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Ethanol Production from Cashew Apple Bagasse: Improvement of Enzymatic Hydrolysis by Microwave-Assisted Alkali Pretreatment

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Abstract In this work, the potential of microwave-assisted alkali pretreatment in order to improve the rupture of the recalcitrant structures of the cashew able bagasse (CAB), lignocellulosic by-product in Brazil with no commercial value, is obtained from cashew apple process to juice production, was studied. First, biomass composition of CAB was determined, and the percentage of glucan and lignin was 20.54±0.70% and 33.80±1.30%, respectively. CAB content in terms of cellulose, hemicelluloses, and lignin, 19.21±0.35%, 12.05±0.37%, and 38.11±0.08%, respectively, was also determined. Results showed that, after enzymatic hydrolysis, alkali concentration exerted influence on glucose formation, after pretreatment with 0.2 and 1.0 mo L^{-1} of NaOH (372±12 and 355±37 mg g_{glucan}^{-1}) when 2% (*w*/*v*) of cashew apple bagasse pretreated by microwave-assisted alkali pretreatment (CAB-M) was used. On the other hand, pretreatment time (15–30 min) and microwave power (600–900 W) exerted no significant effect on hydrolysis. On enzymatic hydrolysis step, improvement on solid percentage (16% *w*/*v*) and enzyme load (30 FPU $g_{CAB\text{-}M}^{-1}$) increased glucose concentration to 15 g L^{-1}. The fermentation of the hydrolyzate by *Saccharomyces cerevesiae* resulted in ethanol concentration and productivity of 5.6 gL^{-1} and 1.41 gL^{-1} h^{-1}, respectively.

Keywords Microwave-Assisted Alkali Pretreatment · Cashew Apple Bagasse · Enzymatic Hydrolysis · *Saccharomyces cerevesiae* and Ethanol

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Nomenclature

CAB	Cashew apple bagasse
CAB-M	Cashew apple bagasse pretreated by microwave-assisted alkali pretreatment
CB	Cellobiose
CBU	Cellobiase activity was expressed as cellobiase units (CBU) per milliliter of enzymatic mixture
CBU/g	Cellobiase activity per gram of raw material
FP	Filter paper Whatman n°1
FPU	Filter paper activity was expressed as filter paper units (FPU) per milliliter of enzymatic mixture
FPU/g	Filter paper activity per gram of raw material
η	Efficiency of fermentation (%)
P_f	Ethanol concentration (g L^{-1})
Q_p	Ethanol productivity (g L^{-1} h^{-1})
S_i	Initial glucose concentration (g L^{-1})
S_f	Final glucose concentration (g L^{-1})
T	Time (h)
$U_{\text{cellobiose}}$	Enzyme amount that converts 1 μmol of cellobiose to 2 μmol of glucose in 1 min on reaction conditions
$Y_{p/s}$	Conversion substrate/product (g g^{-1})

Introduction

Energy consumption has increased steadily as world population has grown and concerns about atmospheric pollution derived from fossil fuels have resulted in a worldwide interest in exploring renewable energy in the form of biomass (bioenergy). The conversion of biomass into biofuels has drawn much attention from the government and researchers, especially fuel ethanol [1–5]. Lignocelluloses represent the most abundant and lowest-cost biomass in the world; thus, their use allows either the production of a valuable biofuel and the utilization of a wide range of residues of domestic, agricultural, or industrial activities [6–8].

Cashew nut is produced in around 20 countries of the world and the major cashew apple-producing countries, and their production figures, in the year of 2006, based on FAO [9] are Vietnam (1.09 million tons), Nigeria (0.64 million tons), India (0.57 million tons), Brazil (0.24 million tons), and Indonesia (0.14 million tons). The production of cashew crop in Brazil in the year of 2009 was around 248,455 tons, according to Brazilian official estimate [10], which accounts for 9.3% of the world production and corresponds to more than 2 billion tons of cashew apple.

In Brazil, especially on Northeast region, the cashew agroindustry has an outstanding role in the local economy. The industrial peduncle processes for juice production results in 40% (*w*/*w*) of bagasse, which represents no commercial value and is usually discarded by the local industry. These facts turns cashew apple bagasse (CAB) an alternative and inexpensive (<$0.50/Kg) raw material for several potential applications [11–14], including the production of fuel ethanol.

In cellulosic ethanol processes, pretreatment of lignocelluloses to disrupt their recalcitrant structures is needed in order to increase the digestibility of materials [7]. Among many pretreatment methods (uncatalyzed steam explosion, liquid hot water, diluted acid, and ammonium fiber/freeze explosion−AFEX), which are usually achieved through a convection

or conduction based heating [1, 15], microwave irradiation appears as an alternative method. It is used in many areas because of its heating efficiency and easy operation [7, 15, 16].

In general, advantages of microwave-based technologies include reduction of process energy requirements, uniform and selective processing, the ability to start and stop the process instantaneously, and reduced equipment size and waste [17–20]. These benefits associated with microwave radiation have led to its numerous applications such as food processing, wood drying, plastic and rubber treating, as well as curing and preheating of ceramics [16, 19]. Furthermore, the main areas of study are minerals processing, waste treatment, contaminated soil remediation, recycling of rubber tires, activated carbon applications, and the treatment of volatile organic compounds [19, 20]. Based on these existing applications, it is reasonable to assert that a microwave-based process can be used for the pretreatment of lignocelluloses and some studies are already available in the literature [7, 15, 16, 21].

