Appl Biochem Biotechnol (2008) 148:45–58
DOI 10.1007/s12010-008-8200-9

A Comparison between Lime and Alkaline Hydrogen Peroxide Pretreatments of Sugarcane Bagasse for Ethanol Production

Sarita C. Rabelo · Rubens Maciel Filho · Aline C. Costa

Received: 8 May 2007 / Accepted: 16 October 2007 /
Published online: 11 April 2008

Abstract Pretreatment procedures of sugarcane bagasse with lime (calcium hydroxide) or alkaline hydrogen peroxide were evaluated and compared. Analyses were performed using 2 × 2 × 2 factorial designs, with pretreatment time, temperature, and lime loading and hydrogen peroxide concentration as factors. The responses evaluated were the yield of total reducing sugars (TRS) and glucose released from pretreated bagasse after enzymatic hydrolysis. Experiments were performed using the bagasse as it comes from an alcohol/sugar factory and bagasse in the size range of 0.248 to 1.397 mm (12–60 mesh). The results show that when hexoses and pentoses are of interest, lime should be the pretreatment agent chosen, as high TRS yields are obtained for nonscreened bagasse using 0.40 g lime/g dry biomass at 70 °C for 36 h. When the product of interest is glucose, the best results were obtained with lime pretreatment of screened bagasse. However, the results for alkaline peroxide and lime pretreatments of nonscreened bagasse are not very different.

Keywords Lignocellulosic materials · Sugarcane bagasse · Pretreatment · Lime · Hydrogen peroxide · Enzymatic hydrolysis · Statistical analysis

Introduction

In recent years, the worldwide trends toward scientific and technological advances in the field of new fuels point to the importance of more efficient utilization of agro-industrial residues as raw material in the ethanol production process. In Brazil, sugarcane bagasse, the major byproduct of the sugar cane industry, seems to be economically viable for the production of environmentally friendly fuels.

In general, lignocellulosic materials are resistant to bioconversion and require pretreatment to increase their biodigestibility and make cellulose more accessible to the

S. C. Rabelo · R. M. Filho · A. C. Costa (✉)
Department of Chemical Process, School of Chemical Engineering,
State University of Campinas (UNICAMP), Campinas, SP, Brazil
e-mail: accosta@feq.unicamp.br

cellulolytic enzymes. Pretreatment methods can be classified into four categories: physical, chemical, biological, and a combination of these. Chemical pretreatments have received more attention because the physical pretreatments are relatively inefficient [1] and the combined pretreatments rarely have improved digestibility when compared with simple treatments [2]; thus, chemical pretreatments have been chosen as the subject in this work.

Lynd et al. [3] has summarized the desireable properties for an ideal lignocellulosic material after chemical pretreatment; that is, it should (a) produce reactive fibers, (b) yield pentoses in nondegraded form, (c) not release the compounds that significantly inhibit fermentation, (d) work in reactors of reasonable size with moderate cost, (f) produce no solid residues, (g) have a high degree of simplicity, and (h) be effective at low moisture contents.

Pretreatment is one of the most expensive and least technologically mature steps in the process for converting biomass to fermentable sugars [4]. Costs are due to the use of steam and chemical products and the need for expensive corrosion resistant reactors; however, pretreatment also has great potential for efficiency improvement and lowering of costs through research and development [5–8].

Enzymatic hydrolysis of cellulosic material by cellulase enzymes is the most promising approach for getting high product yields vital to economic success [3, 9]. The cellulases break down cellulose to cellobiose, which is subsequently cleaved to glucose by β-glucosidase. Enzymatic hydrolysis leads to higher yields of monosaccharides than dilute-acid hydrolysis because cellulase enzymes catalyze only cellulose or hemicellulose hydrolysis reactions and not sugar degradation reactions [10]. Enzymes are naturally occurring compounds that are biodegradable and therefore environmentally friendly.

