ENZYME ADSORPTION AND RECYCLING DURING SACCHARIFICATION OF PRETREATED CASHEWAPPLE FIBER

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ABSTRACT

The recycling of cellulase enzymes is one potential strategy for reducing the cost of the enzymatic hydrolysis step during the bioconversion of lignocellulosics to different products. In this context, the objective of this research was to investigate the recycle of cellulase during enzymatic hydrolysis of cashew apple fiber pretreated with acid followed by alkali. Different concentrations of hydrolyzed solids (HE-CAF) were evaluated in the recycle, and observed that using high quantity of HE-CAF was obtained higher glucose concentration, without enzymatic supplementation. These results indicate that occurred adsorption of free cellulases on pretreated cashew apple fiber and the supplementation of the hydrolysis reaction with fresh substrate was as a potential means of recovering the cellulases. The performances of results achieved with supplementation of enzyme were different, in a sense that it was obtained higher yield than without supplementation.

1. INTRODUCTION

Cashew apple (*Anacardium occidentale* L.) is a tropical pseudo-fruit, which it is widely cultivated and commercially exploited in Brazil and others countries. The industrial process of the pseudo-fruit generates approximately 20-25% of residual fiber (cashew apple fiber) that was almost entirely discarded or used as animal feed supplement (Rodrigues et al., 2011). However, in recent years, there has been an increasing trend towards more efficient utilization these agro-industrial residues. And one way to take advantage is to convert it into carbohydrates (Barros et al., 2017).

Enzymatic conversion of lignocellulosic material, as cashew apple fiber, cheap and readily available into fermentable sugars for production of biofuels and bioproducts has received extensive attention due to the current strong reliance on fossil fuels (Spatari et al., 2010). The bioconversion process of renewable material, involves a pretreatment step that increases the accessibility of enzymes to materials; followed by enzymatic hydrolysis, to release the existing monosaccharide (Hendriks & Zeeman, 2009). However, the high cost of enzymes is one important factors prohibiting the commercialization of chemicals and fuels from lignocellulosic materials (Petersen et al. 2009; Tu et al. 2007). There are some strategies that has as objective to reduce these costs, for example maximizing the enzyme production and improving the enzyme performance, efficient and cost-effective enzymatic saccharification of lignocelluloses (Tu et al., 2007). Another approach for reusing the active enzyme is the recycling of the solid residue after hydrolysis, thereby exploiting the adsorption (Qi et al., 2011). In the context, the objective of this research was to investigate the

recycle of cellulase during enzymatic hydrolysis of cashew apple fiber pretreated with acid followed by alkali.

2. MATERIAL AND METHODS

2.1. Pretreatment of lignocellulosic material

The cashew apple fiber (CAF) used in this study was provided by the Jandaia® Juice Processing Industry in Ceará, Brazil. The CF was washed, dried at 60 °C for 24 h, milled, and particles with size between 0.25–0.84 mm were selected. After, CAF was pretreated with 0.6 M H_2SO_4 at 121 °C for 15 min using 30% w/v of the CAF. Subsequently, the pretreated cashew apple fiber (CAF-H) was washed and subjected to a second treatment with 1 M NaOH at 121 °C for 30 min using a solid ratio of 30% w/v. After, the solid was recovered for vacuum filtered and the solid residue (designated as CAF-HOH) was thoroughly washed with distilled water until the wash water reached a pH of 6.0 ± 0.5; it was then dried at 50 °C for 24 h. Of the remaining solid was selected particles with size between 0.25–0.84 mm and this was used as substrate for enzymatic hydrolysis (Barros et al. 2017).

2.2. Enzymatic Hydrolysis: Recycling

The enzymatic complexes (cellulase (NS22074) and β-glycosidase (NS50010)) were kindly provided by (Novozyme, Bagsvaerd, Denmark). The enzymatic hydrolysis of the pretreated fiber (HOH-CAF) was performed under the following conditions: 10% w/v of cellulose from CAF-HOH with 5 mM citrate buffer at pH 5.0, with cellulase enzymatic activity of 15 FPU/g and β-glycosidase of 60 CBU/g in an orbital shaker (Tecnal - TE 422) at 45 °C, 150 rpm for 72 h (Rodrigues et al., 2011). The experiments were performed in duplicate. 1 mL samples were collected at predetermined times and centrifuged at 6000 rpm for 10 min and filtered, to determine the carbohydrates concentration. The carbohydrates concentration were quantified by HPLC using a column (Bio-Rad, Hercules, CA, USA) at 65 °C, eluent 1 $mmol.L^{-1}$ H_2SO_4 in water (MiliQ Simplicity 185, Millipore, Billerica, MA) with a flow rate of 0.6 $ml.min^{-1}$, using the IR detector (IR DETECTOR SURVEYOR, THERMO SCIENTIFIC, CA, EUA). At the end of the enzymatic hydrolysis, the hydrolyzed solids (HE-CAF)were separated by filtration. New enzymatic hydrolysis using different quantities of hydrolyzed solids (10%, 30% and 50% w/w of HE-CAF) plus fresh solid (CAF-OH), for completing the solid concentration, were investigated. The process was conducted under the same conditions previously mentioned, without supplementation with enzyme. Then, experiments were carried with addition of the enzymes, basing in cellulose load of 10% w/v.

