HYDROTHERMAL PRETREATMENT OF SUGARCANE BAGASSE AIMING ENZYMATIC HYDROLYSIS FOR THE PRODUCTION OF GLUCOSE SYRUPS

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ABSTRACT

The anthropogenic impact on the environment has altered the climate at an unprecedented pace, primarily through the exploitation of fossil reserves, particularly oil, leading to the production of greenhouse gases and contributing significantly to climate change. In this scenario, the use of fuels derived from non-polluting renewable sources is crucial for environmental preservation. Brazil stands out in the utilization of green energy matrices and has been actively investing in their expansion. This study aims to evaluate hydrothermal pretreatment to render the cellulose content of sugarcane bagasse available for enzymatic hydrolysis, ultimately producing glucose syrups for fermentation into the biofuel ethanol. The pretreatment conditions were organized using a 2^2 Central Composite Design (CCD), with treatment time and temperature as independent variables. An increase in temperature resulted in a reduction in hemicellulose concentration and the formation of well-known inhibitors such as furfural, which negatively interfere with subsequent steps of enzymatic hydrolysis and ethanol fermentation. However, treatments performed at 134.5ºC for 17.5 minutes, 134.5ºC for 77.5 minutes, and 120ºC for 47.5 minutes resulted in treated materials with a chemical composition similar to that of untreated sugarcane bagasse. Further statistical analysis and enzymatic hydrolysis to obtain glucose syrups will be conducted in the continuation of this work.

Keywords: Hydrothermal pretreatment, Sugarcane bagasse, Central Composite Design, Cellulose

1 INTRODUCTION

Accessibility to the substrate is a critical requirement for all fermentative processes, and the use of lignocellulosic biomass as raw material faces this technological bottleneck. Despite being rich in fermentable sugars, biomass such as sugarcane bagasse cannot make these substrates available directly, necessitating physical, chemical, or biological treatments[1].

Hydrothermal pretreatment of various feedstocks is a technological option that renders biomass suitable to produce a variety of renewable fuels, such as ethanol and biogas (a mixture of CH_4 and CO_2), via fermentation. This process is conducted in closed systems, under elevated temperatures and pressure, using water as the solvent and catalyst for chemical reactions that expose cellulose and partially remove hemicellulose content[2]. Subsequently, the hydrothermally pretreated biomass can be used to produce monosaccharide syrups through multi-enzymatic reactions and be converted into products of interest through fermentation[3].

2 MATERIAL & METHODS

The hydrothermal pretreatment was carried out through a 2^2 Central Composite Design (CCD) using temperature and treatment time as variables, totaling 11 experiments (4 axial points, 4 factorial points, and 3 central points). Table 1 shows the coded and real values of the factorial, central, and axial points.

Table 1 Matrix for complete Central Composite Design, 2^2.

Variables\Coded values	-1,41	-1	0	1	1,41
Temperature (X_1, ºC)	120	134,5	170	205,5	220
Pretreatment time (X_2 min.)	5	17,5	47,5	77,5	90

The sugarcane bagasse was provided by EMBRAPA Agroindustrial (Brasília/DF) and sent to the Bioethanol Laboratory. Before the hydrothermal pretreatment test, the bagasse was processed in a cutting mill to standardize the material to be treated. The hydrothermal pretreatment was carried out in a Parr Instruments reactor model 4550 with a capacity of 3.78L. For each test, 100 g of biomass (moisture content below 10%) and 1L of distilled water were added to the reactor. The pH was checked and, if necessary, adjusted so that the mixture was in the pH range of 5 to 6. The reactor was then sealed with safety locks, purged with CO_2 to a pressure of 5 bar, and left to drain slowly through the exhaust valve (this procedure was repeated five times consecutively). Then, the reactor was pressurized to a working pressure of 10 bar, the temperature was set according to the experiment, and the stirrer was set to 100 RPM. The treatment start point was considered when the set temperature was reached (Table 1). After the treatment time (Table 1), the reactor was cooled down to 40 °C, and the reactor was opened to recover the pretreated material. The recovered material was separated by filtration using a vacuum pump. The liquid fraction was frozen and stored for later analysis, and the solid phase was washed and dried for its chemical characterization.

