Effect of hydrodynamic cavitation over simultaneous process of pretreatment and enzymatic hydrolysis of sugarcane bagasse

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ABSTRACT

The developing technology for second-generation ethanol production involves three initial steps to convert the carbohydrates in biomass into biofuel: pretreatment, enzymatic hydrolysis, and fermentation. For a sustainable biorefinery, it is essential that these steps be carried out efficiently and in an economically favorable manner. However, several challenges must be overcome, such as the cost of pretreatment and the enzymes used in the process. Therefore, new alternatives have been proposed, and hydrodynamic cavitation (HC)-assisted pretreatment techniques have shown promise. In this work, the pretreatment and enzymatic hydrolysis steps of sugarcane bagasse were simultaneously performed using an HC-assisted process. The process was conducted for 24 hours in a cavitation reactor, with a control experiment performed in the same system but without HC (i.e., without orifice plate). The potential of this new approach was demonstrated, with the HC-assisted process resulting in a 45% hydrolysis yield of glucan in 24 hours of the combined pretreatment/enzymatic hydrolysis process, compared to only a 7% hydrolysis yield in the control experiment.

Keywords: Pretreatment. Enzymatic hydrolysis. Hydrodynamic cavitation. Simultaneous processes. Biorefinery.

1 INTRODUCTION

The intensive use of fossil fuels generates environmental problems, as it induces the production of harmful gases that exacerbate the greenhouse effect.[1] As a result, biorefineries have been receiving attention for their use of renewable raw materials, which are essential for the global transition from fossil fuel-based energy sources to a sustainable bioeconomy. One promising alternative for the development of biofuel production processes is the utilization of lignocellulosic biomass to produce second-generation (2G) ethanol[2].

In this context, an important and highly available material in Brazil is sugarcane bagasse (SCB). Indeed, Brazil is the world's largest producer of sugarcane and, after extracting its juice in the sugar and ethanol industry, a large amount of sugarcane bagasse (SCB), an important lignocellulosic biomass, is generated. Most of the SCB is used for energy production in the plants themselves; however, this biomass is a rich source of carbon for which research into alternative uses is highly desirable. [3]

Aiming to modify the structure of SCB for better utilization of carbohydrate fractions present, a pretreatment step is necessary. The pretreatment is responsible for modify the structure and composition of the material, making the cellulose and hemicellulose more susceptible to enzymatic hydrolysis in a subsequent step.[4] There are many different pretreatments, such as alkaline, acid, organosolv or steam explosion; however, these pretreatment techniques have some drawbacks, for example, harsh process conditions, corrosion of equipment and formation of biological inhibitory compounds. [4, 5] Actually, despite significant progress in advancing research on the conversion of sugars into bioproducts, the pretreatment remains a persistent bottleneck due to the lack of competitive, low-cost processes.[5] An alternative is to assist pretreatment process by hydrodynamic cavitation, which has gained attention in recent years due to its simple system, no timing consuming, and to the fact that it operates under mild conditions and does not form biological inhibitors. [6]

After pretreatment, the cellulose-rich solid material undergoes the biological stages of enzymatic hydrolysis and fermentation. In hydrolysis, a glucose-rich juice is produced, which can be converted into ethanol by the same yeasts traditionally used in 1G ethanol production, followed by alcohol distillation.[3,5] The hemicellulosic fraction is also rich in sugars (pentoses), which can be obtained in their monomeric form either by hydrolysis in the pretreatment or during the enzymatic hydrolysis stage, depending on the pretreatment method employed.[5] If there is hemicellulose in the pretreated solid material, it can be hydrolyzed biotechnologically, with xylanolytic enzymes commonly present in commercial cellulase enzyme preparations. Pentoses can also be converted into ethanol, requiring specific or genetically modified microorganisms for this purpose. Other products of interest can also be obtained, and thus ethanol production has been evaluated within a broader context of multiproduct industrial facilities, known as biorefineries.[7,8] In this case, in addition to ethanol, other higher-value products can be derived from the raw material, enhancing the overall feasibility of the process.

