International Journal of Advanced Engineering Research and Science (IJAERS)
Peer-Reviewed Journal
ISSN: 2349-6495(P) | 2456-1908(O)
Vol-9, Issue-11; Nov, 2022
Journal Home Page Available: https://ijaers.com/
Article DOI: https://dx.doi.org/10.22161/ijaers.911.26

Enzymatic Saccharification of Sugarcane Bagasse Using Ash - Supplemented Hydrogen Peroxide as Pre-Treatment

Estácio Jussie Odisi[1], André Oliveira de Souza Lima[2*], Vivian Colonetti[1], Rômulo Couto Alves[1], Andressa Gilioli[1], Mara Gabriela Novy Quadri[1]

[1]Departamento de Engenharia Química e Engenharia de Alimentos, Universidade Federal de Santa Catarina (UFSC), Campus Universitário, Trindade, CP 476, Florianópolis-Santa Catarina 88040-900, Brazil
[2]Escola do Mar, Ciência e Tecnologia, Universidade do Vale do Itajaí (UNIVALI), Rua Uruguai, 458, Itajaí-SC, 88302-202, Brazil.
*Corresponding author

Received: 05 Oct 2022,

Received in revised form: 24 Oct 2022,

Accepted: 01 Nov 2022,

Available online: 18 Nov 2022

***Keywords*— alkaline hydrolysis, enzyme treatment, physico-chemical characterization, experimental design.**

***Abstract*—** *Purpose: Enzymatic saccharification of sugarcane bagasse with cellulases was investigated after hydrogen peroxide pretreatment. Methods: Two pretreatments, alkaline hydrogen peroxide and hydrogen peroxide supplemented with ash were compared in their performance in the improvement of the susceptibility of bagasse to enzymatic action. The reaction yield was evaluated by the reducing sugar content released from the pretreated bagasse after 48 hours of enzymatic hydrolysis, and the best condition was found for both treatments. Results: The yield, expressed in reducing sugars for the alkaline hydrogen peroxide pretreatment was 217.6 mg g-1 bagasse, and 179.9 mg g-1 bagasse for hydrogen peroxide ash pretreatment; the untreated bagasse provided 74.3 mg g-1 bagasse yield, showing the effectiveness of the two pretreatments. Conclusion: The pretreatment with hydrogen peroxide supplemented with ashes appears more feasible for implementation since alkali addition in the pretreatment delivers many caustic residues that need expensive washing process and generate aggressive effluents into the environment; besides alkali addition promotes partial degradation of hemicellulose.*

Supplementry Materials available at:
https://drive.google.com/file/d/1cK3EEMZ2Y8uViGrF1kbNjGpltY7NID78/view?usp=sharing

I. INTRODUCTION

Lignocellulosic biomass is the only sustainable source of fuels and materials foreseeable to the humanity [1]. Among the different types of biomass, the lignocellulosic residues have awakened a big interest, due to their availability in large scale, the low cost of obtention and the possibility of environmentally correct energy production. The sugarcane bagasse (agro-industrial residue) seems to be an economically viable raw material, since sugar mills generate bagasse at rates as large as 280 kg t^{-1} dry weight of harvested cane [2, 3], which is estimated in 647.6 million tons for 2017/2018 season in Brazil [4]. Part of this amount is used to generate energy in the sugarcane industry, and the other part is considered as waste, which can be used to produce second generation ethanol and other byproducts [3].

Sugarcane bagasse is composed basically of cellulose, hemicellulose and lignin, which represent 94% of its dry weight [2]. Cellulose is a linear homopolysaccharide that consists of glucose (D-glucopyranose) units linked together by β-(1-4) glycosidic bonds (β-D-glucan) [5, 6]. The cellulose hydrolysis may

occur by an enzymatic mechanism that involves synergistic actions by endoglucanase (EC 3.2.1.4), exoglucanase or cellobiohydrolase (EC 3.2.1.91), and β-glucosidase (EC 3.2.1.21) [7–9].

Effective conversion of recalcitrant lignocellulose to fermentable sugars requires three sequential steps: (1) size reduction, (2) pretreatment/fractionation, and (3) enzymatic hydrolysis [7, 10]. The major obstacle and difficult technological challenge to industrial scale production of fuel from lignocellulose is to overcome the recalcitrance of natural lignocellulosic materials, which must be enzymatically hydrolyzed to produce fermentable sugars [10].

Numerous pretreatments of lignocellulosic materials have been described in literature [10–12] in order to loosen lignin and release cellulose and hemicellulose from these materials for subsequent enzymatic hydrolysis, resulting in monomeric sugars of economic wide application [13]. The pretreatment with alkaline hydrogen peroxide has called attention due to the great output found in recent studies [3, 14–16]. Furthermore, hydrogen peroxide (H_2O_2) does not produce waste since it decomposes into water and oxygen, so it does not generate byproducts that could inhibit enzymatic hydrolysis [3]. However, it is known that the elevation of the pH to values as high as 11.5 requires the addition of considerable amounts of sodium hydroxide, resulting in caustic waste production, unsuitable for disposal in the environment [17].

The present work evaluated a process using pretreatment with ashes, alkalizing hydrogen peroxide to ensure efficient oxidation of the bagasse to modify the lignocellulosic structures, aiming to favor enzymatic hydrolysis. Additional benefit comes from this process since ashes can be easily obtained from boilers thus further reducing the waste generated by this industry. For this purpose two pretreatment methods were evaluated: one using alkaline hydrogen peroxide, as a standard process [3, 18], and the other using hydrogen peroxide supplemented with ashes. For both treatments, the best operational conditions were evaluated by experimental design. Chemical composition and morphological structure were analyzed after the modifications promoted on the bagasse.

II. METHOD

Materials preparation

Sugarcane bagasse, obtained from the local market after juice extraction, was grounded, washed with distilled water until the bagasse was sugar free, and dried in two steps: a) at 50°C for 72 h, followed by b) at room temperature for 24 h; samples were stored in plastic bags.

The ashes used in the pretreatment were prepared by drying at 50°C for 72 h, burned, to carbonize, and incinerated at 600°C for 8 hours.

Sugarcane bagasse processing

Pretreatments

The influence of two pretreatments, hydrogen peroxide supplemented with ashes and hydrogen peroxide supplemented with sodium hydroxide (alkaline H_2O_2), was evaluated on the yield of the enzymatic reaction to degrade sugarcane bagasse to fermentable sugars. Pretreatments were performed in suspensions of 2% (w/v) bagasse and H_2O_2 at concentrations specified *a priori*. Details of the pretreatment procedure are described in [19]. The pretreatment optimization was carried out by the surface response methodology where, in the first step, full factorial designs with central points were developed (data not shown here), and the factors studied were H_2O_2 concentration and temperature for both treatments; ash concentration was also studied for former pretreatment, while agitation was for the later. The results were used for the displacement of the response surface. In the second step, composite central designs were carried out. Statistical analyses were performed by Analysis of Variance (ANOVA) and empirical models were developed to describe results.

Enzymatic hydrolysis

Enzymatic hydrolysis was conducted with a mixture of β-glucosidase, exoglucanase and endoglucanase, and hydrolysis was conducted according to the procedure described in [19].

Reducing Sugar Quantification

Reducing sugar contents were determined by the 3,5-dinitrosalicylic acid (DNS) assay [20]. As a nonspecific reagent, it reacts with both five and six carbon reducing sugars, giving readings based on a standard curve of glucose 50 mM in sodium acetate buffer pH 4.0. 100 µL of the sample were added to 100 µL DNS reagent in a microtube (1.5 mL). DNS reactions were carried out in a dry block (MARCONI – MA4004) by heating at 100°C for 15 min, followed by cooling to 4°C for 5 minutes. Then, 1 mL distilled water was added and maintained at 20°C. 100 µL of the completed DNS reaction was added in a flat-bottom microplate and absorbencies were measured at 540 nm.

Chemical analysis

Samples of bagasse, pretreated or not, were prepared for chemical analysis according the TAPPI T 264

cm-97 in order to determine holocellulose content according the method described by Browning [21], cellulose content following the Kurschner-Hoffner approach [21], Klason lignin [21], and ashes content (TAPPI T211 cm07). The hemicellulose was estimated by the difference between holocellulose and cellulose.

