 4.5 – Comparison of energy efficiency during SCB pretreatment under HC- and US

System	Pretreatment condition	Biomass	Efficiency of pretreatment (g de glucose/J)	Reference
HC-NaOH	NaOH (0.48 mol/L) Ratio S/L (4.27%) Time (44.48 min.)	Sugarcane bagasse	6.43×10^{-5}	This study
US-NaOH			2.61×10^{-5}	
HC-NaOH	NaOH (3.0% w/v) Ratio S/L (11.8%) Time (41.1 min.)	Reed	6.51×10^{-5}	Kim et al. (2015)
US-NaOH			2.58×10^{-5}	
HC-SP*	$NaCO_3$ (0.4 mol/L) H_2O_2 (0.6 mol/L) Ratio S/L (4%) Time (60 min.)	Corn stover	2.24×10^{-5}	Nakashima et al. (2016)
US-SP*			0.109×10^{-5}	

*SP: Sodium percarbonate.

Source: Personal archive

4.4 Conclusion

Hydrodynamic cavitation was used to improve the efficiency of alkaline pretreatment of SCB. In an optimum condition, enzymatic hydrolysis of glucan was more than 95%, significantly greater than those with alkaline only and even US-assisted alkaline pretreatment. This technology offers a potential advantage for lignocellulosic bioethanol production in terms of its less energy consumption and easy scalability. The potential of the HC-assisted pretreatment, which is immense, warrants further and extensive investigation in particular in combination with chemical and/or thermal treatments so as to be profitably exploited.

4.5 References

ANGARITA, J. D. et al. Kinetic modeling for enzymatic hydrolysis of pretreated sugarcane straw. **Biochemical Engineering Journal,** v. 104, p.10-19, 2015.

BADVE, M. P. et al. Hydrodynamic cavitation as a novel approach for delignification of wheat straw for paper manufacturing. **Ultrasonics Sonochemistry,** v. 21, p.162-168, 2014.

BAHRANI, S.; RAEISSI, S.; SARSHAR, M. Experimental investigation of ionic liquid pretreatment of sugarcane bagasse with 1,3-dimethylimadazolium dimethyl phosphate. **Bioresource Technology,** v. 185, p.411-415, 2015.

BENJAMIN, Y.; CHENG, H.; GÖRGENS, J. F. Evaluation of bagasse from different varieties of sugarcane by dilute acid pretreatment and enzymatic hydrolysis. **Industrial Crops and Products,** v. 51, p.7-18, 2013.

BOKHARI, A. et al. Optimisation on pretreatment of rubber seed (*Hevea brasiliensis*) oil via esterification reaction in a hydrodynamic cavitation reactor. **Bioresource Technology,** v. 199, p.414-422, 2016.

BOX, G. E. P.; BEHNKEN, D. W. Some new three level designs for the study of quantitative variables. **Technometrics,** v. 2, p.455-464, 1960.

CHANDEL, A. K. et al. Multi-scale structural and chemical analysis of sugarcane bagasse in the process of sequential acid–base pretreatment and ethanol production by Scheffersomyces shehatae and Saccharomyces cerevisiae. **Biotechnology for Biofuels,** v. 7, p.63-69, 2014.

CLAUSER, N. M. et al. Small-sized biorefineries as strategy to add value to sugarcane bagasse. **Chemical Engineering Research and Design,** v. 107, p.137-146, 2016.

GONÇALVES, I. et al. Sonochemical and hydrodynamic cavitation reactors for laccase/hydrogen peroxide cotton bleaching. **Ultrasonics Sonochemistry,** v. 21, p.774-781, 2014.

KIM, H. J. et al. Environmentally friendly pretreatment of plant biomass by planetary and attrition milling. **Bioresource Technology,** v. 144, p.50-56, 2013.

KIM, I. et al. Hydrodynamic cavitation as a novel pretreatment approach for bioethanol production from reed. **Bioresource Technology,** v. 192, p.335-339, 2015.

KIM, J. S.; LEE, Y. Y.; KIM, T. H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. **Bioresource Technology,** v. 199, p.42-48, 2016.

KUMARI, S.; DAS, D. Improvement of gaseous energy recovery from sugarcane bagasse by dark fermentation followed by biomethanation process. **Bioresource Technology,** v. 194, p.354-363, 2015.

LEE, I.; HAN, J-I. Simultaneous treatment (cell disruption and lipid extraction) of wet microalgae using hydrodynamic cavitation for enhancing the lipid yield. **Bioresource Technology,** v. 186, p.246-251, 2015.

LI, P. et al. Enhanced decolorization of methyl orange using zero-valent copper nanoparticles under assistance of hydrodynamic cavitation. **Ultrasonics Sonochemistry,** v. 22, p.132-138, 2015.

MEROUANI, S. et al. Experimental and numerical investigation of the effect of liquid temperature on the sonolytic degradation of some organic dyes in water. **Ultrasonics Sonochemistry,** v. 28, p.382-392, 2016.

NAGULA, K. N.; PANDIT, A. B. Process intensification of delignification and enzymatic hydrolysis of delignified cellulosic biomass using various process intensification techniques including cavitation. **Bioresource Technology,** v. 213, p.162-168, 2016.

NAKASHIMA, K. et al. Hydrodynamic Cavitation Reactor for Efficient Pretreatment of Lignocellulosic Biomass. **Industrial & Engineering Chemistry Research,** v. 55, p.1866-1871, 2016.

OZONEK, J. **Application of Hydrodynamic Cavitation in Environmental Engineering**. New York. CRC Press, 2012.

RAGHAVI, S. et al. Development of a novel sequential pretreatment strategy for the production of bioethanol from sugarcane trash. **Bioresource Technology,** v. 199, p.202-210, 2016.

RAMADOSS, G.; MUTHUKUMAR, K. Mechanistic study on ultrasound assisted pretreatment of sugarcane bagasse using metal salt with hydrogen peroxide for bioethanol production. **Ultrasonics Sonochemistry,** v. 28, p.207-217, 2016.

SAHARAN, V. K. et al. Effect of geometry of hydrodynamically cavitating device on degradation of orange-G. **Ultrasonics Sonochemistry,** v. 20, p.345-353, 2013.

SEGAL, L. et al. An Empirical Method for Estimating the Degree of Crystallinity of Native Cellulose Using the X-Ray Diffractometer. **Textile Research Journal,** v. 29, p.786-794, 1959.

SHARMA, A.et al. Modeling of hydrodynamic cavitation reactors based on orifice plates considering hydrodynamics and chemical reactions occurring in bubble. **Chemical Engineering Journal,** v. 143, p.201-209, 2008.

SILVA, G. T. et al. Sono-assisted alkaline pretreatment of sugarcane bagasse for cellulosic ethanol production. **Catalysis Today,** v. 269, p.21-28, 2016.

SLUITER, A. et al. **Determination of structural carbohydrates and lignin in biomass**. Technical Report NREL/TP-510-42618, version 07-08-2011. National Renewable Energy Laboratory, Golden. 2011.

TAO, Y. et al. Application of Hydrodynamic Cavitation to Wastewater Treatment. **Chemical Engineering & Technology,** v. 39, p.1363-1376, 2016.

TERÁN-HILARES, R. et al. Successive pretreatment and enzymatic saccharification of sugarcane bagasse in a packed bed flow-through column reactor aiming to support biorefineries. **Bioresource Technology,** v. 203, p.42-49, 2016.

VALLEJOS, M. E. et al. Kinetic study of the extraction of hemicellulosic carbohydrates from sugarcane bagasse by hot water treatment. **Industrial Crops and Products,** v. 67, p.1-6, 2015.

VELMURUGAN, R.; MUTHUKUMAR, K. Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: Optimization through response surface methodology. **Bioresource Technology,** v. 112, p.293-299, 2012.

WANG, Z. et al. Sodium hydroxide pretreatment and enzymatic hydrolysis of coastal Bermuda grass. **Bioresource Technology,** v. 101, p.3583-3585, 2010.

CHAPTER V

Hydrodynamic cavitation as an efficient pretreatment method for lignocellulosic biomass: a parametric study

*Chapter previously published: TERÁN HILARES, R. et al. Hydrodynamic cavitation as an efficient pretreatment method for lignocellulosic biomass : A parametric study. **Bioresource Technology**, v. 235, 301–308, 2017.

ABSTRACT

Hydrodynamic cavitation (HC), which is a highly destructive force, was employed for pretreatment of sugarcane bagasse (SCB). The efficacy of HC was studied using response surface methodology (RSM) with determining parameters varied: inlet pressure of 1-3 bar, temperature of 40-70 °C, and alkaline concentration of 0.1-0.3 mol/L. At the best condition (3 bar, 70 °C and 0.3 mol/L of NaOH), 93.05% and 94.45% of hydrolysis yield of cellulose and hemicellulose, respectively, were obtained within 30 min of pretreatment time. Also, pretreatment time higher than 10 min had little to do regarding to SCB composition changes using different orifice plates (16 and 27 holes, with corresponding cavitation number of 0.017 and 0.048, respectively), with higher hydrolysis yield observed at 20 min of process. Therefore, HC-based approach could lead to a high yield of hydrolysis, as long as a treatment condition was right; it could be so at mild conditions and at short running time.

Keywords:

Hydrodynamic cavitation; Lignocellulosic biomass; Sugarcane bagasse; Pretreatment; Enzymatic digestibility

5.1 Introduction

Sugarcane bagasse (SCB) is feedstock that is abundantly available and readily collectable and thus has immense potential as a substrate for the production of bio-products such as biofuels and other value-added products (e.g., polymers, industrial chemicals and additives); and it is particularly true of Brazil. Its commercial use at present, however, is rather surprisingly limited due to the exceptionally resistant nature: the carbohydrate fractions of cellulose and hemicellulose are cemented together by lignin sheath (RAMADOSS; MUTHUKUMAR, 2015). To make the most of SCB, therefore, a treatment able to loosen the complex structure, so-termed pretreatment, must precede any biological processes. Through the pretreatment, biomass will have increased porosity and reduced crystallinity by way of lignin removal and hemicellulose release (WANG et al., 2016).

There are a great number of pretreatment methods developed thus far, such as techniques based on chemical catalysts including acids or bases, and ionic liquid (BAHRANI; RAEISSI; SARSHAR, 2015), and physical means based on microwave (ZHU et al., 2016), extrusion (AHMED et al., 2017), and ultrasound (VELMURUGAN; MUTHUKUMAR, 2012), and also classical thermal treatment; in many cases, more than two methods are combined. All of them have not only merits, but more demerits in terms of reaction time, catalyst amount, energy consumption, and scaling-up (SINDHU; PANDEY; BINOD, 2015).

Hydrodynamic cavitation (HC) is a comparatively less explored yet promising means. Its potential has been proven, though rather briefly, with sugarcane bagasse (TERÁN HILARES et al., 2016a), corn stover (NAKASHIMA et al., 2016) and reed (KIM et al., 2015). Its effectiveness relies on the formation, growing and collapse of micro-bubbles generated by pressure drop in a flowing liquid through cavitating devices (RAJORIYA; BARGOLE; SAHARAN, 2017). This collapse of cavities release a very high amount of energy in localized “hot spots” with transient temperature of about 10,000 K and pressure of 1000 atm (CHAKINALA et al., 2009). These enormous energy, transient at it is, brings about the dissociation of water molecules into oxidative radicals ($OH^{\bullet}$ and $H^{\bullet}$), thereby leading to the oxidation and degradation of organic molecules (RAUT-JADHAV et al., 2016a). Mechanical force, due to high speed microjets (1000 m/s) created by the violent

bubble collapse, also impacts solid surface with pressure of about 1 GPa and shockwaves, destroying organic molecules (OZONEK, 2012).

This power can be displayed likewise with lignocellulose and potentially does so at milder conditions. For example, in our previous study, high enzymatic digestibility (97%) was achieved when SCB was delignified with the alkaline-assisted HC treatment (TERÁN HILARES et al., 2016a). A similar finding was also reported in a technical report of a company E-PIC s.r.l (2016) and in research articles of Kim et al. (2015) and Nakashima et al. (2016).

At the moment, however, there have been only limited studies executed regarding pretreatment efficiency, which depends on various parameters such as temperature, inlet pressure, retention time, particle size and configuration of HC system (GOGATE, 2008; TERÁN HILARES et al., 2016a). In this study, therefore, such key parameters were examined and optimized by using response surface methodology (RSM).

5.2 Materials and Methods

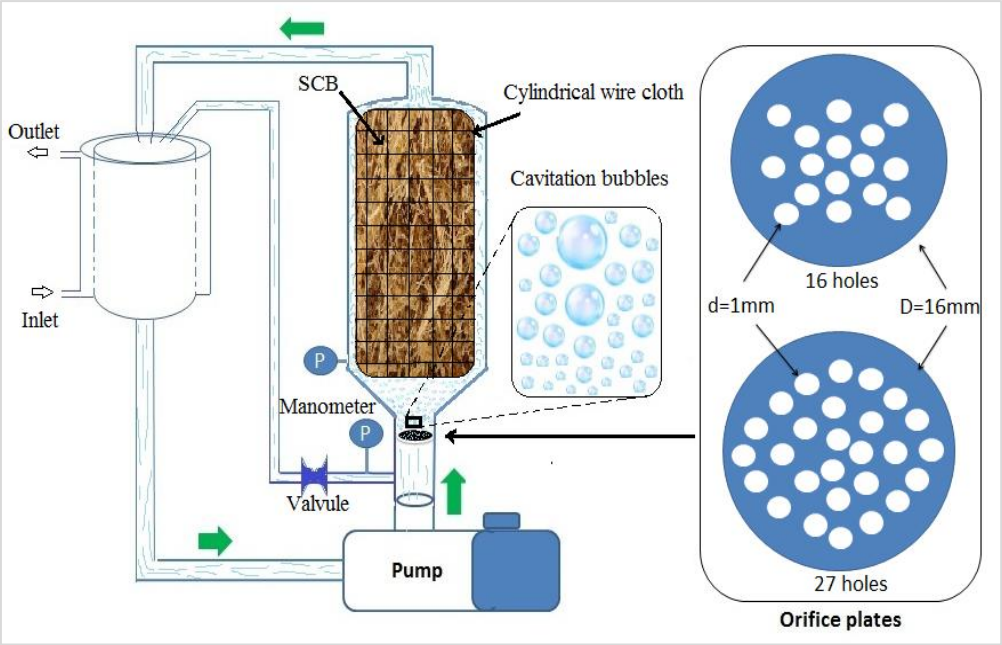
5.2.1 Materials

Sugarcane bagasse was kindly donated by Usina Vale Onda Verde (Onda Verde-SP, Brazil). It was air dried, milled using a Benedetti 270 hammer mill and finally sieved to an average particle size of 4.7 mm prior to experimentation. The compositional analysis revealed 40.15% of cellulose, 25.52% of hemicellulose, 26.17% of lignin and 8.16 % of ash and extractives in raw SCB.

5.2.2 HC-based pretreatment of sugarcane bagasse

A HC experimental set-up was comprised of a 2.5 liter reservoir recirculated through the cavitation zone by a 2 CV centrifugal pump (Fig.5.1). The flow rate of fluid leaving the pump was 5 m^3/h and the fluid velocity through the orifice was 54.49 m/s. Fluid temperature was controlled in all experiments by constant recirculation of cold water through the jacket of the system.

Figure 5.1 – Schematic representation of HC system used for SCB pretreatment.



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For cavitation, an orifice plate having 16 holes of 1 mm diameter was used for all assays of experimental design. Raw SCB was kept in a cylindrical wire cloth (18 mesh) and placed in the cavitation zone (total volume of 1000 ml). Unless otherwise indicated, time of pretreatment was 30 min.

Pretreatment was optimized using a Box-Behnken design for the variables pressure (X_1), temperature (X_2) and NaOH concentration (X_3), considering as responses the pretreated material composition and enzymatic digestibility of carbohydrate fraction (BOX; BEHNKEN, 1960). Three variables (i.e., pressure, temperature and alkali concentration) were selected because they are of prime importance in either HC operation and hydrolysis reaction or both. Their relative effects were studied in the following range: 1-3 bar of pressure, 40 – 70 °C of temperature and 0.1-0.3 mol/L of alkali concentration. The upper limit of 70 °C for temperature was selected because it was the highest possible degree that our HC device could possible reach without external heat supply; and the other conditions were chosen from our previous work (TERÁN HILARES et al., 2016a). Design-Expert software 8.0 (stat-Ease, Inc, USA) was used to compose and evaluate empirical models to describe response variables as a function of temperature, NaOH concentration and inlet pressure. Process optimization was carried out using the numerical optimization feature of the software, based on desirability function. The

goals were to achieve the maximizations of cellulosic hydrolysis yield, lignin removal and carbohydrate contents in the pretreated biomass. After optimization, model prediction was confirmed by experimental runs. Samples (raw and pretreated) were characterized with relation to their main components (cellulose, hemicellulose and lignin), according to methodology described by Sluiter et al. (2012).

5.2.3 Enzymatic hydrolysis

Enzymatic hydrolysis was performed in a 125 mL Erlenmeyer flask at 5% solids loading of the treated SCB in 50 mM sodium citrate solution. A commercial enzyme blend Cellic® CTec2 (kindly donated by Novozymes Latin America Ltda., Araucária-PR, Brazil) was used with a loading of 20 FPU/g of dry biomass. A sample containing the enzyme blend was incubated at 50 °C and 150 rpm for 48 h. Sugar monomers were analyzed by High Performance Liquid Chromatography (HPLC) Agilent 1200 series (Agilent Technologies, Inc., USA) equipped with a Refractive index detector RID-6A and a HPX-87H (300x7.8mm) column (Bio-Rad, USA). Used conditions were the following: 45°C column temperature, 0.01 N H_2SO_4 as the mobile phase, 0.6 mL/min flow rate, and 20 µL injection volume. For furfural and hydroxymethylfurfural (HMF) analysis, the HPLC was equipped with a UV detector (276nm) and a Zorbax Eclipse plus C18 (4.6×150 mm) column (Agilent Technologies, Inc., USA). Used conditions were the following: 25 °C column temperature, acetonitrile: water (1:8) plus 1% of acetic acid as the mobile phase, 0.8 mL/min flow rate, and 20 µL injection volume. For total phenolic analysis, the methodology of Singleton and Rossi (1965) was used. Enzymatic digestibility was calculated for yield of cellulose and hemicellulose hydrolysis (%) on the basis of initial glucan and "xylan + arabinan" contents, respectively.

5.2.4 Evaluation of time of HC-pretreatment and effect of number of holes in orifice plates

At the best operational condition determined by the statistical analysis (3 bar, 0.3 mol/L of NaOH and 70 °C) in the previous step, pretreatment time was evaluated, given its effect in enzymatic digestibility and solid composition of pretreated SCB. In these assays, pretreatment was carried out in batches of 10, 20

and 30 min. Additionally, the effect of the number of holes (16 and 27) in the orifice plate used to create cavitation on the efficiency of pretreatment was also identified (Fig.5.1). After pretreatment, the biomass was washed and dried, and subsequently hydrolyzed and characterized according methodologies above described.

5.2.5 Scanning electron microscopy (SEM) analysis

Any changes on the surface of biomass were evaluated using Scanning Electron Microscope SU5000 (Tokyo, Japan) with acceleration voltage of 10 kV and working distance of 50 µm. The samples of raw and pretreated SCB were fixed with carbon ribbon supporting aluminum and analyzed subsequently.

5.2.6 XRD analysis

Crystallinity indices (CrI) for raw and pretreated SCB were determined by X-ray diffraction (XRD) in a XRD-600 diffractometer (Shimadzu, Tokyo, Japan). The X-ray diffractometer was set at 40 kV and 30 mA. Each sample was pressed into a lamellar container with a 20 mm diameter and scanned over a diffraction angle (2θ°) of 2-60° with a step of 0.02° by Cu radiation (λ =1.54 Å). CrI was determined from a relationship between the intensity of cellulose (I_{002}) peak and the minimum dip (I_{am}) peaks using the Eq.5.1 (SEGAL et al., 1959).

$$CrI\ (\%) = ((I_{002}-I_{am})/I_{002})*100 \qquad \text{Eq. (5.1)}$$

Where I_{002} is the highest peak intensity of the I_{002} is the lattice diffraction and I_{am} is the intensity of the amorphous portion at 18.8°, 2θ degrees.

5.3 Results and discussion

5.3.1 Effect of HC-pretreatment on SCB composition and enzymatic hydrolysis

HC treatment did affect solids composition and enzymatic digestibility of SCB, as shown in Tables 5.1 and 5.2. Solid recovery varied from 77.19% to 87.21% mainly due to varying removal degrees of lignin and hemicellulose that are typical of

the alkaline pretreatment. The highest delignification of 41.83% was observed at run 8 (3 bar of pressure, 0.3 mol/L of NaOH and 55 °C of temperature) with a corresponding increase in cellulose contents up to 47.40%. The response lignin removal was adjusted by a two factor interaction (2FI) model (Eq. 5.2) with R-square value of 0.92. The significance of this model (95% confidence level) was also confirmed by p-value (<0.05), F-value (40.11) and lack of Fit test (p-value>0.05). This lignin removal was quite comparable with previous studies in which about 40% of lignin removal was observed at harsher conditions (KIM et al., 2015). Thus in our system 3 bars of inlet pressure was indeed enough to bring about effective cavitation avoiding supercavitation, phenomenon usually associated to high inlet pressure with consequent reduction in the cavitation efficiency (RAUT-JADHAV et al., 2016b; BARIK; GOGATE, 2016).

$$Y_2\,(\%) = -11.49 + 4.55X_1 + 0.12X_2 - 6.09X_3 + 1.79X_2X_3 \qquad \text{Eq. (5.2)}$$

Where: Y_2 is the response variable "lignin removal" and X_1, X_2 and X_3 correspond to actual values of pressure (bar), temperature (°C) and concentration of sodium hydroxide (mol/L), respectively.

Enzymatic digestibility of glucan fraction varied from 28.94% to 80.98% depending on the process conditions (Table 5.2). Its highest value (80.98%) was observed at 2 bar of inlet pressure, 70 °C of temperature and 0.3 mol/L of sodium hydroxide concentration. This value was fivefold the digestibility of raw SCB (15.36%). Furthermore, the hydrolysis of hemicellulosic fractions showed a maximum value of 81.89%, around 9 times higher than the raw SCB (8.93%) at run 12, proving the efficacy of HC pretreatment (TERÁN HILARES et al., 2016a). This high hydrolysis efficiency could indeed be attributed to the lignin removal by the formation of potential hydroxyl radicals ($HOO^{\bullet}$, $O_2^{\bullet}$ and $OH^{\bullet}$) and structural alterations due to the mechanical effects of cavitation (BADVE et al., 2014; KIM et al., 2015; TERÁN HILARES et al., 2016a).