3. RESULTS AND DISCUSSIONS

Figure 1 shows the glucose concentrations obtained by enzymatic hydrolysis with and without enzyme supplementation. The highest concentration (23.1 g/L) was obtained in the process using a recycle of 50% HE-CAF, indicating that adsorption of enzyme on substrate occurred, because new enzymatic extract was not added (Figure 1A). In the experiment using enzyme supplementation and 10% HE-CAF, the highest glucose concentration was 47.5g/L, but there was no significant difference

using the other solids concentrations. However, a smaller amount of enzyme was necessary when using 50% HE-CAF, making the process more economical. Adsorption of cellulase on substrate is the first step for hydrolyzing lignocellulosic biomass; as the hydrolysis proceeded, adsorbed cellulase would be gradually released into the reaction solution. Then the greater amount of new substrate facilitated this process, as well as more enzyme was added to the process, thus increasing the efficiency of the hydrolysis.

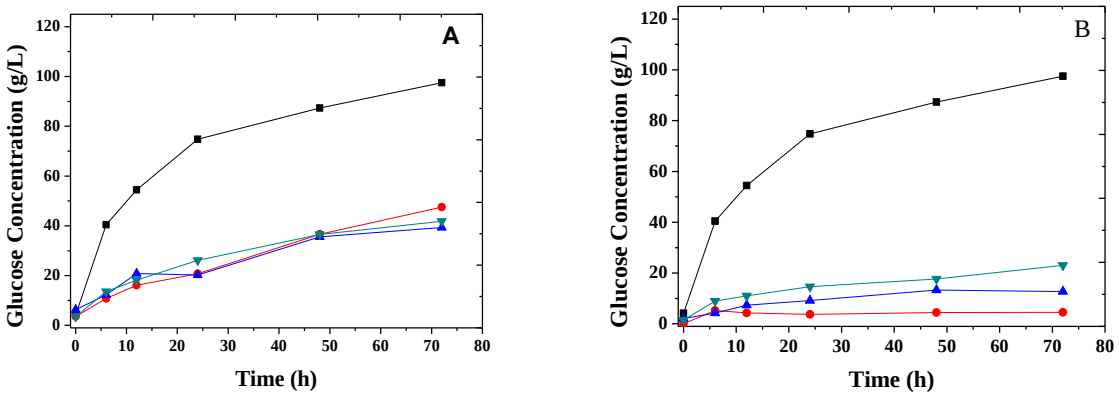

Figure 1: Effect of different percentages of hydrolyzed solids on enzymatic hydrolysis of CAF-HOH in the glucose concentration: (A) with enzymatic supplementation and (B) without enzymatic supplementation. (▪) Initial hydrolysis; (●) 10 % HE-CAF; (▲) 30% HE-CAF and (▼) 50% HE-CAF.

The cellobiose concentrations obtained in all assays were lower than glucose concentration (Figures 1 and 2). In the experiments without addition of new enzymatic extract, there was an accumulation of cellobiose (Figure 2B). Due to the high affinity of cellulase for cellulose, the enzymes present in the hydrolysate could be adsorbed onto fresh substrate (Qi et al., 2011). However, β-glucosidase could not be reused due to the fact that it does not typically adsorb on lignocellulosic substrate to bind to the cellulosic substrate.

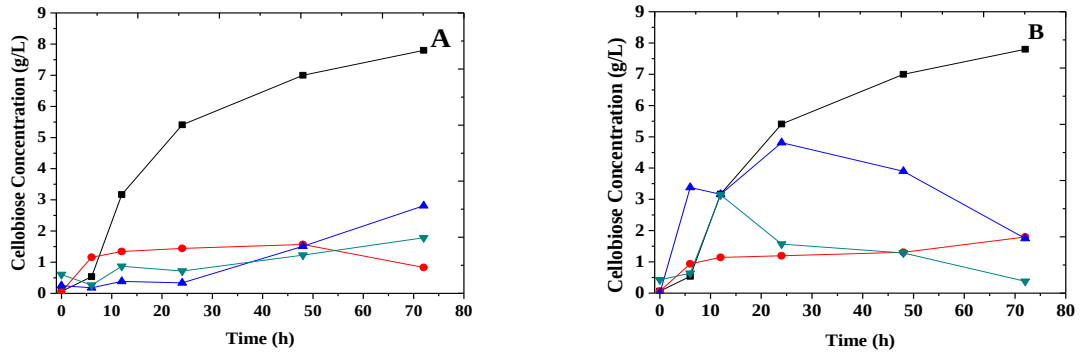

Figure 2: Effect of different percentages of hydrolyzed solids on enzymatic hydrolysis of CAF-HOH in the cellobiose concentration: (A) with enzymatic supplementation and (B) without enzymatic supplementation. (▪) Initial hydrolysis; (●) 10 % HE-CAF; (▲) 30% HE-CAF and (▼) 50% HE-CAF.

