GLUCOSE RELEASE FROM UNTREATED AND ACID PRETREATED CORN COB VIA ACID AND ENZYMATIC HYDROLYSIS

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ABSTRACT

Glucose is a raw material of great importance in different industrial sectors such as food, pharmaceutical, chemical, and textile. This study aims to recover glucose from corn cob, a lignocellulosic biomass composed of hemicellulose, cellulose, and lignin which presents a range of possibilities for use in bioprocesses. For this, physical and chemical pre-treatment processes were used on the material to be exposed to acid and enzymatic hydrolysis. The experiments were conducted to analyze the glucose concentration under different solid loading conditions, 2%, 5%, 8%, 10%, 20%, 40%, and 60% (%w/v). *In natura* corncob and acid pre-treated were exposed to acid hydrolysis. In enzymatic hydrolysis only pre-treated corn cob was used, different enzymatic loads (5 and 10 FPU/g), different amounts of solids, 2%, 5%, 10%, 20%, and 30% (%w/v), and the use of Tween 80 surfactants to analyze their influence on the process. Overall, the results show that both approaches were effective in glucose recovery, with most cellulose to glucose conversion values above 50%, with the process carried out with enzymes and surfactant being the process with the highest concentration of glucose, reaching 92.47 g/L.

Keywords: Biomass. Hydrolysis. Glucose.

1 INTRODUCTION

The search to replace the use of fossil fuels in energy generation with clean and sustainable alternatives is increasingly central in the research and development of new technologies. Brazil has a competitive advantage in the use of biomass due to its vast biodiversity and pioneering in the production of biofuels, such as ethanol and bioethanol[1]. As a result, the use of biomass has gained increasing popularity due to its environmental appeal, both in the production of energy and in raw material products for the development of more sustainable technical evolution. Vegetable biomass can be divided into two distinct fractions: the noble fraction, which contains grains, sucrose and vegetable oils, normally intended for food production; and the fibrous fraction, subdivided into cellulose, hemicellulose and lignin[1]. Cellulose, a polysaccharide composed of long glucose chains, is widely used in the manufacture of paper, cloth and bioplastics. Hemicellulose made up of a set of polysaccharides that include sugars such as xylose, arabinose and mannose, can be used in the production of biofuels[1]. Certain procedures are essential for the separation of polymers from the fibrous fraction of lignocellulosic biomass. These procedures, called pretreatment, cover a series of chemical, physical or biological processes applied to the raw material before its conversion. The objective of these procedures is to remove or separate components that present resistance or that may hinder the efficiency of conversion[2]. In this study, the main objective was to recover glucose from corn cobs by performing two types of pretreatments on the biomass used: a physical treatment, involving grinding the corn cobs to increase its interfacial area, and an acid pre-treatment at high temperature in a part of the biomass to decrease the level of hemicellulose in the biomass. The acid and enzymatic hydrolysis processes were then used to find the ideal condition for the glucose concentration obtained and the conversion in each process.

2 MATERIAL & METHODS

Ground corncob was used as a source of cellulose. Part of the biomass was subjected to acid pretreatment with sulfuric acid following a consolidated methodology. In 1000 mL Erlenmeyer flasks using 1% (v/v) sulfuric acid mixed with ground corn cob with a solids load of 10% (w/v) and placed in an autoclave at 121 °C for 60 minutes[3]. After cooling, the mixture was filtered with a cloth and the solids were washed with tap water at least 3 times. After this process, the solids were separated and dried in the oven for 24 hours and then stored in a dark bag. Table 1 presents the chemical composition of the corn cob under both conditions.

Table 1: Chemical composition of corn cob treated and untreated

Samples	% Cellulose	% Hemicellulose	% Lignin
Untreated	32.15	34.37	17.23
Pretreated	63.92	3.87	20.72

In analyzing the processes, the percentage of glucose recovery was calculated for each condition according to Equation 1 below. Where [G] represents the concentration of glucose in grams per liter, V is the final volume of hydrolysis in liters, M is the mass of corn cob used in each condition, %cellulose is the percentage of cellulose present in the biomass and 1.1 is the cellulose to glucose conversion factor.

