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Evaluation of dilute acid pretreatment on cashew apple bagasse for ethanol and xylitol production

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HIGHLIGHTS

- Dilute acid pretreatment on cashew apple bagasse (CAB) to solubilize hemicellulose.
- Achievement of the hemicellulose and cellulose-derived sugars from CAB.
- Hydrolyzate was used as a fermentation medium for ethanol and xylitol production.
- Concentrations of the inhibitors in hydrolyzate cause no toxicity to yeasts.

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ABSTRACT

Pretreatment conditions such as the concentration of sulfuric acid added, solid concentration and the contact time were studied to maximize the yields from the bagasse. The best condition for dilute acid pretreatment was obtained when using 0.6 mol/L H_2SO_4 for 15 min at 121 °C. At this pretreatment condition, no detectable quantities of furfural or hydroxymethyl furfural were observed in the hydrolyzate fraction after treatment with $Ca(OH)_2$. Scanning electron microscopy imaging showed the physical structural changes of cashew apple bagasse when it was pretreated with dilute sulfuric acid. Dilute-sulfuric acid pretreatment of CAB released hemicellulose-derived sugars to the liquid and these carbohydrates can be used to ethanol and xylitol production. An ethanol concentration of 10.0 g/L was obtained after 4 h of fermentation by *Saccharomyces cerevisiae* in agitated Flasks, with a yield of 0.49 $g/g_{glucose}$ and a productivity of 1.43 g/(L h) In a 4 L bench-scale bioreactor using *S. cerevisiae*, the maximum ethanol concentration obtained was 9.59 ± 1.74 g/L, and the maximum productivity obtained was 1.22 ± 0.06 g/(L h) *Kluyveromyces marxianus* CCA 510 produced ethanol and xylitol, reaching a concentration of 11.89 ± 0.34 g/L and 6.76 ± 0.28 g/L, respectively, at 96 h.

1. Introduction

The economy of the northeastern Brazilian state of Ceará is heavily dependent on the cashew agroindustry. Cashew apple bagasse represents approximately 20% of the total peduncle weight [1] and is one of the main residual products of the cashew agronomic industry. Cashew apple bagasse (CAB) is a lignocellulosic by-product of the cashew apple juice industry and its main components are cellulose, hemicellulose and lignin [2,3]. Its hemicellulosic fraction can be hydrolyzed into glucose and xylose, which may be fermented to produce bioproducts, such as ethanol and/or xylitol. Especially in the last years, research on the use of agroindustrial residues as raw materials for ethanol production has deserved great attention [4].

The production of ethanol from cashew apple bagasse is attractive in terms of the energy balance and reducing emissions and may therefore be used as a Supplementary material for ethanol production in Brazil. Xylitol, a high-value polyalcohol, may be produced by the reduction of D-xylose derived from hemicellulose fraction of lignocellulose, which has applications in the medical, odontological, food and cosmetic industries [5,6]. It has a similar sweetness to sucrose [7] and can be employed in specific diets for people with special caloric needs, such as diabetics [8].

Studies on lignocellulose pretreatment are required to convert the cellulose and the hemicellulose into fermentable sugars or to improve the availability of the cellulose for enzymatic hydrolysis [7–12]. Pretreatment methods mainly include physical methods such as mechanical or thermal methods [13–15], chemical

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methods [3,15–21], biological methods [9] and a combination of these methods. The mechanism of physical pretreatment is targeted at increasing the accessible surface area and decreasing the crystallinity of lignocellulose by chipping, milling, grinding or irradiation [9,13,14]. Thermal pretreatment methods such as steam explosion and pretreatment with hot water are also effective in improving the enzymatic hydrolysis process, as they are able to remove most of the hemicellulose [22–24]. Among others pretreatment, have been developed for decreasing the biomass recalcitrance, but only a few of them seem to be promising [12].

Dilute-acid hydrolysis is probably the most commonly applied method among the chemical pretreatment methods that are used. The main objectives of the acid hydrolysis pretreatment stage are to solubilize the hemicellulosic fraction of the biomass and to increase the accessibility of the cellulose to the enzymes [16,25,26]. It can be used either to pretreat the lignocellulose prior to enzymatic hydrolysis or to directly hydrolyze the lignocellulose to fermentable sugars [9,16,25].

Acid hydrolysis pretreatment can be performed with concentrated or diluted acid, but the use of concentrated acids for ethanol and xylitol production is less desirable because inhibitory compounds are formed when concentrated acids are used. Furthermore, concentrated acid pretreatment methods generate more issues with equipment corrosion and acid recovery. Hence, these methods are associated with high operational and maintenance costs, which result in a reduced interest in the application of concentrated acid pretreatment methods at a commercial scale [9,10,21,25].

In this context, the present study aims to investigate the effects of different pretreatment parameters on the fermentability of the hemicellulose and cellulose-derived sugars from dilute acid pretreated cashew apple bagasse. In Addition, the effect of these parameters on the composition and structure of lignocellulose was observed.

2. Experimental

2.1. Raw material

Cashew apple (*Anacardium occidentalis* L.) bagasse (CAB) was kindly donated by Jandaia Industry of Juice (Ceará, Brazil). It was dried at 60 °C for 24 h and milled in a hammer mill to produce particles that were sized on average between 20 and 80 mesh (0.25–0.84 mm). The milled CAB was stored at 30 °C.