Table 5.1 – Composition of HC-pretreated SCB and percentage of removal of components during pretreatment

Run	Experimental variables*			Solid recovery (%)	Solid composition after pretreatment (%)			Removal of components (%)	
	Pressure (bar)	Temperature (°C)	NaOH (mol/L)		Cellulose	Hemicellulose	Lignin	Hemicellulose	Lignin
1	1 (-1)	40 (-1)	0.2 (0)	87.91	42.57	21.05	27.02	27.49	9.23
2	3 (+1)	40 (-1)	0.2 (0)	84.92	43.23	21.24	24.89	29.33	19.23
3	1 (-1)	70 (+1)	0.2 (0)	80.07	45.39	21.01	23.65	34.09	27.64
4	3 (+1)	70 (+1)	0.2 (0)	81.73	46.11	23.42	21.43	25.01	33.07
5	1 (-1)	55 (0)	0.1 (-1)	85.94	42.98	20.28	26.55	31.71	12.81
6	3 (+1)	55 (0)	0.1 (-1)	83.01	43.68	21.06	25.18	31.50	20.13
7	1 (-1)	55 (0)	0.3 (+1)	80.96	46.67	22.32	23.23	29.20	28.15
8	3 (+1)	55 (0)	0.3 (+1)	77.19	47.40	23.73	19.72	28.21	41.83
9	2 (0)	40 (-1)	0.1 (-1)	88.12	41.55	22.07	27.01	23.81	9.05
10	2 (0)	70 (+1)	0.1 (-1)	85.51	43.51	22.33	25.56	25.19	16.49
11	2 (0)	40 (-1)	0.3 (+1)	84.14	45.00	21.71	24.23	28.43	22.10
12	2 (0)	70 (+1)	0.3 (+1)	77.99	46.92	23.54	20.04	28.07	40.27
13	2 (0)	55 (0)	0.2 (0)	82.48	44.30	22.23	25.26	28.15	20.39
14	2 (0)	55 (0)	0.2 (0)	82.03	44.93	23.01	24.32	26.05	23.77
15	2 (0)	55 (0)	0.2 (0)	83.47	43.80	22.73	25.26	25.64	19.43

*coded levels in parenthesis
Source: Personal archive

Table 5.2 – Cellulose enzymatic digestibility of HC-pretreated SCB

Run	Experimental variables*			Enzymatic digestibility (%)	
	Pressure (bar)	Temperature (°C)	Concentration of sodium hydroxide (mol/L)	Experimental	Predicted
1	1 (-1)	40 (-1)	0.2 (0)	44.31	45.77
2	3 (+1)	40 (-1)	0.2 (0)	51.39	51.28
3	1 (-1)	70 (+1)	0.2 (0)	69.46	68.70
4	3 (+1)	70 (+1)	0.2 (0)	74.80	74.21
5	1 (-1)	55 (0)	0.1 (-1)	37.65	38.02
6	3 (+1)	55 (0)	0.1 (-1)	32.86	33.93
7	1 (-1)	55 (0)	0.3 (+1)	61.39	60.32
8	3 (+1)	55 (0)	0.3 (+1)	75.80	75.43
9	2 (0)	40 (-1)	0.1 (-1)	28.94	27.54
10	2 (0)	70 (+1)	0.1 (-1)	46.42	46.37
11	2 (0)	40 (-1)	0.3 (+1)	55.30	55.35
12	2 (0)	70 (+1)	0.3 (+1)	80.98	82.38
13	2 (0)	55 (0)	0.2 (0)	48.14	48.81
14	2 (0)	55 (0)	0.2 (0)	49.31	48.81
15	2 (0)	55 (0)	0.2 (0)	48.97	48.81

* Coded values in parenthesis

Source: Personal archive

5.3.2 Pretreatment optimization and model confirmation

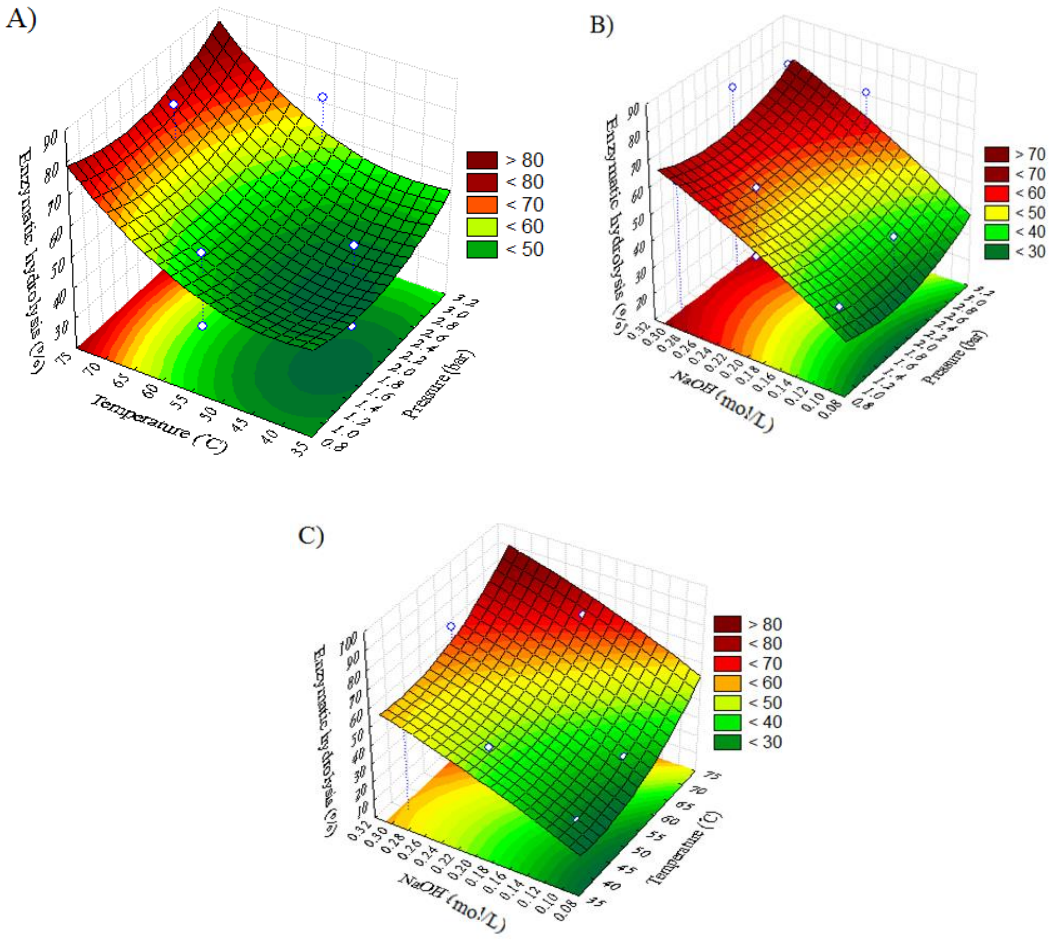
An ANOVA test was also performed to optimize the enzymatic digestibility of cellulose and results are presented in Table 5.3. As shown in the table, the model (Eq. 5.3) has quite high R-square value of 0.99 and was significant at 95% of confidence level. It was also confirmed by p-value (<0.05), F-value (333.69) and non-significant lack of Fit test (p-value>0.05). The quality of the quadratic model was also verified by comparing the observed enzymatic digestibility (75.80%) with the predicted value (75.45%) for run 8 (Table 5.2). A similar value was predicted by the quadratic model reported by Terán Hilares et al. (2016a) for cellulose digestibility of SCB processed by the HC at harsher conditions (3 bar, 0.3 mol/L of NaOH and 45 min).

$$Y3\ (\%) = 100.26 - 27.86X_1 - 2.55X_2 + 72.51X_3 + 48.02X_1X_3 + 1.37X_2X_3 + 5.22X_1^2 + 0.028X_2^2 - 210.53X_3^2 \quad \text{Eq. (5.3)}$$

Where: Y_3 is the response variable "enzymatic digestibility of cellulose" and X_1, X_2 and X_3 correspond to actual values of pressure (bar), temperature (°C) and concentration of sodium hydroxide (mol/L), respectively.

3D response surface plots were also drawn to observe the effects of pretreatment variables on the digestibility. As it is obvious from Fig. 5.2, both temperature and alkali concentration had a positive effect on the enzymatic digestibility of cellulosic fraction (KIM et al., 2015). This behavior is well-known in the alkaline pretreatment as a higher alkali concentration and temperature favour lignin removal and subsequently enhance the digestibility (SINDHU; PANDEY; BINOD, 2015; TERÁN-HILARES et al., 2016b).

Figure 5.2 – Response surface for enzymatic digestibility of cellulose: A- Pressure vs Temperature, B- NaOH vs Pressure, C- NaOH vs Temperature



Source: Personal archive

Table 5.3 – Analysis of variance (ANOVA) for the adjusted quadratic model for cellulose enzymatic digestibility of SCB pretreated with hydrodynamic cavitation (HC)

Source	Sum of Squares	Degrees of freedom	Mean Square	F-Value	*p*-value (Prob > F)	
Model	3487.021	8	435.8776	333.6894	< 0.0001	Significant
Pressure (X_1)	55.30505	1	55.30505	42.33921	0.0006	
Temperature (X_2)	1028.88	1	1028.88	787.6672	< 0.0001	
Concentration of NaOH (X_3)	2035.893	1	2035.893	1558.593	< 0.0001	
X_1X_3	92.22292	1	92.22292	70.60196	0.0002	
X_2X_3	16.8228	1	16.8228	12.87883	0.0115	
X_1^2	100.746	1	100.746	77.1269	0.0001	
X_2^2	142.3623	1	142.3623	108.9865	< 0.0001	
X_3^2	16.36633	1	16.36633	12.52937	0.0122	
Residual	7.837424	6	1.306237			
Lack of Fit	7.107292	4	1.776823	4.86713	0.1776	not significant
Pure Error	0.730132	2	0.365066			
Total	3494.858	14				
Adjusted R-square: 0.9948						

Source: Personal archive

The effect of temperature on the performance of HC was significant in that pretreatment at 70 °C led to greater enzymatic digestibility than that at 40 °C, quite different from other HC applications such as degradation of methyl parathion that occurred highest at 39 °C (PATIL; GOGATE, 2012) and degradation of imidacloprid at 34°C (PATIL; BOTE; GOGATE, 2014). This discrepancy was likely attributable both to the more rigid structure of lignocellulose and to the reactivity of chemical catalysts. It was plausible that HC acted more effectively in disrupting the touch biomass in the presence of an alkaline especially at elevated temperatures. Besides, controlling temperature at 70 °C and thus improved pretreatment efficiency would allow shorted process time and decreased alkaline loading, thereby resulting in more economically competitive at least than the temperature=uncontrolled treatment done in our previous study (TERÁN HILARES et al., 2016a).

The lignin removal and enzymatic digestibility, each as a response, were optimized and defined according to statistical analysis (adjusted models for pretreated SCB composition are shown in supplementary materials), under the following conditions: inlet pressure of 3 bar, temperature of 70 °C and sodium hydroxide concentration of 0.3 mol/L. Experimental results obtained with these conditions were compared with predicted values and are presented in Table 5.4. As expected, all the obtained experimental results are in good agreement with the predicted values.

Table 5.4 – Experimental confirmation of values predicted by models in optimized HC-pretreatment conditions

Response variables (%)	Experimental value* ($\dot{X}$ ± SD)	Predicted value** (CI ± 95%)
Cellulose ***	48.23 ± 0.39	48.03 ± 0.68
Hemicellulose***	22.26 ± 0.43	23.94 ± 1.12
Lignin ***	18.20 ± 0.53	17.57 ± 0.85
Lignin removal	51.52 ± 2.23	46.61 ± 5.26
Cellulose enzymatic hydrolysis	93.05 ± 4.33	94.90 ± 2.98

*Experimental values corresponding to average of three values ± standard deviation (SD). ** Predicted values ± 95% confidence interval (CI). *** Model reported in supplementary material.

Source: Personal archive

This study showed a high hydrolysis efficiency of both the cellulosic (93.05%) and hemicellulosic (94.45%) fractions with rather shorter pretreatment time (30 min) and mild alkaline concentration (0.3 mol/L of NaOH) compared to a previous report in which optimized conditions corresponded to 44.8 min of time employing 0.48 mol/L NaOH concentration (TERÁN HILARES et al., 2016a). In another study (KIM et al., 2015), reed biomass showed a maximum cellulose hydrolysis yield of 85% with 3% NaOH and 40 min of process time, a result lower than our results. In the work reported by Nakashima et al. (2016), when a Venturi tube was employed in HC system for corn stover pretreatment, total monomeric sugars yield (275 g/kg of biomass) was lower than our (559 g /kg of biomass) and, even considering the use of different biomass, this fact can indicate a less severe cavitation intensity obtained using venture devices. Cavitation intensity in our system (based on the orifice plate) appeared to be better suited for pretreatment process due to a combination of stable oscillatory and transient motion as a result of two pressure gradients (linear mean pressure gradient and oscillatory turbulent pressure gradient) (MOHOLKAR; PANDIT, 2001).

Inhibitory compounds such as furfural, hydroxymethylfurfural (HMF) and total phenolics were also analyzed in the hydrolysate obtained from SCB pretreated at optimum conditions. They were found to be 0.02 g/L, $2x10^{-4}$ g/L and 0.09 g/L for furfural, HMF and total phenolics respectively; these values were lower than works that we did on the basis of alkaline only or HC-assisted alkaline pretreatments (TERÁN HILARES et al., 2016a,b) and lower than considered inhibitory for the fermentation process (WIKANDARI et al., 2010).

5.3.3 Effect of cavitation number on pretreatment efficacy

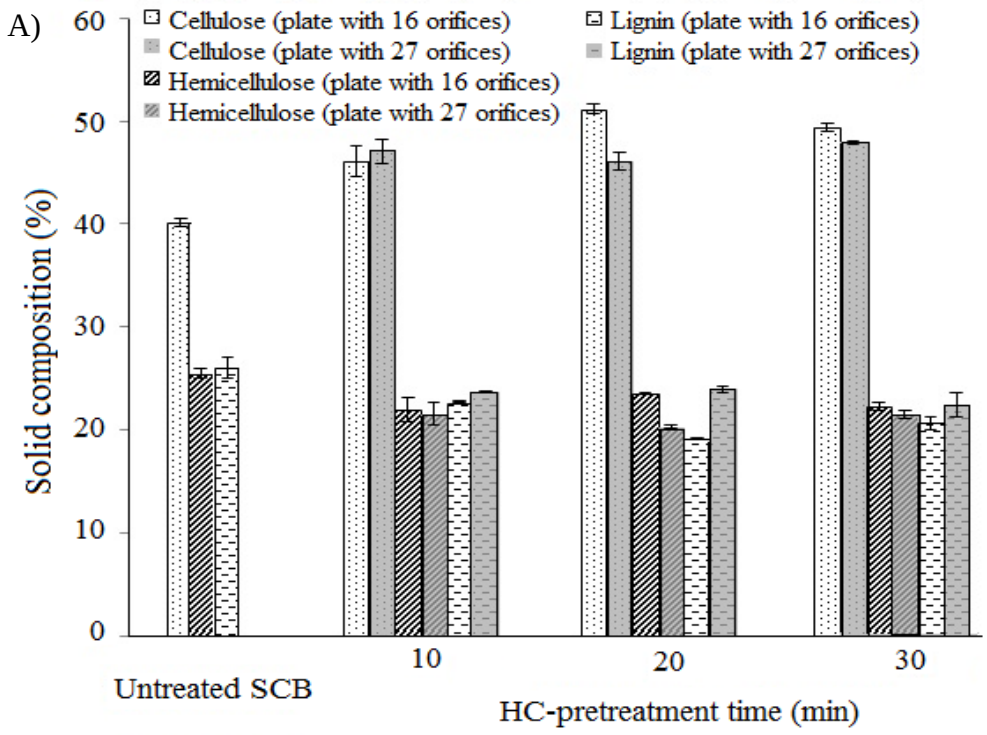
For the sake of comparison, an additional pretreatment study was performed employing two different cavitation numbers (C_v), namely 0.017 and 0.048 (Eq. 5.4) (CAPOCELLI et al., 2014), obtained by using different orifice plates.

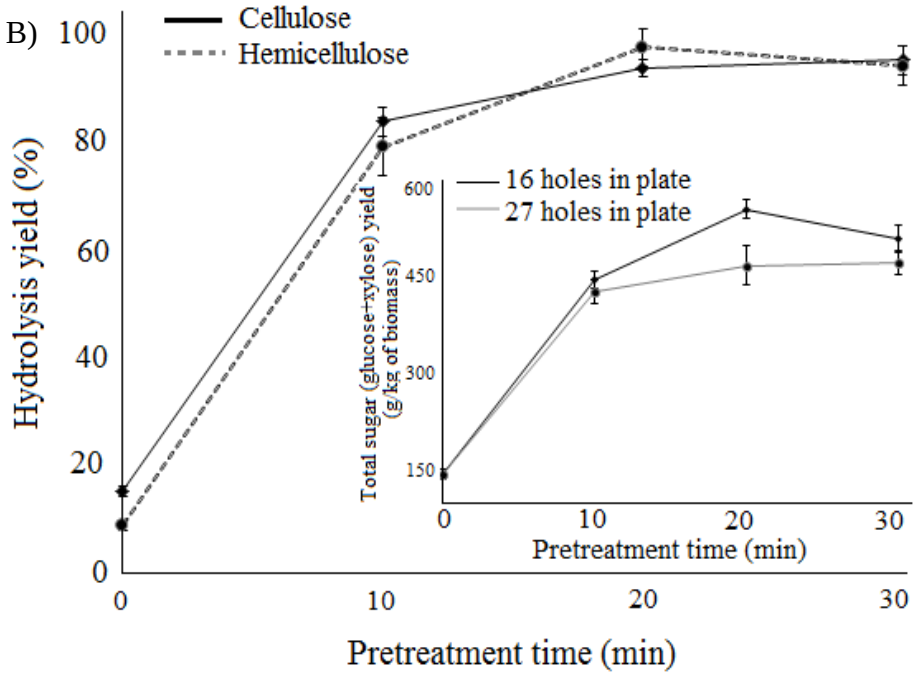
$$C_v = 2(P_2 - P_v)/\rho * V_{th}^2 \qquad \text{Eq. 5.4}$$

Where P_2 is downstream pressure (Pa), P_v corresponds to vapor pressure of alkaline solution (Pa), ρ is density of alkaline solution (kg/m^3) and V_{th} is the velocity of fluid through the orifice (m/s)

Figure 5.3A shows the solids composition of SCB treated with either 16 or 27 orifice plates (corresponding to Cv values of 0.017 and 0.048, respectively) for different time intervals (10, 20 and 30 min). As shown, for initial 10 min of HC pretreatment, considerable increases in the cellulose contents (46.10%) and reduction in hemicellulose (22.26%) and lignin contents (20.69%) resulted using 16 holes in plate. No significant changes in compositions were observed when the HC time was prolonged to 30 min. The cellulose hydrolysis yield, on the other hand, showed the highest value of 94.18% at 20 min of pretreatment time and was not increased for 30 min of process (Fig. 3B). Thus, a reduced time, with 16 orifice plate, was enough to yield a high amount of total monomeric sugars of 559 g/kg of biomass, which was considerably higher than previous studies (KIM et al., 2015; TERÁN HILARES et al., 2016a; NAKASHIMA et al., 2016).

Figure 5.3 – Effect of HC-pretreatment time (3 bar, 70 °C and 0.3 mol/L of NaOH) on the biomass, by using two different orifice plates. A-Variation in the solid composition, B- Enzymatic digestibility of carbohydrate fraction (only for plate with 16 orifices) and total sugars (glucose + xylose) yield (both plates)





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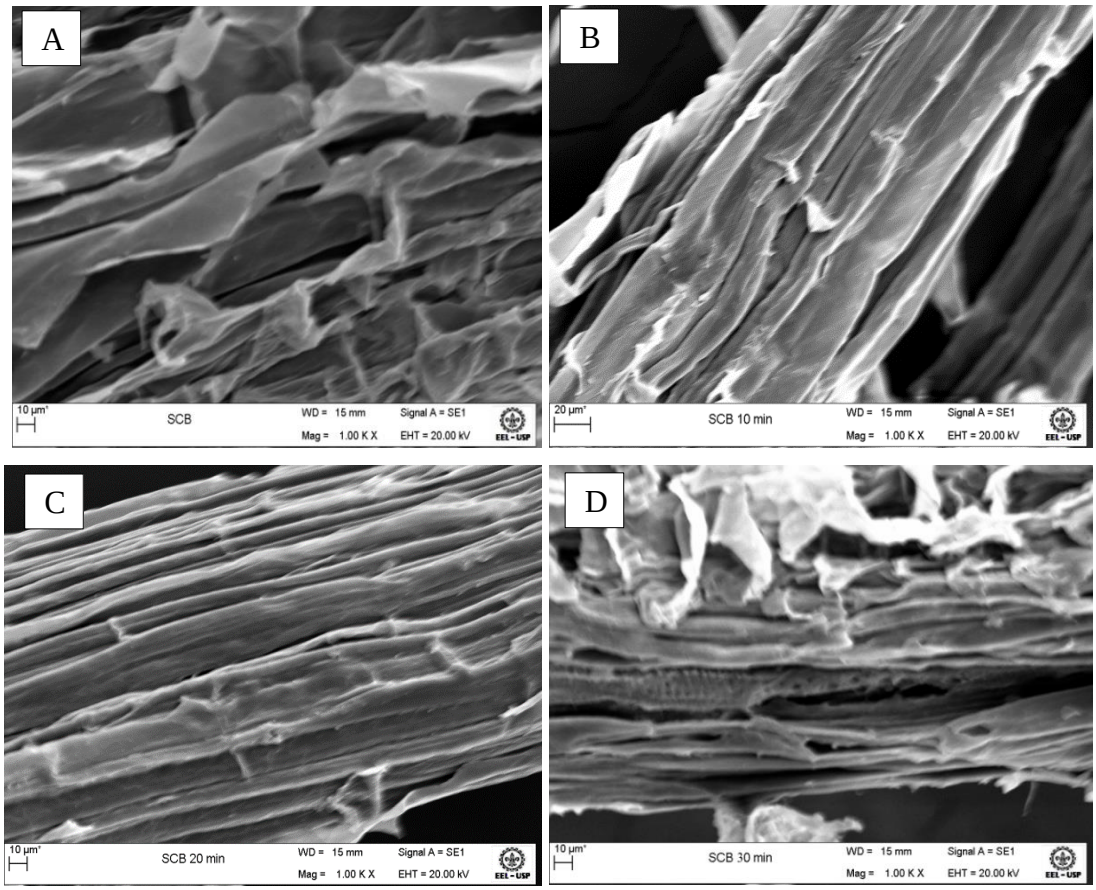
Used cavitation numbers were lower than the previous reports: e.g. 1.04 for cells disruption and lipid extraction (LEE; HAN, 2015), 0.38-0.89 for biogas production (HABASHI et al., 2016) and 0.095-0.21 for degradation of reactive orange-4 dye (GORE et al., 2014). Influence of inlet pressure, number and diameter of orifices on the cavitation number (Cv) were previously evaluated by Vichare, Gogate and Pandit (2000) that reported Cv increased to 0.59 when the pressure gradually dropped to 1.4 bar. They also observed the effective decomposition of iodine even with lower values of Cv (<0.1). These results were in agreement with the proposition that SCB pretreatment show high efficiency by using low cavitation number. Although a low Cv is associated with supercavitation phenomenon, our value was still higher than the critical cavitation number. In a previous report of Yan and Thorpe (1990), the critical cavitation number in a system using a single hole was 0.026 for 0.005 of β value (ratio of the hole areas on the orifice plate and the cross sectional area of the pipe). Our HC system had multiple holes and it presumably helped to decrease the critical value compared to the investigation of Yan and Thorpe (1990).