$$REC\ (\%) = \frac{[G] \times V}{M \times \%cellulose \times 1.1} \quad (1)$$

Using glass tubes closed with screw caps, untreated and pretreated corn cobs were placed for different solids loads 2%, 5%, 8%, 10%, 20%, 40% and 60% (w/v); and 1.5 mL of 72% sulfuric acid. The tubes were homogenized and kept at room temperature for 1 hour, followed by the addition of distilled water to dilute the acid to a concentration of 30% and heated with water at a temperature between 90 °C and 100 °C for 2 hours. After heating, 10 mL of distilled water was added to each tube and placed in the freezer to stop the hydrolysis process. Samples were transferred to 15 mL falcon tubes and centrifuged for 3 minutes at 3000 rpm, the supernatant was filtered through 0.44 µm membrane syringe filters and placed in 2 mL Eppendorf tubes for liquid chromatographic analysis (HPLC). Enzymatic hydrolysis experiments were conducted in 25 mL Erlenmeyer flasks containing acid pretreated corn cob with solids loadings of 5%, 10%, 20% and 30% (%m/v), sodium citrate buffer (50 mM, pH 4.8), cellulases (5 and 10 FPU/g), 2% (m/m) Tween 80 and 0.01% (m/v) sodium azide for a final volume of 3 mL. Under conditions of 20% and 30% (m/v) solids loading, a 10% fed-batch system was used every 12 hours. To evaluate the effects of the surfactant, experiments were also carried out in the absence of Tween 80 and with the replacement of the sodium citrate buffer (50 mM, pH 4.8). All flasks were incubated in a shaker at 50 °C and 150 rpm for 96 h and the resulting supernatants were collected, diluted and filtered for analysis. As with acid hydrolysis, the results obtained through HPLC analysis, calibration curves and the dilution factor, allowed us to find the concentrations in grams per liter of glucose and xylose released.

Sugars (cellobiose, xylobiose, glucose, xylose, arabinose), organic acids (formic acid, acetic acid, levulinic acid) were quantified by HPLC analysis using SCR101-H column (7.9 x 300 mm) and refractive index detector. The mobile phase was prepared with 5 mM sulfuric acid, with a flow rate of 0.6 mL/min, and the oven temperature was 50 °C[3].

3 RESULTS & DISCUSSION

Samples with different solid loads were analyzed for the same volume of sulfuric acid and contact and heating time. In the experiments carried out after the analysis, the presence of glucose, xylose, cellobiose and arabinose, as shown in Figure 1.

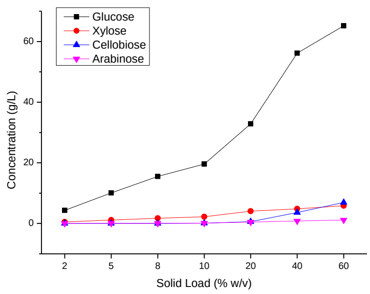
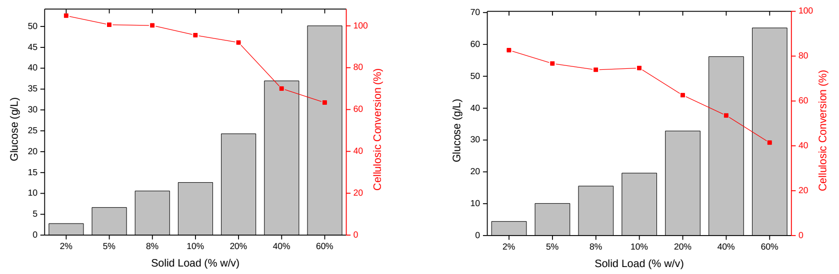


Figure 1: Concentration of sugars released after acid hydrolysis process using pretreated corn cob and 30% diluted sulfuric acid.

Both pretreated and untreated biomass showed an increase in glucose concentration with increasing solids load. Figure 2 shows the concentrations found in both types of corn cob used. In corn cobs without pre-treatment, the concentration ranged from 2.77 g/L, at a solids load of 2%, to 50.16 g/L, with a solids load of 60%. In the pretreated corn cob, under the same solids loading conditions the variation was from 4.43 g/L to 65.19 g/L. Although this is expected and satisfactory behavior, along with the increase in glucose, other sugars are also released, despite less intensity, which can be harmful to some processes.