Compared to conventional (conduction/convection) heating, which is based on superficial heat transfer, microwave irradiation induces an interaction between a heated object and an applied electromagnetic field to create heat. When microwave is used to treat lignocelluloses, it selectively heats the more polar part and creates a "hot spot" with the inhomogeneous materials. It results in an "explosion" effect among the particles, and improves the disruption of the recalcitrant structures of lignocellulose [7, 21, 22]. Some authors [23] observed that the hydrolysate obtained from the microwave/chemical pre-treated rice straw after enzymatic hydrolysis had relatively high pentose content although, it is much lower than that from the conventional heating chemical pre-treated rice straw enzymatic hydrolysis. Hu and Wen [7] observed that, although microwave treatment resulted in a much higher switchgrass digestibility than conventional heating, the absolute sugar yield was still relatively low, which suggests that microwave pretreatment can facilitate, but cannot totally, break down the recalcitrant structures of switchgrass. To improve the proposed microwave pretreatment, these authors [7, 23] studied the combination of microwave irradiation and alkali pretreatment technique, since that alkali is capable of effectively removing the lignin barrier of lignocelluloses and, thus, enhance the sugar yield [7].

In this work, the combination of microwave and alkali pretreatment of CAB and its enzymatic hydrolysis were investigated. After that, the hydrolyzed obtained was fermentated to ethanol production using *Saccharomyces cerevisiae*.

Material and Methods

Raw Material

CAB (*Anacardium occidentale* L.) was kindly donated by Jandaia Industry of Juice (Ceará, Brazil). It was washed five times with water and dried at 60°C for 24 h, and milled for 10 min in a domestic blender (or hammer mill). The particles that passed through a 20# mesh sieve but were retained by a 115# mesh sieve were used as raw material. The milled CAB was stored at room temperature.

Determination of Structural Carbohydrates and Lignin in Cashew Apple Bagasse

The biomass composition of CAB was firstly determined according to the National Renewable Energy Laboratory (NREL) laboratory analytical procedures (LAP) [24–26] in terms of glucans and lignin.

The methodology of Gouveia et al. [27], which was validated for sugarcane bagasse, was also used to determinate cellulose, hemicelluloses, and lignin present at CAB. The methodology consisted in the hydrolysis of the material with H_2SO_4 at 72% (*v*/*v*). Afterwards, carbohydrates, organic acid, furfural, and hydroxymethylfurfural were determined by high-performance liquid chromatography (HPLC), insoluble lignin by gravimetry, and soluble lignin by spectrophotometry.

Pretreatment of Cashew Apple Bagasse

Pretreatment of CAB particles was conducted in a LG-MS/114 ML domestic microwave at a frequency of 2.45 GHz. The microwave had a maximum power of 1,150 W with 10 discrete settings. The microwave/alkali pretreatment was carried out as follows: 30 g of CAB was slurried in 100 mL either pure water or NaOH solutions (0.2 and 1.0 mol L^{-1}) at 28°C in an Erlenmeyer flask of 250 mL for 10 min. Then, the flask was placed in the microwave oven at a given power (600 or 900 W) for 15 or 30 min. When the pretreatment was completed, the residues were collected and washed with distilled water to neutralize the pH. It was dried at 60°C for 24 h, milled for 10 min in a domestic blender (or hammer mill). The particles that passed through a 20# mesh sieve but were retained by a 80# mesh sieve and stored for enzymatic hydrolysis. This resulting solid was called cashew apple bagasse pretreated by microwave-assisted alkali pretreatment (CAB-M).

Enzymatic Hydrolysis

The saccharification of pretreated CAB-M with a commercial enzyme extract, Celluclast 1.5 L (Novozyme, Bagsvaerd, Denmark), was performed in triplicate, incubated at 45°C under mild agitation (150 rpm), in 250 mL Erlenmeyer flasks with screw caps to avoid volume loss due to evaporation. Samples (1 mL) were taken from the reaction mixture at different times, centrifuged at 6,000 rpm for 15 min. The supernatants were stored for sugar analysis. The influence of solid content (2% and 16% *w*/*v*) and enzyme load (15 and 30 filter paper units (FPU) $g_{CAB\text{-}M}^{-1}$) was investigated. Enzyme was added to the flasks in diluted form, using 50 mM citrate buffer and pH 5.0, slurried and incubated for 72 h.

Enzyme Activity

Filter paper activity was determined as recommend by Ghose (1987) [28] and it was expressed as FPU per milliliter of mixture. The activity of enzyme Celluclast 1.5 L used was 134.63 FPU/mL of mixture. β-glucosidases activity, 5.0 $U_{cellobiose}$/mL [29], was defined as the enzyme amount that converts 1 μmol of cellobiose to 2 μmol of glucose in 1 min on reaction conditions.

Glucose Conversion

In this work, glucose conversion was defined as the amount of glucose released, expressed in milligram,, per gram of glucans present in the substrate (CAB *in natura*), mg g_{glucan}^{-1}.