Studies on the combined processes of hydrolysis and fermentation are commonly found; however, the simultaneous process of enzymatic hydrolysis and pretreatment are still relatively underreported.[9] This alternative can be advantageous, potentially favoring the viability of the process by combining two steps in one equipment. Until now, there are no previous report regarding to the use of HC to assist a combined pretreatment and enzymatic hydrolysis process.

This work aimed to evaluate the simultaneous pretreatment and enzymatic hydrolysis assisted by hydrodynamic cavitation. Untreated SCB was charged in a hydrodynamic cavitation reactor, together with a commercially available cellulase preparation. Control experiment was conducted using the same amount of enzyme but without the hydrodynamic cavitation phenomenon, which was correspondent to perform the process without using the cavitation generator device (orifice plate).

2 MATERIAL & METHODS

The sugarcane bagasse sample donated by Usina Ipiranga Agroindustrial (Descalvado, Sao Paulo, Brazil) was previously dried to approximately 10% moisture content using the oven Thermosolda Ltda (VF2x2x2, series 46322), and then stored. The bagasse was milled using a knife mill (Mill Benedetti 270) and classified in Tyler series sieves (-14/+20 MESH). The biomass pretreatment simultaneous with enzymatic hydrolysis was conducted in a hydrodynamic cavitation reactor designed at the LBBSIM/EEL-USP (Figure 1). The main components of the system are the centrifugal pump (1.5 HP), the cavitation zone, and the recirculation tank. The cavitation phenomenon is generated using an orifice plate (16 holes with a 1 mm diameter). The system also includes accessories such as pressure gauges and valves for controlling the pressure and temperature of the fluid.

Pretreatment along with enzymatic hydrolysis assisted by hydrodynamic cavitation was conducted in a system with a total volume of 3.2 liters, loaded with 32g of bagasse (dry mass), pressure of 4 atm upstream of the cavitation generator device (orifice plate), and maintained for 24 hours. During the 24-hour reaction, 6 samples were taken (2h, 4h, 6h, 8, 12h, and 24h), which were subsequently analyzed by high-performance liquid chromatography (HPLC) (Agilent Technologies 1200 series). The medium used in the system had a pH value of 4.8, obtained using a 0.05 M citrate buffer. The process was maintained at a temperature of 50°C, and performed using 1% of H_2O_2 and 20 FPU.$g_{dry\ mass}^{-1}$ of the enzymatic preparation Cellic Ctec2 (purchased from Merck S.A., São Paulo, Brazil). Before the onset of cavitation, the SCB was placed in an Erlenmeyer flask along with 800mL of previously prepared buffer solution. Following, flask was taken to an ultrasonic bath (Unique USC-2800) with vacuum application for 30 minutes. This step was carried out to ensure that the used bagasse was deaerated. Control experiments were conducted applying the same process conditions, however without the orifice plate, to evidence the hydrodynamic cavitation effect.

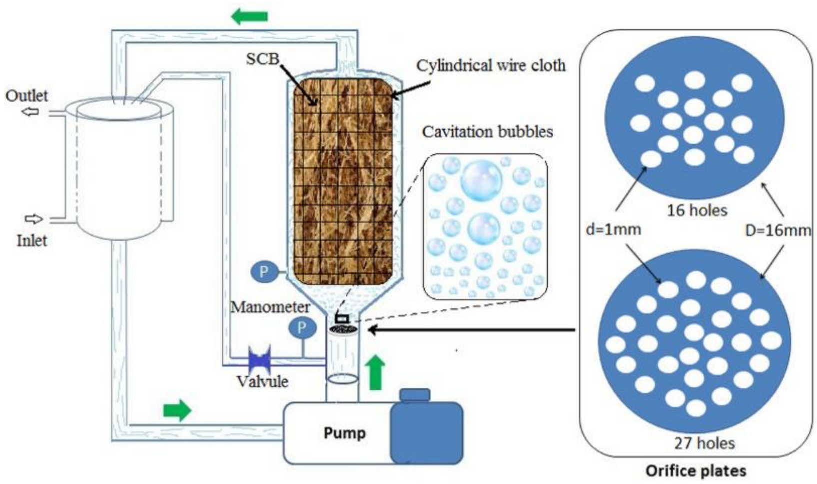

Figure 1– Schematic representation of Hydrodynamic Cavitation system.[10]

3 RESULTS & DISCUSSION

The characterization of SCB was carried out and its chemical composition is shown in Table 1. Based on data from biomass characterization and HPLC sugar concentration, it was to determine the Kinect profile of hydrolysis yield of glucan and xylan along the process, showed in Figure 2.