5.3.4 SEM and XRD analysis

The surface morphology of HC-treated SCB was also visualized by SEM images (Figure 5.4). As expected, pretreated SCB had a characteristic loosened structure due to the destructive effects of cavitation (i.e., high speed microjets and shockwaves). Similar physical changes were also noticed in wheat straw and SCB treated with the HC system reported by E-PIC s.r.l (2016) and Terán Hilares et al. (2016a), respectively.

Figure 5.4 – SEM analysis of SCB surface. A-raw SCB, B- 10 min of pretreatment, C- 20 min of pretreatment and D-30 min of pretreatment. Pretreatment conditions: 3 bar, 70 °C and 0.3 mol/L of NaOH. Orifice plate with 16 holes



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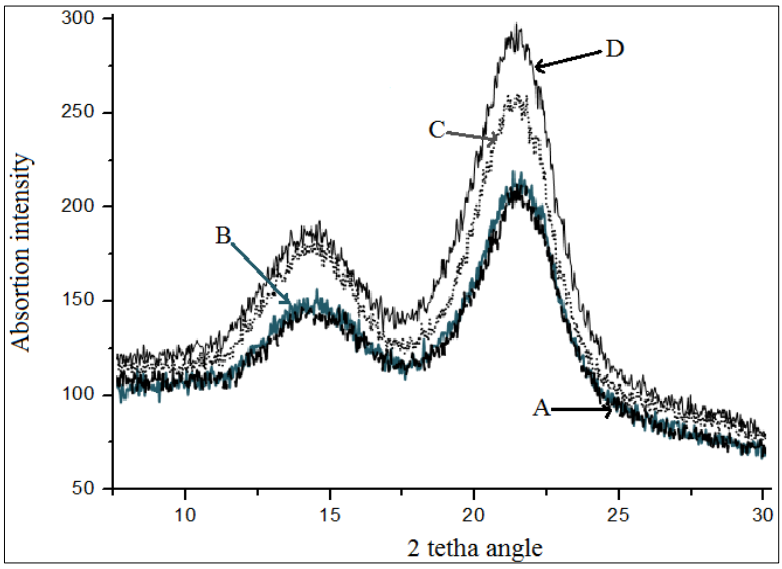
Crystallinity index (CrI) was also calculated for raw and HC-pretreated SCB using data from XRD analysis (Figure 5.5). This analysis, albeit imperfect, is used as an indicator of crystallinity, which is influenced by the solid composition, as lignin and hemicellulose fraction (amorphous compounds) and cellulose content

(crystalline fraction) (CHANDEL et al., 2014). This value increases as a result of lignin and hemicellulose removal (VELMURUGAN; MUTHUKUMAR, 2012). CrI was found to increase from 50.5% of raw material to 60.3% for HC-pretreated SCB during 10 min and to 65.7% for 20 min of pretreatment. No difference was observed in CrI values for 20 and 30 min of pretreatment. This observed behavior was probably due to no significant lignin and hemicellulose removal (Fig. 5.3) and also no remaining crystalline portion of cellulose after 20 min.

Figure 5.5 – XRD analysis of SCB. A-raw SCB, B- 10 min of pretreatment, C- 20 min of pretreatment and D-30 min of pretreatment. Pretreatment conditions: 3 bar, 70 °C and 0.3 mol/L of NaOH. Orifice plate with 16 holes



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5.4 Conclusion

Cavitation energy was used to improve the efficiency of SCB pretreatment under controlled conditions of temperature and pressure. It was found that as long as a treatment condition was right, a high yield of cellulose hydrolysis (>90%) was achievable; it could be so at mild conditions and at short running time. This result was accompanied by the drastic change of biomass structure created by the immensely destructive cavitation. HC appears to be indeed a surely promising yet less explored means for the pretreatment of lignocellulose.

5.5 References

AHMED, M. A. et al. Optimization of twin gear-based pretreatment of rice straw for bioethanol production. **Energy Conversion and Management,** v. 141, p.120-125, 2017.

BADVE, M. P. et al. Hydrodynamic cavitation as a novel approach for delignification of wheat straw for paper manufacturing. **Ultrasonics Sonochemistry,** v. 21, p.162-168, 2014.

BAHRANI, S.; RAEISSI, S.; SARSHAR, M. Experimental investigation of ionic liquid pretreatment of sugarcane bagasse with 1,3-dimethylimadazolium dimethyl phosphate. **Bioresource Technology,** v. 185, p.411-415, 2015.

BARIK, A. J.; GOGATE, P. R. Degradation of 4-chloro 2-aminophenol using a novel combined process based on hydrodynamic cavitation, UV photolysis and ozone. **Ultrasonics Sonochemistry,** v. 30, p.70-78, 2016.

BOX, G. E. P.; BEHNKEN, D. W. Some new three level designs for the study of quantitative variables. **Technometrics,** v. 2, p.455-462, 1960.

CAPOCELLI, M. et al. Hydrodynamic cavitation of p-nitrophenol: A theoretical and experimental insight. **Chemical Engineering Journal,** v. 254, p.1-8, 2014.

CHAKINALA, A. G. et al. Industrial wastewater treatment using hydrodynamic cavitation and heterogeneous advanced fenton processing. **Chemical Engineering Journal,** v. 152, n. 2-3, p.498-502, 2009.

CHANDEL, A. K et al. Multi-scale structural and chemical analysis of sugarcane bagasse in the process of sequential acid–base pretreatment and ethanol production by Scheffersomyces shehatae and Saccharomyces cerevisiae. **Biotechnology for Biofuels,** v. 7, p.63-70, 2014.

E-PIC S.r.l. Biomass pretreatment (BIOGAS), 2016. Available in: http://www.epic-srl.com/en/cavitation-technologies/biomass-pretreatment-biogas. Accessed in: 8 Oct. 2017.

GOGATE, P. R. Cavitational reactors for process intensification of chemical processing applications: A critical review. **Chemical Engineering and Processing: Process Intensification,** v. 47, p.515-527, 2008.

GORE, M. M. et al. Degradation of reactive orange 4 dye using hydrodynamic cavitation based hybrid techniques. **Ultrasonics Sonochemistry,** v. 21, n. 3, p.1075-1082, 2014.

HABASHI, N. et al. Hydrodynamic cavitation as a novel approach for pretreatment of oily wastewater for anaerobic co-digestion with waste activated sludge. **Ultrasonics Sonochemistry,** v. 31, p.362-370, 2016.

KIM, I. et al. Hydrodynamic cavitation as a novel pretreatment approach for bioethanol production from reed. **Bioresource Technology,** v. 192, p.335-339, 2015.

LEE, I.; HAN, J-I. Simultaneous treatment (cell disruption and lipid extraction) of wet microalgae using hydrodynamic cavitation for enhancing the lipid yield. **Bioresource Technology,** v. 186, p.246-251, 2015.

MOHOLKAR, V. S.; PANDIT, A. B. Modeling of hydrodynamic cavitation reactors: a unified approach. **Chemical Engineering Science,** v. 56, p.6295-6302, 2001.

NAKASHIMA, K. et al. Hydrodynamic Cavitation Reactor for Efficient Pretreatment of Lignocellulosic Biomass. **Industrial & Engineering Chemistry Research,** v. 55, n. 7, p.1866-1871, 2016.

OZONEK, J. **Application of Hydrodynamic Cavitation in Environmental Engineering**. New York, CRC Press, 2012.

PATIL, P. N.; BOTE, S. D.; GOGATE, P. R. Degradation of imidacloprid using combined advanced oxidation processes based on hydrodynamic cavitation. **Ultrasonics Sonochemistry,** v. 21, p.1770-1777, 2014.

PATIL, P. N.; GOGATE, P. R. Degradation of methyl parathion using hydrodynamic cavitation: Effect of operating parameters and intensification using additives. **Separation and Purification Technology,** v. 95, p.172-179, 2012.

RAJORIYA, S.; BARGOLE, S.; SAHARAN, V. K. Degradation of a cationic dye (Rhodamine 6G) using hydrodynamic cavitation coupled with other oxidative agents: Reaction mechanism and pathway. **Ultrasonics Sonochemistry,** v. 34, p.183-194, 2017.

RAMADOSS, G.; MUTHUKUMAR, K. Influence of dual salt on the pretreatment of sugarcane bagasse with hydrogen peroxide for bioethanol production. **Chemical Engineering Journal,** v. 260, p.178-187, 2015.

RAUT-JADHAV, S. et al. Intensification of degradation of methomyl (carbamate group pesticide) by using the combination of ultrasonic cavitation and process intensifying additives. **Ultrasonics Sonochemistry,** v. 31, p.135-142, 2016a.

RAUT-JADHAV, S. et al. Effect of process intensifying parameters on the hydrodynamic cavitation based degradation of commercial pesticide (methomyl) in the aqueous solution. **Ultrasonics Sonochemistry,** v. 28, p.283-293, 2016b.

SEGAL, L. et al. An empirical method for estimating the degree of crystallinity of native cellulose using the X-ray diffractometer. **Textile Research Journal,** v. 29, p.786-794, 1959.

SINDHU, R.; PANDEY, A.; BINOD, P. Alkaline Treatment. In: Pandey, A.; Negi, S.; Binod, P.; Larroche, C. (Eds). **Pretreatment of Biomass: processes and technologies**. Massachusetts, Elsevier Inc. p. 51–60. 2015.

SINGLETON, V. L.; ROSSI, J. A. Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. **American Journal Enology and Viticulture**, v. 16, p. 144-158, 1965.

SLUITER, A. et al. Determination of Structural Carbohydrates and Lignin in Biomass. National Renewable Energy Laboratory, 2012. Available in: http://www.nrel.gov/docs/gen/fy13/42618.pdf. Accessed in: 3 Nov. 20, 2016.

TERÁN HILARES, R. et al. Hydrodynamic cavitation-assisted alkaline pretreatment as a new approach for sugarcane bagasse biorefineries. **Bioresource Technology,** v. 214, p.609-614, 2016a.

TERÁN-HILARES, R. et al. Successive pretreatment and enzymatic saccharification of sugarcane bagasse in a packed bed flow-through column reactor aiming to support biorefineries. **Bioresource Technology,** v. 203, p.42-49, 2016b.

VELMURUGAN, R.; MUTHUKUMAR, K. Ultrasound-assisted alkaline pretreatment of sugarcane bagasse for fermentable sugar production: Optimization through response surface methodology. **Bioresource Technology,** v. 112, p.293-299, 2012.

VICHARE, N. P.; GOGATE, P. R.; PANDIT, A. B. Optimization of Hydrodynamic Cavitation Using a Model Reaction. **Chemical Engineering & Technology,** v. 23, n. 8, p.683-690, 2000.

WANG, W. et al. High conversion of sugarcane bagasse into monosaccharides based on sodium hydroxide pretreatment at low water consumption and wastewater generation. **Bioresource Technology,** v. 218, p.1230-1236, 2016.

WIKANDARI, R. et al. Effect of furfural, hydroxymethylfurfural and acetic acid on indigeneous microbial isolate for bioethanol production. **Agricultural Journal,** v. 5, p.105-109, 2010.

YAN, Y.; THORPE, R. B. Flow regime transitions due to cavitation in the flow through an orifice. **International Journal of Multiphase Flow,** v. 16, p.1023-1045, 1990.

ZHU, Z. et al. Efficient sugar production from sugarcane bagasse by microwave assisted acid and alkali pretreatment. **Biomass and Bioenergy,** v. 93, p.269-278, 2016

CHAPTER VI

Ethanol production in a simultaneous saccharification and fermentation process with interconnected reactors employing hydrodynamic cavitation-pretreated sugarcane bagasse as raw material

*Chapter published previously: TERÁN HILARES, R. et al. Ethanol production in a simultaneous saccharification and fermentation process with interconnected reactors employing hydrodynamic cavitation-pretreated sugarcane bagasse as raw material. **Bioresource Technology**, v. 243, p. 652-659, 2017.

Abstract

In this study, sugarcane bagasse (SCB) pretreated with alkali assisted hydrodynamic cavitation (HC) was investigated for simultaneous saccharification and fermentation (SSF) process for bioethanol production in interconnected column reactors using immobilized *Scheffersomyces stipitis* NRRL-Y7124. Initially, HC was employed for the evaluation of the reagent used in alkaline pretreatment. Alkalis (NaOH, KOH, Na_2CO_3, $Ca(OH)_2$) and NaOH recycled black liquor (successive batches) were used and their pretreatment effectiveness was assessed considering the solid composition and its enzymatic digestibility. In SSF process using NaOH-HC pretreatment SCB, 62.33% of total carbohydrate fractions were hydrolyzed and 17.26 g/L of ethanol production (0.48 g of ethanol/g of glucose and xylose consumed) was achieved. This proposed scheme of HC-assisted NaOH pretreatment together with our interconnected column reactors showed to be an interesting new approach for biorefineries.

Keywords:

Hydrodynamic cavitation; Alkaline pretreatment; Sugarcane bagasse; Interconnected column reactors; SSF process

6.1 Introduction

Global climate change and energy sustainability are concerns that have received great attention around the world. Within this current context, renewable sources of energy and chemicals have been extensively explored since last decades, in order to reduce the petroleum global dependence.

Lignocelluloses, the most abundant sustainable global feedstock, are precursors for the production of a number of different bio-based compounds, such as ethanol, xylitol, adhesives, bio-polymers, bioplastics, organic acids and others (SANTOS et al., 2005; GHATAK, 2011; TERÁN HILARES et al., 2017a). However, a typical prerequisite to overcome the inherited biomass recalcitrance is a pretreatment step. Among recently reported new approaches, hydrodynamic cavitation (HC) has emerged as promising as previously reported for reed (KIM et al., 2015), corn stover (NAKASHIMA et al., 2016) and sugarcane bagasse (TERÁN HILARES et al., 2016a;2017b; MADISON et al., 2017).

In its typical mechanism, hydrodynamic cavitation corresponds to the generation of cavities (micro-bubbles), growth and ultimately their collapse at the expanse of pressure drop induced by a constriction of either orifice plate or venture tube (GOGATE; PANDIT, 2005; GOGATE; BHOSALE, 2013). These cavities upon their collapse create a localized temperature of ~5000 K and a pressure of ~500 atm to further produce strong oxidative radicals ($OH^{\bullet}$, $O_2^{\bullet}$) reinforcing thus the chemical effect of cavitation to disrupt the carbohydrate-lignin matrix (BADVE et al., 2014; TERÁN HILARES et al., 2016a). Moreover, the induced mechanical effects generated by high speed microjets and shockwaves also contribute to the structural disintegration as well an increase in the porosity of the native biomass, as reported in a technical report of the company E-PIC s.r.l. (2016), making it more susceptible to enzymatic hydrolysis (NAKASHIMA et al., 2016; TERÁN HILARES et al., 2017b).

Usually, after lignocellulosic pretreatment, hydrolysis and fermentation are the subsequent steps for biofuel and biomaterials production. For bioethanol production, simultaneous saccharification and fermentation (SSF) offer several advantages as compared to the traditional separate hydrolysis and fermentation (SHF) process; e.g. less enzymatic inhibition by hydrolysis products, no sugar loss, and economical process intensification (DAHNUM et al., 2015; SHADBAHR; KHAN; ZHANG, 2017). However, the SSF process is usually carried out in only one vessel, resulting

in the use of an un-optimized temperature for enzymatic hydrolysis and fermentation process. Moreover, reuse of microorganisms in an SSF process is complicated due to its separation from the residual biomass (OLOFSSON; BERTILSSON; LIDÉN, 2008). Recently, some alternatives for SSF have been evaluated particularly incorporating the use of separate vessels interconnected with membranes to help retain the cells in the fermentation section (VIOLA et al., 2013; ISHOLA et al., 2013). But there is another possible route by using immobilized cells in the fermentation section, confining the microorganism in a reactor and ultimately allowing to use another vessel with adequate conditions to enzymatic hydrolysis (configuration not previously reported). This immobilization offers many advantages such as the possibility of easy recovery and reuse of cells in the process. In this regard, the encapsulation in calcium alginate has been widely reported strategy for different substrates, e.g., for ethanol (DUARTE et al., 2013; ANTUNES et al., 2015) and xylitol production (CARVALHO et al., 2003).

For efficient ethanol production in SSF process containing sugars (pentoses and hexoses), selection of an adequate microorganism for conversion of both sugars into ethanol is necessary. In this way, e.g. microorganisms as *Candida shehatae* and *Scheffersomyces stipitis* were reported as promising yeasts for co-fermentation of lignocellulosic hydrolysates into ethanol (CHANDEL et al., 2007; TANIMURA et al., 2012; TERÁN-HILARES et al., 2016b; YUVADETKUN; LEKSAWASDI; BOONMEE, 2017).

Another fundamental aspect that could favor the economic viability of biorefineries is the operation at high solids contents (higher than normal loading of 10%). Such high solids loading are perceived to yield high sugar and ethanol titers along with a reduced distillation cost ultimately enhancing the economics of the overall process. However, such high solids loading suffer from mass transfer limitations and offer difficulties for homogenization during the process (SURIYACHAI et al., 2013).

These typical constraints of high solid slurries can be overcome using special configurations of reactors; e.g. Terán-Hilares et al. (2016b) reported the use of packed bed column reactor for enzymatic hydrolysis process using high solid loading (17.1%) of alkaline pretreated sugarcane bagasse, achieving high concentration of sugars. In these specially designed packed bed column reactors, the constant recirculation of liquid through the reactor produce a homogenous distribution of

enzymes in the system, facilitating their access to catalytic site (BORREGA; SIXTA, 2015).

Therefore, in this work we studied the feasibility of bioethanol production by SSF process with immobilized cells using *Scheffersomyces stipitis* NRRL-Y7124 employing high solids loading in designed interconnected reactors system. Sugarcane bagasse was chosen as a model feedstock and the most efficient pretreatment catalyst was selected through the evaluation of different alkalis (NaOH, KOH, Na_2CO_3, $Ca(OH)_2$) and recycled black liquor using hydrodynamic cavitation (HC).

6.2 Materials and methods

6.2.1 Sugarcane bagasse

Sugarcane bagasse (SCB) was kindly donated by Usina Vale Onda Verde (Onda Verde-SP, Brazil). It was dried and milled using a Benedetti 270 hammer mill and finally sieved to an average particle size of 4.7 mm prior to assays.

6.2.2 HC-system to assist different alkaline pretreatment processes

A 3 L of total volume HC system was used in the experimental process (TERÁN HILARES et al., 2017b). The system was composed by a centrifugal pump (1.5 CV), cavitation zone and recirculation tank. The flow rate of the alkaline solution was 4 m^3/h with a fluid velocity through the orifice plate of 88 m/s and cavitation number of 0.017. Fluid temperature was controlled with the help of water jackets maintaining a constant recirculation of water.

Alkaline solution was constantly recirculated in the system at 60 °C for 20 min. Raw SCB was kept inside a cylindrical wire cloth (18 mesh) and placed in the cavitation zone (total volume of 1000 ml). Cavitation was generated by pressure drop from 3 bar to 0.3 bar in the flowing fluid through orifice plate (16 holes with 1 mm of diameters). The alkalis sodium carbonate Na_2CO_3 (0.5, 1.0 and 1.5 mol/L), calcium hydroxide $Ca(OH)_2$ (0.5 mol/L), potassium hydroxide KOH (0.1, 0.3 and 0.5 mol/L) and sodium hydroxide NaOH (0.3 mol/L) were chosen based on previous studies of Salehi et al. (2012), Khor, Rabaey and Vervaeren (2015), Sharma et al. (2013) and Terán Hilares et al. (2016a), respectively. Additionally, experiments in

Erlenmeyer flasks using each alkali separately (60 °C, 20 min and 5% of S/L ratio) were performed in order to compare with the cavitation effect on the solid composition and enzymatic digestibility. After pretreatment, all the samples were washed, dried and finally characterized with relation to quantify their main constituents (cellulose, hemicellulose and lignin).

The lignin removal in the process was calculated considering the lignin content before and after pretreatment process and the solid recovery values; similar calculation was also carried out for cellulose and for hemicellulose removal. Also, samples were enzymatically hydrolyzed according to methodology described in the section 6.2.4.

6.2.3 Reuse of black liquor for pretreatment in sequential HC-batches

The black liquor obtained in the first batch of NaOH/HC-pretreatment was further reused in additional batches in a sequential HC pretreatment process using fresh biomass. In these experiments, black liquor from the previous batch was used for pretreatment of the following one. The solids composition and enzymatic digestibility of this sequentially treated biomass was further assessed to observe the pretreatment efficacy. Tukey's range test was performed for comparison in the solid composition and hydrolysis yield obtained in the different batches, aided by the software STATISTICA 8.0 (StatSoft, Inc., Tulsa, OK, USA).

6.2.4 Enzymatic hydrolysis of pretreated SCB

Enzymatic hydrolysis was carried out in 125 mL Erlenmeyer flasks at 5% solids loading in 50 mM sodium citrate solution (pH 4.8). A commercial cellulases enzyme blend Cellic® CTec2, kindly donated by Novozymes Latin America Ltda. (Araucária-PR, Brazil) was used at 20 FPU/g of dry pretreated SCB. Samples were incubated at 50 °C and 150 rpm in an Innova® 44/44R rotary shaker (New Brunswick Scientific Co., Inc., USA) for 24 h. The sugar contents were analyzed by High Performance Liquid Chromatography (HPLC) as described in section 6.2.6. Enzymatic digestibility was calculated for yield of cellulose and hemicellulose hydrolysis (%) on the basis of initial glucan and ‘‘xylan + arabinan" contents, respectively.

6.2.5 Simultaneous saccharification and fermentation (SSF) process

6.2.5.1 Microorganism and encapsulation

Scheffersomyces stipitis NRRL-Y7124 was obtained from stock cultures available in the Applied Microbiology and Bioprocess Laboratory (GMBio) at the Engineering School of Lorena – University of Sao Paulo, Brazil.

