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High solid loading enzymatic hydrolysis of alkaline pretreated corn cob for fermentable sugars production

Anderson G. Corrêa[1]*, Daniel Pasquini[2] & Milla Alves Baffi[3]

[1] Postgraduate in Biofuels, Federal University of Uberlândia (IQ-UFU), Institute of Chemistry, Uberlândia, Brazil.
[2] Federal University of Uberlândia (IQ-UFU), Institute of Chemistry.
[3] Federal University of Uberlândia (ICIAG-UFU), Institute of Agricultural Sciences.
** Corresponding author's email address: anderson.correa@ufu.br*

ABSTRACT

Agroindustrial wastes, such as corn cob (CC) are lignocellulosic biomasses mainly composed by cellulose, hemicellulose and lignin, characterized by a recalcitrant structure. In order to overcome this complex matrix and improve the digestibility of the biomass, a pretreatment stage is required for further bioconversions. In this study, an alkaline pretreatment with 2 % sodium hydroxide in mild conditions (100 ºC under reflux for 4 h) was applied to enhance the enzymatic hydrolysis of CC. Afterwards, saccharification using high solids load of pretreated CC was performed to release fermentable sugars. The alkaline pretreatment achieved remarkable delignification (69.8%) as well as an increase in the cellulosic fraction (33.1 to 61.0%) with preservation of hemicellulose (35.1 to 34.1%). The highest total reducing sugars (TRS) were obtained after the enzymatic hydrolysis performed with 25% solid, resulting in a hydrolysate rich in glucose (61.6 g/L) and xylose (33.2 g/L), with potential applications in several bioconversion routes, specially second generation (2G) ethanol production.

Keywords: Batch Hydrolysis. Sodium Hydroxide. Glucose. Xylose. Bioethanol.

1 INTRODUCTION

Lignocellulosic biomass is widely available throughout the world, generally considered as a waste material from agricultural, forestry and industrial activities. It is mostly composed by hemicellulose, cellulose, and lignin, with variable proportions depending on the kind of biomass (Arora et al., 2023). Lignocellulose is characterized by a recalcitrant structure, which requires the application of pretreatments to facilitate the accessibility of the hydrolytic enzymes to polysaccharides, for their subsequent conversion into bioproducts and biofuels (Buyukoztekin and Buyukkilecib, 2024).

Enzymatic hydrolysis with high solids load is a viable approach to improve the economic and technical feasibility of cellulosic ethanol production, with reduction in the use of inputs, such as water and enzyme load, presenting lower energy consumption (Arora et al., 2023). In addition, the use of high concentrations of substrates (15 > 30%, w/w) can provide optimum enzymatic yields and high concentrations of fermentable sugars (Barba et al., 2022). However, its efficiency depends on a number of factors, such as the pretreatment method, hydrolysis conditions, reactor, enzyme load, among others (Raj and Krishnan, 2019).

The recovery of corn cob (CC) is currently highlighted for many biotechnological applications, such as the production of biohydrogen from alkaline and oxidative pretreatment (Kucharska et al., 2020), in enzymatic prospecting (Capetti et al., 2023; Ismail et al., 2022) and for ethanol and furfural generation (Qiao et al., 2022). In this context, the present study investigated the application of alkaline pretreatment in the enzymatic hydrolysis of corn cob with high solids load, aiming the improvement of fermentable sugar release and 2G ethanol production.

2 MATERIAL & METHODS

Alkaline pretreatment was carried out in 2 L round-bottomed flask loaded with 25.0 g of crushed CC and 0.625 mL of aqueous 2% NaOH solution (w v^{-1}). A reflux condenser was attached to the flask, under blanket heating to the boiling point (~100 °C) for 4 h (Rodrigues et al., 2017). The pretreated material was filtered and washed with distilled water to neutral pH. Afterwards, it was dried at 50 °C for 12 h and stored for chemical characterization and enzymatic hydrolysis.