Metal content was analyzed in the ashes provided from sugarcane bagasse. The samples were weighed accurately (approximately to 0.130g) in Teflon decomposition vessels. Five milliliters of ultrapure nitric acid and one milliliter of hydrogen peroxide (pro analysis) were added. The samples were decomposed in a microwave oven (Milestone MLS 1200) and the metals Ca, Na, Mn and Mg were determined by inductively coupled plasma mass spectrometry (Perkin Elmer - NexION 300 D). The concentrations were given as dry weights.

FTIR- analysis

FTIR spectroscopic analysis was carried out to detect changes in functional groups that may have been promoted by pretreatments and enzymatic hydrolysis. FTIR spectrum was recorded between 4000 and 400 cm^{-1} using a Shimadzu (IRPrestige-21) spectrometer with detector at 4 cm^{-1} resolution and 120 scan per sample. Discs have been prepared by mixing 3 mg of dried sample with 300 mg of KBr (spectroscopic grade) in an agate mortar. The resulting mixture was successfully pressed at 10 MPa for 3 min.

Scanning electron microscopy (SEM)

Physical changes in the native and pretreated sugarcane bagasse were observed by Scanning electron microscopy (SEM). Images of the surfaces of the native and pretreated sugarcane bagasse were taken using a JEOL - JSM-6390LV SEM. The specimens were coated with gold palladium using a LEICA SCD 500 fine coater and observed using a voltage of 15 KV.

GLC conditions

The samples after enzymatic hydrolysis (50 mL) were lyophilized. The reducing sugars were converted to their persilyl derivatives by the addition of 0.1 mL of pyridine with 0.1 mL of BSTFA [N,O-bis-(trimethylsilyl)-trifluoracetamide] and heated at 70°C for 20 min. Gas-liquid chromatography analysis of alditol acetates was carried out on a Shimadzu GC 2014 gas chromatograph equipped with a capillary column Restek (30 m long, 0.25 mm internal diameter, 0.25 µm film thickness) and a temperature program consisting of 2 min at 140°C followed by heating at a rate of 10°C min^{-1} up to 260°C. Detection was carried out with a flame ionization detector (FID) at 250ºC. N_2 was used as a carrier gas (56 psi; 1.4 mL min^{-1} flow) and the injection split ratio was 1:20.

III. RESULTS AND DISCUSSION

Optimization of the alkaline hydrogen peroxide pretreatment

Alkaline hydrogen peroxide is a known treatment to release lignin from lignocellulosic materials and was used as a standard treatment in this paper. A 2^3 full factorial experimental design with triplicated central point (data not shown here) was used to evaluate the influence of the hydrogen peroxide concentration, temperature and agitation during the pretreatment. Enzymatic hydrolysis was carried out after each bagasse pretreatment specified by the experimental design, and the reducing sugar yield response was obtained after 48 hours of enzymatic hydrolysis. The influencing factors were statistically evaluated by the ANOVA in a 5% significance p-level. From the F-test, the model showed a calculated F_{calc} value (597.8) much higher than the listed one (3.45), indicating that the model is highly significant within 95% of confidence level [22]. No evidences of lack of fit were observed since the F_{calc} (2.26) was smaller than the one listed (9.16). The model's regression coefficients have shown a significant curvature coefficient which evidences a nonlinear model; the main effects of temperature and agitation, followed by the interactions H_2O_2 concentration x temperature and temperature x agitation negatively influenced the reaction, which means that lower temperatures and agitation gave better yields.

Figure 1 shows the level curves of the reducing sugar yield as a function of H_2O_2 concentration-temperature, and temperature-agitation. At low H_2O_2 concentrations, the influence of temperature is very small, but at higher H_2O_2 concentration, the influence of temperature is large. The best hydrolysis condition was found in high H_2O_2 concentration and low temperature pretreatment. It was observed that at high H_2O_2 concentration and high temperature the bagasse particles agglomerate in a form similar to a paper, becoming less susceptible to the enzymatic attack, as evidenced by the low yield results. It was also observed that the agitation influence was negative, and low agitation speed adequately improves the process in the range of values studied.

Although ANOVA had indicated the main effect of alkaline hydrogen peroxide concentration as not significant, it was positive, suggesting that other experimental ranges could maximize the reducing sugars yield while temperature was maintained around the central point and agitation at low values.

New experiments, with a central composite factorial design, were performed increasing peroxide concentration at different temperatures (Table 1). ANOVA (Table 2) shows that the model (Equation 1) described the

results very well, and can be used for prediction, since calculated F-value is more than 5 times the obtained F-value from statistical tables; the lack of fit is non-significant as calculated F-value is lower than the obtained F-value from statistical tables.

$$RS = - 590.6155 + 25.6824 \times P - 0.2585 \times P^2 + 38.7489 \times T - 1.5881 \times T^2 - 0.1932 \times P \times T \quad (1)$$

where RS is the Reducing sugars yield (mg g^{-1} bagasse), P is H_2O_2 concentration (%), T is the Temperature (°C).

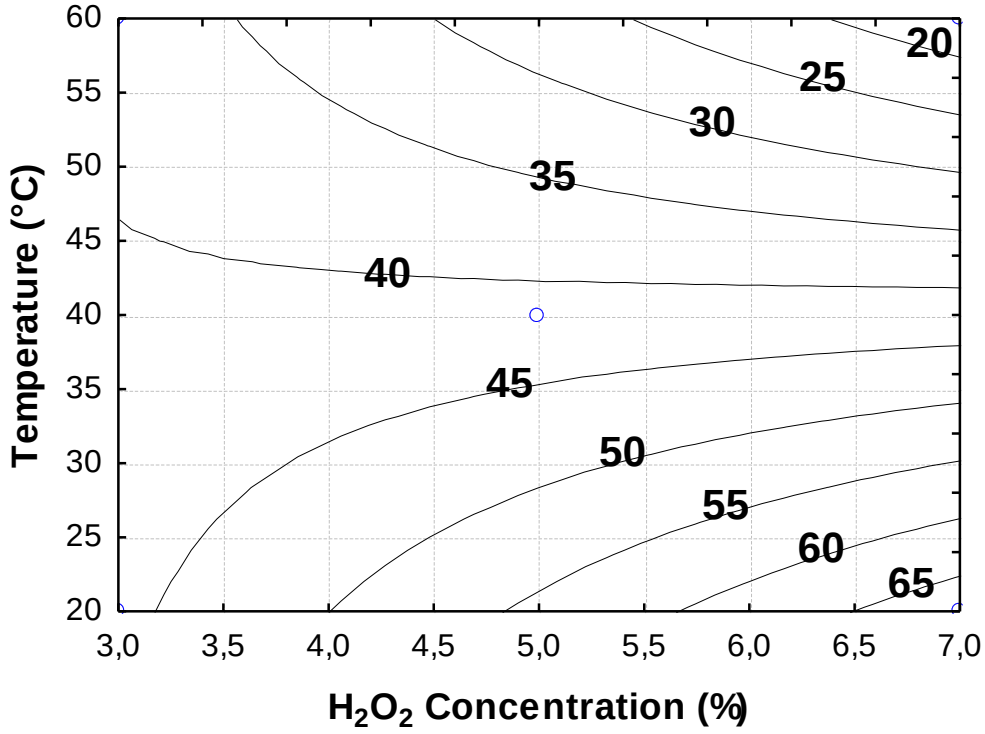

Fig.1. Level curves of reducing sugar yields after hydrolysis of alkaline hydrogen peroxide pretreatment. The numbers in the contour lines represent the reducing sugar yields (mg g^{-1} raw bagasse).

Table 1. 2^2 Central composite design for the sugarcane bagasse pretreatment with alkaline hydrogen peroxide.

Assay	H_2O_2 (%)	Temperature (°C)	Reducing sugars (mg g^{-1})
1	5	38	127.8
2	9	38	167.3
3	9	52	144.2
4	5	52	172.9
5	7	35	137.8
6	7	55	164.4
7	4.17	45	143.6
8	9.83	45	183.8
9	7	45	175.6
10	7	45	179.5
11	7	45	173.6
12	7	45	172.0
13	7	45	170.6
Control	-	-	42.2

An optimal operational condition was found at a concentration of 9.39% (v/v) H_2O_2 at 46°C. It differs from values obtained in other studies in the literature [3, 14, 15] due to the levels used in the experimental design. The kinetics of the reaction can be observed in Figure 2, and the best reaction time is 40 minutes of pretreatment. After 1 hour pretreatment, the reducing sugars yield decreases suggesting that excess of time reaction makes the bagasse less susceptible to enzymatic hydrolysis.