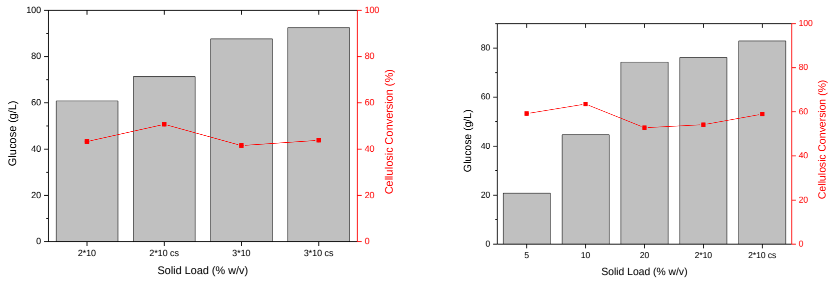
Regarding the conversion of this experiment, the interest is to discover how much of the cellulose present in the biomass used is being converted into glucose, which is the product of interest. In the case of corn cob without pretreatment, as it has a lower percentage of cellulose, it is acceptable for the conversion to reach high values, especially in the initial cases where the quantity of solids is low and the acid does not find it difficult to permeate through the solid and the hydrolysis process occurs. The percentage values found in the initial cases are approximately 100%, and the minimum conversion found in the 60% solids load condition was 63.35%, still a considerably good result for this process, in this case, due to the glassware used, the greater the amount of solids present, the greater the difficulty for the acid to homogenize. These same difficulties are also found in the case of pretreated biomass, in addition, in its chemical composition, the percentage of cellulose is even higher due to the decrease in hemicellulose during the acid pre-treatment, therefore, it is expected that the values of conversion in this case are lower when compared to untreated biomass, even though the cellulose is more exposed. The formation of acids such as formic and levulinic acids during the process can degrade glucose, and thus reduce conversion. For the highest solids load, the conversion was 41.1%, for the 40% solids load it was higher, exceeding 50% and being a condition suitable for use in processes.



(a) (b)

Figure 2: (a) Glucose concentration and cellulosic conversion in acid hydrolysis of ground corn cob; (b) Glucose concentration and cellulosic conversion in acid hydrolysis of ground and acid pretreated corn cob.

In some conditions of enzymatic hydrolysis, the solid was added continuously to the process in steps of 10% (conditions: 2*10% and 3*10%). Furthermore, in some samples of this condition, Tween 80 (cs, with surfactant) was added, a surfactant that prevents cellulases from adhering to other polymers and not being able to break down cellulose more efficiently[2]. Even with a solids load of 5%, the concentration of glucose released was 20.8 g/L, an amount already consistent with the amount of substrate. The increase in the amount of solids also generated an increase in the concentration of sugars, reaching 74.27 g/L at 20% (w/v). Figure 3 presents the glucose concentration results found in this hydrolysis. The samples containing Tween 80 proved to be even more efficient, with a difference of more than 10 g/L of glucose when comparing the conditions of 2*10% with and without surfactant, for both amounts of cellulases used. In the 3*10% condition, the samples with surfactant still showed a greater advantage, but with a difference of approximately 5 g/L of glucose.



(a) (b)

Figure 3: (a) Glucose concentration and cellulosic conversion in an enzymatic hydrolysis with enzymatic load of 5 FPU/g; (b) Glucose concentration and cellulosic conversion in an enzymatic hydrolysis with enzymatic load of 10 FPU/g.

When comparing hydrolysis under conditions of 20% solids load, enzymatic hydrolysis presents a higher efficiency than acid hydrolysis, generating around 74.27 g/L of glucose, due to the enzymes being able to break the specific cellulose bonds, releasing glucose, without permeability obstacles or degrading other polymers as in the case of acid. Despite this, the use of enzymes requires a cost both for their use and for their recovery, which can make the acid hydrolysis process more viable in some cases depending on the cost.

4 CONCLUSION

Enzymatic hydrolysis, despite achieving higher concentrations of glucose, faces the challenge of the additional cost associated with enzymes. Therefore, to define an ideal condition in this type it is necessary to understand the financial viability of the process, however, in general, the conditions of 10 and 20% solids load have high glucose concentrations and conversion, even without the use of surfactant. The ideal condition found for acid hydrolysis is a solids load of 40%, a condition in which significant concentrations of glucose and a low concentration of substances that can degrade and hinder its conversion as acids are obtained. The use of ground corn cob showed better responses in the release of glucose. The concentration of sugars from the hydrolysis of pretreated cob is around 20% higher than that not treated for acid hydrolysis. In enzymatic hydrolysis, cellulose conversion proved to be more efficient at 10 FPU/g, almost 10% more, despite the lower final glucose concentration. Homogenization becomes a critical issue at higher solids loads, complicating the hydrolysis process and impairing conversion. When using glucose in processes based on chemical hydrolysis, extreme acidity requires neutralization, which can lead to the degradation of sugars.

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