Cellobiose Conversion

In this work, cellobiose conversion was defined as the amount of cellobiose released, expressed in milligram, per gram of glucans present in the substrate (CAB *in natura*), mg g_{glucan}^{-1}.

Microorganism and Innoculum Preparation

S. cerevisiae cells were isolated from a commercial baker's yeast Saf-momento (SAF Argentina, Buenos Aires) in the Bioengineering Laboratory at University Federal of Ceará, Brazil. The culture was inoculated on agar *Sabouraud* Acumedia (dextrose 40 gL^{-1}, casein 5 gL^{-1}, animal tissue 5 gL^{-1} e agar 15 gL^{-1}), and incubated at 30°C for 48 h. Inocullum preparation was performed in 250 mL Erlenmeyers flask with a medium volume of 100 mL, consisting of (g L^{-1}): glucose, 30; yeast extract, 5; $(NH_4)_2SO_4$ 10; KH_2PO_4 4.5; $MgSO_4.7H_2O$ 1.0; $ZnSO_4$ 0.65. The growth was carried out at 30°C and 150 rpm on an orbital shaker for 24 h. After that, cells were centrifuged at 10,000×*g* for 10 min to obtain the initial biomass used in fermentation assays.

Fermentation Assays

Experiments were conducted in 250 mL Erlenmeyer flasks at 30°C, 150 rpm and pH 4.5 and initial cell concentration of 13 gL^{-1}. The hydrolyzed products obtained after the enzymatic hydrolysis at 45°C, using an enzyme load of 30 $FPU/g_{bagasse}$ and solid percentage of 16% (*w*/*v*), were used as fermentation medium without any nutritional supplements.

Fermentation Parameters

The fermentation parameters studied were productivity (Q_p), efficiency of fermentation (η), and conversion of substrate (glucose)/product ($Y_{P/S}$). Ethanol volumetric productivity (Q_p, g L^{-1} h^{-1}) was calculated as the ratio of ethanol concentration of the fermentation (P_f, g L^{-1}) with the corresponding time (t, h), defined according to Eq. 1:

$$Q_p = \frac{P_f}{t} \tag{1}$$

The yield of ethanol based on consumed sugar ($Y_{P/S}$, g g^{-1}) was defined according to Eq. 2:

$$Y_{P/S} = \frac{P_f}{(S_0 - S_f)} \tag{2}$$

Where, S_0 ($g.L^{-1}$) and S_f ($g.L^{-1}$) are the initial and the final glucose concentration, respectively.

The efficiency of sugar conversion to ethanol (η, %) has been estimated by the relationship described in Eq. 3:

$$\eta = \frac{Y_{P/S}}{0.511} \times 100 \tag{3}$$

Where, 0.511 gg^{-1} is the theoretical value for stoichiometry conversion of glucose into ethanol.

Analytical Methods

Biomass

Cell concentration was determined by dry weight [30]. Samples (1 mL) were taken from the reaction mixture at different times, centrifuged at 6,000 rpm for 15 min a BE-6000 centrifuge (BIO ENG, Piracicaba, SP, Brazil). The biomass content was dried at 60°C on a Tecnal TE-397/4 stove (Tecnal, Piracicaba, SP, Brazil) until constant weight.

Sugars, Glycerol, and Ethanol Concentrations

Sugars (glucose, celobiose, and xylose), glycerol and ethanol were analyzed by HPLC using a Waters HPLC system (Waters, Milford, MA, USA) equipped with a refractive index Waters 2414 detector using an Aminex HPX-87 H column (Bio-Rad, Hercules, CA, USA). The eluent was 5 mmoL L^{-1} H_2SO_4 in Water MiliQ (simplicity 185, Millipore, Billerica, MA) in flow rate of 0.5 mL min^{-1} at 65°C. Samples were identified by comparing the retention times with those of carbohydrate, glycerol, and ethanol standards.

Results and Discussion

Cashew Apple Bagasse Composition

The lignocellulosic content of cashew apple bagasse *in natura* used in this study was experimentally determined according to two different methodologies. CAB was analyzed according to NREL LAP [24–26] in terms of glucan and lignin contents and results are pictured in Table 1. Table 2 shows the results of biomass content according to Gouveia et al. [27], an analytical procedure validated in Brazil to sugarcane bagasse, in terms of cellulose, hemicelluloses, and lignin.

By comparing the results of glucan and cellulose on Tables 1 and 2, it can be observed that they are similar. Lignin content, on the other hand, was a little different, but lignin content obtained by the NREL LAP was only 1.13-fold higher than the results obtained by using the procedure proposed by Gouveia et al. [27]. Therefore, results of glucose and cellobiose yield were calculated based on initial glucan content determined by the NREL LAP. These yields were further used to evaluate the overall pretreatment efficiency.

