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Enhanced enzymatic hydrolysis and ethanol production from cashew apple bagasse pretreated with alkaline hydrogen peroxide

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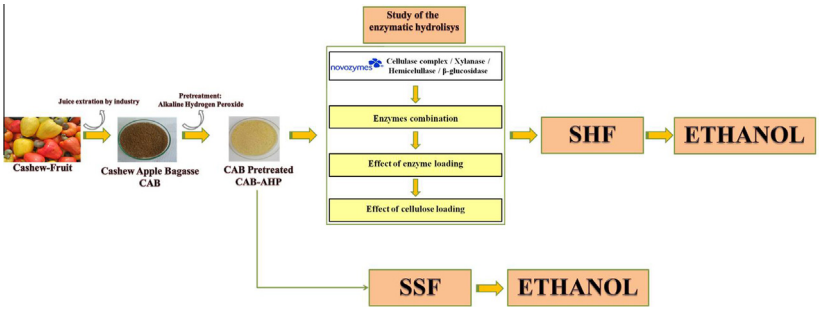
HIGHLIGHTS

- Conversion of cashew apple bagasse to fuel ethanol.
- The combination between different enzymes was evaluated during enzymatic hydrolysis.
- Cellulase and β-glucosidase showed the more effective interactions.
- The study demonstrates an insignificant impact of xylanase on the hydrolysis yield.
- The ethanol production by SHF and SSF processes showed promising using CAB-AHP.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of combinations and ratios between different enzymes has been investigated in order to assess the optimal conditions for hydrolysis of cashew apple bagasse pretreated with alkaline hydrogen peroxide (the solids named CAB-AHP). The separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF) processes were evaluated in the ethanol production. The enzymatic hydrolysis conducted with cellulase complex and β-glucosidase in a ratio of 0.61:0.39, enzyme loading of 30 FPU/$g_{CAB\text{-}AHP}$ and 66 CBU/$g_{CAB\text{-}AHP}$, respectively, using 4% cellulose from CAB-AHP, turned out to be the most effective conditions, with glucose and xylose yields of 511.68 mg/$g_{CAB\text{-}AHP}$ and 237.8 mg/$g_{CAB\text{-}AHP}$, respectively. Fermentation of the pure hydrolysate by *Kluyveromyces marxianus* ATCC 36907 led to an ethanol yield of 61.8 kg/ton_{CAB}, corresponding to 15 g/L ethanol and productivity of 3.75 g/(L h). The ethanol production obtained for SSF process using *K. marxianus* ATCC 36907 was 18 g/L corresponding to 80% yield and 74.2 kg/ton_{CAB}.

1. Introduction

Ethanol production using lignocellulosic materials as substrates represents one of the main routes for second-generation biofuel production. Cashew apple bagasse (CAB), a by-product of the cashew apple juice industry, represents approximately 20% of the total peduncle weight and is mainly composed of cellulose, hemicellulose and lignin. The industrial peduncle processing for juice production results in 15% (w/w) of bagasse, which has essentially no commercial value and is usually discarded by local industries (Rocha et al., 2011; Correia et al., 2013).

Cashew apple bagasse can be converted into bioethanol by performing the following operations: pretreatment, hydrolysis, fermentation, and distillation. Enzymatic hydrolysis is one of the most common and effective methods employed to generate

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fermentable sugars due to the high yields of sugars that can be obtained, with subsequent fermentation of these sugars to produce ethanol (Saha et al., 2011; Van-Dyk and Pletschke, 2012; Meng and Ragauskas, 2014). For that to become possible, the raw material needs to be pretreated so that the cellulose in the plant fibers is exposed to enzymatic action (Rocha et al., 2009).

Pretreatment is a crucial step to generate fermentable sugars. It breaks open the polymeric structures of lignin and carbohydrates in the lignocellulosic biomass and enhances the accessibility of enzymes to the solid substrate during the enzymatic hydrolysis step (Saha et al., 2011). A pretreatment that has been studied to prepare lignocellulosic substrates from a wide range of raw materials for subsequent bioconversion is performed with alkaline hydrogen peroxide (AHP) (Correia et al., 2013; Karagöz et al., 2012; Banerjee et al., 2011). It is known to decrystallize cellulose and with the oxidative action of the H_2O_2-derived radicals, it is also thought to contribute to the depolymerization and high solubilization of lignin (Correia et al., 2013; Karagöz et al., 2012; Selig et al., 2009).

The effectiveness of hydrolysis in the polysaccharides present in the lignocellulose substrates, therefore, is determined by an appropriate pretreatment, good selection of enzymatic complexes and cellulose accessibility (Meng and Ragauskas, 2014).

Enzymatic hydrolysis is an environment-friendly process that consists in the use of enzymes from groups of cellulases and hemicellulases, which are capable of degrading polysaccharides (cellulose and hemicellulose) with high-specificity catalyst activity (Kinnarinen and Häkkinen, 2014). The optimization of the composition of enzymatic complexes aims to boost the yield and reduce hydrolysis cost. But primarily, it should provide a high content of glucose in the hydrolysate under the action of the available enzymes combined (Zhou et al., 2009).