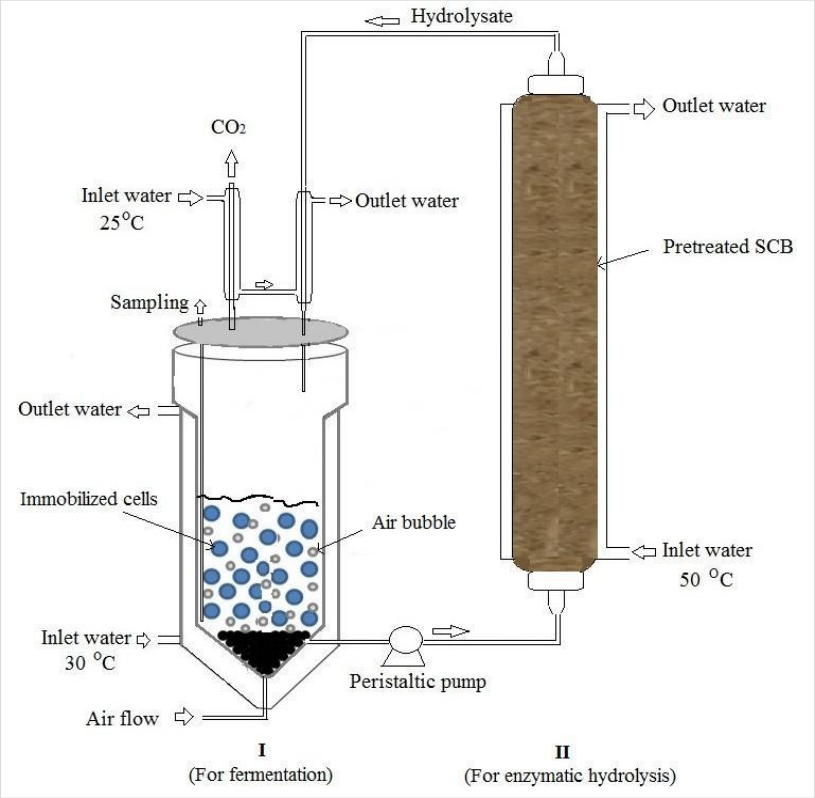
Inoculum medium was composed of: glucose 20 g/L, xylose 10 g/L, peptone 20 g/L and yeast extract 10 g/L. Inoculum was growth in 125 mL Erlenmeyer flasks containing 50 mL of medium in an Innova® 44/44R rotary shaker (New Brunswick Scientific Co., Inc., USA) at 30 °C, 200 rpm and 48 h. The cells were centrifuged (3000 x g, 10 min) and washed using distilled water, and the recovered yeast were mixed with sodium alginate solution (1%) previously sterilized in order to obtain 5 g/L of cells. Immobilization was carried out according to the methodology described by Antunes et al. (2016), by dripping the solution containing cells into sterilized calcium chloride solution (0.2 mol/L) using a peristaltic pump ALITEA-XV (Bioengineering AG –Wald, Switzerland). The immobilized cells were used in SSF process.

6.2.5.2 Ethanol production in SSF process

Figure 6.1 shows a representative scheme of SSF system adapted in this study. Initially, 1000 mL of medium was prepared in column reactor I (bubble column reactor used for fermentation, 80 x 6 cm) and it was composed of 3 g/L of yeast extract, 5 g/L of peptone, 0.1 g/L of calcium chloride and 2 g/L of ammonium sulphate, diluted in sodium citrate buffer (50 mM, pH of 4.8). The spheres of alginate containing cells (average diameter of spheres of 0.29 mm) and a commercial enzyme blend Cellic® CTec2 (Novozymes Latin America Ltda., Araucária-PR, Brazil) were added in the reactor I with an enzyme loading of 20 FPU/g of the dry biomass contained in the reactor II (90 x 5 cm), which was filled with 110 g of dry SCB. The solution was recirculated through the reactors aided by a peristaltic pump ALITEA-XV (Bioengineering AG-Wald, Switzerland) in a flowrate of 26 mL/min. Temperature was kept at 30 °C and 50 °C in reactor I and II, respectively. The reactor I was continuously supplied by air at flowrate of 0.26 vvm. SSF process was

carried out for 32 h and then all the liquid was transferred to the reactor I for fermentation. Samples were periodically taken to monitor the sugars consumption and ethanol production.

Figure 6.1 – Representative scheme of the system used for ethanol production in SSF configuration in interdependent reactors. Fermentation vessel (I) and hydrolysis vessel (II).



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6.2.6 Analytical methods

Biomass composition was characterized according to methodology described by Sluiter et al. (2012). The sugars (glucose, xylose, arabinose and cellobiose) and ethanol concentration were analyzed by High Performance Liquid Chromatography (HPLC) Agilent 1200 series (Agilent Technologies, Inc., USA) equipped with a Refractive index detector RID-6A and a HPX-87H (300x7.8mm) column (Bio-Rad, USA). The analysis conditions were following: 45°C column temperature, 0.01 N H_2SO_4 as the mobile phase, 0.6 mL/min flow rate, and 20 µL injection volume.

6.3 Results and discussion

6.3.5 HC-assisted pretreatment using different alkalis

6.3.5.1 Effects of sodium carbonate on pretreatment and enzymatic hydrolysis

The raw SCB characterization resulted in the following composition: 40.15% of cellulose, 25.52% of hemicellulose, 26.17% of lignin and 8.16 % of others compounds as ash and extractives, all on dry basis. Based on these results, carbohydrates and lignin removal could be calculated in the pretreated material.

The effects of different alkalis in HC-assisted process, particularly in terms of solid recovery and solid composition, are shown in Table 6.1. Specifically, for Na_2CO_3 the solid recovery after pretreatment ranged from 87.08 to 92.58%; but no significant difference was observed in solid composition, according to Tukey's range test (95% of confidence level), for the pretreated material obtained using different sodium carbonate concentrations.

A further assessment of this alkali was performed by doing the enzymatic digestibility test. For the sake of comparison, samples of raw biomass and without HC treatment (control) were also performed. As delineated in Fig. 6.2A, using Na_2CO_3 (1 mol/L) with 20 min of HC process, 43.84% and 22.83% of cellulose and hemicellulose hydrolysis yields were achieved. These values are considerably higher than raw SCB (12.72% and 8.82%) and control (20.69% and 8.21%, respectively). This result can be attributed to an increase in the porosity by mechanical effect of cavitation favoring the diffusion of enzymes into substrate (NAKASHIMA et al., 2016; TERÁN HILARES et al., 2017b).

As compared with other studies, the achieved enzymatic digestibility is low, as in one of our previous work, 93.05% of cellulose and 94.45% of hemicellulose were hydrolyzed with SCB at conditions of: 3 bar of inlet pressure, 70 °C, 30 min and 0.3 mol/L of NaOH (TERÁN HILARES et al., 2017b).

Table 6.1 – Solid composition of pretreated SCB using different alkalis in process assisted by HC (3 bar of inlet pressure, 60 °C, 20 min)

Alkaline	Alkali concentration (mol/L)	Solid recovery* %	Solid composition** (%)			Removal of compounds** (%)	
			Cellulose	Hemicellulose	Lignin	Hemicellulose	Lignin
Na_2CO_3	0.5	92.58	39.71 ± 1.65^{a}	28.31 ± 4.40^{a}	26.45 ± 3.54^{a}	13.60 ± 2.75^{a}	17.88 ± 0.62^{a}
	1.0	89.63	40.76 ± 1.44^{a}	26.75 ± 1.17^{a}	24.72 ± 0.08^{a}	11.19 ± 3.88^{a}	17.94 ± 0.25^{a}
	1.5	87.08	42.30 ± 0.13^{a}	$27.75 \pm 0.01_{a}$	23.85 ± 0.30^{a}	10.50 ± 0.32^{a}	23.07 ± 0.97^{a}
KOH	0.1	88.24	44.20 ± 0.10^{b}	29.02 ± 0.49^{b}	22.42 ± 0.72^{b}	5.16 ± 1.59^{b}	26.72 ± 2.37^{b}
	0.3	78.49	46.19 ± 0.04^{c}	29.92 ± 0.03^{b}	19.93 ± 0.39^{c}	13.02 ± 0.08^{c}	42.07 ± 1.15^{c}
	0.5	76.44	47.69 ± 0.18^{d}	28.32 ± 0.31^{b}	18.26 ± 0.58^{d}	19.81 ± 0.88^{d}	48.31 ± 1.64^{d}
NaOH	0.3	78.84	44.34 ± 0.01	26.72 ± 0.03	18.20 ± 0.01	21.98 ± 0.32	45.57 ± 1.25
$Ca(OH)_2$	0.5	92.56	41.27 ± 0.61	27.44 ± 0.70	25.02 ± 1.99	5.92 ± 2.39	14.24 ± 6.83

* Ratio between solid mass after and before pretreatment procedure; ** Results corresponding to average of triplicates ± standard deviation. Same letter in column indicates no difference according to Tukey's range test (95% of confidence level), that was carried out separately for each alkali.

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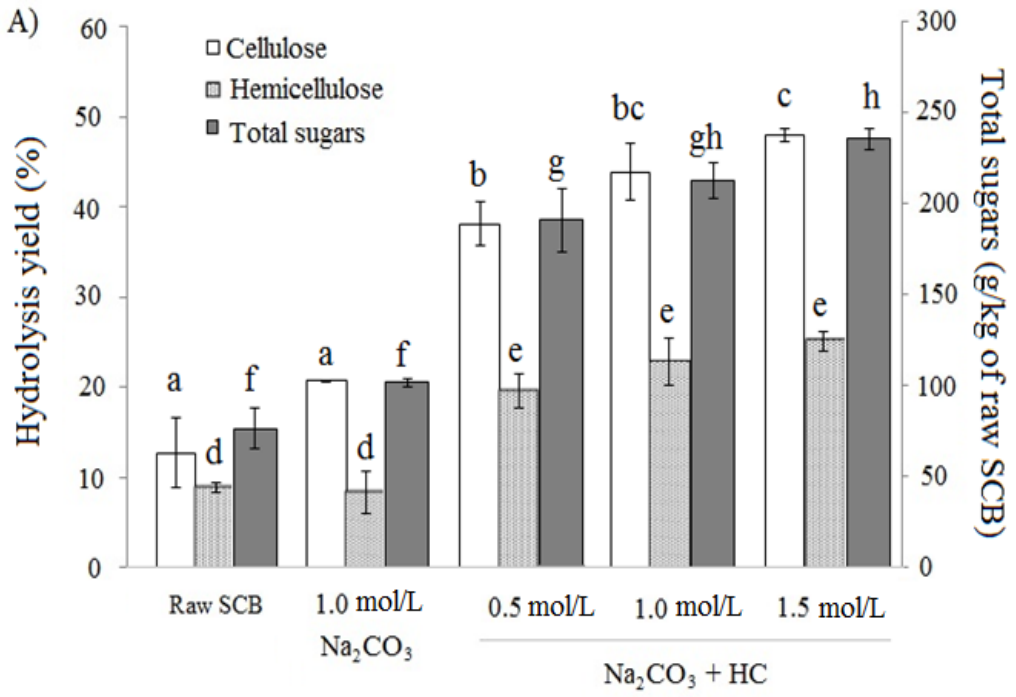
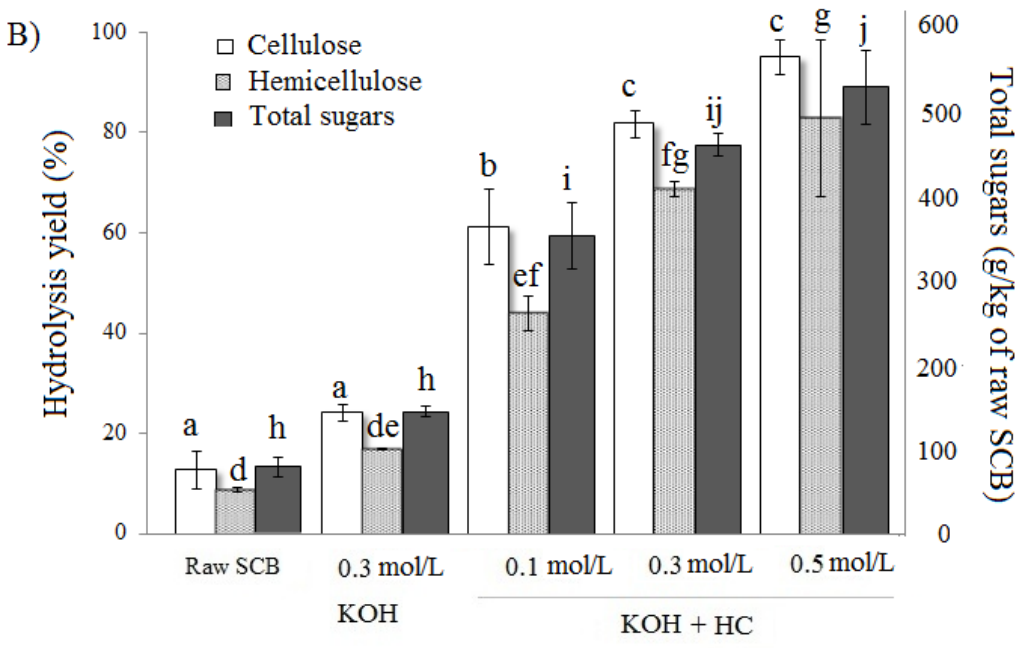
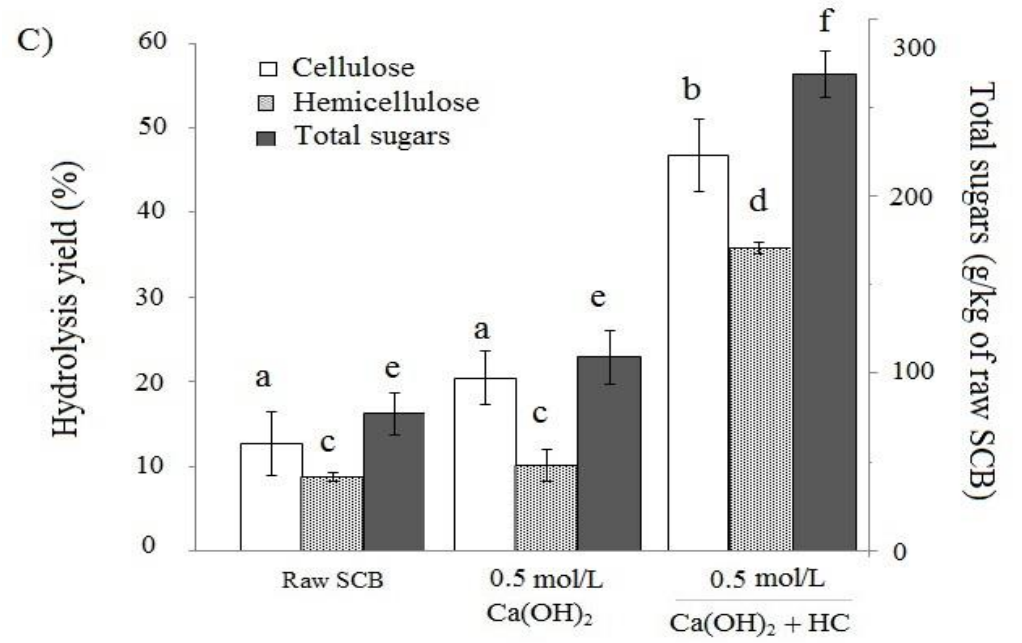
Sodium carbonate has been used for lignocellulosic biomass pretreatment by other authors. For example, pretreatment of *Miscanthus* using Na_2CO_3 was reported by Mirmohamadsadeghi, Chen and Wan (2016). In their study, the enzymatic digestibility was threefold the obtained with raw biomass (21%) with pretreatment conditions of: 0.5 mol/L of Na_2CO_3, 80°C and 3 h. In another study, sodium carbonate was used for rice straw pretreatment by Yang et al. (2012). In that study, the biomass was soaked in Na_2CO_3 (20% on dry biomass) for 30 min at 80°C and then heated to 150 °C, resulting in 25.7% and 11.9% of enzymatic digestibility of glucan and xylan, respectively. Therefore, considering the milder conditions (low temperature and short process time) used for HC-assisted process, a significant increase in the efficiency of pretreatment was observed compared to previous reports that requires harsher conditions.

Figure 6.2 – Comparative of cellulose and hemicellulose digestibility in 24 h of hydrolysis time of SCB pretreated with Na_2CO_3 (A), KOH (B) and $Ca(OH)_2$ (C) in process assisted by HC. Bars correspond to standard deviation (SD) calculated for triplicates. Same letters above error bars indicate homogeneous subsets according to Tukey's range test at 95% of confidence level.







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6.3.5.2 Effects of potassium hydroxide on pretreatment and enzymatic hydrolysis

For the evaluation of potassium hydroxide in HC-assisted pretreatment, similar conditions of temperature and inlet pressure were maintained but with different KOH concentrations, as shown in Table 6.1. In terms of solids composition, it exhibited comparatively better pretreatment performance even with 0.5 mol/L concentration

(lower than Na_2CO_3 = 1.5 mol/L), heightening glucan contents up to 47.69% with a corresponding lignin removal of 48.31%. For the enzymatic hydrolysis, as expected, the treated SCB using 0.3 mol/L of NaOH-HC showed quite distinct yield (82%) as compared with raw and control (without HC) as shown in Fig. 6.2B. In terms of total sugars production, a significant amount of 525.58 and 473.06 g/kg of biomass were produced within 24 h of hydrolysis time when SCB pretreated using 0.5 and 0.3 mol/L of NaOH were used, respectively. This value is higher than both the raw and control samples, but lower than 559.24 g/kg of raw SCB obtained with NaOH-HC assisted process when a similar concentration of 0.3 mol/L was employed during pretreatment but slightly higher temperature of 70 °C, as reported by Terán Hilares et al. (2017b). Actually, this HC-assisted process is promising even to work effectively at mild temperature (60 °C) and with short process time (20 min).

6.3.5.3 Effects of calcium hydroxide on pretreatment and enzymatic hydrolysis

Regarding calcium hydroxide, its effects on both the solid composition and subsequent enzymatic hydrolysis are shown in Table 6.1 and Figure 6.2C, respectively.

Under mild HC-assisted pretreatment conditions (3 bar of inlet pressure, 0.5 mol/L of $Ca(OH)_2$, 60 °C and 20 min), only small differences were observed in solid composition (percentage of cellulose, hemicellulose and lignin) for SCB pretreated in HC-process as compared with raw and control (without HC) SCB. For the enzymatic hydrolysis, a lower hydrolysis yield of cellulosic fraction (46.75%) was observed, although higher than the cellulose hydrolysis yield in control test (20.49%) and in the raw biomass (12.72%).

These results suggest that digestibility of biomass is not solely dependent on changes in the composition of pretreated biomass, but has a strong correlation with structural changes. Therefore, an increase in digestibility of biomass could be attributed to increase the porosity of the material produced by mechanical effect of cavitation (E-PIC S.R.L, 2016; NAKASHIMA et al., 2016; TERÁN HILARES et al., 2017b).

On the other hand, for efficient lignocellulosic pretreatment, specifically using $Ca(OH)_2$, more severe process conditions and long retention times are required. For example, pretreatment of SCB using lime was reported by Fuentes et al. (2011). In

that study, 228.45 g/kg of raw SCB and 409.9 g of total reducing sugars/kg of raw SCB biomass were obtained in 72 h of enzymatic hydrolysis using pretreated SCB (90 °C, 90 h and 0.4 g of alkali/g of dry biomass). In our study, the maximal total reducing sugar achieved was 269.30 g/kg of raw SCB, a value lower than reported by Fuentes et al. (2011). The difference can be attributed to short process time and low temperature used in HC process.

6.3.5.4 Sodium hydroxide-HC assisted pretreatment

NaOH-HC assisted pretreatment was performed at 3 bar of inlet pressure, 0.3 mol/L of conc., 60 °C of temperature and 20 min of time. Table 6.2 and Fig. 6.3 show the effects of this pretreatment in terms of solids composition and enzymatic hydrolysis, respectively. As shown, this pretreatment led to an increase of cellulose contents from 40 to 43.86% with a corresponding lignin removal of 43.63%. These pretreatment conditions yielded a total sugar of 487.72 g/kg of biomass after an enzymatic hydrolysis for 24 h. These values corresponded to 82.69% of cellulose hydrolysis and 74.29% of hemicellulose hydrolysis yield. This performance of sodium hydroxide-HC assisted pretreatment was a bit lower than the one obtained in our previous work (TERÁN HILARES et al., 2017b), that was 559 g/kg of raw biomass in SCB pretreated with NaOH-HC process (3 bar of inlet pressure, 0.3 mol/L, 70 °C and 20 min). The difference in the total sugar contents can be attributed to lower temperature now used (60 °C) compared with previously reported work. Indeed, this lower temperature was effective to mitigate the foaming phenomena (that was higher at 70 °C) observed during preliminary trials with reuse of liquor (data not shown).

Although sodium hydroxide is more expensive alkali ($300-480/metric ton) than calcium hydroxide ($90-150/metric ton) and sodium carbonate ($200-240/metric ton), this alkali indeed showed a better performance in pretreatment of biomass with cost effective sugar production due to high cellulose conversion, as reported by Rodrigues, Jackson e Montross (2016). NaOH in combination with HC process also present high pretreatment efficiency under milder condition and short process time (KIM et al., 2015; TERÁN HILARES et al., 2016a). Besides, it is cheaper when compared to potassium hydroxide ($700-1000/metric ton), the other alkali that showed similar performance compared to NaOH.