Ash-supplemented Hydrogen peroxide pretreatment optimization

Pretreatment optimization with ash-supplemented hydrogen peroxide followed the same procedure as described above. The influence of the factors temperature and hydrogen peroxide and ash concentrations was evaluated by a 2^3 full factorial experimental design in triplicates at the central point; it was statistically analyzed by ANOVA in a significance level of 5% (data not shown here). The value for the F_{calc} (29.94) was greater than the listed one (5.050), generating a significant model for prediction. For the lack of fit, the F_{calc} value (11.59) is somewhat smaller than the listed value (19.16), and the model was well fit [22]. Results had a significant curvature coefficient, which means that the model is nonlinear. The significant effects were ash concentration and temperature, followed by the second order interactions of the H_2O_2 concentration×ash concentration and ash concentration×temperature. Additionally, two control assays were carried out in order to evaluate the pretreatment effect on the enzyme reaction: one, with no ash-H_2O_2 pretreatment showed 49.0 mg g^{-1} yield, and the other, just with 3% ash and no H_2O_2 have shown 60.1 mg g^{-1} yield, suggesting a cellulase activity increase by the presence of ashes.

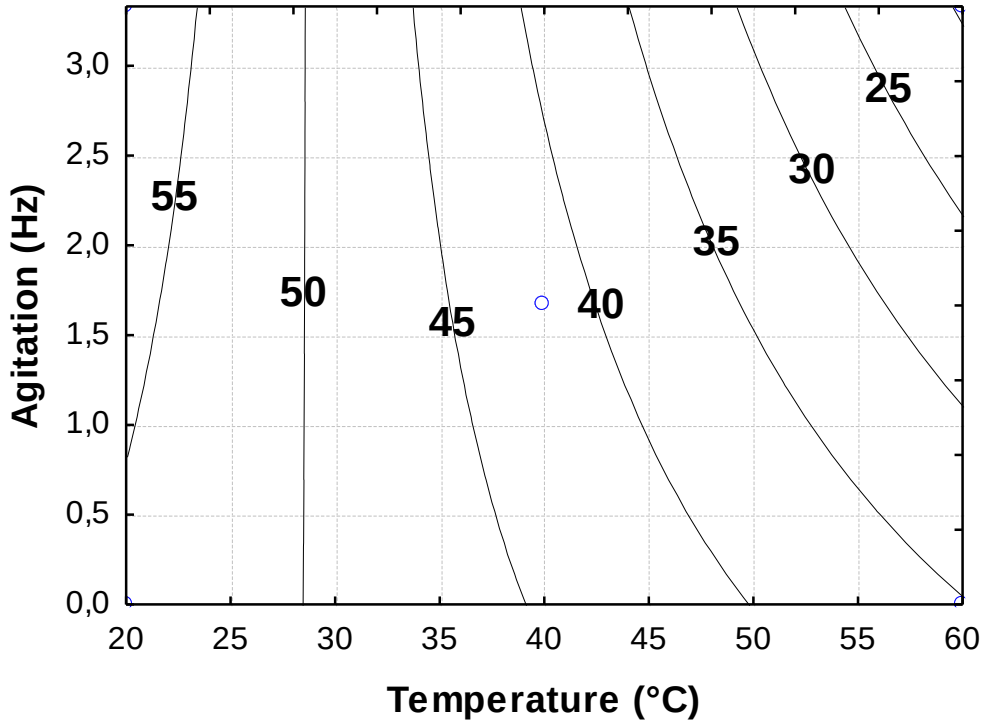

Fig.2. Level curves of reducing sugars yields after hydrolysis of alkaline hydrogen peroxide pretreatment. The numbers in the contours represent the reducing sugar yields (mg g^{-1} raw bagasse).

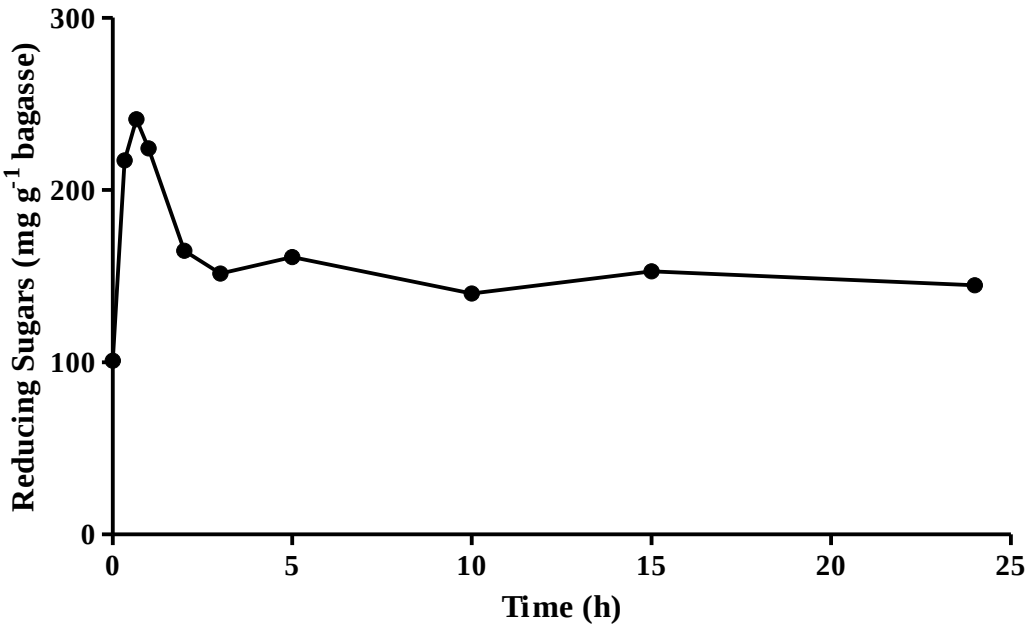

Fig.3. Kinetics of reducing sugar yield after hydrolysis as a function of pretreatment time at optimal conditions of alkaline-pretreatment.

Figure 3 shows the level curves of the reducing sugar yield as a function of the H_2O_2 concentration-ash concentration and ash concentration-temperature. The concentration of H_2O_2 does not influence the reaction in a great extension. On the other hand, the higher the ash concentration the higher the reducing sugars conversion. Temperature has a better yield at 60°C, the highest temperature tested.

Displacement of the response surface was performed with new experiments given by a composite factorial design by increasing ash concentration and peroxide concentration at 60°C (Table 3). The ANOVA (Table 4) attests that a good model (Equation 2) was built; it can be used for prediction, with no lack of fit.

$$RS = 44.2143 + 54.0467\times A - 7.5981\times A^2 + 3.2051\times P - 0.1543\times P^2 - 0.3687\times A\times P \quad (2)$$

where A is the Ash concentration (%).

Table 2. ANOVA of the alkaline hydrogen peroxide pretreatment model.

Source	Sum of Square	Degree of freedom	Mean of Square	F_{calc} [a]	F_{tab} [b]
Regression	3801	4	950.2	46.90 [a]	3.838
Residue	162.1	8	20.26		
Lack of Fit	114.4	4	28.60	2.399 [b]	6.388
Pure Error	47.69	4	11.93		
Total	3963	12			

[a] F_{calc} is the calculated F-value; [b] F_{tab} is the obtained F-value from tables. Explained variation=95.91%; Maximum explained variation=98.80%.

Table 3. Central composite design 2^2 for the pretreatment of sugarcane bagasse with ash supplemented hydrogen peroxide.

Assay	Ash (%)	H_2O_2 (%)	Reducing sugar (mg g^{-1})
1	3	5	151.1
2	3	9	138.6
3	7	5	46.1
4	7	9	27.7
5	2.17	7	132.6
6	7.83	7	7.9
7	5	4.17	123.4
8	5	9.83	136.2
9	5	7	110.3
10	5	7	125.1
11	5	7	110.7
12	5	7	143.7
13	5	7	142.5
Control	-	-	21.5

Table 4. ANOVA for the model from hydrogen peroxide pretreatment supplemented with ashes.