Table 1 Lignocellulosic (glucan and lignin) content of cashew apple bagasse *in natura* analyzed according to NREL laboratory analytical procedures [24–26]

Raw material	Glucans (%)	Lignin (%)
CAB *in natura*	20.54±0.70	33.80±1.30

Table 2 Lignocellulosic (cellulose, hemicellulose and lignin) content of cashew apple bagasse *in natura* analyzed according to Gouveia et al. [27]

Raw material	Cellulose (%)	Hemicellulose (%)	Lignin (%)
CAB *in natura*	19.21±0.35	12.05±0.37	38.11±0.08

Microwave-Assisted Alkali Pretreatment of Cashew Apple Bagasse

No information about the effect of solid content on pretreatment of CAB is available in the literature. But, to other raw materials, the effect of enhancing solid content may vary. Some authors [31] that studied corn stover pretreatment by lime, observed that sugar yields from enzymatic hydrolysis was independent of the solid content within a range from 60 to 200 gL^{-1}. However, Hu and Wen [7] studied a similar range of solid concentration for switchgrass microwave alkali pretreatment, and high sugar yields was obtained at low solid content. De la Hoz et al. [22] reports that for samples with low solid content, a relatively high proportion of water will favor the adsorption of microwave irradiation energy because the energy adsorption is based on oscilation of lossy water molecules. Therefore, in this work, the solid content on pretreatment stage was kept constant on 300 gL^{-1} and the effects of alkali concentration, microwave power, and pretreatment time on glucose and cellobiose yields were investigated.

Effects of Alkali Concentration on Glucose and Cellobiose Yields

Among many methods, microwave-assisted pretreatment have been studied because of its high heating efficiency to disrupt the ultrastructure of cellulose. The combination with alkali pretreatment can accelerate the chemical reaction rate by lignin removal and partial hemicellulose degradation [3, 7, 15, 16]. Although lime (calcium hydroxide) has the additional benefits of low reagent cost and safety [32, 33] and being recoverable from water as insoluble calcium carbonate by reaction with carbon dioxide [34], sodium hydroxide has received the most attention [35–38], especially combined with microwave pretreatment [7, 39–42]. Therefore, the influence of alkali concentration on the pretreatment of CAB was investigated using different concentrations of sodium hydroxide. For that purpose, before pretreatment with microwave irradiation, CAB was presoaked in NaOH solutions, concentrations ranging from 0.2 to 1.0 mol L^{-1}. After pretreatment, a sample of the liquid fraction, after the first wash, was collected and analyzed by HPLC. However, no glucose, or any other sugar, was detected. Other authors [7] observed only the presence of oligosacharides in the liquid fraction obtained after pretreatment, which are not easily quantified. Sugars yield, glucose and cellobiose, obtained after enzymatic hydrolysis are presented in Figs. 1 and 2, respectively.

As shown in Fig. 1, high glucose yields (372±12 and 355±37 mg g_{glucan}^{-1}) were obtained after pretreatment with 0.2 and 1.0 mol L^{-1} alkali concentration. It is 1.3-fold higher when compared with the control, in the absence of alkali. For a level of 99% of significance, using Microcal Origin, version 8.1, the addition of NaOH (0.2 and 1.0 mol L^{-1}) improved the glucose levels after enzymatic hydrolysis; however, the increase on alkali concentration (0.2–1.0 mol L^{-1}) did not increase glucose yield. Rocha et al. [14], who evaluated the diluted sulfuric acid pretreatment of CAB-coded as CAB-H, obtained a maximum glucose yield of 47±2 mg.$g_{CAB\text{-}H}^{-1}$ after enzymatic hydrolysis, which

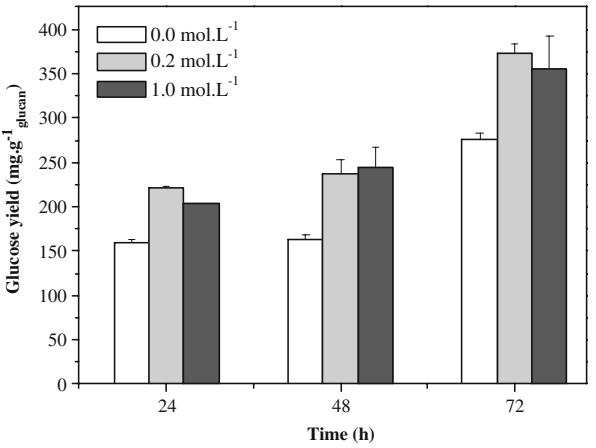

Fig. 1 Glucose yields after enzymatic hydrolysis (15 FPU $g_{CAB\text{-}M}^{-1}$) of CAB-M (2% *w*/*v*) microwave pretreated with NaOH solutions at 600 W for 15 min

corresponds to 229 mg of glucose per gram of glucan. The yields obtained in this work were higher than the obtained by Rocha et al. [14].

Hu and Wen [7] studied similar alkali load in switchgrass microwave-assisted pretreatment. These authors pointed that glucose yield increased with increasing alkali load from 0 to 0.1 $g g_{biomass}^{-1}$ (0.2 mol L^{-1} of NaOH) treated by microwave at 190°C during 30 min., and they obtained 315 mg $g_{switchgrass}^{-1}$ after enzymatic hydrolysis at 12 FPU/$g_{biomass}$ of Celluclast 1.5 L and β-glucosidase activity of 21 U/$g_{biomass}$. However, glucose yield decreased at high alkali load, from (0.2–0.3 g alkali/g biomass). The authors associated the decrease in yield to partial degradation of the hemicellulose at high alkali load.

On the other hand, Keshwani [16] has studied microwave pretreatment of switchgrass (250 W during 10 min) and the maximum glucose yield (289 mg $g_{switchgrass}^{-1}$) was obtained with 0.5 mol L^{-1} of NaOH concentration. The author pointed that celulase (40 FPU/g) and cellobiase activity (70 CBU/g) was necessary to mitigate cellobiose inhibition of cellulose.