Table 1 – Chemical characterization of raw sugarcane bagasse

Components	Content (%)
Lignin	22.0 ± 0.5
Glucan	44.1 ± 0.9
Xylan	26.0 ± 0.4
Arabinosyl	1.9 ± 0.2
Acetyl	0.8 ± 0.3
Extractive	3.3 ± 0.9
Ash	1.8 ± 0.1

As shown in Figure 2, hydrodynamic cavitation had a positive effect on the simultaneous process of pretreatment and enzymatic hydrolysis, resulting in an increase in the observed percentage of final hydrolysis yield of glucan and xylan by 6.7 and 2.7 times, respectively, when compared to control. As also shown in Figure 2, a higher hydrolysis rate was observed in the first 8h of process, with slower reactor rate after this time. This behavior can be attributed to a fast hydrolysis of the glucan and xylan fractions more accessible to the enzymes at the beginning of the process, remaining a more recalcitrant fraction (cellulose more crystalline, for example) after 8h. Another hypothesis can be related to enzymes inactivation along the process, which must be investigated in future work.

HC-assisted pretreatment has been demonstrated as beneficial for pretreatment of biomass, which is due to both chemical and physical effects of HC.[10,11] HC can generate oxidative radicals •OH, which results in break of linkages of the structure of biomass, particularly lignin, increasing the enzymatic accessibility of the carbohydrate fractions of the material. Additionally, high velocity microjets are generated when cavitation microbubbles implode, modifying the porous structure of the biomass. Both chemical and physical effects of HC were already shown as beneficial for SCB pretreatment,[8,10,11] but simultaneous pretreatment and enzymatic hydrolysis has not been previously reported. As shown, HC effects were also beneficial in this case, resulting in enhanced enzymatic hydrolysis yield compared to the control.

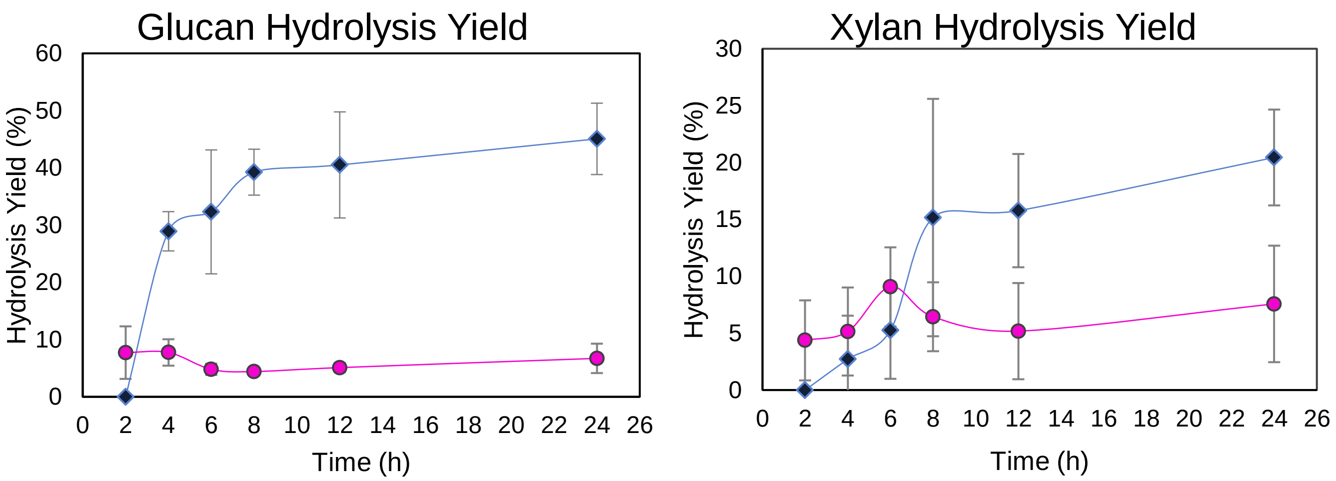

Figure 2 Kinect profile of glucan and xylan hydrolysis yield applying (-♦-) HC Assisted Process, (-•-) Non- HC Assisted Process (control)

Considering the simultaneous process was not previously reported in literature, after showing the potential of the technique, as performed in the present work, future studies should be directed to process optimization aiming to increase the hydrolysis yield. In this case, variables as concentration of peroxide, loading of enzymes and biomass should be evaluated, besides extending the process time, also evaluating a possible enzyme activity loss along the process due to HC effects.