Source	Sum of Square	Degree of freedom	Mean of Square	F_{calc} [a]	F_{tab} [b]
Regression	25658	2	12829	72.86 [a]	4.103
Residue	1760	10	176.1		
Lack of Fit	694.9	6	115.8	0.435 [b]	6.163
Pure Error	1066	4	266.5		
Total	27419	12			

[a] F_{calc} is the calculated F-value; [b] F_{tab} is the obtained F-value from tables. Explained variation=93.58%; Maximum explained variation=96.11%.

Table 5. Chemical composition of the sugarcane bagasse after the pretreatments.

	Without pretreatment		Alkaline hydrogen peroxide pretreatment		Ash-supplemented Hydrogen peroxide pretreatment	
	Mass (g)	Yield (%)	Mass (g)	Yield (%)	Mass (g)	Yield (%)
Inicial	5.00	100	3.02 ±0.09	60.47 ±1.8	4.51 ±0.08	90.16 ±1.69
Cellulose	2.08 ±0.04	41.6 ±0.9	2.01 ±0.1	40.2 ±2.0	2.03 ±0.04	40.8 ±0.9
Hemicellulose	1.05 ±0.04	20.90 ±0.8	0.70 ±0.05	13.9 ±0.9	0.96 ±0.07	19.3 ±1.3
Lignin	1.4 ±0.01	28.1 ±0.3	0.28 ±0.01	9.2 ±0.2	0.78 ±0.02	17.3 ±0.4
Ash	0.19 ±0.01	3.7 ±0.3	0.08 ±0.01	2.8 ±0.3	0.15 ±0.02	3.4 ±0.6
Extractives	0.035 ±0.008	0.7 ±0.2				

The optimal conditions found were 3.40% (v/v) ash and 6.32% (v/v) H_2O_2 concentration. By the kinetic procedure (Figure 6), 2 hours of pretreatment is the best reaction time. Longer pretreatment time reduces the

production of reducing sugars for the alkaline pretreatment.

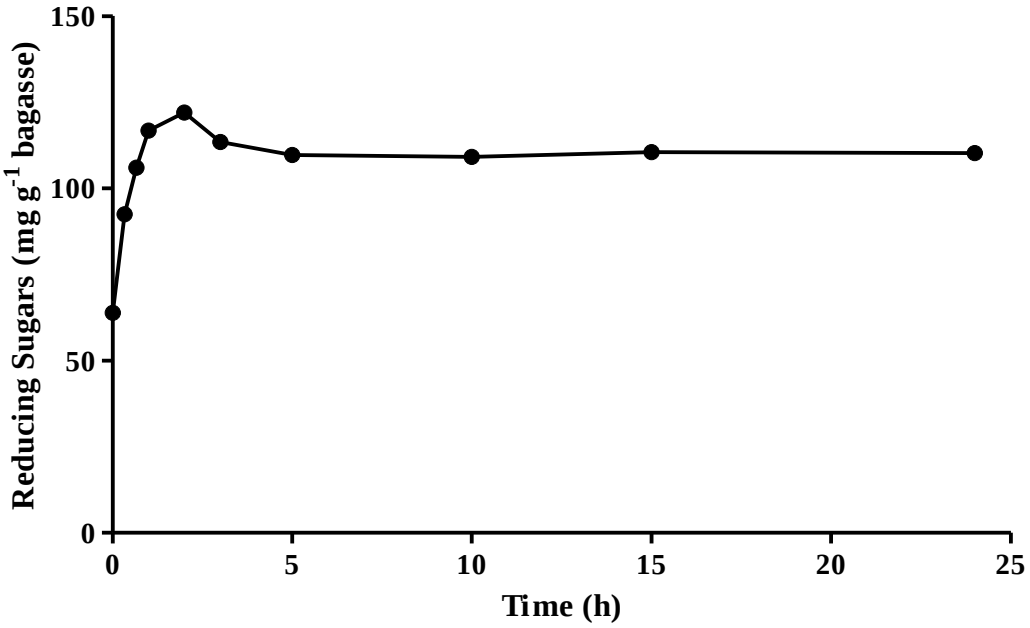

Fig.6. Kinetics of reducing sugars yield after hydrolysis as a function of pretreatment time at optimal conditions of ashes-pretreatment.

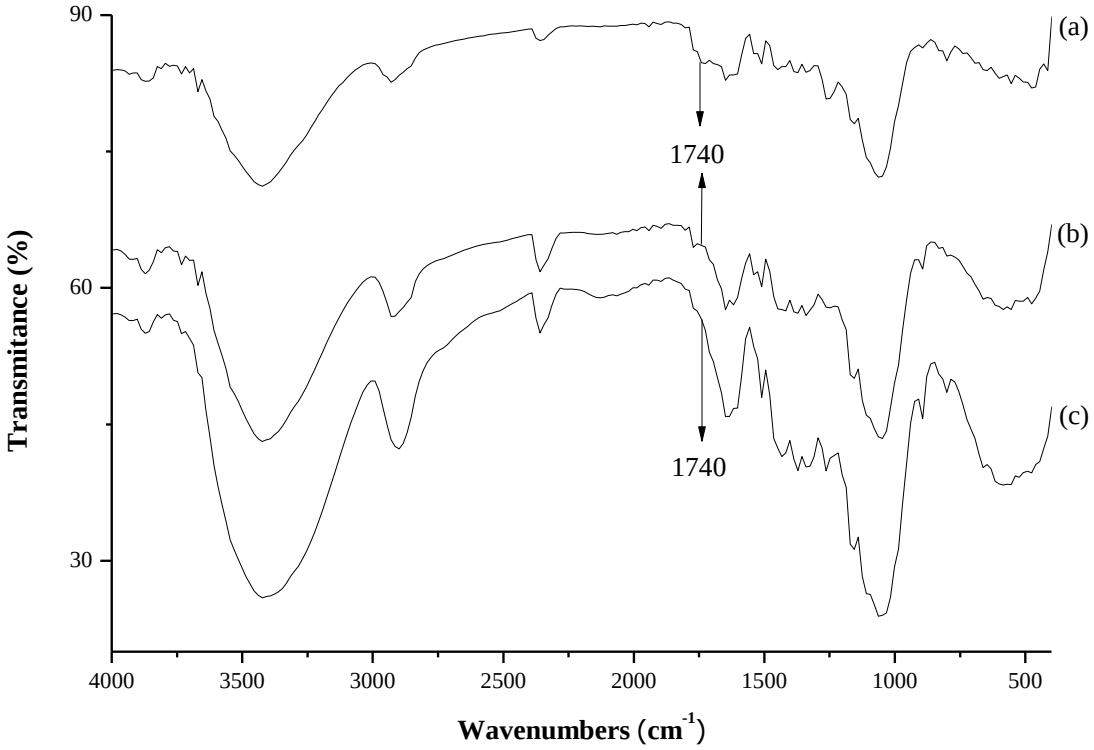

Fig.7. Infrared spectra from (a) Bagasse. (b) Bagasse pretreated with alkaline hydrogen peroxide. (c) Bagasse pretreated with alkaline hydrogen peroxide after enzymatic hydrolysis.