Figure 2 shows cellobiose yields during enzymatic hydrolysis of CAB-M. Cellobiose is a product formed by cellulase attack on cellulose chain [43]. Maximum cellobiose yield

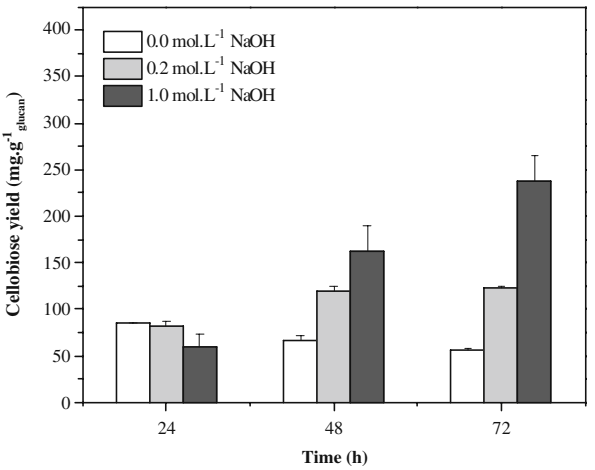

Fig. 2 Cellobiose yields after enzymatic hydrolysis (15 FPU $g_{CAB\text{-}M}^{-1}$) of CAB-M (2% *w*/*v*) pretreated with NaOH solutions at power of 600 W for 15 min

was obtained after pretreatment with 1.0 mol L^{-1} of alkali concentration (238± 28 mg g_{glucan}^{-1}). It is 1.9-fold higher than 0.2 mol L^{-1} (123±1 mg g_{glucan}^{-1}) and 4.3-fold higher than the control (56±2 mg g_{glucan}^{-1}). Thus, the increase on cellobiose yield during enzymatic hydrolysis of CAB-M can be attributed to the low cellobiase activity, 5.0 $U_{cellobiose}$/mL [29], present in Celluclast 1.5 L. Based on cellobiose levels, from the point of view the optimization of enzymatic hydrolysis for fermentation, the subsequent stages were performed using 1.0 mol L^{-1} of NaOH.

Effects of Microwave Power

Since the degree of heating was increased when microwave power is increased [42], the influence of microwave power on glucose yields during enzymatic hydrolysis was evaluated and the results are presented on Fig. 3. The pretreatment was carried out with alkali concentration of 1.0 mol L^{-1} for 15 min. It can be observed that increasing power from 600 to 900 W lead to a slight decrease on glucose yields after enzymatic hydrolysis of CAB-M, from 355±37 to 307±30 mg g_{glucan}^{-1}, respectively. Moisture loss may have occurred during the pretreatment and it may have increased with microwave power [42]. However, no significant difference ($p>0.1$) on glucose levels after enzymatic hydrolysis was observed by increasing microwave power. Other authors [42] observed no significant difference for 300, 450, and 550 W microwave pretreatments of a mixed substrate (wheat bran and rice hulls), while the substrates pretreated by 700 W microwave gave the similar enzyme production to that of untreated substrates. Results obtained by Zhu et al. [15] in a domestic microwave oven, at microwave power ranging from 300–700 W, showed that the rice straw pretreated by microwave/alkali presented almost the same final cellulose composition when the irradiation was set at 300, 500, and 700 W for 15 min. However, the increase on the pretreatment time (30 min) resulted on the increase of cellulose content. Based on this, the pretreatment time of CAB was studied maintained the power of on 600 W.

Effects of Pretreatment Time

The effects of pretreatment time (15 and 30 min) were investigated by maintaining the alkali concentration at 1.0 mol L^{-1} and power of 600 W. As shown in Table 3, the

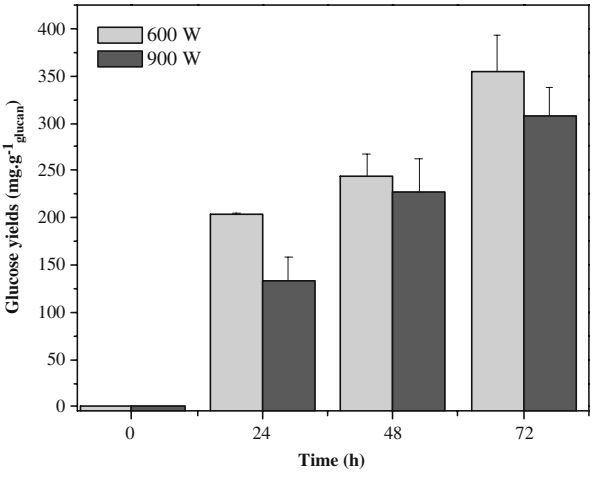

Fig. 3 Glucose yields after enzymatic hydrolysis (15 FPU $g_{CAB\text{-}M}^{-1}$) of CAB-M (2% *w*/*v*) pretreated with 1.0 mol L^{-1} of NaOH for 15 min

Table 3 Glucose yields after enzymatic hydrolysis (15 FPU.$g_{CAB\text{-}M}^{-1}$) of CAB-M (2% *w*/*v*) pretreated with 1.0 mol L^{-1} of NaOH at power of 600 W