The saccharification was performed in Erlenmeyer 50 mL, containing sodium citrate buffer (0.05 mol/L, pH 4.8), a liquid/solid ratio of 10 mL/g (10% substrate) and the commercial solution Cellic CTec3 (Novozymes) with an enzyme load of 12.5 FPU/g of cellulose (filter paper units). This enzyme cocktail was characterized and exhibited 140 FPU/mL (Ghose, 1987). The tests were conducted in triplicate at 150 rpm and 50 ºC for 72 h in order to determine the reaction time for sugar release. Then, the fed-batch assays were carried out with additions of 5% substrate every 12 h (at 12, 24, 36 and 48 h) until a total of 30% solids was reached. After 72 h, the liquid fractions containing the released sugars were filtered and centrifuged.

The concentration of total reducing sugars (TRS) in the hydrolysates was determined by the 3,5- dinitrosalicylic acid (DNS) method (Miller, 1959), and the monosaccharides, acetic acid, formic acid, furfural (FF) and 5-hydroxymethylfurfural (HMF) released were determined by high performance liquid chromatography (HPLC). The samples were diluted with a mobile phase (aqueous solution of 0.1% phosphoric acid (v/v), filtered (0.20 µm, Chromafil® Xtra CA-20/25) and introduced into the chromatographic system (Shimadzu™ LC-20A Prominence). The system was equipped with a column of Supelcogel™ C-610H maintained at 32 °C, using isocratic elution at a flow rate of 0.5 mL/min. Ultraviolet detectors (operating at 210 nm) and refractive index were used. The analysis was based on patterns of glucose, xylose, formic acid, acetic acid, furfural (FF) and 5-hydroxymethylfurfural (HMF), according to Lamounier et al., (2020).

3 RESULTS & DISCUSSION

The main goal of a pretreatment step is to increase the accessibility of cellulose by hydrolytic enzymes through the removal of the lignin barrier. Alkaline pretreated CC exhibited delignification of 69,8% in comparison to *in natura* CC (Table 1). On the other hand, the cellulosic fraction exhibited a significant increase (33,1 to 61,0%), with preservation of the hemicellulose fraction (35,1 to 34,1). These results demonstrated that the alkaline pretreatment conditions maintained the polysaccharidic fractions in pretreated CC, with remarkable decrease of lignin, which are favorable to enzymatic hydrolysis. These findings are in accordance to Gatt et al., (2019), who demonstrated that after the alkaline pretreatment with NaOH of the corn straw and corn cob, more cellulose became available on the surface of the fiber, with a significant increase of 78%, while lignin was reduced around 65%.

Table 1: Corn cob mass characterization (%).

Biomass	Cellulose	Hemicellulose	Lignin	Delignification
in natura CC	33,18 ±1,15	35,15 ±1,05	19,53 ±1,62	-
NaOH pretreated CC	61,03 ±2,1	34,10 ±5,2	10,72 ± 2,59	69,89

The saccharifications with 10% of pretreated CC achieved a maximum TRS release of around 50 g/L after 72 h (Figure 1a), with no significant differences between 24 and 48 h (around 40 g/L). The data also proved the positive role of the pretreatment in comparison to *in natura* CC hydrolysate, which reached only 3.7 g/L of TRS (Figure 1b), evidencing the importance of the application of pretreatments for the enhancement of enzymatic hydrolyses.