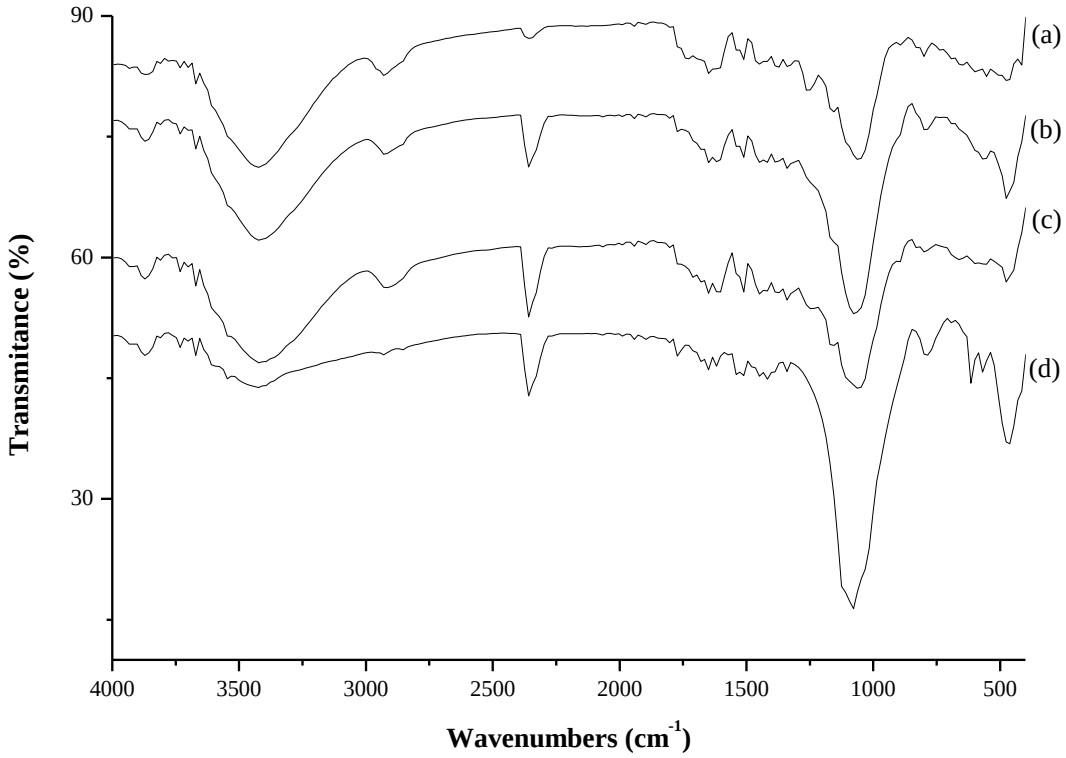

Fig.8. Infrared spectra from (a) Bagasse. (b) Bagasse pretreated with hydrogen peroxide with ash. (c) Bagasse pretreated with hydrogen peroxide with ash after enzymatic hydrolysis. (d) Ash.

Chemical Composition

The sugarcane bagasse from each pretreatment was characterized for its chemical composition (Table 5). According to the results, after the alkaline hydrogen peroxide pretreatment, the amount of lignin was much lower than that of non-pretreated bagasse. Thus it is clear that this method detaches lignin off the lignocellulosic material, causing its removal from cellulose and hemicellulose, which are now available for enzymatic hydrolysis [3, 14, 15, 18]. The pretreatment supplemented with ashes was not so effective as the alkaline pretreatment since about half of lignin still remains in the vegetable matrix. On the other hand, after alkaline pretreatment, a mass loss of about 35% of hemicellulose from the original bagasse was observed, whereas in the pretreatment supplemented with ashes, the loss was of about 10%. This result shows that alkaline pretreatment was aggressive to the vegetable fibers, performing not only the lignin release but also partially degrading the components. Monte et al. [14] reported a hemicellulose loss of 36% after alkaline pretreatment.

The ashes from sugarcane bagasse have shown a great amount of the metals Ca (14.66 ± 0.06 g/Kg) and Mg (11.04 ± 0.06 g/Kg) in their composition, and a small quantity of Mn (2.98 ± 0.01 g/Kg) and Na (1.73 ± 0.01 g/Kg). Some studies show that some metals, such as Mn, act as catalysts to oxidize the H_2O_2 by peroxidase, performing delignification of lignocellulosic materials [23–25]. They can also be cofactors for cellulase enzymes, which has its activity increased [26]. This can explain the activity increase of cellulases when ashes were added.

Functional groups analysis

The FTIR spectrum of the sugarcane bagasse shows the same basic structure as all wood samples: wide OH stretching (3300–4000 cm^{-1}), C–H stretching in methyl and methylene groups (2800–3000 cm^{-1}), and a wide superposition with sharp and discrete absorptions in the region from 1000 to 1750 cm^{-1} [27]. Figure 5 shows the FTIR spectra for samples pretreated with alkaline hydrogen peroxide and after the enzymatic hydrolysis, also. Pretreated and hydrolyzed samples showed no significant change compared to the sample of untreated bagasse. It is possible to identify only a slight change in intensity of some bands in which one cannot affirm a real decrease of these components, as it can only be related to the concentration and homogeneity from the preparation of the KBr pellets. However, it is possible to observe that the band formed in the region of 1740-1720 cm^{-1} starts to reduce its intensity after pretreatment and almost completely disappears after the enzymatic hydrolysis [27].

Moreover, this region is responsible for the stretching of the carbonyl (C=O bond), and a decrease of these groupings may indicate the decrease of xylans from hemicellulose [27, 28].

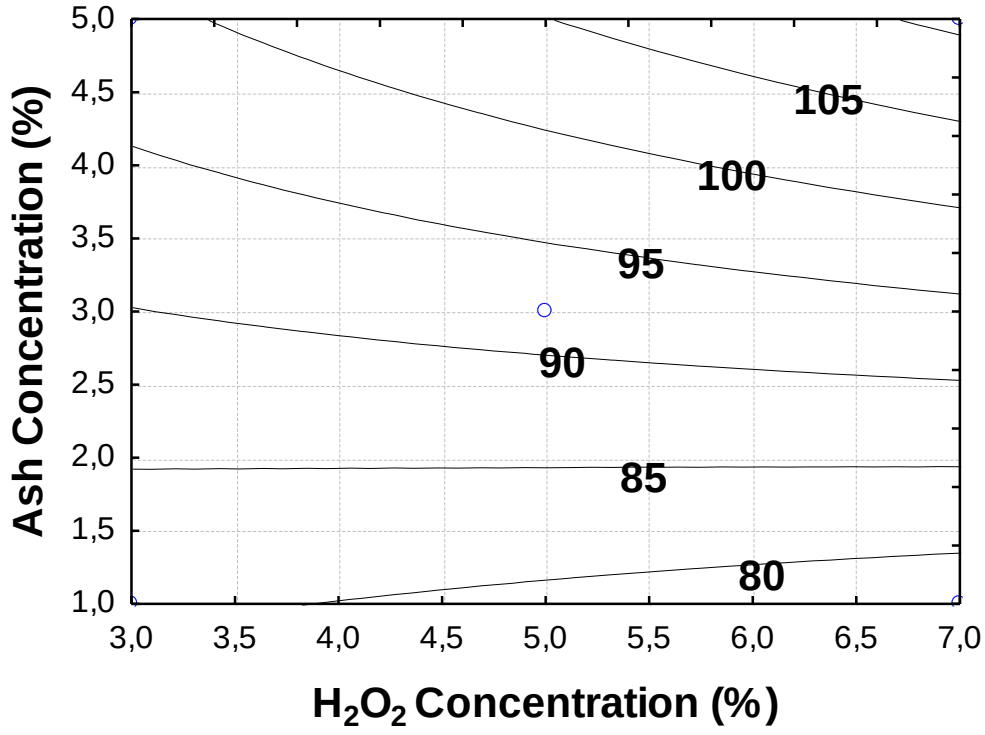

Fig.4. Level curves of reducing sugar yield after hydrolysis of the hydrogen peroxide pretreatment supplemented with ash. The numbers in the contours represents the reducing sugar yields (mg g^{-1} raw bagasse).

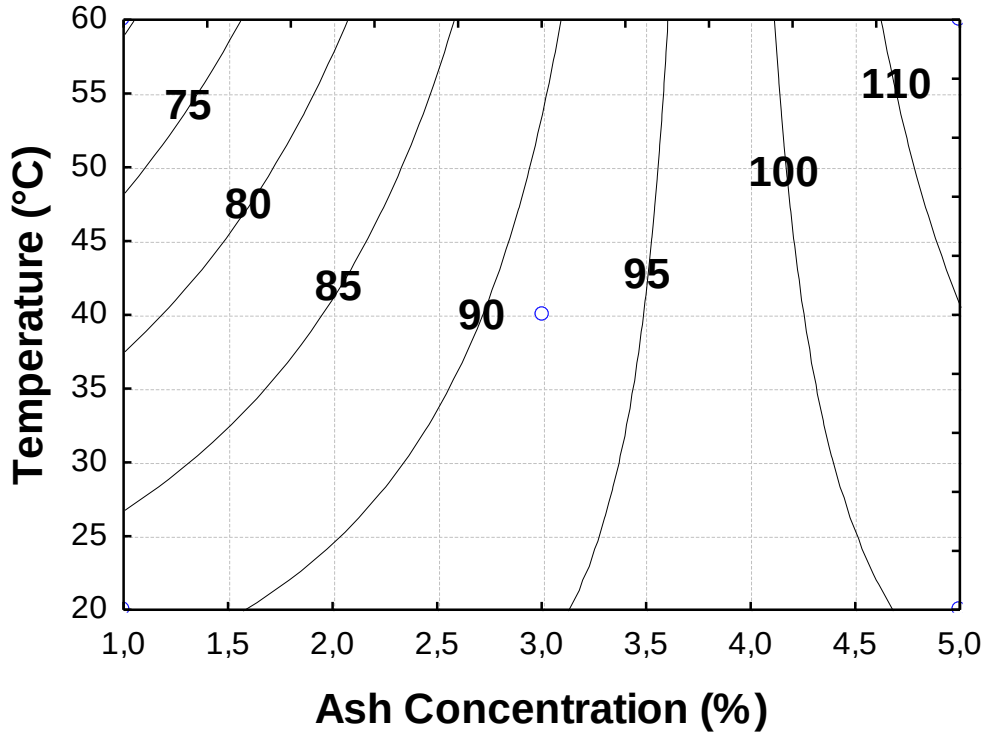

Fig.5. Level curves of reducing sugar yields after hydrolysis of hydrogen peroxide pretreatment supplemented with ashes. The numbers in the contours represents the reducing sugars yields (mg g^{-1} raw bagasse).