Table 6.2 – NaOH-HC pretreatment reusing black liquor on the solid composition, removal of compounds and total sugar released during enzymatic hydrolysis

HC process	Solid recovery (%)	Solid composition (%)			Removal of compounds (%)*		Total sugar released after enzymatic hydrolysis** (g/kg de SCB)***
		Cellulose	Hemicellulose	Lignin	Hemicellulose	Lignin	
Fresh solution	78.84	44.34 ± 0.01[a]	26.72 ± 0.03[a]	18.20 ± 0.01[a]	21.98 ± 0.32[a]	45.57 ± 1.25[a]	487.72 ± 16.68[a]
1st reuse	78.84	44.99 ± 0.83[a]	25.55 ± 1.65[a]	18.55 ± 0.41[a]	21.10 ± 3.69[a]	43.83 ± 2.33[a]	458.65 ± 6.87[a]
2nd reuse	80.24	44.81 ± 1.25[a]	24.40 ± 0.94[a]	20.42 ± 0.06[a]	23.27 ± 3.63[a]	37.22 ± 0.50[a]	488.39 ± 23.32[a]
3th reuse	78.68	44.04 ± 2.09[a]	22.58 ± 2.06[a]	19.75 ± 0.34[a]	30.53 ± 3.22[a]	40.99 ± 1.63[a]	462.23 ± 39.55[a]
4th reuse	82.52	43.78 ± 1.90[a]	22.49 ± 2.98[a]	17.16 ± 4.61[a]	27.27 ± 9.64[a]	45.46 ± 14.69[a]	471.95 ± 2.23[a]
5th reuse	80.05	42.44 ± 0.03[a]	23.45 ± 0.46[a]	22.40 ± 0.89[a]	26.43 ± 1.44[a]	31.15 ± 2.73[a]	504.03 ± 24.63[a]
6th reuse	80.51	43.51 ± 1.50[a]	24.07 ± 1.15[a]	21.51 ± 1.51[a]	24.07 ± 3.62[a]	33.48 ± 4.66[a]	462.90 ± 13.64[a]
7th reuse	81.06	43.79 ± 0.43[a]	22.02 ± 2.60[a]	21.67 ± 0.55[a]	30.06 ± 8.26[a]	32.69 ± 1.97[a]	475.92 ± 1.15[a]
8th reuse	81.34	41.70 ± 0.86[a]	22.37 ± 1.46[a]	21.74 ± 0.60[a]	28.70 ± 4.66[a]	32.25 ± 2.10[a]	474.87 ± 51.62[a]
9th reuse	82.82	41.45 ± 0.12[a]	25.79 ± 0.01[a]	18.30 ± 2.61[a]	16.29 ± 3.60[a]	42.11 ± 5.55[a]	447.33 ± 7.18[a]

Results correspond to average of triplicates ± standard deviation. Same letter in column indicates no difference according to Tukey's range test (95% of confidence level). *In all cases the cellulose fraction removal was lower than 10%. **Total sugars correspond to "glucose + xylose + cellobiose + arabinose" released in 24 h of enzymatic hydrolysis of SCB. *** g of sugars per kg of raw SCB. Source:

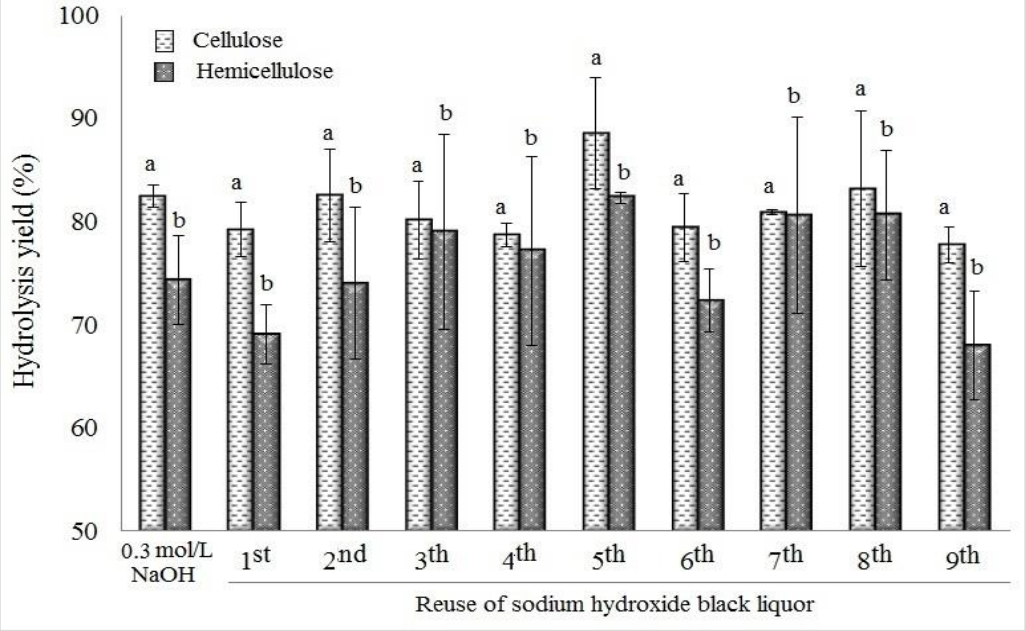
Source: Personal archive

Thus, sodium hydroxide was chosen to be used in next experiments and, in order to reduce the alkali consumption, the black liquor obtained after first HC pretreatment was reused in 9 additional batches, and results are discussed in the following section.

6.3.6 Reuse of black liquor in NaOH-HC assisted pretreatment in repeated batches

As shown in Figure 6.3, about 80% and 70% of cellulose and hemicellulose hydrolysis yields were achieved in SCB pretreated with black liquor in nine successive repeated batches (1^{st} - 9^{th}). Furthermore, no significant difference was observed in the hydrolysis of carbohydrate fractions (Fig. 6.3) and solid composition (Table 6.2) according to Tukey's range test. In Table 6.2, total sugars released after 24 h of enzymatic hydrolysis are shown. On average, 472.8 g of sugars/kg of raw SCB were achieved.

Figure 6.3 – Cellulose and hemicellulose hydrolysis yield (%) in 24 h of hydrolysis time of SCB pretreated under HC-system using fresh sodium hydroxide solution and reused black liquor in successive batches. Bars correspond to standard deviation (SD) calculated for triplicates. Same letters above error bars indicate homogeneous subsets according to Tukey's range test at 95% of confidence level.



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This result stands out as important to favor economic feasibility of biomass pretreatment e.g reducing the alkali consumption (g of alkali/g of biomass). Moreover, with an adequate design of HC reactor, alkali consumption could be even more reduced, pretreating higher quantity of biomass in each 20 min batch. The pretreated SCB with NaOH and reused black liquor in HC-assisted process were used to produce ethanol in a new proposal of SSF process, as presented in following section.

6.3.7 Ethanol production in SSF configuration

Figure 6.4 shows ethanol production profile in SSF process with interconnected column reactors, by using immobilized cells and HC-pretreated SCB. Figure 6.5 shows a schematic representation of mass balance corresponding to the SSF process for the estimation of ethanol yield based on the total sugars consumption.

Figure 6.4 – Sugar releasing and consumption, and ethanol production by *Scheffersomyces stipitis* NRRL-Y7124 during SSF process in interconnected columns by using HC-pretreated SCB.

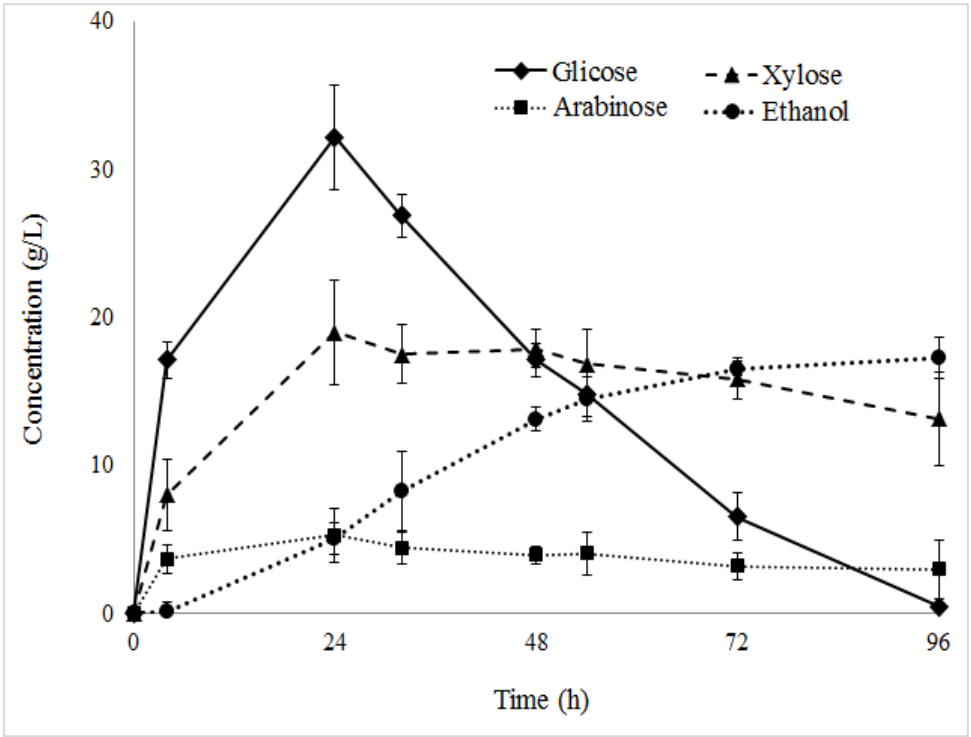

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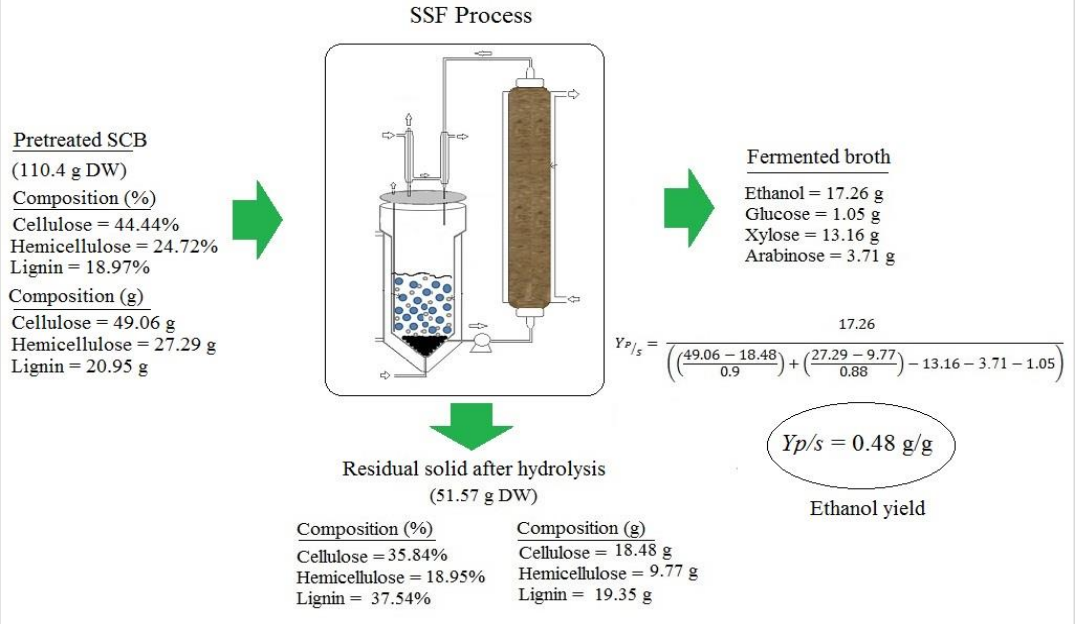
Figure 6.5 – Mass balance for ethanol production in SSF by immobilized *Scheffersomyces stipitis* NRRL-Y7124 using SCB pretreated with hydrodynamic cavitation as carbon source



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As exhibited in the aforementioned Fig. 6.4 and Fig. 6.5, after 96 h of fermentation, 17.26 g/L of ethanol production (0.48 g of ethanol/g of total sugar consumed, corresponding to a 94% of efficiency considering a theoretical Y*p/s* value of 0.51 g/g) was achieved.

Although not measured, inhibitors concentration in the hydrolysate can be supposed as low, considering previous reported results described by TERÁN-HILARES et al. (2016b). In that work, using harsher HC-pretreatment conditions (0.48 mol/L of NaOH, 4.24% of solid/liquid ratio and 44.48 min of HC process), a low concentration of inhibitors were observed (0.1 g/L of furfural, 0.06 g/L of acid acetic and 0.39 g/L of HMF) in enzymatic hydrolysate. Additionally, biomass after pretreatment was washed in order to remove the residual alkali and other compounds.

As observed in Fig. 6.4, glucose and xylose released were higher in first 24h of process, decreasing after this time. Thus, considering the hydrolysis rate was decreased and the sugars consumption was increased, the hydrolysis process was stopped and all liquid contained in reactor II was transferred to reactor I used for fermentation (Fig. 6.1), carrying out the fermentation process until 96 h of total time. In same figure (Fig. 6.4) can be also observed a concomitant production of ethanol

during the first 24 h (8 g/L) of SSF process. In 96 h of fermentation process, 96.9% of the total glucose released (33.97 g/L) was consumed by immobilized cells; however, xylose was only slightly consumed after glucose consumption. The rate of sugars consumption could be explained by limited transference of sugars inside the calcium alginate spheres, as also verified by Antunes et al. (2016).

Our new approach is surely promising even with the comparison of other studies; for example, Kim et al. (2015), reported the ethanol production by *S. cerevisiae* 7928 in SSF process using alkali-HC assisted pretreated reed (3% of NaOH, inlet pressure of 6 bar, temperature attained of 77 °C and 41.1 min of pretreatment time). In that study, 35.6 g/L of glucose was released in pre-hydrolysis step carried out in Erlenmeyer flask (250 ml) during 24h at 50 °C; after this time, the hydrolysate was supplemented and inoculated at a total volume of 100 ml, and fermentation was carried out for 96h at 38 °C. 25.9 g/L of ethanol (corresponding to 90.01% of theoretical efficiency) was achieved after 72h. The efficiency of fermentation in that study was lower than obtained in our study (94%), which could be associated to optimal temperature used for fermentation process. In our work, temperature was maintained at 30 °C in the fermentation column during the whole process and this temperature has to do with the ethanol yield by *Saccharomyces cerevisiae* G1 as reported by Nadeem et al. (2015). It was reported that the maximal yield (0.14 g/g) was achieved at 30 °C, it further reduced in 2% and 32.6% with an increase to 35°C and 40°C, respectively.

Regarding glucose preference by different microorganisms for ethanol production in the presence of hexoses and pentoses, this behavior was also related in other works. For example, simultaneous saccharification and co-fermentation of pretreated rice straw for ethanol production by *Saccharomyces cerevisiae* and *Scheffersomyces stipitis* co-culture was reported by Suriyachai et al. (2013). In that study, rice straw pretreated (5% sodium hydroxide at 90 °C for 20 min) was pre-hydrolyzed during 6h (25 FPU/g of biomass) at 5% of solid loading in a 2 L bioreactor; after this, the medium was inoculated with free cells at a ratio of 0.31:0.69 (OD_{600} approximately 1) and fermented at 33 °C and 116 rpm during 72 h. In that investigation, 14.8 g/L and of ethanol and 0.49 g/g of ethanol yield were achieved after 72h, representing 96% of efficiency of fermentation. Also, the preference by glucose was observed in both microorganisms, with significant increase in xylose consumption when the glucose was completely consumed. Similar

behavior of sugars consumption by *Scheffersomyces stipitis* NRRL-Y7124 was reported by Terán-Hilares et al. (2016b). In that study, the xylose present in SCB hydrolysate was consumed after complete consumption of glucose, phenomenon known as diauxic shift (BARI et al., 2013; SANTOS et al., 2015).

Our proposed system has potential advantages in terms of overcoming the innate mass transfer limitations typically associated with high solids loading (>11%) which would otherwise hinder the process efficiency (SURIYACHAI et al., 2013). Actually, our approach is likely capable to overcome the initial diffusion limitations due to the uniqueness of its aforementioned units. For instance, during the enzymatic hydrolysis, the fluid recirculation helps to overcome this concern by circulating the mixture as we have already reported the efficacy of such system in one of our previous publication (TERÁN-HILARES et al., 2016b). Moreover, it is well known that the rheology of biomass slurry, specifically during high solid enzymatic hydrolysis, improves with the progress of the reaction (JØRGENSEN; KRISTENSEN; FELBY, 2007). This improvement is attributed to the continuous saccharification of the pretreated biomass helping thus to enhance the hydrolysis kinetics. For the fermentation process, the unit is provided with an orifice locus at the bottom of the tank for the introduction of air. This aeration is itself playing a dual role both for fermentation medium as well as aiding to improve the mixing during fermentation albeit to lower extent. An additional advantage can be related to the application of immobilized cells, which can favors the reuse of the microbial biomass in repeated batches eliminating the need of membrane separation process as reported by Viola et al. (2013) and Ishola et al. (2015).

6.4 Conclusion

NaOH assisted HC pretreatment is undoubtedly an interesting option offering an added advantage of the potential use of the recycled black liquor in the pretreatment process. Our newly designed interconnected column reactor system helped to promote the fermentation efficiency even with high solids contents of SCB. Moreover, the use of immobilized cells is attractive and can be used in future works for repeated cycles of fermentation to help reduce the recovery cost. Therefore, it can be anticipated that this process is promising for large-scale applications due to the simplicity, efficiency and possibility of reuse of cells in subsequent batches.

6.4 Reference

ANTUNES, F. A. F. et al. Hemicellulosic Ethanol Production By Immobilized Wild Brazilian Yeast *Scheffersomyces Shehatae* UFMG-HM 52.2: Effects of cell concentration and stirring rate. **Current Microbiology**, v. 72, p. 133–138, 2016.

BADVE, M. P. et al. Hydrodynamic cavitation as a novel approach for delignification of wheat straw for paper manufacturing. **Ultrasonics Sonochemistry,** v. 21, p.162-168, 2014.

BARI, I. et al. Bioethanol production from mixed sugars by Scheffersomyces stipitis free and immobilized cells, and co-cultures with Saccharomyces cerevisiae. **New Biotechnology,** v. 30, p.591-597, 2013.

BORREGA, M.; SIXTA, H. Water prehydrolysis of birch wood chips and meal in batch and flow-through systems: a comparative evaluation. **Industrial Engineering Chemistry Research**, v. 54, p. 6075–6084, 2015.

CARVALHO, W. et al. Xylitol Production by Ca-alginate entrapped cells: comparison of different fermentation systems. **Enzyme and Microbial Technology**, v. 32, p. 553–559, 2003.

CHANDEL, A. K. et al. Detoxification of sugarcane bagasse hydrolysate improves ethanol production by *Candida Shehatae* NCIM 3501. **Bioresource Technology**, v. 98, p. 1947-1950, 2007.

DAHNUM, D. et al. Comparison of SHF and SSF processes using enzyme and dry yeast for optimization of bioethanol production from empty fruit bunch. **Energy Procedia,** v. 68, p.107-116, 2015.

DUARTE, J. C. Et Al. Effect of immobilized cells in calcium alginate beads in alcoholic fermentation. ***Amb Express***, v. 3, p. 31, 2013.

E-PIC S.R.L. Biomass Pretreatment (Biogas), 2016. Available in: Http://Www.Epic-Srl.Com/En/Cavitationtechnologies/ Biomass-Pretreatment-Biogas. Accessed in: 8 Apr. 2016.

FUENTES, L. L. G. et al. Kinetics of lime pretreatment of sugarcane bagasse to enhance enzymatic hydrolysis. **Applied Biochemistry and Biotechnology,** v. 163, p.612-625, 2010.

GHATAK, H. R. Biorefineries from the perspective of sustainability: feedstocks, products, and processes. **Renewable and Sustainable Energy Reviews,** v. 15, p.4042-4052, 2011.

GOGATE, P. R.; BHOSALE, G. S. Comparison of effectiveness of acoustic and hydrodynamic cavitation in combined treatment schemes for degradation of dye wastewaters. **Chemical Engineering and Processing: Process Intensification, v**. 71, p.59-69, 2013.

GOGATE, P. R.; PANDIT, A. B. A review and assessment of hydrodynamic cavitation as a technology for the future. **Ultrasonics Sonochemistry,** v. 12, p.21-27, 2005.

ISHOLA, M. M. et al. Simultaneous Saccharification, Filtration and Fermentation (SSFF): A novel method for bioethanol production from lignocellulosic biomass. **Bioresource Technology**, v. 133, p. 68–73, 2013.

JØRGENSEN, H.; KRISTENSEN, J, B.; FELBY, C. Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. **Biofuels, Bioproducts and Biorefining,** v. 1, p.119-134, 2007.

KHOR, W. C.; RABAEY, K.; VERVAEREN, H. Low temperature calcium hydroxide treatment enhances anaerobic methane production from (extruded) biomass. **Bioresource Technology,** v. 176, p.181-188, 2015.

KIM, I. et al. Hydrodynamic cavitation as a novel pretreatment approach for bioethanol production from reed. **Bioresource Technology**, v. 192, p. 335–339, 2015.

MADISON, M. J. et al. Mechanical pretreatment of biomass – part i: acoustic and hydrodynamic cavitation. **Biomass And Bioenergy**, v. 98, p.135–141, 2017.

MIRMOHAMADSADEGHI, S.; CHEN, Z.; WAN, C. Reducing biomass recalcitrance via mild sodium carbonate pretreatment. **Bioresource Technology,** v. 209, p.386-390, 2016.

NADEEM, M. et al. Production of ethanol from alkali-pretreated sugarcane bagasse under the influence of different process parameters. **Frontiers In Life Science,** v. 8, p.358-362, 2015.

NAKASHIMA, K. et al. Hydrodynamic cavitation reactor for efficient pretreatment of lignocellulosic biomass. **Industrial Engineering Chemistry Research**, v. 55, p.1866–1871, 2016.

OLOFSSON, K.; BERTILSSON, M.; LIDÉN, G. A short review on ssf – an interesting process option for ethanol production from lignocellulosic feedstocks. **Biotechnology for Biofuels,** v. 1, p.7-14, 2008.

RODRIGUES, C. I. S.; JACKSON, J. J.; MONTROSS, M. D. A molar basis comparison of calcium hydroxide, sodium hydroxide, and potassium hydroxide on the pretreatment of switchgrass and miscanthus under high solids conditions. **Industrial Crops and Products,** v. 92, p.165-173, 2016.

SALEHI, S. M. A. et al. Efficient conversion of rice straw to bioethanol using sodium carbonate pretreatment. **Energy & Fuels,** v. 26, p.7354-7361, 2012

SANTOS, J. C. et al. Sugarcane Bagasse As Raw Material And Immobilization Support For Xylitol Production. **Applied Biochemistry and Biotechnology**, v. 122, p.0673-0684, 2005.

SANTOS, S. C. et al. Fermentation of xylose and glucose mixture in intensified reactors by *Scheffersomyces Stipitis* to produce ethanol. **International Scholarly and Scientific Research & Innovation**, v. 9, p. 539–544, 2015.

Shadbahr, J.; Khan, F.; Zhang, Y. Kinetic modeling and dynamic analysis of simultaneous saccharification and fermentation of cellulose to bioethanol. **Energy Conversion and Management,** v. 141, p.236-243, 2017.

SHARMA, R. et al. Potential of potassium hydroxide pretreatment of switchgrass for fermentable sugar production. **Applied Biochemistry and Biotechnology,** v. 169, p.761-772, 2013.

SLUITER, A. et al. Determination of structural carbohydrates and lignin in biomass. Technical Report Nrel/Tp-510-42618 Prepared For National Renewable Energy Laboratory, Golden Co, 2012.

SURIYACHAI, N. et al. Optimized simultaneous saccharification and co-fermentation of rice straw for ethanol production by *Saccharomyces Cerevisiae* and *Scheffersomyces Stipitis* co-culture using design of experiments. **Bioresource Technology**, v. 142, p. 171–178, 2013.