Pretreatment time (min)	Glucose yields after enzymatic hydrolysis (mg g_{glucan}^{-1})		
	24 h	48 h	72 h
15	203.4±0.7	243.8±23.7	355.3±37.1
30	187.0±5.2	209.6±13.8	380.7±13.8

increasing on pretreatment time exerted no significant influence ($p>0.1$) on glucose yields after enzymatic hydrolysis of CAB-M. The glucose yields were 355.3±37.1 and 380.7± 13.8 mg g_{glucan}^{-1} after 15 and 30 min of pretreatment, respectively. Similar results were obtained by Hu and Wen [7], when studying different pretreatment times (5–40 min) for switchgrass. They reported that the yields of glucose and total sugars during the pretreatment and hydrolysis stages remained at almost constants levels, except for xylose yield at the pretreatment stage that dropped when the pretreatment time was 40 min. On the other hand, Zhu et al. [15] studied different pretreatment time conditions and cellulose composition on rice straw was influenced with time at all power evaluated by the authors (300, 500, and 700 W).

Effect of Solid Contente and Enzyme Load on the Enzymatic hydrolysis of CAB-M

Figure 4 shows the effects of solid content (2% and 16% *w*/*v*) on sugar yield after enzymatic hydrolysis of CAB-M. It can be observed that maximum glucose production was achieved at 16% *w*/*v*, 264±40 mg g_{glucan}^{-1}, while at 2% *w*/*v*, glucose production was 355± 37 mg g_{glucan}^{-1}. For a level of 99% of significance, using Microcal Origin, version 8.1, increasing solid content exerted a negative influence on glucose yield after enzymatic hydrolysis. However, the glucose concentration was 5.9-fold higher when 16% *w*/*v* of solids was used instead of 2% *w*/*v*, 8.8 and 1.5 gL^{-1}, respectively. It is noteworthy that mixing problems between the hydrolysis mixtures could have negatively influenced glucose

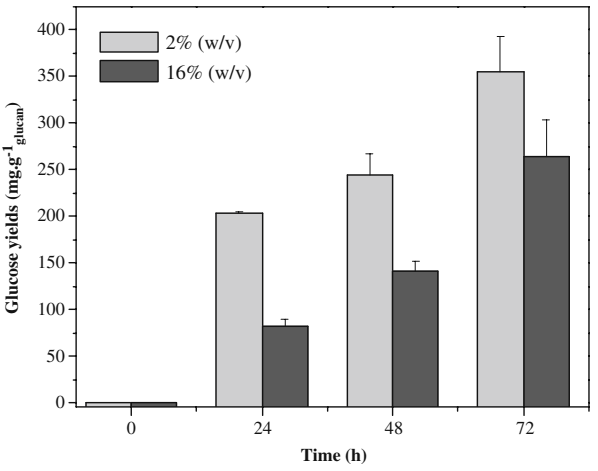

Fig. 4 Glucose yields after enzymatic hydrolysis (15 FPU $g_{CAB\text{-}M}^{-1}$) of CAB-M (2 and 16% *w*/*v*) pretreated with 1.0 mol L^{-1} of NaOH at power of 600 W

levels during enzymatic hydrolysis, especially for high solid percentage. Those mixing problems could have harmed the contact of enzyme with CAB-M.

A relatively large difference in the cellulose conversion was observed by Tengborg et al. [43] at low substrate concentrations of steam-pretreated softwood (2–5% *w/v*). However, an increase in the substrate concentration of pretreated solid (10% *w/v*) resulted in almost the same degree of cellulose conversion, but glucose concentration was doubled. Vásquez et al. [44] studied the interaction of some factors such as temperature, enzyme load, and solid percentage on conversion to glucose of sugarcane bagasse. In general, the negative influence on solid percentage over the conversion to glucose was attributed to enzymatic inhibition caused by the increase on glucose concentration. In spite of the low reducing sugar yields obtained when high substrate concentration is used, from the point of view of the process (fermentation), a high reducing sugar concentration is needed to ethanol production, which is only achieved when using high solid content. Therefore, several authors [14, 15, 43, 44] have studied some factors during enzymatic hydrolysis that can influence reducing sugar yield at high substrate concentration. They pointed the following: decrease on reactivity of the substrate in the course of hydrolysis, enzyme inactivation by cellobiose content, no-specific adsorption of the enzyme to lignin, and inhibition by high end product (glucose).

According this, to improve glucose concentration on hydrolysate obtained after microwave pretreatment of CAB to the fermentation step, the enzyme load was increased to 30 FPU $g_{CAB\text{-}M}^{-1}$.

Figure 5 show the influence of enzyme load after enzymatic hydrolysis of CAB-M at 16% (*w/v*). The maximum glucose yield (478±28 mg g_{glucan}^{-1} or 15 gL^{-1}) was obtained after 72 h of hydrolysis at 30 FPU $g_{CAB\text{-}M}^{-1}$. When 15 FPU $g_{CAB\text{-}M}^{-1}$ of bagasse was used instead, the conversion was approximately 1.8 times smaller. For a level of 99% of significance, using Microcal Origin, version 8.1, increasing enzyme load increased glucose yield after enzymatic hydrolysis. Similar results were obtained by Rocha et al. [14] after diluted sulfuric acid pretreatment. The authors achieved 19 gL^{-1} of glucose when 16% *w/v* and 30 FPU(g bagasse)$^{-1}$ was used.