Figure 6 shows the FTIR spectra for samples pretreated with hydrogen peroxide supplemented with ashes. Pretreated and hydrolyzed samples showed no significant change compared to the crude bagasse; only that the former behaves somewhat differently from untreated bagasse and after hydrolysis, which occurs in the region 900-400 cm^{-1} where a large variation of the peaks is observed. This behavior is similar to that presented by the ash spectrum, suggesting that these bands come from the ash's compounds. The ash spectrum shows a large decrease in OH stretching band (3423 cm^{-1}), and the absence of the band at 2930 cm^{-1}; a significant increase in asymmetrical CO_2 stretching band (2358 cm^{-1}) was observed. In the region of 1750-1000 cm^{-1} the bands are smaller compared to the others' spectra. These modifications were associated with the combustion process that virtually volatilized almost all organic matter [29]. The band at 1078 cm^{-1}, which responds for deformations of CH and CO groups [30], greatly increased its intensity indicating that practically only saturated compounds remained. The region between 800 and 400 cm^{-1} in the ash spectrum presents several intensity bands associated to silicon compounds [31]; according to Belini et al. [32], the ash from sugarcane bagasse is mostly comprised of silicon dioxide.

Morphological analysis

Scanning electron microscopy (SEM) of the untreated bagasse has shown consistent fibers which, in a close view, are large fibers built of several small void channels (Figure 7.A), tightly joined by a compact material (Figure 7.B). The pretreatment with alkaline hydrogen peroxide destroys the cement among the void channels and the edges of the fibers (Figures 7.C and 7.D). Finally, after the enzymatic hydrolysis of bagasse has frayed the fibers into smaller units – microfibrils (Figure 7.E); an approximation of these fibers shows a high incidence of holes, creating a surface that resembles a network (Figure 7.F). Therefore, these micrographs show that the pretreatment contributed to a physical disruption of fiber, providing greater accessibility to the enzymes. Similar observations were made on micrographs of sugarcane bagasse pretreated with formic acid [33], SO_2 and CO_2 [34].

The bagasse pretreated with hydrogen peroxide supplemented with ashes presented a surface where the presence of small plates of irregular edges (fragments in white color) on the lignocellulosic structures can be observed (Figure 8.A), which were identified as the ashes used in the pretreatment. It is possible to see the evolution of agglomerate (Figure 8.C) that is loosening after the enzymatic hydrolysis (Figures 8.D and 8.E). It may also be noted that the fibers are also slightly shredded (Figure 8.F), after the stages of pretreatment and enzymatic hydrolysis.

Comparison among micrographs of the two pretreatments (Figures 7 and 8) shows that pretreatment with alkaline hydrogen peroxide is more aggressive, with greater disruption of lignocellulosic fibers. However, the manner in which the fibers of pretreatment with alkaline hydrogen peroxide are disposed closely resembles the form in which the pulp is treated with NaOH [35]. This reinforces the hypothesis that pretreatment with alkaline hydrogen peroxide, is basically a caustic and oxidative treatment, suffering the same consequences of caustic treatment with strong bases generating aggressive waste, which causes the loss of carbohydrates with partial degradation of hemicellulose.

Sugar Contents

Figure 9 shows the yield expressed in reducing sugars content released during enzymatic hydrolysis. Pretreatment with hydrogen peroxide supplemented with ash has shown a yield (179.9 mg g^{-1} bagasse) 142% higher than the non-treated bagasse (74.3 mg g^{-1} bagasse), and 17% lower than the pretreatment with alkaline hydrogen peroxide (217.6 mg g^{-1} bagasse).

In spite of the inferior result, the ash-supplemented pretreatment has several favorable characteristics. Alkaline hydrogen peroxide involves large amounts of NaOH. To adjust the pH to a value of 11.5 in a solution containing 9.39% (v/v) H_2O_2 7.30% (w/v) NaOH is needed, on average [3, 18]. In addition, pretreatment with alkaline hydrogen peroxide has a higher cost of reagents since 7.30% NaOH solution uses 30% more of H_2O_2. In a 10 m^3 reactor 775 L more H_2O_2 (130 volumes) would be used as compared to ash-supplemented pretreatment, as well an addition of 730 Kg of NaOH. Otherwise the ash-supplemented pretreatment has lower washing step, maintaining pH around 8.5, consuming lower chemical reagents (6.32% (v/v) H_2O_2 and no NaOH), without much caustic waste.

IV. CONCLUSIONS

The use of ash-supplemented pretreatment has shown to be viable; it was proved to be eco-friendly since it uses less hydrogen peroxide as reagent, uses industrial lignocellulosic residues to lightly alkalize the reaction and practically does not generate waste. The slightly slower yield and higher time and temperature pre-treatment conditions are largely compensated by these advantages over the alkaline hydrogen peroxide. On the other hand, alkaline pretreatment gave higher yield in a shorter processing time interval, and effectively enhanced the

recovery of cellulose and hemicellulose removing lignin, but the reaction time must be monitored accurately, since extended time affects the process thereby degrading part of the fermentative sugars. Also, unlike reported in several literatures, it generates large amounts of caustic waste.