TANIMURA, A. et al. Isolation of a novel strain of Candida shehatae for ethanol production at elevated temperature. **Springerplus**, v. 1, p. 27, 2012.

TERÁN HILARES, R. et al. Hydrodynamic cavitation-assisted alkaline pretreatment as a new approach for sugarcane bagasse biorefineries. **Bioresource Technology**, v. 214, p. 609–614, 2016a.

TERÁN-HILARES, R. et al. Successive pretreatment and enzymatic saccharification of sugarcane bagasse in a packed bed flow-through column reactor aiming to support biorefineries. **Bioresource Technology**, v. 203, p.42–49, 2016b.

TERÁN HILARES, R. et al. Low-Melanin containing pullulan production from sugarcane bagasse hydrolysate by *Aureobasidium Pullulans* in fermentations assisted by light-emitting diode. **Bioresource Technology**, v. 230, p. 76–81, 2017a.

TERÁN HILARES, R. et al. Hydrodynamic cavitation as an efficient pretreatment method for lignocellulosic biomass : a parametric study. **Bioresource Technology**, v. 235, p. 301–308, 2017b.

VIOLA, E. et al. Use of a two-chamber reactor to improve enzymatic hydrolysis and fermentation of lignocellulosic materials. **Applied Energy**, v. 102, p. 198–203, 2013.

YANG, L. et al. Effects of sodium carbonate pretreatment on the chemical compositions and enzymatic saccharification of rice straw. **Bioresource Technology**, v. 124, p. 283–291, 2012.

YUVADETKUN, P.; LEKSAWASDI, N.; BOONMEE, M. Kinetic modeling of *Candida shehatae* ATCC 22984 on xylose and glucose for ethanol production. **Preparative Biochemistry and Biotechnology,** v. 47, p.268-275, 2016.

CHAPTER VII

Intensification of alkaline hydrogen peroxide pretreatment of sugarcane bagasse by hydrodynamic cavitation and use of column reactors for ethanol production

Abstract

Hydrodynamic cavitation (HC) was used to assist alkaline hydrogen peroxide pretreatment to disrupt the rigid biomass structure even at mild process conditions and so with a short span of time. Initially pretreatment was optimized in terms of time, H_2O_2 and NaOH concentrations and the established conditions were: 0.29 mol/L of NaOH, 0.78 % v/v of H_2O_2 and 9.95 min. Under these conditions, 95.43% and 81.34% of digestibility of cellulosic and hemicellulosic fractions were reached, respectively. Subsequently, a process intensification approach was applied first to the enzymatic hydrolysis for handling high dry mass contents (20% solids loading) in a packed bed flow-through column reactor yielding appreciable conversions of both the cellulose and hemicellulose conversions up to 74.7% and 75% respectively. Ultimately, a bubble column reactor was employed for the fermentation of SCB into ethanol by *Scheffersomyces stipitis* NRRL-Y7124, producing 31.50 g/L of ethanol, 0.49 g/g of ethanol yield and 0.68 g/L.h of productivity. Obtained results showed that our HC assisted NaOH-H_2O_2 pretreatment strategy along with the process intensification approach of different reactors (cavitation and columns) might favor biorefineries in industrial scale.

Keywords:

Hydrodynamic cavitation; Intensification of process; Hydrogen peroxide; Alkaline pretreatment; Sugarcane bagasse; Ethanol production

7.1 Introduction

The inherited recalcitrance of lignocelluloses is a barrier offering difficulties for enzymatic hydrolysis of its carbohydrate fractions (HIMMEL et al., 2007; CHEN; FU et al., 2017). Thus, a prior structural disintegration named as pretreatment is a vital prerequisite to improve the enzyme access for an efficient release of fermentable sugars (JØRGENSEN; KRISTENSEN; FELBY, 2007). The main changes, usually promoted in pretreatment, correspond to lignin and/or hemicellulose removal and destructuration of the well-organized biomass structure, resulting in the reduction of crystallinity and an increase in the porosity of the substrate (TERÁN-HILARES et al., 2016a).

Despite the existence of many pretreatment methods, this step is still the current technological bottleneck for lignocellulosic biorefineries (NOVACANA, 2016). Among from these such options, alkaline pretreatment stands prominent due to efficient delignification, high energy-efficiency and low severity required during pretreatment (KIM; LEE; KIM, 2016). However, long process time and high concentration of chemicals are required when the process is performed at milder conditions as reported previously, e.g. in studies of Terán-Hilares et al. (2016a) and Antunes et al. (2017).

An alternative pretreatment approach corresponds to the application of hydrogen peroxide as an environmentally benign pretreatment reagent. Thus far, it has been extensively used for delignification and bleaching purposes in pulp and paper industries (SIXTA et al., 2006). However, for this approach to be efficient enough a long process time (even up to 4 h) (CORREIA et al. 2013) along with a high dose of H_2O_2 e.g. 16% w/v (HIDENO, 2017) is deemed necessary. In order to reduce the treatment severity yet enhancing its efficiency different combined processes have been proposed such as (RABELO et al., 2014; VALIM et al., 2017), metal salt with ultrasound (RAMADOSS; MUTHUKUMAR, 2016) and the use of microwaves (SINGH et al., 2014). Another potential strategy in this regard could be a process integration of alkaline hydrogen peroxide with hydrodynamic cavitation (HC) (KIM et al., 2015; TERÁN HILARES et al., 2016b:2017a).

Typically, HC corresponds to the formation, growth and consequently collapse of micro bubbles generated in the process fluid at the expense of pressure gradient maintained along an orifice or a venture tube. The immense localized pressure and

temperature inside these micro-bubbles, dissociate water molecules into strong oxidants of hydroxyl ($OH^{\cdot}$) and superoxide ($O2^{\cdot-}$) radicals (BADVE et al., 2014; CAI et al., 2016). Moreover, under alkaline conditions hydrogen peroxide (H_2O_2) and hydroperoxy anion (HOO^-) can also be produced (BADVE et al., 2014). The synergetic action of these radicals, during lignocellulosic oxidative pretreatment, is well known and the typically degradation of lignin is attributed to the hydroxylation, hydroxyl substitution, side chain oxidation, cleavage of the ether linkage, side chain cleavage, aromatic ring cleavage and oxidative coupling (GIERER, 1993; BADVE et al., 2014).

Therefore, in HC-assisted pretreatments, the oxidative radical concentration is escalated to consequently reinforce the pretreatment severity, resulting thus a porous biomass more susceptible to enzyme attack. Mechanical effects of cavitation is another feature of HC pretreatment process. The erosion (small perforations) produced by the impact of high speed micro-jets on solids and shockwaves generated by violent collapse of cavities produce an increase in the specific surface area, total pore volume and total micropore volume, which turns the carbohydrate fraction even more susceptible to enzyme attack (KIM et al., 2015; E-PIC S.R.L., 2016; NAKASHIMA et al., 2016; TERÁN HILARES et al., 2016b, 2017a, 2017b).

Besides pretreatment, another challenge for economical operation of biorefineries corresponds to enzymatic hydrolysis specifically at high solids loading. However, such high biomass loading has a negative correlation with the heat and mass transport intensifying thus the problems of feedback inhibition (FOCKINK et al., 2016). To this end, percolate column reactors were successfully used with solid loading higher than 10% for pretreatment and enzymatic hydrolysis of sugarcane bagasse (SCB) (TERÁN HILARES et al., 2016b) and for ethanol production in a simultaneous hydrolysis and fermentation process (TERÁN HILARES et al., 2017b), this last with potential to avoid inhibition of enzymes by end-products.

In this work, the feasibility of bio-refinery process intensification for all main steps of 2G ethanol production process was studied. Initially, HC was used for intensification of alkaline hydrogen peroxide pretreatment in order to reduce the process time under milder conditions. Subsequently, percolate column reactor was employed for hydrolysis steps using high solid loading (20% S/L) and finally a bubble column reactor was used for ethanol production by *Scheffersomyces stipitis* NRRL-Y7124.

7.2 Materials and methods

7.2.1 Sugarcane bagasse

Sugarcane bagasse (SCB) was kindly donated by Usina Vale Onda Verde (Onda Verde-SP, Brazil). Biomass was dried, milled and classified through a standard Tyler sieve of 2 1/2 mesh to be retained in a 16 mesh sieve for experimental runs.

7.2.2 Alkaline hydrogen peroxide-HC assisted pretreatment

A 3 L (total volume) HC system was used in the experimental procedure. This system was composed of a centrifugal pump (1.5 CV), a cavitation zone and a recirculation tank, as previously described (TERÁN HILARES et al., 2017a). The liquid was recirculated through the system at a flowrate adjusted to 5 m^3/h, corresponding to a fluid velocity through the orifice (16 holes, 1 mm of diameter) of 88 m/s and cavitation number of 0.017, as described in the previous work of TERÁN HILARES et al. (2017a). Process fluid temperature was maintained at 60 °C during pretreatment with the help of water-jackets and the upstream pressure for generation of cavities was set to be at 3 bar.

Alkaline hydrogen peroxide-HC assisted pretreatment experiments were carried out using 20 g of dry SCB which were kept inside a cylindrical wire cloth (20 mesh) and placed in the cavitation zone. A Box-Benhken desing was used to evaluate the influence for the variables sodium hydroxide concentration (0.1 - 0.3 mol/L), hydrogen peroxide concentration (0.2 – 1.0%v/v) and HC process time (2 - 10 min). Under optimized conditions, additional experiments were performed without NaOH and without H_2O_2 to generate data for comparative discussion.

After pretreatment, the biomass was collected, washed with distilled water, dried and finally analyzed for cellulose, hemicellulose and lignin contents. The treated samples were also subjected to the enzymatic hydrolysis according to the methodology described in section 7.2.3.

7.2.3 Enzymatic hydrolysis of pretreated SCB

Enzymatic hydrolysis was carried out in 125 mL Erlenmeyer flasks at 5% of solid loading (%S/L) in 50 mM sodium citrate solution (pH 4.8). Commercial cellulases enzyme blend Cellic® CTec2, kindly donated by Novozymes Latin America Ltda. (Araucária-PR, Brazil), was used at 20 FPU/g of dry pretreated SCB. Samples were incubated at 50 °C and 200 rpm in an Innova® 44/44R rotary shaker (New Brunswick Scientific Co., Inc., USA) during 24 h. The sugar content in the hydrolysate was analyzed by High Performance Liquid Chromatography (HPLC) as described in section 7.2.7. Enzymatic digestibility was calculated for the yield of cellulose and hemicellulose hydrolysis (%) on the basis of initial glucan and ‘‘xylan + arabinan” contents, respectively.

Design-Expert software 8.0 (stat-Ease, Inc, USA) was used to compose and evaluate empirical model to describe response variable as a function of NaOH and H_2O_2 concentration and process time. Process optimization was carried out using the numerical optimization feature of the software, based on desirability function. The maximization of cellulosic hydrolysis yield was considered as a goal for optimization process. After optimization, predicted model was confirmed by experimental means.

7.2.4 Enzymatic hydrolysis at high solids loading in packed bed flow-through column reactor

The SCB pretreated in optimized condition was enzymatically hydrolysed in a packed bed flow-through column reactor (PBFTCR, 35 cm length x 2.5 cm internal diameter), at high solids loading (20 %) in the system with constant recirculation through the reactor of 120 mL of buffer solution containing commercial cellulases Cellic® CTec2 (Novozyme, Brazil) at two different enzyme loading (10 and 20 FPU/g of SCB). The flowrate of buffer solution during the hydrolysis process was kept in 8 mL/min by using a peristaltic pump. The process was carried out during 24h at 50 °C and samples were periodically taken to monitor the enzymatic digestibility of cellulose fraction based in released glucose (%). The resulted hydrolysate containing high sugars concentration was used for ethanol production as described in the following section.

7.2.5 Ethanol production

Scheffersomyces stipitis NRRL-Y7124 was obtained from stock cultures available in the Applied Microbiology and Bioprocess Laboratory (GMBio) of the Engineering School of Lorena – University of Sao Paulo, Brazil.

Inoculum was grown in 125 mL Erlenmeyer flasks containing 50 mL of medium composed of: glucose 30 g/L, peptone 20 g/L and yeast extract 10 g/L. Medium containing cells was kept in a shaker at 30 °C and 200 rpm during 48 h. Then, cells were centrifuged at 2000 x g during 15 min and added in the fermentation medium containing enzymatic hydrolysate in quantity enough to result in 3.5 g/L of initial biomass concentration.

The hydrolysate obtained in the packed flow-thought column reactor from SCB pretreated under optimized condition was used for ethanol production in a 1.5 L bubble column reactor using free cells of *Scheffersomyces stipitis* NRRL-Y7124. Fermentation medium (300 mL) containing dilute enzymatic hydrolysate was supplemented with 3 g/L of yeast extract, 5 g/L of peptone, 0.1 g/L of calcium chloride and 2 g/L of ammonium sulphate. During the fermentation process, air was continuously supplied into the reactor at 0.3 vvm (PORTUGAL-NUNES et al., 2015). Samples were periodically taken to monitor the sugar consumption, ethanol production and biomass concentration as described in the section 7.2.7.

7.2.6 Characterization of pretreated biomass

7.2.6.1 Surface features of pretreated SCB

Surface morphology of biomass samples were observed using Scanning Electron Microscope (SEM) SU5000 (Tokyo, Japan). The samples of raw and pretreated SCB under optimized condition were fixed with carbon ribbon supporting aluminum and subsequently analyzed.

7.2.6.2 Solid-state NMR

A qualitative identification of the main chemical changes taking place in samples as a consequent of alkaline hydrogen peroxide-HC assisted pretreatment was

determined by solid-state ^{13}C nuclear magnetic resonance (NMR) spectroscopy (REZENDE et al., 2011). Samples were analyzed using an Agilent-NMR-vnmrs400 spectrophotometer (Agilent Technologies, USA) at ^{13}C and ^{1}H frequencies of 20 and 50 kHz. Radio frequency ramped cross-polarizations under magic angle spinning (CPMAS) combined with total suppression of spinning sidebands (TOSS) and hetero-nuclear ^{1}H decoupling (CPMAS-TOSS) was used to acquire the ^{13}C spectra.

7.2.7 Analytical methods

SCB composition was characterized according to methodology described by Sluiter et al. (2012). The sugars (glucose, xylose, arabinose and cellobiose) and ethanol concentration were analyzed by High Performance Liquid Chromatography (HPLC) Agilent 1200 series (Agilent Technologies, Inc., USA) equipped with a Refractive index detector RID-6A and a HPX-87H (300x7.8mm) column (Bio-Rad, USA). Conditions used in the analysis were the following: 45°C column temperature, 0.01 N H_2SO_4 as the mobile phase, 0.6 mL/min flow rate, and 20 µL injection volume.

Microbial biomass was determined by turbidity using Beckman DU640B Spectrophotometer (Minnesota, USA) at 600 nm and absorbance values were converted in g/L by using a previously established standard curve.

7.3 Results and discussion

7.3.1 Alkaline hydrogen peroxide-HC assisted pretreatment: compositional alterations in SCB

The raw SCB had the following composition: 40.7% of cellulose, 26.02% of hemicellulose, 27.18% of lignin and 7.1% of others minor components. The experimental design for sodium hydroxide/hydrogen peroxide ($NaOH/H_2O_2$)–HC assisted pretreatment with respective results of solid composition and removal of compounds are shown in Table 7.1.

Table 7.1 – Solid composition and removal of components in SCB pretreated using $NaOH/H_2O_2/HC$

Run	Experimental variables			Solid recovery (%)	Solid composition			Removal (%)*	
	Process time (min)	Hydrogen peroxide (%v/v/)	Sodium hydroxide (mol/L)		Cellulose (%)	Hemicellulose (%)	Lignin (%)	Hemicellulose	Lignin
1	2	0.2	0.2	84.25	42.74	30.24	19.66	5.64	36.29
2	10	0.2	0.2	78.76	46.40	28.20	19.55	17.74	40.78
3	2	1.0	0.2	83.97	43.68	27.90	21.92	13.23	29.21
4	10	1.0	0.2	78.14	49.88	28.78	16.25	16.71	51.16
5	2	0.6	0.1	86.55	41.84	27.95	22.16	10.40	26.23
6	10	0.6	0.1	83.73	44.49	27.03	21.57	16.18	30.54
7	2	0.6	0.3	81.09	43.10	25.54	20.40	23.29	36.38
8	10	0.6	0.3	70.51	46.76	29.92	14.33	26.30	63.34
9	6	0.2	0.1	80.14	45.44	28.71	23.10	14.78	28.80
10	6	1.0	0.1	84.14	42.97	27.62	22.35	13.93	27.67
11	6	0.2	0.3	75.68	50.35	29.41	18.71	21.92	48.42
12	6	1.0	0.3	66.00	44.86	27.45	16.66	32.90	57.71
13	6	0.6	0.2	77.50	47.29	29.49	19.23	15.35	42.68
14	6	0.6	0.2	73.39	43.07	28.55	19.23	22.40	45.72
15	6	0.6	0.2	75.89	45.48	27.24	20.00	23.44	41.62

*Cellulose removal was lower than 10%.

Source: Personal archive

As can be observed in Table 7.1, the cellulose contents in biomass increased from 40.7 to 50.3% after treatment, with the highest value obtained at experimental run 11 (6 min process time, 0.2%v/v of H_2O_2 and 0.3 mol/L of NaOH). However, the lignin contents in the biomass was strongly modified by pretreatment, decreasing from 27.20 to 14.33%, as observed at run 8 (10 min, 0.6%v/v of H_2O_2 and 0.3 mol/L of NaOH) which corresponds to 63.34% of lignin removal. This removal value was slightly higher than 60% observed in alkaline-HC assisted pretreatment of SCB (0.48 mol/L of NaOH, 4.24% of solid/liquid ratio and 44.48 min, process without temperature control) previously report by Terán Hilares et al. (2016b). That value was also higher than 45.57% achieved in SCB pretreated with sodium hydroxide-HC assisted process (0.3 mol/L of NaOH, 70 °C, 20 min of process) reported by Terán Hilares et al. (2017a) and 57% achieved in rice straw pretreated with sodium hydroxide-ultrasound assisted process (1% w/v of NaOH, 50 °C, by 1h) reported by Wu et al. (2017).

In another report, a value of about 60% of delignification of SCB was achieved at 1% of H_2O_2, 1:100 molar ratio of metal salt and H_2O_2, 100 °C, 50% ultrasound amplitude, 70% of ultrasound duty cycle and 60 min of process time) (RAMADOSS; MUTHUKUMAR, 2016). In that work, an increased concentration of $OH^{\bullet}$ was observed due to the presence of H_2O_2 and the cavitation phenomenon of ultrasound. Following a similar way, the highest lignin removal achieved in our work can be associated to the synergistic effect of alkali and potential oxidative radicals ($OH^{\bullet}$) generated by the dissociation of water molecules due to HC phenomenon and produced by reaction of hydroperoxy radical ($HOO^{\bullet}$) with hydrogen peroxide (BADVE et al., 2014).

As also shown in Table 7.1, only small differences were observed in hemicellulose content of the material after pretreatment. Despite of this fact, only 33% of hemicellulosic fraction was removed by pretreatment, as observed in run 12 (6 min, 1% v/v of H_2O_2 and 0.3 mol/L of NaOH). That value was similar to 34% of removal efficiency obtained in SCB pretreated with NaOH-HC assisted pretreatment reported previously by Terán Hilares et al. (2017b) and lower than the value of 61% obtained during alkaline hydrogen peroxide pretreatment (35 °C, 4.3% v/v H_2O_2, pH of 11.5, by 6 h) of cashew apple bagasse, reported by Correia et al. (2013). For all pretreatment conditions, cellulosic fraction removal was lower than 10%.

7.3.2 Optimization of pretreatment for enzymatic hydrolysis yield

All the treated SCB samples were enzymatically hydrolyzed and results particularly in terms of glucose release are shown in Table 7.2. As observed, the higher cellulose hydrolysis yield (94.96%) was achieved in biomass pretreated at run 8 (10 min, 0.6% v/v H_2O_2 and 0.3 mol/L of NaOH). That value is 6 times higher than the obtained for untreated biomass (16%) and similar to the value of 93.05% obtained for SCB pretreated with NaOH-HC assisted process (0.3 mol/L, 30 min, 70 °C) reported by Terán Hilares et al. (2017a).

Table 7.2 – Cellulose hydrolysis yield in pretreated biomass with NaOH/H_2O_2/HC

Run	Pretreatment experimental variables			Cellulose hydrolysis yield values in 24h (%)	
	Process time (min)	H_2O_2 (% v/v)	NaOH (mol/L)	Experimental	Predicted
1	2	0.2	0.2	55.39	54.78
2	10	0.2	0.2	67.61	68.74
3	2	1.0	0.2	41.60	36.81
4	10	1.0	0.2	83.99	80.93
5	2	0.6	0.1	38.13	36.03
6	10	0.6	0.1	48.37	44.54
7	2	0.6	0.3	43.66	44.05
8	10	0.6	0.3	94.96	93.62
9	6	0.2	0.1	46.74	47.61
10	6	1.0	0.1	27.90	32.96
11	6	0.2	0.3	66.02	64.40
12	6	1.0	0.3	70.71	73.28
13	6	0.6	0.2	58.78	60.32
14	6	0.6	0.2	56.11	60.32
15	6	0.6	0.2	58.73	60.32

Source: Personal archive

This rather milder and shorter process time resulted in the same cellulose hydrolysis yield compared to other pretreatment methods reported using more severe conditions. For example, 94% of cellulose hydrolysis yield was achieved in SCB pretreated under ozonolysis combined with ultrasound process (32 mg O_3/minute during 60 min, follow by alkaline process using 0.1 mol/L of NaOH during 2h and finally submitted to ultrasound process during 5 min.) reported by Perrone et al. (2016). Also, near to 98.25% of cellulose hydrolysis was obtained using 2% of solid loading during 72 h in SCB pretreated with NaOH (0.5 mol/L, 80 °C and 2h) followed by microwave assisted process (600 W by 5 min), reported by Liu et al. (2016).

An ANOVA test was carried out for the response cellulose hydrolysis yield and results are shown in Table 7.3. Values were adjusted to quadratic model (Eq. 7.1) with correlation coefficient (R^2) of 0.94; the adequate fit of response values to compose equation was determined by the significance of model (*p*-value < 0.0001) and non-significance of lack of fit test (p-value > 0.05) at 95% of confidence level. Also, predicted values for cellulose hydrolysis yield were near to the experimental, as shown in Table 7.2.

$$Y_1 = 54.55 - 4.33X_1 - 61.31X_2 + 130.73X_3 + 4.71X_1X_2 + 25.66X_1X_3 + 147.06X_2X_3 - 575.45X_3^2 \quad \text{Eq. (7.1)}$$

Where, Y_1 is the cellulose hydrolysis yield (%), X_1 is process time (min), X_2 and X_3 correspond to concentration to concentration of hydrogen peroxide (%v/v) and sodium hydroxide (mol/L), respectively.