4 CONCLUSION

HC-assisted pretreatment and enzymatic hydrolysis simultaneous process had shown better results when compared to control experiments (non- HC assisted process), highlighting the positive effect of hydrodynamic cavitation. It was observed an enhancement by 6.7 and 2.7 times of glucan and xylan hydrolysis yield, respectively, when HC was used. Simultaneous pretreatment and enzymatic hydrolysis process is promising because, in addition to reducing total costs with equipment and utilities, the reaction time of the upstream stages is reduced by combining two processes into a single reactor. This was a pioneer study about simultaneous pretreatment and enzymatic hydrolysis assisted by HC and, after showing the potential of the technique, future studies are indicated aiming to optimize the process, also evaluating possible loss of enzymes activity along the process.

REFERENCES

[1] SHARMA, B., LARROCHE, C., DUSSAP, G. C. 2020. Bioresour. Technol. 313.
[2] LOAIZA S. S., GARCIA C. E., CARDONA. A. C. 2018. Bioresour. Technol. 126. 92-102.
[3] SHIBUKAWA, V. P., REIS, C. E. R., dos SANTOS, J. C., da RÓS, P. C. M. 2023. Braz. J. Chem. Eng.
[4] KARP G. S., WOICIECHOWSKI L. A., SOCCOL T. V., SOCCOL R. C. 2013. Braz. Arch. Biol. Techn. 56(4). 679-689.
[5] VASCONCELOS, M. H., MENDES, F. M., RAMOS, L., DIAS, M. O. S., BONOMI, A., JESUS, C. D. F., WATANABE, M. D. B., JUNQUEIRA, T. L., MILAGRES, A. M. F., FERRAZ, A., dos SANTOS, J. C. 2020. Energy. 199.
[6] TERÁN-HILARES, R., RAMOS, L. da SILVA, S. S., DRAGONE, G., MUSSATTO, S. I., dos SANTOS, J. C. 2017. Crit. Rev. Biotechnol. 38(4). 483-493.
[7] GIULIANO, A., POLETTO, M., BARLETTA, D. 2016. Chem. Eng. Res. Des. 107. 236-252.
[8] ANTUNES, F. A. F., CHANDEL, A. K., TERÁN-HILARES, R., MILESSI, T. S. S., TRAVALIA, B. M., FERRARI, F. A., HERNANDEZ-PÉREZ, A. F., RAMOS, L., MARCELINO, P. F., BRUMANO, L. P., SILVA, G. M., FORTE, M. B. S., SANTOS, J. C., FELIPE, M. G. A., da SILVA, S. S. 2019. Sugarcane Biofuels. *In*: Biofuel Production from Sugarcane in Brazil. KHAN, M., KHAN, I. 1st ed. Springer, Cham. Germany. 99-121.
[9] SUDIYANY, Y., DAHNUM, D., PUTRI, H. M. A. 2019. Evaluation and comparison between simultaneous saccharification and fermentation and separated hydrolysis and fermentation process. *In:* Second and third Generation of Feedstocks: The Evolution of Biofuels. BASILE, A., DALENA, F. 1st ed. Science Direct, Netherlands. 273-290.
[10] TERÁN-HILARES, R., ALMEIRDA, G. F., AHMED, A. A., ANTUNES, F. A. F., da SILVA, S., HAN, J. I., dos SANTOS, J. C. 2017. Bioresour. Technol. 235. 301-308.
[11] PRADO, C. A., ANTUNES, F. F., ROCHA, T. M., MUÑOZ, S. S., BARBOSA, F. G., TERÁN-HILARES, R., CRUZ-SANTOS, M. M., ARRUDA, G. L., da SILVA, S. S., SANTOS, J. C. 2022. Bioresour. Technol. 345.

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