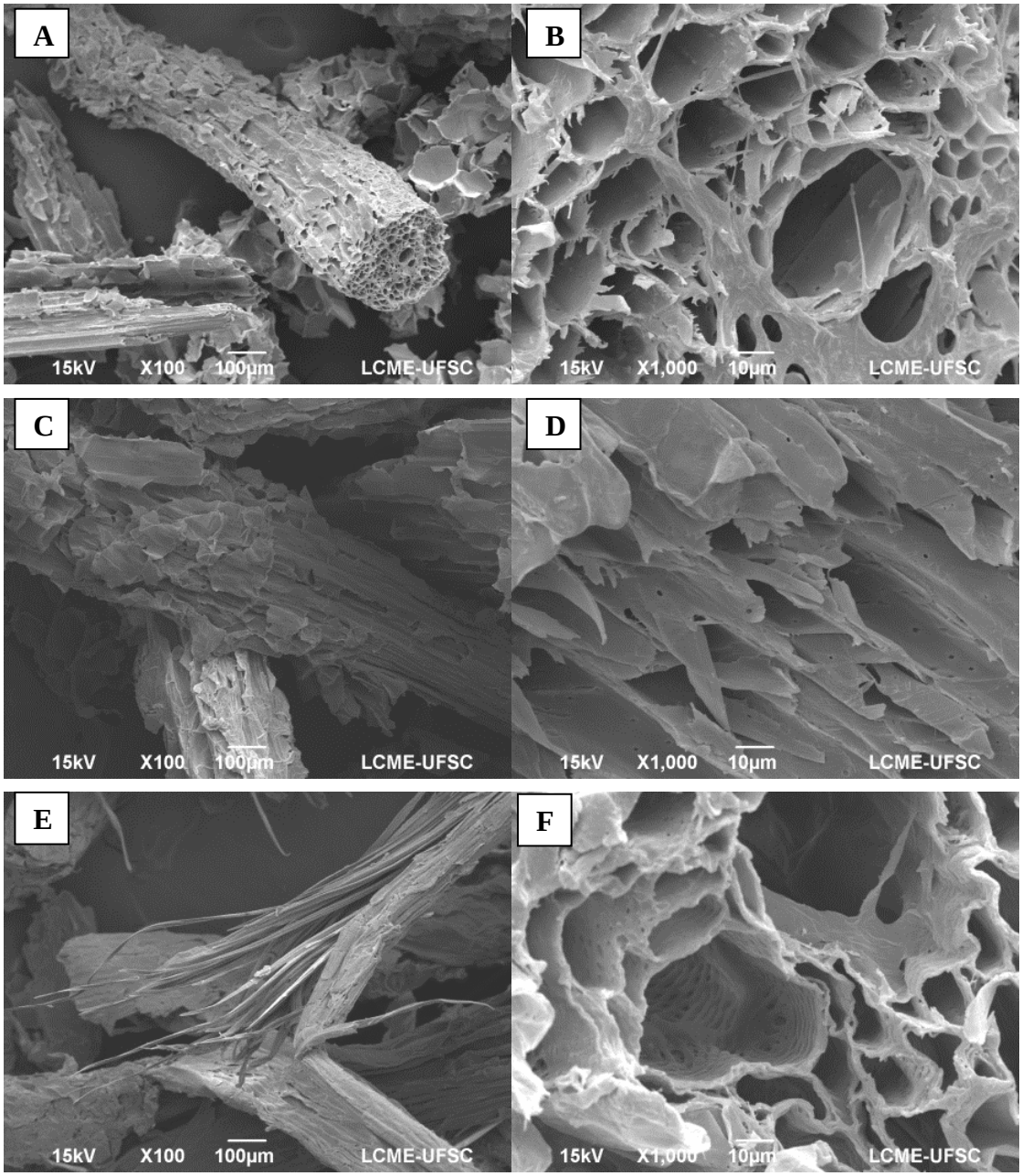

Fig.9. Micrograph of the bagasse fibers. A and B - Untreated bagasse. C and D - Bagasse pretreated with alkaline hydrogen peroxide. E and F - Bagasse pretreated with alkaline hydrogen peroxide after enzymatic hydrolysis.

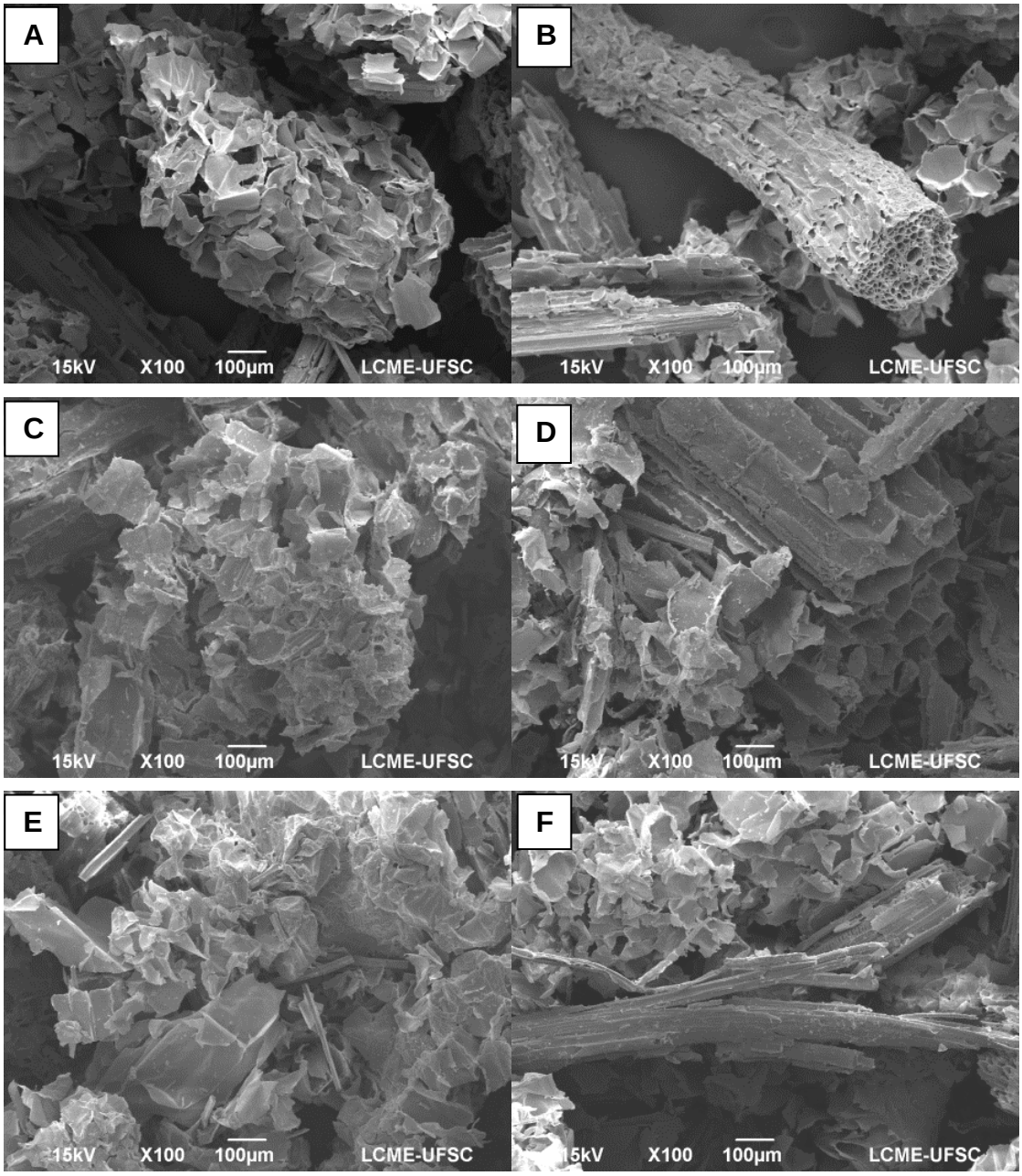

Fig.10. Micrograph of the bagasse fibers. A and B - Untreated bagasse. C and D - Bagasse pretreated with hydrogen peroxide with ash. E and F - Bagasse pretreated with hydrogen peroxide with ash after enzymatic hydrolysis.

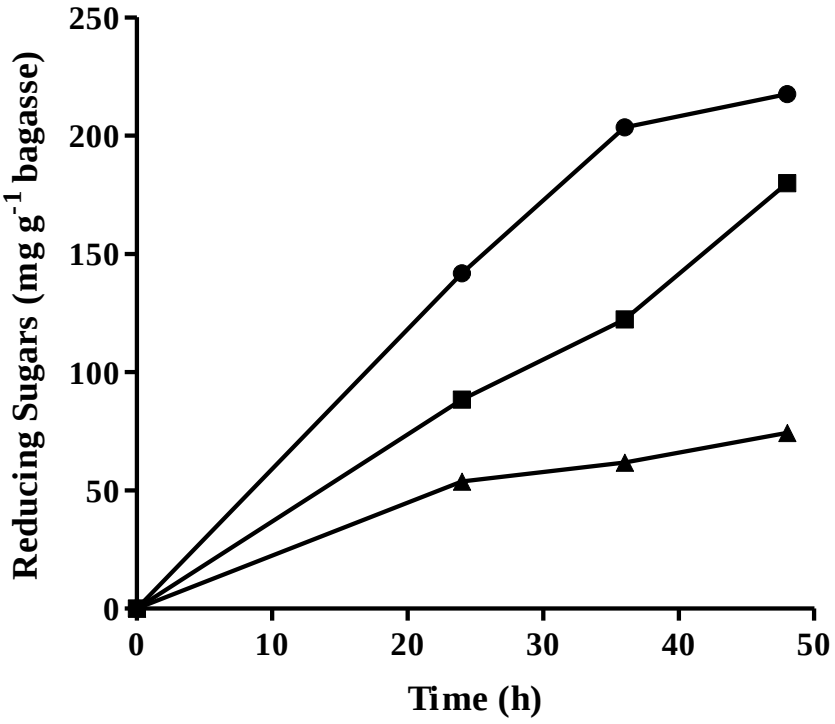

Fig.11. Reducing sugars yield after hydrolysis. (▲) Without pretreatment. (●) Alkaline hydrogen peroxide pretreatment. (■) Hydrogen peroxide supplemented with ash pretreatment.

ACKNOWLEDGEMENTS

Thanks are due to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) for the financial support and to LCME-UFSC and LCP-UFSC for their collaboration. Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil) and CNPq are acknowledged for scholarships provided to E. J. O. (Process 151545/2010-3) and A. O. S. L. (Process 312363/2018-4), respectively.