As delineated in the Table 7.3, the enzymatic digestibility of cellulose was strongly influenced by sodium hydroxide concentration and the process time which coefficients in the model showed p-values lower than 0.0001. No significant main effect of hydrogen peroxide on cellulose hydrolysis yield was observed; however, interaction of this variable with others shown significance at 95% of confidential level. 3D response surface plots (Figure 7.1) were also plotted to observe the effect of pretreatment variables on the cellulose hydrolysis yield. As observed in the Figure 1, increasing the alkaline concentration can improve the susceptibility of biomass to hydrolysis yield due to the structural and compositional changes. (SILVA et al., 2015; CARVALHO; QUEIROZ; COLODETTE, 2016; TERÁN HILARES et al., 2016b).

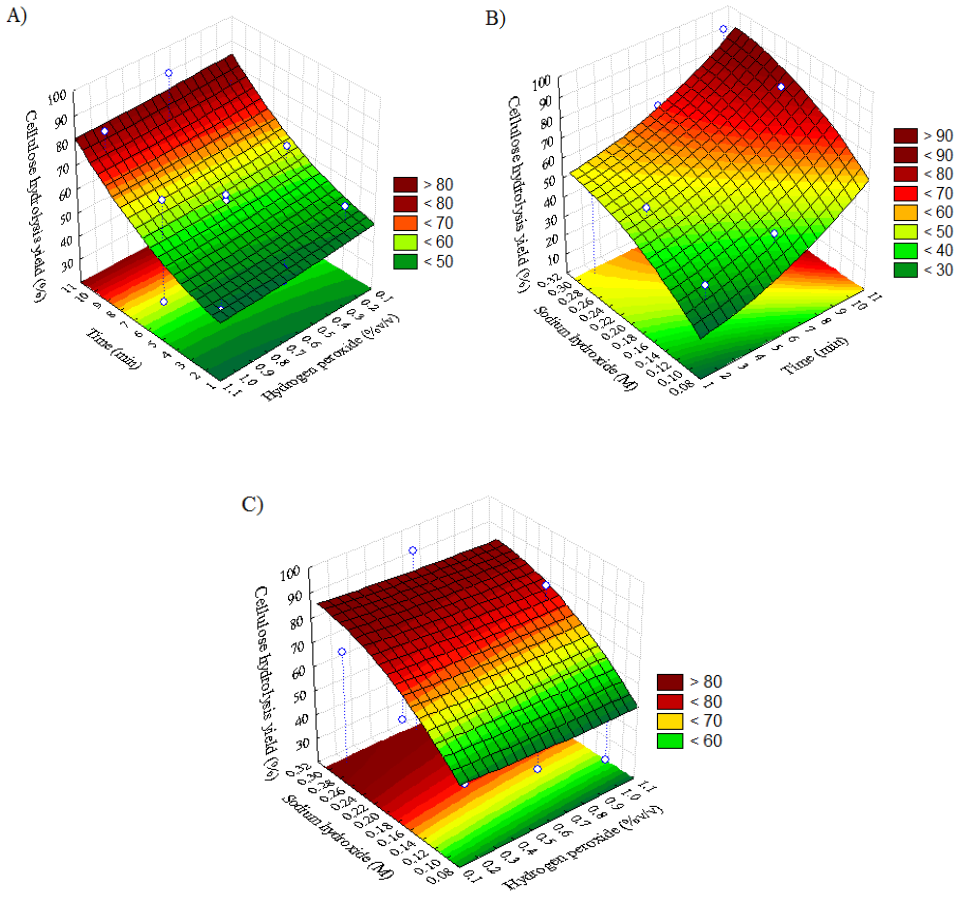
Table 7.3 – ANOVA for a second order model composed for cellulose hydrolysis yield after 24h of enzymatic hydrolysis in sugarcane bagasse pretreated with H_2O_2/NaOH/HC

Source	Sum of squares	Df	Mean square	*F*-Value	*p*-Value	
Model	4244.63	7	606.38	37.54	< 0.0001	Significant
Time (X1)	1686.35	1	1686.35	104.40	< 0.0001	
Hydrogen peroxide (X2)	16.70	1	16.70	1.03	0.3430	
Sodium hydroxide (X3)	1630.49	1	1630.49	100.94	< 0.0001	
X_1X_2	227.56	1	227.56	14.09	0.0071	
X_1X_3	421.48	1	421.48	26.09	0.0014	
X_2X_3	138.42	1	138.42	8.57	0.0221	
X_3^2	123.63	1	123.63	7.65	0.0278	
Residual	113.07	7	16.15			
Lack of Fit	108.41	5	21.68	9.29	0.1000	Not significant
Pure Error	4.67	2	2.33			
Total	4357.70	14				
R^2 adjusted = 0.9487						

Source: Personal archive

In Fig. 7.1a and 7.1b is shown that no significantly increase in the hydrolysis yield was promoted by increasing the hydrogen peroxide from 0.2% to 1 %v/v, which can be attributed to the small H_2O_2 concentrations evaluated. For example, in the work of Correia et al. (2013), the cellulose hydrolysis yield of alkaline hydrogen peroxide (AHP) pretreated cashew apple bagasse (CAB) was improved from 35% to 50% by increasing the concentration of H_2O_2 from 0.654% v/v to 5%v/v for pretreatment. In the report of Saha and Cotta (2006), the effect of concentration of H_2O_2 used for pretreatment of wheat straw on the released sugar was also reported. In that work, the sugar yield in 120 h increased from 200 to 350 mg/g of biomass when H_2O_2 concentration was increased from 0.5% to 2.15 %v/v in pretreatment process.

Figure 7.1 – Response Surface Graphs for optimization of enzymatic hydrolysis yield. Interaction of variables: (A) time and hydrogen peroxide concentration, (B) time and sodium hydroxide concentration, (C) hydrogen peroxide and sodium hydroxide concentration.



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The pretreatment variables were optimized aiming at maximizing the cellulose hydrolysis yield. At the optimized conditions (9.95 min, 0.78 %v/v of H_2O_2 and 0.29 mol/L of NaOH), 96.27±7.31% of cellulose hydrolysis yield was predicted by model. This value was also confirmed by experimental result which was 95.43% of hydrolysis in 24 h (Table 7.4). Also, in the optimized pretreatment conditions, 81.34% of hemicellulose hydrolysis yield was achieved, which can be attributed to the presence of xylanases in commercial blend of cellulases.

Table 7.4 – Experimental confirmation of predicted values by the model for cellulose hydrolysis yield in the optimized pretreatment HC condition: 9.95 min, 0.78 %v/v of H_2O_2 and 0.29 mol/L of NaOH

Responses	Experimental value (X ± SD)*	Predicted value (X ± CI)**
Cellulose hydrolysis yield***	95.43 ± 4.51	96.27 ± 7.31
Hemicellulose hydrolysis yield	81.34 ± 6.36	-
Cellulose content	49.16 ± 1.14	-
Hemicellulose content	25.33 ± 0.75	-
Lignin content	12.96 ± 0.89	-
Total sugars content (g/kg of raw biomass)	780.45 ± 10.85	-

*Experimental values correspond to average of three obtained values ± standard deviation (SD).

** Predicted value ± 95% confidence interval (CI).

*** The maximal cellulose hydrolysis yield in 24h was considered as optimization criteria.

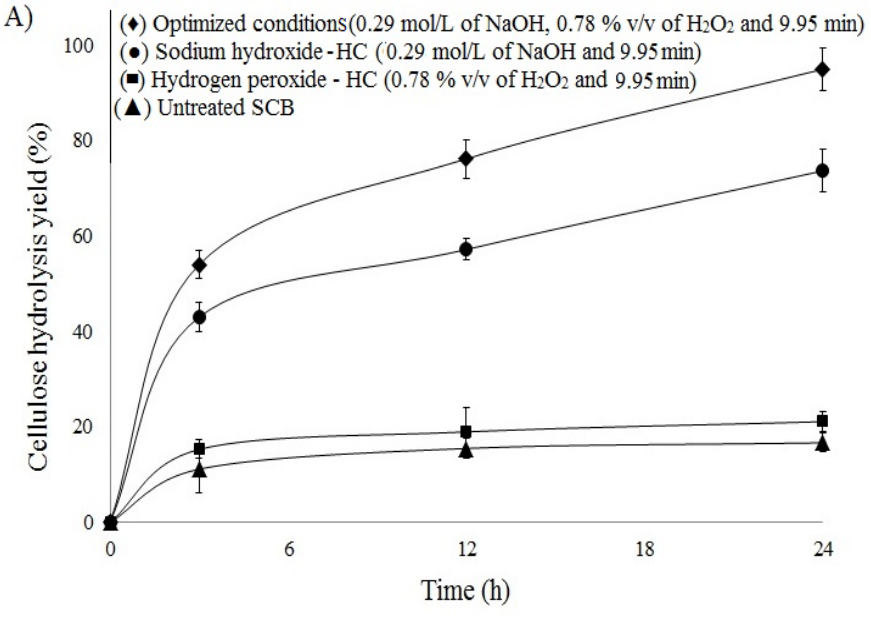
Source: Personal archive

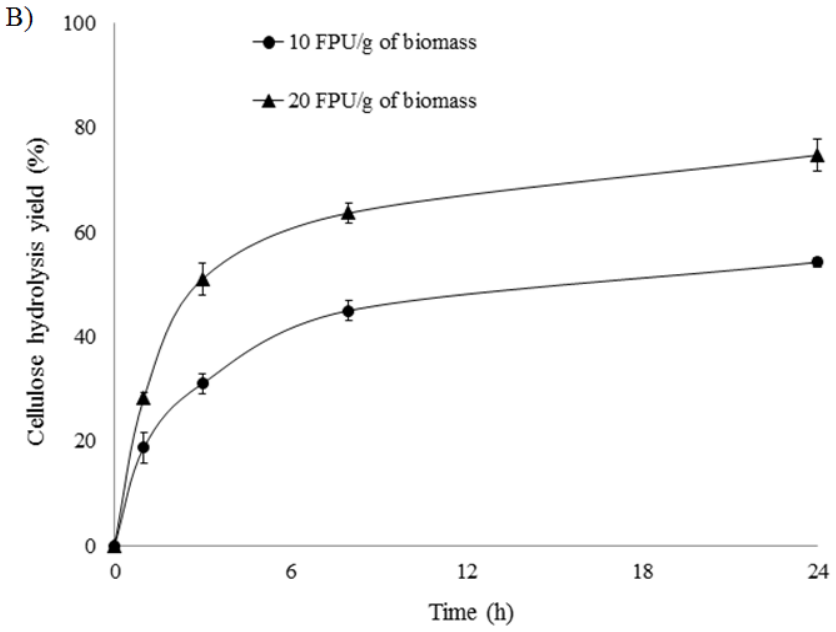
Figure 7.2A shows the profile of cellulose hydrolysis yield during 24h of process. As can be observed, the optimized pretreatment process significantly improved the susceptibility of SCB to enzyme attack compared to untreated biomass or pretreated with NaOH-HC and H_2O_2-HC pretreatment. In all cases, the hydrolysis yield rate was higher in the first 4h of process, with no increase after this time for untreated material or pretreated with H_2O_2-HC and a slower increase observed for biomass pretreated with NaOH-HC or NaOH- H_2O_2-HC. This fact can be related to the lower hydrolysis rate of recalcitrant cellulosic fraction, which can be performed only for efficiently pretreated biomass.

As also can be observed in Fig. 7.2A, the use of H_2O_2-HC process without NaOH did not result any noticeable increase in the SCB cellulose hydrolysis yield compared to untreated material. Indeed, hydrogen peroxide requires alkaline conditions to be effective during biomass pretreatment (ALVAREZ-VASCO; ZHANG, 2017). Besides, another important observation is, despite the non-significance of model coefficient correspondent to the main effect of hydrogen peroxide concentration (Table 7.3), interaction effect of this compound with alkali assisted by HC was beneficial to the hydrolysis yield.

As discussed, this kind of pretreatment efficiently improved the carbohydrate digestibility in the biomass. Besides the synergetic effects of chemicals and HC, the beneficial results of the proposed pretreatment can be attributed to mechanical effects of microjets and shockwaves generated during violent collapse of cavities that improve the porosity of material (E-PIC s.r.l, 2016; TERÁN HILARES et al., 2017a, 2017b).

Figure 7.2 - Cellulose hydrolysis yield profile observed in SCB pretreated and hydrolyzed under different conditions. A) Hydrolysis yield in Erlenmeyer flasks using biomass pretreated with NaOH-H_2O_2-HC under optimized conditions compared with untreated SCB or pretreated using NaOH-HC and H_2O_2-HC process and B) Hydrolysis yield of cellulose fraction of SCB pretreated under optimized condition using two different enzymes loading at high solid loading (20%) in column reactor.





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7.3.3 Enzymatic hydrolysis in column flow-through column reactor

The enzymatic hydrolysis profile of cellulose fraction using high solid loading (20%) in a packed bed flow-thought column reactor (PBFTCR) at two different enzymes loading also is observed in Figure 7.2B. As expected, using high enzymes loading (20 FPU/g of dry SCB), higher hydrolysis yield (74.7%) was achieved in 24h compared to the process using 10 FPU/g. Also, employing an enzyme loading of 20 FPU/g, the resulted hydrolysate showed a composition of 80 g/L glucose, 38 g/L xylose, 20 g/L cellobiose and 4 g/L arabinose, which is adequate to subsequent fermentation process for ethanol production employing co-fermentative yeasts, e.g. *Scheffersomyces stipitis* and *Candida shehatae* (SÁNCHEZ et al., 2002; GUTIÉRREZ-RIVERA et al., 2011; TERÁN-HILARES et al., 2016b). Similarly to previously discussed, enzymatic hydrolysis rate was higher in the first 4 h of reaction, reaching a value of about 60 g/L of glucose, 25 g/L of xylose and 22 g/L of cellobiose and 3 g/L of arabinose.

The reduction in the hydrolysis yield obtained using 20% of solid loading (Fig. 7.2B) compared to the one using 5% loading in Erlenmeyer flasks (Fig. 7.2A) could be associated to several factors as enzyme activity inhibition by end-products (e.g. cellobiose), adsorption of enzyme on the lignin surface and limited mass and heat

transference in the system due to low water content (ROCHE et al., 2009; MODENBACH; NOKES, 2013; LIN et al., 2017).

7.3.4 Ethanol production in a bubble column reactor

The fermentability of enzymatic hydrolysate obtained from SCB pretreated under optimized conditions with sodium hydroxide/hydrogen peroxide-HC assisted process, was evaluated for ethanol production by yeast *S. stipitis* NRRL-Y7124 in a bubble column reactor. The obtained results are summarized in terms of production of ethanol and biomass in Table 7.5 and Figure 7.3.

Table 7.5 – Results obtained in fermentation performed for Ethanol production from SCB hydrolysate in a bubble column reactor using *S. stipitis* NRRL-Y7124

Parameters of fermentation	Value
Fermentation time (h)	46
Initial glucose concentration (g/L)	41.76 ± 2.08
Initial xylose concentration (g/L)	18.28 ± 1.82
Initial arabinose concentration (g/L)	2.54 ± 2.71
Initial cellobiose concentration (g/L)	7.40 ± 1.98
Glucose consumption (%)	100 ± 0.00
Xylose consumption (%)	100 ± 0.00
Arabinose consumption (%)	NC*
Cellobiose consumption (%)	32.43 ± 5.06
Ethanol production (g/L)	31.50 ± 1.57
Ethanol yield (g/g)**	0.49 ± 0.10
Volumetric productivity (g/L.h)	0.68 ±0.12
Biomass production (g/L)	10.41 ± 1.20

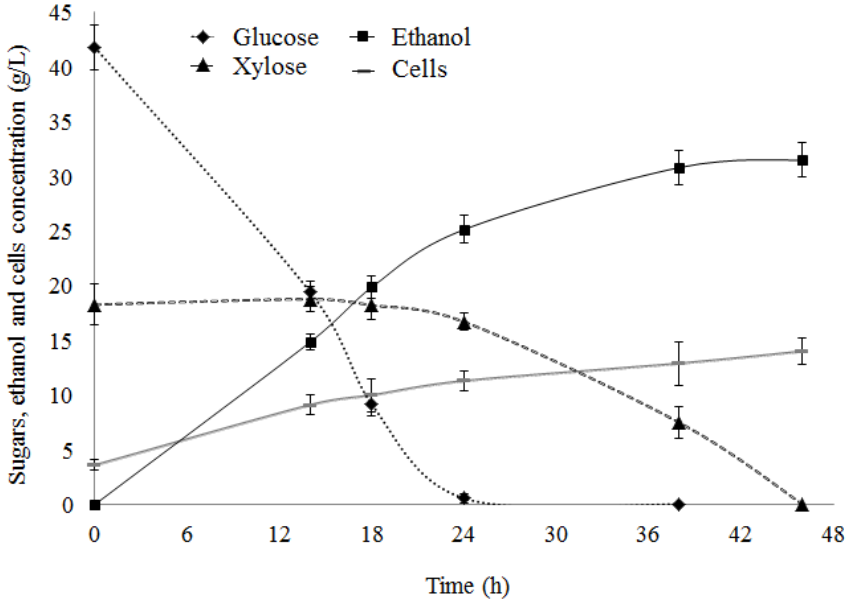
*NC: not consumed, **calculated considering total sugars consumption

Source: Personal archive

As observed in Table 7.5, 31.50 g/L of ethanol were produced, corresponding to 0.49 g/g of yield and 0.68 g/L.h of volumetric productivity. A complete, but non-simultaneous, consumption of glucose and xylose was observed in 46 h of fermentation (Figure 7.3). The glucose was quickly consumed in the first 24 h and,

after this time, xylose consumption was started. This behavior is attributed to a phenomenon known as diauxic shift (repression of D-xylose reductase (XR) and xylitol dehydrogenase (XD) enzymes involved in xylose metabolism by the presence of glucose), as reported by different authors (GUTIÉRREZ-RIVERA et al., 2012; BARI et al., 2013; SANTOS et al., 2015).

Figure 7.3 – Ethanol production, sugars consumption and biomass production profile during fermentation process using SCB hydrolysate in a bubble column reactor.



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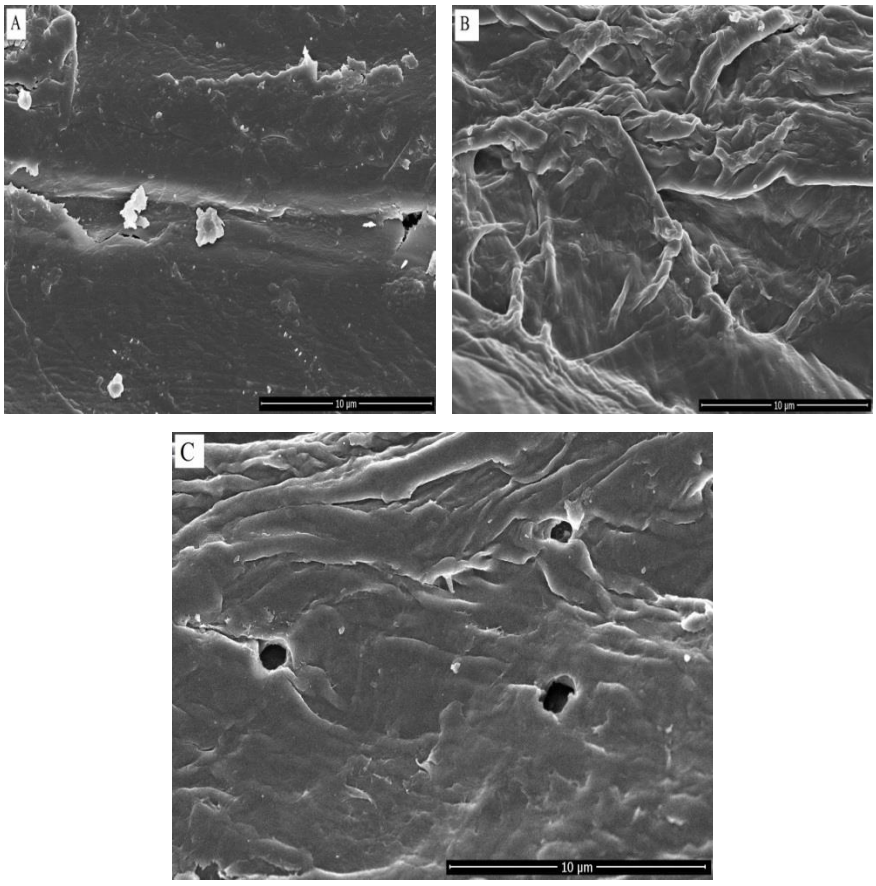
The results obtained using bubble column reactor showed better performance compared to the reported in Erlenmeyer flasks by Terán-Hilares et al. (2016a) using enzymatic hydrolysate of SCB. In that work, 0.4 g/g of ethanol yield and 0.6 g/L.h of productivity were achieved. Results were also higher than from the obtained using different kinds of reactors reported in literature. For example, Nakanishi et al. (2017), e.g., reported the use of stirred tank reactor (STR) in fed batch process and observed a production of 18.52 g/L of ethanol (yield of 0.32 g/g and 0.36 g/L.h of productivity) using *S. stipitis*. In another work, Antunes et al. (2017) reported, for *S. shehatae* cultured in a fluidized bed reactor, production of 5.56 g/L of ethanol, corresponding to 0.34 g/g ethanol yield and 0.18 g/L.h of productivity using enzymatic hydrolysate of SCB as raw material.

7.3.5 Structural characteristics of pretreated SCB

The changes on the surface morphology before and after pretreatment of SCB are shown in Figure 7.4. In this Fig. (7.4A), shows a characteristic surface for untreated biomass which has a rigid and ordered structure with a smooth morphology. On the other hand, surface of pretreated biomass (Fig. 7.4B-C) displayed characteristic roughness. This altered, quite loosened structure on surface of pretreated biomass can be also attributed to mechanical effects of cavitation which corresponds to high speed microjets and shockwaves that are generated during violent collapse of cavities (YUSOF et al., 2016; EBRAHIMI et al., 2017). Therefore, the highest enzymatic digestibility of SCB pretreated under optimized condition is favored by the changes in the structure which increase the surface area and the accessibility of enzyme to carbohydrate fraction.

Figure 7.4 – SEM images of SCB. A) Untreated SCB and B-C) Pretreated SCB under optimized pretreatment conditions (0.29 mol/L of NaOH, 0.78%v/v of H_2O_2 and 9.95 min of HC process).