Table 4 presents the profile of cellobiose yields at 16% (*w/v*) of CAB-M and 30 FPU $g_{CAB\text{-}M}^{-1}$. After 72 h of hydrolysis, cellobiose yield was 1.3-fold higher when enzyme load was enhanced from 15 to 30 FPU $g_{CAB\text{-}M}^{-1}$, 101.9±10.0 and 132.9±

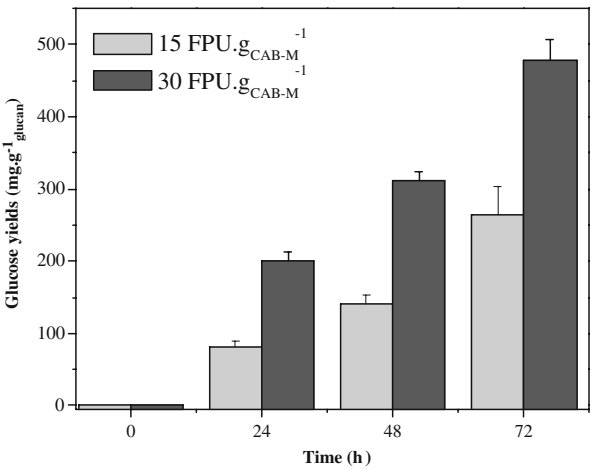

Fig. 5 Glucose yields after enzymatic hydrolysis (15 and 30 FPU $g_{CAB\text{-}M}^{-1}$) of CAB-M (16% *w/v*) pretreated with 1.0 mol L^{-1} of NaOH at power of 600 W for 15 min

Table 4 Cellobiose yields after enzymatic hydrolysis (15 and 30 FPU $g_{CAB\text{-}M}^{-1}$) of CAB-M (16% *w/v*) pretreated with 1.0 mol L^{-1} of NaOH at power of 600 W for 15 min

Enzyme load (FPU $g_{CAB\text{-}M}^{-1}$)	Cellobiose yields (mg g_{glucan}^{-1})		
	24 h	48 h	72 h
15	54.8±10.7	82.5±6.5	101.9±10.0
30	72.7±6.4	121.6±1.7	132.9±0.0

0.0 mg g_{glucan}^{-1}, respectively. Furthermore, the increase in reaction time, from 24 to 72 h, promoted an increase in cellobiose yields for both enzyme load investigated. For instance, when using 30 FPU $g_{CAB\text{-}M}^{-1}$, the yield at 72 h was 132.9±0.0 mg g_{glucan}^{-1} and at 24 h, 72.7±6.4 mg g_{glucan}^{-1}. In this case, the yield at 72 h was 1.8-fold higher than at 24 h. Regarding cellobiose yield, the increase in reaction time is more efficient than increasing enzyme load. However, the supplementation with enzyme was important to increase glucose concentration (1.7-fold higher; Fig. 05) for further ethanol production. It can also be observed that cellobiose yields at the end of enzymatic hydrolysis (15 and 30 FPU $g_{CAB\text{-}M}^{-1}$) are similar, indicating that and increase on enzyme load did not cause an increase in cellobiose yields, which may indicate that the end product (cellobiose) does not cause a enzymatic inhibition.

Fermentation of Cashew Apple Bagasse Hydrolyzates

Microwave-alkali pretreatment of cashew apple bagasse was evaluated in order to improve the glucose yield after its enzymatic hydrolysis. The pretreatment conditions were fixed at 1.0 mol L^{-1} of alkali concentration, 15 min and power of 600 W. Enzymatic hydrolysis of CAB-M was conducted with solid percentage of 16% (*w/v*), 30 FPU $g_{CAB\text{-}M}^{-1}$, at 45°C and 72 h. The hydrolyzate obtained was used as substrate for ethanol production by *S. cerevisiae*.

Fermentation was conducted using 13 gL^{-1} of initial biomass and the profiles of glucose, ethanol, biomass, glycerol, and xylose concentrations are presented in Fig. 6. *S.*

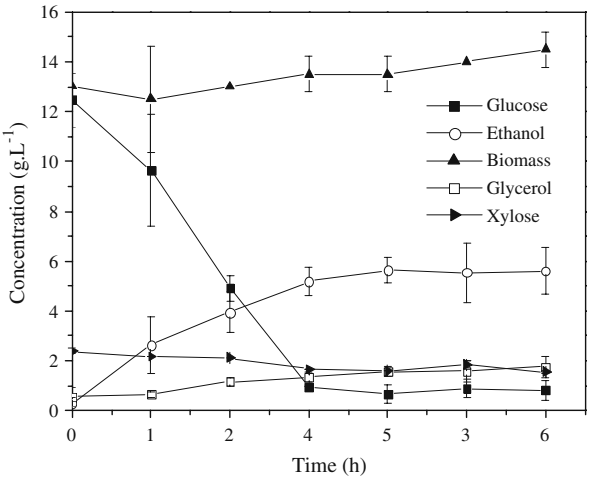

Fig. 6 Fermentation assay of the hydrolyzate obtained after enzymatic hydrolysis (30 FPU $g_{CAB\text{-}M}^{-1}$) of CAB-M (16% *w/v*) pretreated with 1.0 mol L^{-1} of NaOH at power of 600 W for 15 min by *S. cerevisiae* at 30°C and 150 rpm. Glucose (*filled square*), ethanol (*empty circle*), biomass (*filled triangle*), glycerol (*empty square*) and xylose (*filled right-pointing arrow*)

cerevisiae was able to grow and to produce ethanol when cultivated in CAB-M hydrolyzates without any nutritional supplements.