Cellulose accessibility has been proposed as a key factor in the effectiveness of bio-conversion of lignocellulosic biomass into fermentable sugars. Different factors affecting the cellulose accessibility, i.e., the substrate type, chemical composition (such as lignin/hemicellulose content), and the biomass structure (Meng and Ragauskas, 2014; Kinnarinen and Häkkinen, 2014; Van-Dyk and Pletschke, 2012). Other factors affect the enzymes and the optimization of the hydrolysis process, such as enzyme ratio, substrate and enzyme loadings and inhibitors (Van-Dyk and Pletschke, 2012; Rocha et al., 2009). The enzyme loadings are determined by financial factors, such as the cost of enzymes and final products, and should be optimized (Kinnarinen and Häkkinen, 2014). In addition to enzyme loadings, substrate loadings are a major factor. It has to allow the bioconversion to be economical and, at the same time, provide sufficient sugar levels for fermentation. Thus, optimal enzyme and substrate loadings have to be identified for maximum efficiency and economy (Van-Dyk and Pletschke, 2012). In the case of industrial processes, in which a high sugar concentration must be obtained, the initial suspended solid concentration in the hydrolysis should be high. However, high solid loadings are known to reduce the obtainable yield (Rocha et al., 2009), which adversely affects the process economy.

In this context, the aim of this study was to determine the feasibility of employing several complexes of cellulolytic and hemicellulolytic enzymatic preparations, the ratio of these complexes, and the enzyme and cellulose loadings for efficient hydrolysis of polysaccharides in pretreated cashew apple bagasse. The effects of hydrolysis were assessed based on the quantity of released sugars. The pretreatment was conducted with CAB 5% (w/v), 4.3% (v/v) AHP at 35 °C and 250 rpm for 6 h, the solid fraction was then separated for experiments of enzymatic hydrolysis. Afterwards, ethanol production in both Separate Hydrolysis and Fermentation (SHF) and Simultaneous Saccharification and Fermentation (SSF) processes using the yeast *Kluyveromyces marxianus* ATCC36907 was also evaluated.

2. Methods

2.1. Lignocellulosic material

Cashew apple (*Anacardium occidentalis* L.) bagasse (CAB) was kindly donated by Jandaia Juice Industry (Ceará, Brazil). The initial treatment of CAB was conducted of according to Correia et al. (2013), in which CAB was washed three times with water, dried at 60 °C for 24 h and milled in a hammer mill in order to obtain an average particle size of 20–80 mesh (0.25–0.84 mm).

2.2. Pretreatment of cashew apple bagasse

The milled cashew apple bagasse was pretreated by alkaline hydrogen peroxide according to the best conditions obtained in the study of Correia et al. (2013). The cashew apple bagasse, with a concentration of solids of 5% (w/v), was slurred in hydrogen peroxide H_2O_2 (4.3% v/v) with the H_2O_2 solution adjusted to pH 11.5 using 6 mol L^{-1} NaOH. The pretreatment was conducted in an orbital shaker (Tecnal – TE 422, SP, Brazil) at 35 °C for 6 h and 250 rpm. After the pretreatment, both solid and liquid fractions were separated by filtration. The solid fraction was washed with distilled water and oven dried at 60 °C for 24 h. This solid fraction, named CAB-AHP, was used as substrate for the subsequent enzymatic hydrolysis.

2.3. Characterization of untreated and pretreated CAB

Compositional analyses (cellulose, hemicellulose and lignin) of the CAB and CAB-AHP raw materials were determined according to Gouveia et al. (2009). The extractables, total solids and ash were analyzed according to the NREL Laboratory Analytical Procedures – LAP (Sluiter et al., 2008).

2.4. Lignin recovery

Lignin samples generated in the pretreatment were recovered from the hydrolysate liquor by precipitation with acidification at pH 2 using 50% v/v H_2SO_4. The mass of precipitated lignin was calculated in dry mass basis.

2.5. Enzymes

The cellulase complex (NS22074), as well as the xylanase (NS22036), hemicellulase (NS22002) and β-glucosidase (NS50010) preparations were kindly supplied by Novozymes. The enzymatic activities of the cellulase complex and β-glucosidase enzymes were determined as recommended by Ghose (1987) and expressed as follows: 1 FPU – the quantity of enzyme releasing 1 μmol of glucose from blotting-paper Whatman No. 1 within 1 min and 1 CBU – the quantity of enzyme transforming 1 μmol of cellobiose into 2 μmol of glucose within 1 min (reaction conditions: pH 4.8, temperature 50 °C) for the cellulase complex and the β-glucosidase, respectively. The enzymatic activities of xylanase and hemicellulase were determined according to Bailey et al. (1992) and the values were expressed as 1 U – the quantity of enzyme releasing 1 μmol of xylose from xylan within 1 min (reaction conditions: pH 4.8, temperature 50 °C). The protein content of the liquid enzyme preparations was determined using the Bradford method (Bradford, 1976). The enzymatic activity and the protein concentration of each of these enzymes are shown in the Table 1.