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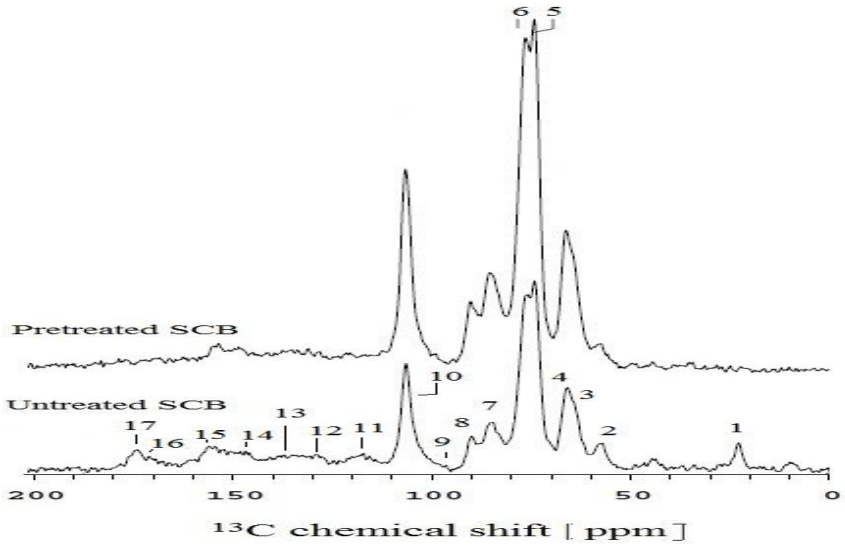
Furthermore, the solid-state ^{13}C NMR spectra of SCB samples was also performed and is shown in Figure 7.5. The chemical shift assignments for lines 1 to 17 (indicated in Fig. 7.5) were based on the comparison with the ^{13}C NMR spectra from sample as cotton (EARL; VANDERHART, 1980), birch pulp (WICKHOLM; LARSSON; IVERSEN, 1998) and sugarcane bagasse (REZENDE et al., 2011; CHANDEL et al., 2014). For our samples, the most intense lines between 50 and 120 ppm region correspond to cellulose carbons (δ: 102–108, 80–92 and 57–67 ppm attributed to the C_1, C_4, and $C_{2,3,5,6}$ signals of cellulose, respectively), but also with contributions from hemicellulose and lignin signals (FOSTON, 2014; IDSTRÖM et al., 2016). A reduction in the hemicellulosic and lignin lines for pretreated sample compared to untreated SCB can be observed; for example, reduction in line 1 (21.5 ppm: CH3 in acetyl groups) and 17 (173.6 ppm: carboxyl groups) could be attributed to hemicellulose removal (REZENDE et al., 2011; FOSTON, 2014). Moreover, reduction in line 2 (56.2 ppm: aryl methoxyl carbons of lignin), 11 (110-115 ppm: C2 and C6 aromatic carbons of syringyl and C5 and C6 aromatic carbons of guaiacyl in lignin) and 16 (163-180 ppm: Carboxyl groups of lignin) can be also attributed to lignin removal by pretreatment (REZENDE et al., 2011; CHANDEL et al., 2014). Besides, reduction in line 12 (126.6 ppm), 13 (134.5 ppm), 14 (148 ppm) and 15 (153.5 ppm) described in the work of Rezende et al. (2014) also confirms the changes in lignin structure due to the pretreatment effects.

Figure 7.5 – Solid state 13C CPMAS-TOSS nuclear magnetic resonance (NMR) spectra of untreated and pretreated SCB under optimized condition



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7.4 Conclusion

In this study, the synergetic effects of alkaline hydrogen peroxide and hydrodynamic cavitation were found effective even at mild treatment conditions for an improved subsequent bioprocessing of SCB fractions. Moreover, a process intensification approach was used for hydrolyzing pretreated SCB at high solids loading in a packed bed flow-through column reactor to yield an appreciable amount of reducing sugars. For the fermentability of hydrolysates into ethanol by *S. stipites*, a bubble column bioreactor was evaluated yielding higher ethanol titers and productivity. Results showed that the evaluated strategy of alkaline-assisted HC pretreatment along with process intensification approach is an interesting potential option for application in lignocellulosic biorefineries.

7.5 Reference

ALVAREZ-VASCO, C.; ZHANG, X. Alkaline hydrogen peroxide (AHP) pretreatment of softwood: Enhanced enzymatic hydrolysability at low peroxide loadings. **Biomass and Bioenergy,** v. 96, p.96-102, 2017.

ANTUNES, F.A.F. et al. A novel process intensification strategy for second-generation ethanol production from sugarcane bagasse in fluidized bed reactor. **Renewable Energy**, *In press*. 2017. http://dx.doi.org/10.1016/j.renene.2017.06.004.

BADVE, M. P. et al. Hydrodynamic cavitation as a novel approach for delignification of wheat straw for paper manufacturing. **Ultrasonics Sonochemistry,** v. 21, p.162-168, 2014.

BARI, I. et al. Bioethanol production from mixed sugars by *Scheffersomyces stipitis* free and immobilized cells, and co-cultures with *Saccharomyces cerevisiae*. **New Biotechnology**, v. 30, p.591-597, 2013.

CAI, M. et al. Decolorization of azo dyes Orange G using hydrodynamic cavitation coupled with heterogeneous fenton process. **Ultrasonics Sonochemistry,** v. 28, p.302-310, 2016.

CARVALHO, D. M.; QUEIROZ, J. H.; COLODETTE, J. L. Assessment of alkaline pretreatment for the production of bioethanol from eucalyptus, sugarcane bagasse and sugarcane straw. **Industrial Crops and Products**, v. 94, p.932-941, 2016.

CHANDEL, A. K et al. Multi-scale structural and chemical analysis of sugarcane bagasse in the process of sequential acid–base pretreatment and ethanol production by Scheffersomyces shehatae and Saccharomyces cerevisiae. **Biotechnology for Biofuels**, v. 7, p.63-73, 2014.

CHEN, H.; FU, X. Industrial technologies for bioethanol production from lignocellulosic biomass. **Renewable and Sustainable Energy Reviews**, v. 57, p.468-478, 2016.

CORREIA, J. A. C. et al. Alkaline hydrogen peroxide pretreatment of cashew apple bagasse for ethanol production: Study of parameters. **Bioresource Technology,** v. 139, p.249-256, 2013.

EARL, W. L.; VANDERHART, D. L. Observations by High-Resolution Carbon- 13 Nuclear Magnetic Resonance of cellulose i related to morphology and crystal structure. **Macromolecules**, v. 14, p.570-574, 1981.

EBRAHIMI, B. et al. Characterization of high-pressure cavitating flow through a thick orifice plate in a pipe of constant cross section. **International Journal of Thermal Sciences**, v. 114, p.229-240, 2017.

E-PIC S.r.l. Biomass pretreatment (BIOGAS), 2016. Available in: http://www.epic-srl.com/ en/cavitationtechnologies/ biomass-pretreatment-biogas. Accessed in 06 Apr. 2017.

FOCKINK D.H. et al. Principles and challenges involved in the enzymatic hydrolysis of cellulosic materials at high total solids. In: SOCCOL C., BRAR S., FAULDS C. (Eds) **Green Fuels Technology**. Green Energy and Technology. Springer, Cham, 2016.

FOSTON, M. Advances in solid-state NMR of cellulose. **Current Opinion in Biotechnology**, v. 27, p.176-184, 2014.

GIERER, J. The reaction of lignin with oxygen-containing species. **In Proceedings of the International Symposium of Wood and Pulping Chemistry**, v. 1, p. 301, 1993.

GUTIÉRREZ-RIVERA, B. et al. Conversion efficiency of glucose/xylose mixtures for ethanol production using *Saccharomyces cerevisiae* ITV01 and *Pichia stipitis* NRRL Y-7124. **Journal of Chemical Technology & Biotechnology**, v. 87, p.263-270, 2011.

HIDENO, A. Short-time alkaline peroxide pretreatment for rapid pulping and efficient enzymatic hydrolysis of rice straw. **Bioresource Technology**, v. 230, p.140-142, 2017.

HIMMEL, M. E. et al. Biomass recalcitrance: engineering plants and enzymes for biofuels production. **Science**, v. 315, p.804-807, 2007.

IDSTRÖM, A. et al. 13C NMR assignments of regenerated cellulose from solid-state 2D NMR spectroscopy. **Carbohydrate Polymers**, v. 151, p.480-487, 2016.

JØRGENSEN, H.; KRISTENSEN, J. B.; FELBY, C. Enzymatic conversion of lignocellulose into fermentable sugars: challenges and opportunities. **Biofuels, Bioproducts and Biorefining,** v. 1, p.119-134, out. 2007.

KIM, I. et al. Hydrodynamic cavitation as a novel pretreatment approach for bioethanol production from reed. **Bioresource Technology,** v. 192, p.335-339, 2015.

KIM, J. S.; LEE, Y. Y.; KIM, T. H. A review on alkaline pretreatment technology for bioconversion of lignocellulosic biomass. **Bioresource Technology**, v. 199, p.42-48, 2016.

LIN, X. et al. Effect of cationic surfactant cetyltrimethylammonium bromide on the enzymatic hydrolysis of cellulose. **Cellulose**, v. 24, p.61-68, 2016.

LIU, Y. et al. Reinforced alkali-pretreatment for enhancing enzymatic hydrolysis of sugarcane bagasse. **Fuel Processing Technology**, v. 143, p.1-6, 2016.

LIU, Y. et al. Reinforced alkali-pretreatment for enhancing enzymatic hydrolysis of sugarcane bagasse. **Fuel Processing Technology,** v. 143, p.1-6, 2016.

MODENBACH, A. A.; NOKES, S. E. Enzymatic hydrolysis of biomass at high-solids loadings – A review. **Biomass and Bioenergy**, v. 56, p.526-544, 2013.

NAKANISHI, S. C. et al. Fermentation strategy for second generation ethanol production from sugarcane bagasse hydrolyzate by *Spathaspora passalidarum* and *Scheffersomyces stipitis*. **Biotechnology and Bioengineering**, *In press.* 2017. Wiley-Blackwell. http://dx.doi.org/10.1002/bit.26357.

NAKASHIMA, K. et al. Hydrodynamic cavitation reactor for efficient pretreatment of lignocellulosic biomass. **Industrial & Engineering Chemistry Research,** v. 55, p.1866-1871, 2016.

NOVACANA. Granbio paralisa temporariamente sua usina de etanol celulósico, 2016. Available in: https://www.novacana.com/n/etanol/2-geracao-celulose/granbio-paralisa-usina-etanol-celulosico-050416/. Accessed in: 1 Aug. 2017.

PERRONE, O. M. et al. Ozonolysis combined with ultrasound as a pretreatment of sugarcane bagasse: Effect on the enzymatic saccharification and the physical and chemical characteristics of the substrate. **Bioresource Technology,** v. 218, p.69-76, 2016.

PORTUGAL-NUNES, D. et al. Effect of cell immobilization and pH on *Scheffersomyces stipitis* growth and fermentation capacity in rich and inhibitory media. **Bioresources and Bioprocessing,** v. 2, p.1-9, 2015.

RABELO, S. C. et al. Alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis and fermentation of sugarcane bagasse to ethanol. **Fuel**, v. 136, p.349-357, 2014.

RAMADOSS, G.; MUTHUKUMAR, K. Mechanistic study on ultrasound assisted pretreatment of sugarcane bagasse using metal salt with hydrogen peroxide for bioethanol production. **Ultrasonics Sonochemistry,** v. 28, p.207-217, 2016.

REZENDE, C. et al. Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility. **Biotechnology for Biofuels**, v. 4, p.54-60, 2011.

ROCHE, C. M. et al. Particle concentration and yield stress of biomass slurries during enzymatic hydrolysis at high-solids loadings. **Biotechnology and Bioengineering**, v. 104, p.290-300, 2009.

SAHA, B. C.; COTTA, M. A. Ethanol production from alkaline peroxide pretreated enzymatically saccharified wheat straw. **Biotechnology Progress**, v. 22, p.449-453, 2006.

SÁNCHEZ, S. et al. The fermentation of mixtures of D-glucose and D-xylose by *Candida shehatae*, *Pichia stipitisor* or *Pachysolen tannophilus* to produce ethanol. **Journal Of Chemical Technology & Biotechnology**, v. 77, p.641-648, 2002.

SANTOS, S. C. et al. Fermentation of Xylose and Glucose Mixture in Intensified Reactors by *Scheffersomyces stipitis* to Produce Ethanol. **International Journal of Biological, Biomolecular, Agricultural, Food and Biotechnological Engineering**, v. 9, p. 539-544, 2015.

SILVA, G. T. et al. Sono-assisted alkaline pretreatment of sugarcane bagasse for cellulosic ethanol production. **Catalysis Today**, v. 269, p.21-28, 2016.

SINGH, R. et al. Experimental study on the performance of microwave assisted Hydrogen peroxide (H_2O_2) pretreatment of rice straw. **Agricultural Engineering International: CIGR Journal**, v. 16, 173-181, 2014.

SIXTA, H. **Handbook of Pulp**. Weinheim, Wiley-VCH, 2006.

SLUITER, A. et al. Determination of Structural Carbohydrates and Lignin in Biomass. Technical report NREL/TP-510-42618 prepared for National Renewable Energy Laboratory, Golden Co., 2011. Available in: http://www.nrel.gov/docs/gen/fy13/42618.pdf. Accessed in 14 Apr. 2017.

TERÁN-HILARES, R. et al. Successive pretreatment and enzymatic saccharification of sugarcane bagasse in a packed bed flow-through column reactor aiming to support biorefineries. **Bioresource Technology,** v. 203, p.42-49, 2016a.

TERÁN HILARES, R. et al. Hydrodynamic cavitation-assisted alkaline pretreatment as a new approach for sugarcane bagasse biorefineries. **Bioresource Technology,** v. 214, p.609-614, 2016b.

TERÁN HILARES, R. et al. Hydrodynamic cavitation as an efficient pretreatment method for lignocellulosic biomass: a parametric study. **Bioresource Technology,** v. 235, p.301-308, 2017a.

TERÁN HILARES, R. et al. Ethanol production in a simultaneous saccharification and fermentation process with interconnected reactors employing hydrodynamic cavitation-pretreated sugarcane bagasse as raw material. **Bioresource Technology,** v. 243, p.652-659, 2017b.

VALIM, I. C. et al. Neural network modeling to support an experimental study of the delignification process of sugarcane bagasse after alkaline hydrogen peroxide pre-treatment. **Bioresource Technology**, v. 243, p. 760–770, 2017.

WICKHOLM, K.; LARSSON, P. T.; IVERSEN, T. Assignment of non-crystalline forms in cellulose I by CP/MAS 13C NMR spectroscopy. **Carbohydrate Research**, v. 312, p.123-129, 1998.

WU, H. et al. Ultrasound-assisted alkaline pretreatment for enhancing the enzymatic hydrolysis of rice straw by using the heat energy dissipated from ultrasonication. **Bioresource Technology**, v. 241, p.70-74, 2017.

YUSOF, N. S. M. et al. Physical and chemical effects of acoustic cavitation in selected ultrasonic cleaning applications. **Ultrasonics Sonochemistry**, v. 29, p.568-576, 2016.

CHAPTER VIII

Conclusion and future works

8.1 Conclusions

The main conclusions are following listed:

— Optimum NaOH- HC pretreatment conditions (0.3mol/L of NaOH, 3 bar and 44.48 min) allow high enzymatic hydrolysis of glucan (more than 95%), which was significantly greater than those obtained using only NaOH and even US-assisted alkaline pretreatment. Moreover, this technology offers a potential advantage compared to US-assisted ones for lignocellulosic bioethanol production process in terms of its lower energy consumption and easier scalability;

— HC-assisted alkaline pretreatment under controlled conditions of temperature (70 °C) and pressure (3 bar) present high efficiency for SCB pretreatment, resulting in high enzymatic cellulose hydrolysis yield (>90%) of material pretreated during 30 min. Moreover, significant changes in the composition and structure of biomass were observed after pretreatment;

— Hydrodynamic cavitation improves the pretreatment efficiency of different alkalis ($Ca(OH)_2$, Na_2CO_3, NaOH, KOH), resulting in high enzymatic digestibility of carbohydrate fractions. Among the evaluated alkali options, potassium hydroxide- and sodium hydroxide-HC assisted processes resulted in highest lignin removal and enzymatic hydrolysis yield for pretreated biomass;

— Total sugars released during enzymatic hydrolysis of NaOH-HC pretreated SCB (pretreatment conditions: 70 °C, 3 bar and 20 min) was slightly higher when cavitation number of 0.017 was used in pretreatment, compared to the result obtained using Cv of 0.048;

— Non-significant reduction in enzymatic hydrolysis yield was observed when the biomass was pretreated using recycled sodium hydroxide black liquor in 10 successive batches with pretreatment process assisted by HC;

— The use of interconnected columns reactor system helped to promote the fermentation efficiency even using high solids contents (11%) of SCB in SSF configuration process, allowing to use optimized conditions of temperature for both biological processes;

— Addition of hydrogen peroxide to sodium hydroxide-HC assisted process significantly improves the pretreatment efficiency in milder condition of temperature and significantly reduces the process time. Besides, significantly improve in lignin removal was also observed in the process;

— Packed bed column reactor presents promising performance to carry out enzymatic hydrolysis of carbohydrate fractions of pretreated SCB at high solids loading (20%), resulting in high fermentable sugars concentration (about 138 g/L of total sugars) and cellulose hydrolysis yield (74.7%) in 24h;

— High conversion yield of sugars into ethanol (Yp/s) by *S. stipitis* was achieved in a bubble column reactor, besides a high volumetric productivity value (Qp). Moreover, both sugars (glucose and xylose) were efficiently metabolized by *S. stipitis* in 46h of fermentation.

8.2 Suggestions for future works

— Evaluation of the combined effect of hydrodynamic cavitation with other potential oxidants besides hydrogen peroxide for pretreatment of sugarcane bagasse;

— Development of continuous process based in hydrodynamic cavitation technology for pretreatment of lignocellulosic biomass;

— Evaluation of profile of pressure, fluid velocity and temperature in different sections of cavitation device aided by Computational Fluid Dynamics (CFD);

— Evaluation of the potential of hydrodynamic cavitation for accelerate the enzymatic hydrolysis process of pretreated SCB or untreated SCB;

— Scale up of hydrodynamic cavitation system and techno-economic evaluation of this technology for pretreatment process.

APPENDIX

Appendix 1 – List of publications, book chapter and patent

1. Terán Hilares, R., Ramos, L., Silva, S. S., Dragone, G., Mussatto, S. I., Santos, J. C. Hydrodynamic cavitation as a strategy to enhance the efficiency of lignocellulosic biomass pretreatment. **Critical Review in Biotechnology**, *in press*. 2017.

2. Terán Hilares, R., Ienny, J. V., Marcelino, P. F., Ahmed, M. A., Antunes, F. A. F., Silva, S. S., Santos, J. C. Ethanol production in a simultaneous saccharification and fermentation process with interconnected reactors employing hydrodynamic cavitation-pretreated sugarcane bagasse as raw material. **Bioresource Technology**, v. 243, p. 652-659, 2017.

3. Terán Hilares, R., Faria de Almeida, G., Ahmed, M. A., Antunes, F. A. F., Silva, S. S., Han, J-I., Santos, J. C. Hydrodynamic cavitation as an efficient pretreatment method for lignocellulosic biomass: a parametric study. **Bioresource Technology**, v. 235, p. 301-308. 2017.

4. Terán Hilares, R., Orsi, C. A., Ahmed, M. A., Marcelino, P. F., Menegatti, C. R., Silva, S. S., Santos, J. C. Low-melanin containing pullulan production from sugarcane bagasse hydrolysate by *Aureobasidium pullulans* in fermentations assisted by light-emitting diode. **Bioresource Technology**, v. 230, p. 76-81, 2017.

5. Terán Hilares, R., Swerts, M. P., Ahmed, M. A., Ramos, L., Silva, S. S., Santos, J. C. Organosolv pretreatment of sugarcane bagasse for bioethanol production. **Industrial Engineering Chemistry Research**, v. 56, p. 3833-3838, 2017.

6. Terán-Hilares, R., Chirinos, R., Pedreschi, R., Campos, D. Enhanced antioxidant properties of tara gallotannins by thermal hydrolysis and its synergistic effects with α-tocopherol, ascorbyl palmitate and citric acid on sacha inchi oil. **Journal of Food Process Engineering**. *In press*, 2017.

7. Terán-Hilares, R., Reséndiz, A. L., Martínez, R. T., Silva, S. S., Santos, J. C. Successive pretreatment and enzymatic saccharification of sugarcane bagasse in a packed bed flow-through column reactor aiming to support biorefineries. **Bioresource Technology**, v. 203, p. 42-49. 2016.

8. Terán Hilares, R., Santos, J. C., Ahmed, M. A., Jeon, S. H., Silva, S. S., Han, J-I. Hydrodynamic cavitation-assisted alkaline pretreatment as a new approach for sugarcane bagasse biorefineries. **Bioresource Technology**, v. 214, p. 609-614. 2016.

9. Ahmed, M. A., Seo, Y. H., Terán-Hilares, R., Rehman, M. S. U., Han, J-I. Persulfate based pretreatment to enhance the enzymatic digestibility of rice straw. **Bioresource Technology**, v. 220, p. 523-526. 2016.

10. Ahmed, M. A., Rehman, M. S. U., Terán-Hilares, R., Khalid, S., Han, J-I. Optimization of twin gear-based pretreatment of rice straw for bioethanol production. **Energy Conversion and Management**, v. 141, p. 120-125. 2016.

11. Antunes, F.A. F.; Chandel., A. K.; Brumano, L.P.B.; Terán Hilares R.; Peres, G. F. D.; Ayabe, L. E. S.; Sorato, V. S.; Santos. J. R., Santos, J.C.; Da Silva, S.S. A novel process intensification strategy for second-generation ethanol production from sugarcane bagasse in fluidized bed reactor. **Renewable Energy**. *In press*.

12. Antunes, F. A. F.; Santos, Júlio César. ; Cunha, M. A. A.; Brumano, l. P.; Milessi, Thaís Suzane dos Santos; Terán Hilares, R; Peres, Guilherme Fernando Dias; Dussan, K. J.; Silva, D.D.V.; Dalli, S. S. ; Gaikwad, S.; Da Silva, Silvio S . Biotechnological production of xylitol from biomass. In: Fang Z; Smith; Qi. (Editors). Biofuels and biorefineries. Springer International Publishing AG: 2017, p 311-342.

13. Pedido de patente depositado (Nº BR 10 2017 010734 5) no Instituto Nacional da Propriedade Industrial - I.N.P.I./S.P. "Processo para a produção de pululana isenta de pigmento melanina por *Aureobasidium pullulans* em processos fermentativos auxiliados por luz visível, pululana isenta de pigmento melanina, e usos da mesma". Ruly Terán Hilares, Camila Ayres Orsi, Carlos Renato Menegatti, Silvio Silvério da Silva, Júlio César dos Santos. 2017.