The CAB-M hydrolyzate has an initial glucose concentration of 13.0 gL^{-1} and after 4 h of fermentation, ethanol concentration, and productivity were 5.6 gL^{-1} and 1.41 g L^{-1} h^{-1}, respectively. After this time, glucose concentration was 1.0 gL^{-1}. Xylose was identified on media at concentration of 2.0 gL^{-1} and it was not consumed by *S. cerevisiae*. Maximum glycerol concentration (1.5 gL^{-1}) was quantified after 4 h of fermentation. Ethanol yields obtained in this study based on the amount of glucose was 0.48 g ethanol g^{-1} glucose. In addition, no influence of the inhibitors that are formed during pretreatment were observed in the fermentation of the hydrolyzate. It is believed that they were most likely removed after the washing step, what promoted the growth and ethanol production by *S. cerevisiae*.

Zhu et al. [3] evaluated ethanol production from wheat straw pretreated with microwave/alkali in simultaneous saccharification and fermentation (SSF) process. Maximum ethanol concentration (34.3 gL^{-1}) was obtained after 72 h of fermentation, which corresponds to a productivity of 0.48 gL^{-1} h^{-1}. The same authors pointed that for SSF process, the microwave-assisted alkali pretreated wheat straw had higher ethanol productivity and yield promoted by higher hydrolysis rate when compared to conventional alkali pretreatment.

In a previous work, Rocha et al. [14] studied diluted acid pretreatment of cashew apple bagasse alone and followed by alkali pretreatment, and the solid residues were coded CAB-H and CAB-OH, respectively. The fermentation of hydrolyzates after enzymatic hydrolysis resulted in ethanol concentration and productivity of 8.2 gL^{-1} and 2.7 gL^{-1} h^{-1} for CAB-H and 20.0 gL^{-1} and 3.33 gL^{-1} h^{-1} for CAB-OH.

Table 5 presents the data for Q_p, η, and $Y_{p/s}$ obtained in this work and others from literature for alcoholic fermentation of cashew apple juice (glucose and fructose) and CAB-H hydrolyzate (glucose).

It is observed on Table 5 that the values of η and $Y_{p/s}$ obtained in this work are similar when compared with the literature, being close to the values for cashew apple juice and CAB-H hydrolyzate. This result turns the hydrolysate obtained from CAB-M an interesting alternative for ethanol production by fermentation. Although the productivity of this work was inferior to the others, it is pointed that the total sugars concentration in cashew apple juice and CAB-H hydrolyzate were around to 70 and 17.4 gL^{-1}, respectively. However, some aspects of microwave alkali pretreatment have to be further investigated as well as other pretreatments to increase glucose concentration after enzymatic hydrolysis.

Table 5 Fermentation parameters with *S. cerevisiae* at 30°C and 150 rpm for several substrates from this study and literature

Substrate	Productivity (g L^{-1} h^{-1})	Efficiency (%)	Conversion substrate/product $Y_{P/S}$ (g/g)
CAB-H hydrolyzate (glucose)[a]	2.7	91.98	0.47
Cashew apple juice (glucose+fructose)[b]	4.29	88.95	0.47
Present study (glucose)	1.41	93.93	0.48

[a] Rocha et al. [14]

[b] Pacheco et al. [45]

Conclusion

This work shows that the used of microwave is an efficient heating method in the pretreatment of CAB to increase its enzymatic hydrolysis rate, observed by the increase on glucose and cellobiose levels. The parameters evaluated here that influenced the glucose production after enzymatic hydrolysis were: alkali concentration (0.2 and 1.0 mol L^{-1}), solid percentage (16% *w*/*v*), and enzyme load (30 FPU $g_{CAB\text{-}M}{}^{-1}$). Pretreatment time and microwave power had no significant effect on glucose concentration. Increasing the solid content and enzyme load improved glucose concentration to 15 gL^{-1} (98±8 mg $g_{CAB\text{-}M}{}^{-1}$) at 30 FPU $g_{CAB\text{-}M}{}^{-1}$ and 16% (*w*/*v*) of CAB-M, after 72 h of hydrolysis. Fermentation of the hydrolyzate obtained after enzymatic hydrolysis of CAB-M by *S. cerevisiae* cells resulted in an ethanol concentration of 5.6 gL^{-1} in 4 h of fermentation. Ethanol yields based on the amount of glucose was 0.48 $g_{ethanol}g_{glucose}{}^{-1}$. Therefore, based on the results provided, the microwave/alkali pre-treatment was an efficient pre-treatment method of CAB aiming at ethanol production; however, some aspects of microwave alkali pretreatment have to be further investigated to increase glucose concentration after enzymatic hydrolysis.

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