The chemical composition of the solid phase was determined via acid hydrolysis in triplicate to achieve complete hydrolysis of cellulose and hemicellulose. Approximately 300 mg of pretreated dry biomass (moisture content below 10%) was placed in hydrolysis flasks, added with 3 mL of 72% sulfuric acid, and maintained under constant agitation for 1 hour at 30°C. After this time, 84 mL of distilled water was added to achieve a final concentration of 4% sulfuric acid. After this adjustment, the hydrolysis flasks were autoclaved at 1 atm, 121°C for 1 hour and cooled at room temperature. In the end, the samples were filtered, the solid fractions were dried and weighed for insoluble residue determination (lignin and ashes), while the liquid fractions were used for UV-spectroscopy determination of soluble lignin and biomass-derived monosaccharides quantified by HPLC.

3 RESULTS & DISCUSSION

Table 2 presents the treated material chemical composition as for cellulose, hemicellulose, and lignin content in comparison to that of untreated sugarcane bagasse. The increase in temperature from 134ºC to 220ºC reduced the normalized hemicellulose concentration and increased lignin concentration without a considerable effect on the cellulose content compared to untreated sugarcane bagasse. The decrease in hemicellulose content was likely due to thermal degradation that leads to the formation of inhibitors such as furfural and the acids acetic and formic[2].

The highest cellulose content (over 50%), in the treated material, was observed for central points (170ºC/47.5 min) and condition 8 (170ºC/90 min). However, it was also observed that the hemicellulose content was around 30% of that for the untreated bagasse, indicating hemicellulose degradation and the formation of inhibitors that would negatively impact the subsequent stages of this study. Therefore, considering that the objective of this work is to produce, via enzymatic hydrolysis, fermentable glucose syrups the treatment conditions 1 (134.5ºC/17.5 min), 3 (134.5ºC/77.5 min), and 5 (120ºC/47.5 min) that show chemical composition similar to that of untreated bagasse and possibly lower inhibitor formation, would be selected as most viable for the continuation of the study.

Table 2 Chemical composition of sugarcane bagasse in different conditions proposed by 2^2 CCD.

Experiment	Temperature (X_1, ºC)	Pretreatment time (X_2 min.)	Cellulose (%)	Hemicellulose (%)	Lignin (%)
1	134.5	17.5	28.59	30.55	22.67
2	205.5	17.5	32.03	0.73	37.63
3	134.5	77.5	36.91	29.91	23.57
4	205.5	77.5	39.73	0.00	42.54
5	120	47.5	41.68	30.32	21.78
6	220	47.5	41.06	0.00	51.71
7	170	5.0	25.48	18.14	25.24
8	170	90.0	53.27	5.71	32.05
9	170	47.5	56.52	9.48	28.86
10	170	47.5	54.18	8.47	31.47
11	170	47.5	55.64	8.80	29.10
Untreated sugarcane bagasse			38.96	27.81	19.95

The observed results are consistent with the literature and suggest that temperature has a greater effect on chemical composition than treatment time. Furfural and organic acids presence in enzymatic hydrolysis stage can inhibits directly by enzyme binding, altering its conformation, or indirectly by changing the pH of the reaction medium, making it less favorable for enzymatic activity. During the fermentation stage, these compounds can interfere with cell-substrate interactions or cause damage to the fermentation agents. These data will still undergo statistical analysis, and subsequently, the pretreated materials of conditions 1, 3 and 5 will be subjected to enzymatic hydrolysis with glucose concentration monitoring[3,4] as well as sugars yields and recovery parameters.

4 CONCLUSION

Temperature increasing is probably a determinant factor for hemicellulose degradation and the normalized lignin higher content. Hemicellulose thermal degradation leads to the formation of inhibitors such as furfural and the acids acetic and formic, conditions that should be avoided in the sugarcane bagasse hydrothermal treatment. As such, the sugarcane bagasse hydrothermal pretreatment conditions 1 (134.5ºC/17.5 min), 3 (134.5ºC/77.5 min), and 5 (120ºC/47.5 min) are promising to be further studied in enzymatic hydrolysis experiments to produce glucose syrups.

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