REFERENCES

[1] Ferreira-Leitao V, Gottschalk LMF, Ferrara MA, et al (2010) Biomass residues in Brazil: Availability and potential uses. Waste and Biomass Valorization 1:65–76. https://doi.org/10.1007/s12649-010-9008-8

[2] Bhattacharyya S, Datta S, Bhattacharjee C (2012) Sonication boost the total reducing sugar (TRS) extraction from sugarcane bagasse after dilute acid hydrolysis. Waste and Biomass Valorization 3:81–87. https://doi.org/10.1007/s12649-011-9078-2

[3] Rabelo SC, Amezquita Fonseca NA, Andrade RR, et al (2011) Ethanol production from enzymatic hydrolysis of sugarcane bagasse pretreated with lime and alkaline hydrogen peroxide. Biomass and Bioenergy 35:2600–2607. https://doi.org/10.1016/j.biombioe.2011.02.042

[4] Hernández-Pérez AF, Chaves-Villamil AC, de Arruda PV, et al (2019) Sugarcane Syrup Improves Xylitol Bioproduction from Sugarcane Bagasse and Straw Hemicellulosic Hydrolysate. Waste and Biomass Valorization. https://doi.org/10.1007/s12649-019-00742-6

[5] Pereira Ramos L (2003) The chemistry involved in the steam treatment of lignocellulosic materials. Quim. Nova 26:863–871

[6] Zhao X, Zhang L, Liu D (2012) Biomass recalcitrance. Part I: the chemical compositions and physical structures affecting the enzymatic hydrolysis of lignocellulose. Biofuels, Bioprod Biorefining 6:465–482. https://doi.org/10.1002/bbb.1331

[7] Percival Zhang YH, Himmel ME, Mielenz JR (2006) Outlook for cellulase improvement: Screening and selection strategies. Biotechnol. Adv. 24:452–481

[8] Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS (2002) Microbial Cellulose Utilization: Fundamentals and Biotechnology. Microbiol Mol Biol Rev 66:506–577. https://doi.org/10.1128/mmbr.66.3.506-577.2002

[9] Odisi EJ, Silvestrin MB, Takahashi RYU, et al (2012) Bioprospection of cellulolytic and lipolytic south atlantic deep-sea bacteria. Electron J Biotechnol 15:18. https://doi.org/10.2225/vol15-issue5-fulltext-17

[10] Robak K, Balcerek M (2018) Review of second generation bioethanol production from residual biomass. Food Technol Biotechnol 56:174–187. https://doi.org/10.17113/ftb.56.02.18.5428

[11] Mosier N, Wyman C, Dale B, et al (2005) Features of promising technologies for pretreatment of lignocellulosic biomass. Bioresour Technol 96:673–686. https://doi.org/10.1016/j.biortech.2004.06.025
[12] Alvira P, Tomás-Pejó E, Ballesteros M, Negro MJ (2010) Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: A review. Bioresour Technol 101:4851–4861. https://doi.org/10.1016/j.biortech.2009.11.093
[13] Yang B, Wyman CE (2008) Pretreatment: the key to unlocking low-cost cellulosic ethanol. Biofuels, Bioprod Biorefining 2:26–40. https://doi.org/10.1002/bbb.49
[14] Monte JR, Brienzo M, Milagres AMF (2011) Utilization of pineapple stem juice to enhance enzyme-hydrolytic efficiency for sugarcane bagasse after an optimized pre-treatment with alkaline peroxide. Appl Energy 88:403–408. https://doi.org/10.1016/j.apenergy.2010.08.009
[15] Karagöz P, Rocha I V., Özkan M, Angelidaki I (2012) Alkaline peroxide pretreatment of rapeseed straw for enhancing bioethanol production by Same Vessel Saccharification and Co-Fermentation. Bioresour Technol 104:349–357. https://doi.org/10.1016/j.biortech.2011.10.075
[16] Bolado-Rodríguez S, Toquero C, Martín-Juárez J, et al (2016) Effect of thermal, acid, alkaline and alkaline-peroxide pretreatments on the biochemical methane potential and kinetics of the anaerobic digestion of wheat straw and sugarcane bagasse. Bioresour Technol 201:182–190. https://doi.org/10.1016/j.biortech.2015.11.047
[17] Keramati N, Moheb A, Ehsani MR (2010) Effect of operating parameters on NaOH recovery from waste stream of Merox tower using membrane systems: Electrodialysis and electrodeionization processes. Desalination 259:97–102. https://doi.org/10.1016/j.desal.2010.04.027
[18] Gould JM (1985) Studies on the mechanism of alkaline peroxide delignification of agricultural residues. Biotechnol Bioeng 27:225–231. https://doi.org/10.1002/bit.260270303
[19] Quadri MGN, Odisi EJ, Lima AOS (2012) Método de pré-tratamento e hidrólise de materiais lignocelulósicos para obtenção de açúcares monoméricos. 13
[20] Ghose TK (1987) Measurement of cellulase activities. Pure Appl Chem 59:257–268. https://doi.org/10.1351/pac198759020257
[21] Browning BL (1967) Methods of wood chemistry, 2nd ed. John Wiley & Sons Inc, New York
[22] Montgomery DC (2012) Design and Analysis of Experiments, 8th ed
[23] Martínez-Huitle CA, Brillas E (2009) Decontamination of wastewaters containing synthetic organic dyes by electrochemical methods: A general review. Appl. Catal. B Environ. 87:105–145
[24] Crestini C, Crucianelli M, Orlandi M, Saladino R (2010) Oxidative strategies in lignin chemistry: A new environmental friendly approach for the functionalisation of lignin and lignocellulosic fibers. Catal Today 156:8–22. https://doi.org/10.1016/j.cattod.2010.03.057
[25] Lucas M, Hanson SK, Wagner GL, et al (2012) Evidence for room temperature delignification of wood using hydrogen peroxide and manganese acetate as a catalyst. Bioresour Technol 119:174–180. https://doi.org/10.1016/j.biortech.2012.05.086
[26] Lima AOS, Quecine MC, Fungaro MHP, et al (2005) Molecular characterization of a β-1,4-endoglucanase from an endophytic Bacillus pumilus strain. Appl Microbiol Biotechnol 68:57–65. https://doi.org/10.1007/s00253-004-1740-1
[27] Bodîrlău R, Bodîrlău R, Teacă CA (2008) Fourier transform infrared spectroscopy and thermal analysis of lignocellulose filters treated with organic anhydrides. Rom J Phys 93--104
[28] Colom X, Carrillo F, Nogués F, Garriga P (2003) Structural analysis of photodegraded wood by means of FTIR spectroscopy. Polym Degrad Stab 80:543–549. https://doi.org/10.1016/S0141-3910(03)00051-X
[29] De Paula MO, De I, Tinôco FF, et al Potencial da cinza do bagaço da cana-de-açúcar como material de substituição parcial de cimento Portland
[30] Fengel D, Wegener G (2003) Wood Chemistry, Ultrastructure, Reactions. Berlin
[31] Silverstein RA, Chen Y, Sharma-Shivappa RR, et al (2007) A comparison of chemical pretreatment methods for improving saccharification of cotton stalks. Bioresour Technol 98:3000–3011. https://doi.org/10.1016/j.biortech.2006.10.022
[32] Belini UL, Tomazello M, Mendes LM, et al (2012) Teor de Sílica em Compósitos Confeccionados com Bagaço deCana-de-Açúcar e Eucalipto. Floresta e Ambient 19:250–256. https://doi.org/10.4322/floram.2012.030
[33] Sindhu R, Binod P, Satyanagalakshmi K, et al (2010) Formic acid as a potential pretreatment agent for the conversion of sugarcane bagasse to bioethanol. Appl Biochem Biotechnol 162:2313–2323. https://doi.org/10.1007/s12010-010-9004-2
[34] Corrales RCNR, Mendes FMT, Perrone CC, et al (2012) Structural evaluation of sugar cane bagasse steam pretreated in the presence of CO 2 and SO 2. Biotechnol Biofuels 5:36. https://doi.org/10.1186/1754-6834-5-36
[35] Rezende CA, De Lima M, Maziero P, et al (2011) Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility. Biotechnol Biofuels 4:54. https://doi.org/10.1186/1754-6834-4-54