2.2. Microorganisms and inoculum preparation

The yeast *Saccharomyces cerevisiae* was inoculated on *Sabouraud* agar consisting of 5.0 g/L peptone, 15.0 g/L glucose and 15.0 g/L agar and incubated at 30 °C for 48 h. Then, three colonies were transferred from the stock culture to a 500 mL Erlenmeyer flask containing 200 mL of inoculum medium. The medium had a composition of 30 g/L glucose, 5 g/L yeast extract, 10 g/L $(NH_4)_2SO_4$, 4.5 g/L KH_2PO_4, 1 g/L $MgSO_4{\cdot}7H_2O$ and 0.65 g/L $ZnSO_4$. The pH of the medium was 5.0, and it had been sterilized at 110 °C for 10 min prior to use. The flasks were incubated at 30 °C and 150 rpm for 24 h to grow the inocula. After 24 h of incubation, the medium was centrifuged (Hettich Zentrifugen Rotina 38R) at 10000*g* for 10 min and the solid cells were removed after centrifugation to obtain the inoculum that would be used in the fermentation assays.

Kluyveromyces marxianus CCA510 was obtained from LAMAM – Laboratório de Microbiologia Agrícola e Molecular, Federal University of São Carlos – UFSCar, São Carlos, Brazil. The yeast was inoculated in complex YEPD (Yeast Extract Peptone Dextrose) medium (composition: 20.0 g/L glucose, 10.0 g/L yeast extract, 20.0 g/L peptone, 1.2 g/L KH_2PO_4, 0.18 g/L Na_2HPO_4, pH 6.0) and incubated at 30 °C and 200 rpm for 24 h. Afterwards, aliquots of medium, corresponding to 1.0 g/L of cells, were centrifuged and the cell pellet was used on ethanol and xylitol production.

2.3. Dilute acid pretreatment of cashew apple bagasse

The milled CAB was added to dilute H_2SO_4 (Vetec, Rio de Janeiro, Brazil) of different concentrations (0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 mol/L) to form a slurry with a solid concentration of 15% w/v. In this work, a treatment in water without H_2SO_4 (no acid was added) was conducted, here in named control set up. This slurry was homogenized for 5 min to ensure that the solids were uniformly dispersed. The pulp was then pretreated in an autoclave for 30 min at 121 °C and 1 atm and vacuum filtered (GAST Manufacturing, Inc., Model DOA-P704, Michigan, USA). The solid residue (denoted as CAB – H) was washed with water until the wash water reached a pH of 6.0 ± 0.5; it was then dried at 50 °C for 24 h. The filtered liquid hydrolyzate fraction was adjusted to a pH of 6.5 ± 0.2 with $Ca(OH)_2$ and filtered to remove the precipitate that was formed.

After evaluating the optimal acid concentration, the quantity of solids added to the slurry was varied at 15%, 20%, 25%, 30% and 35% w/v, with pretreatment carried out at 121 °C for 30 min. The variation of contact time (15, 30 and 45 min) used for hydrolysis was also investigated using a slurry containing 30% w/v of CAB and sulfuric acid concentrations of 0.6, 0.7 and 0.8 mol/L. Assays were performed in triplicate, and results represent the mean ± standard deviations of the three independent experiments.

2.4. Fermentation medium

The liquid fraction of the cashew apple bagasse sample (denoted as MCAB-H hydrolyzate) pretreated using 0.6 mol/L H_2SO_4 with a solids content of 30% w/v CAB for 15 min at 121 °C was used for ethanol and/or xylitol production. The pH of the hydrolyzate was adjusted with $Ca(OH)_2$ to pH 5.0, when using *S. cerevisiae*, or to pH 6.0, when using *K. marxianus*.

2.5. Ethanol production by S. cerevisiae using MCAB-H

2.5.1. Fermentation assays in agitated flasks

Batch fermentation experiments were conducted in 250 mL Erlenmeyer flasks containing 100 mL of fermentation medium at a temperature of 30 °C and an agitation speed of 150 rpm. The flasks were inoculated with *S. cerevisiae* to a cell concentration of 10 g/L for ethanol production. Medium supplementation with a nitrogen source was evaluated with an addition of 5.0 g/L of $(NH_4)_2SO_4$ to the hydrolyzate. Cell growth, pH, substrate concentration and ethanol concentration were monitored with respect to time.

2.5.2. Fermentation assays on a bench-scale bioreactor

Fermentation was conducted in a 4 L bench-scale bioreactor (Marconi, São Paulo, Brazil) at a temperature of 30 °C and an agitation speed of 150 rpm, with a working volume of 2 L. The bioreactor was inoculated with *S. cerevisiae* to a cell concentration of 10 g/L for ethanol production. Cell growth, pH, dissolved oxygen concentration, substrate concentration and ethanol concentration were monitored with respect to time.

2.6. Ethanol and xylitol production by K. marxianus CCA510 using MCAB-H

All assays were conducted in 250 mL-Erlenmeyer flasks of 250 mL containing 100 mL of culture medium (MCAB-H) on a