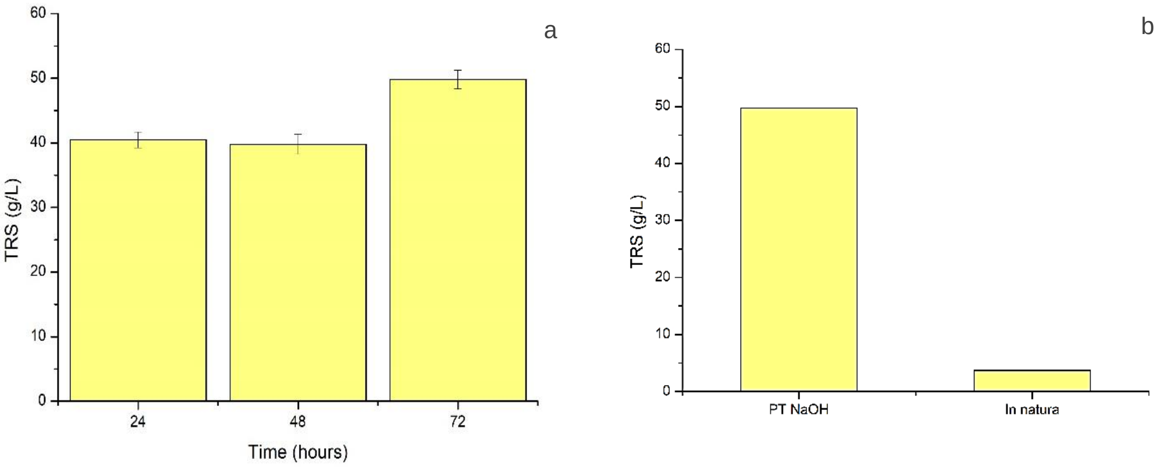

Figure 1. Concentrations of TRS (g/L) after enzymatic hydrolysis of corn cob.

Considering the cost of enzymatic processes, the saccharifications with high solids load are industrially favored and economically viable. In this sense, the hydrolyses carried out in different concentrations of solids (15 – 30%) achieved an increase in TRS release with maximum concentration of 99,7 g/L in the hydrolysate containing 25% solids (Figure 2). The HPLC analysis of the hydrolysate with 25% solids revealed elevated content of monomeric sugars (61.6 g/L of glucose, 33.2 g/L of xylose, 2.0 g/L of arabinose and 1.9 g/L of cellobiose), in a total of 98.7 g/L, corroborating the TRS results.

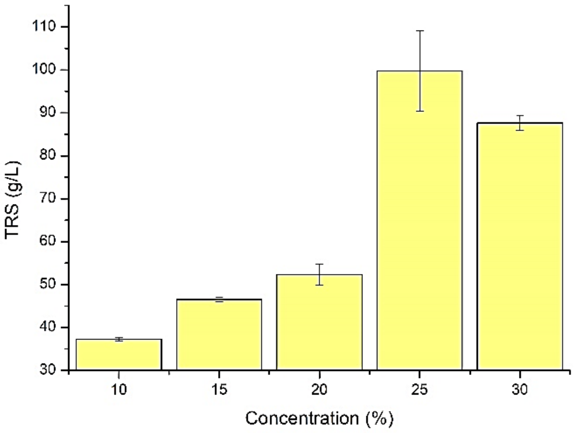

Figure 2. Concentrations of TRS (g/L) after hydrolysis of alkaline pretreated CC with high solid loading.

Cai et al., (2021) investigated fed-batch hydrolysis of CC from the alkaline pretreatment and obtained similar results to the present study, with a total of 113.7 g/L of fermentable sugars after 72 h of saccharification with 25% of solids, also demonstrating the positive effect of saccharifications carried out with high solids content. In addition, enzymatic hydrolysis with high solids content can be an efficient and economically viable way to produce high concentrations of monosaccharides. Kadhum et al., (2019), determined that capital costs that would decrease by more than 15% with solid content increasing by only 3% (12 to 15%). This increase resulted in significant reductions of 20% in energy consumption from distillation and wastewater generation, along with savings of 33% in steam consumption.

Another important factor in bioconversions of lignocellulosic biomass is the possible formation of inhibitors after the pretreatment. In the present study, acetic and formic acid were not detected and 5-hydroxymethylfurfural (HMF) and furfural were detected in very low concentrations, reinforcing the advantageous of the applied pretreatment for the success of hydrolytic process. The current data also indicate that the alkaline pretreatment can remove the acetyl groups, and especially lignin, the main inhibitor of hydrolysis, achieving high yields of fermentable sugars via enzymatic hydrolysis and 2G ethanol (Zhang et al., 2021).

4 CONCLUSION

This study demonstrated the efficacy of alkaline pretreatment of corn cob in mild conditions to achieve remarkable delignification and preservation of the polysaccharides. In addition, elevated concentrations of TRS were obtained in the enzymatic hydrolysis carried out with 25% solid, resulting in a hydrolysate rich in glucose (61,6 g/L) and xylose (33,2 g/L), with potential use in ethanol production and biorefineries.

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