The Figure 3A and 3B show the hydrolysis yield. There was a considerable decrease in the ability of cellulase to hydrolyze acid-alkali treated CAF (CAF-HOH) when the rounds of hydrolysis were conducted without supplementation of new enzymatic extract and with low loads of hydrolyzed solids (HE-CAF). For example, the hydrolysis yield of initial round was 90%; however, the yield for the first recycle (using 50% HE-CAF) decreased to 40%, that is, a decrease of 54%. In contrast to the hydrolysis using enzyme supplementation and addition of fresh substrate, cellulase still possessed the capacity to hydrolyze 77% of polysaccharides in the same load of hydrolyzed solid (HE-CAF). The enzyme yield calculations were made based on the amount of cellulose placed in each assay.

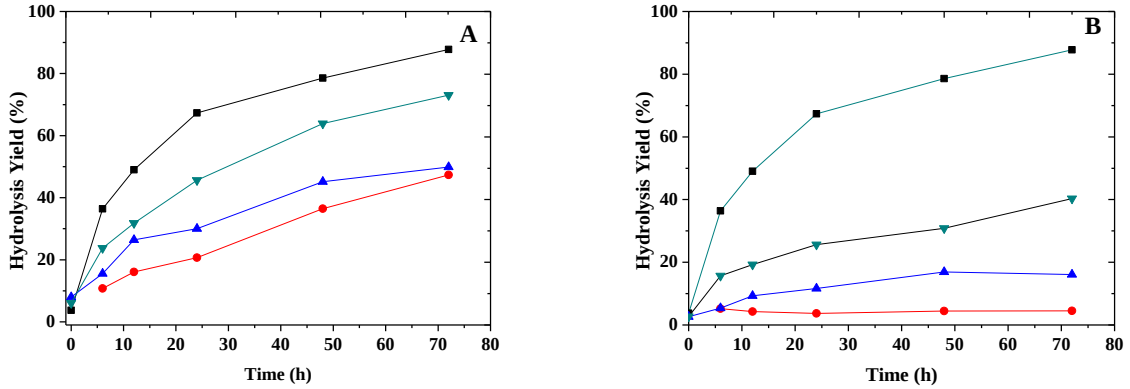

Figure 3: Effect of different percentages of hydrolyzed solids on yield of enzymatic hydrolysis of CAF-HOH: (A) with enzymatic supplementation and (B) without enzymatic supplementation. (▪) Initial hydrolysis; (●) 10 % HE-CAF; (▲) 30% HE-CAF and (▼) 50% HE-CAF.

4. CONCLUSIONS

The results from this study showed that it was possible to recycle a substantial amount of cellulase during hydrolysis of cashew apple fiber pretreated with acid followed by alkali.This strategy can be useful to reduce the cost of lignocellulose-to-bioproduct process.

5. REFERENCES

Barros, E.M. et al., 2017. Comparison of strategies for the simultaneous saccharification and fermentation of cashew apple bagasse using a thermotolerant *Kluyveromyces marxianus* to enhance cellulosic ethanol production. *Chemical Engineering Journal*, v. 307, p.939–947.

Hendriks, A.T.W.M. & Zeeman, G., 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Technology*, v. 100, p.10–18.

Petersen, O., M., et al., 2009. Optimization of hydrothermal pretreatment of wheat straw for production of bioethanol at low water consumption without addition of chemicals. Biomass and Bioenergy, v. 33 (3), p. 834–840.

Qi, B. et al., 2011. Enzyme adsorption and recycling during hydrolysis of wheat straw lignocellulose. *Bioresource Technology*, 102 (3), p.2881–2889.

Rodrigues, S., et al., 2011. Ethanol Production from Cashew Apple Bagasse: Improvement of Enzymatic Hydrolysis by Microwave-Assisted Alkali Pretreatment. *Applied Biochemistry and Biotechnology*, 164 (6), p.929–943.

Spatari, S., Bagley, D.M. & Maclean, H.L., 2010. Life cycle evaluation of emerging lignocellulosic ethanol conversion technologies. *Bioresource Technology*, 101 (2), p.654–667.

Tu, M., Chandra, R.P. & Saddler, J.N., 2007. Evaluating the Distribution of Cellulases and the Recycling of Free Cellulases during the Hydrolysis of Lignocellulosic Substrates. *Biotechnology Progress*, 23 (2), p.398–406.