In this work, two promising pretreatment technologies are compared. They were chosen for occurring under mild conditions (temperature, pressure, and absence of acids). Both are alkaline processes, which are expected to cause less sugar degradation than acid processes [11]. The first is the pretreatment with alkaline hydrogen peroxide [12–17], which is a well-known reagent in the paper and cellulose industry, being used as a bleach agent. It has also the great advantage of not leaving residues in the biomass, as it degrades into oxygen and water. Furthermore, the formation of secondary products is practically inexistent.

The other pretreatment agent considered is lime (calcium hydroxide) [18–27], which is an inexpensive reagent and can be easily recovered as calcium carbonate by neutralization with carbon dioxide. The calcium hydroxide can be subsequently regenerated using established lime kiln technology [26].

Analyses were performed using 2 × 2 × 2 factorial designs. The factors considered were pretreatment time, temperature, and lime loading or hydrogen peroxide concentration. The responses evaluated were the total reducing sugar (TRS) and glucose yield from the pretreated bagasse after enzymatic hydrolysis. Experiments were performed using the bagasse as it comes from an alcohol/sugar factory and bagasse with a screened size of 0.248 to 1.397 mm (12–60 mesh) to evaluate the possibility of using the bagasse as it comes from the mills.

Materials and Methods

Substrate

Fresh sugarcane bagasse was obtained from the sugar plant Usina São Luiz—Dedini S/A, (Pirassununga/SP, Brazil). It was dried at 45 °C for 48 h, left for 48 h at room temperature,

put into plastic bags, and kept in a freezer until used. The dry matter content of the bagasse after being dried was 95%. The bagasse used in the tests was divided into two parts. One part was used as it came from the mill, without prior screening, and presented highly heterogeneous particle sizes. This part will be called nonscreened bagasse throughout this article. The other part was screened in the size range of 0.248 to 1.397 mm (12–60 mesh). Smaller particles were discarded because they corresponded mainly to sand. Figure 1 shows samples of the screened and nonscreened bagasse.

Chemical Analysis of Bagasse Samples

Samples of the screened and nonscreened bagasse were milled to pass through a 0.75-mm screen. Approximately 3 g of milled sample was extracted with 95% ethanol for 6 h in a Soxhlet apparatus. Ash content was determined after burning of the samples in a muffle 600 °C for 4 h [28]. Extracted bagasse samples were hydrolyzed with 72% sulfuric acid at 30 °C for 1 h (300 mg of sample and 3 ml of sulfuric acid). The acid was diluted to a final concentration of 3% (addition of 79 ml of water), and the mixture was heated at 125 °C/1atm for 1 h. The residual material was cooled and filtered through porous glass filter number 3. The solids were dried to constant weight at 105 °C and determined as insoluble lignin. The soluble lignin concentration in the filtrate was determined by measuring absorbance at 205 nm and using the value of 105 l g^{-1} cm^{-1} as the absorptivity of soluble lignin [29]. The concentrations of monomeric sugars in the soluble fraction were determined by high-performance liquid chromatography (HPLC) using a BIORAD HPX87H column at 45 °C, eluted at the 0.6 ml/min with 0.005 mol/l sulfuric acid. Sugars were detected in a 30 °C temperature-controlled refractive index detector (Knauer HPLC pump and detector). Under these conditions, xylose, mannose, and galactose eluted at the same retention time were integrated as a single peak. Glucose, xylose, arabinose, and acetic acid were used as external calibration standards. No corrections were performed due to sugar degradation reactions during acid hydrolysis. The factors used to convert sugar monomers to anhydromonomers were 0.90 for glucose and 0.88 for xylose and arabinose. Acetyl content was calculated as the acetic acid content multiplied by 0.7. These factors were calculated based on water addition to polysaccharides during acid hydrolysis [30–33]. Table 1 shows the composition of the screened and nonscreened bagasse.

Pretreatment

The pretreatment agents evaluated were alkaline hydrogen peroxide and lime (calcium hydroxide). Pretreatment time, temperature, and lime loading or hydrogen peroxide

Fig. 1 **a** Nonscreened bagasse, **b** screened bagasse (12–60 mesh)

Table 1 Composition of the nonscreened and screened sugarcane bagasse.

	Nonscreened bagasse, %	Screened bagasse. %
Glucan	39.6±0.9	34.1±0.9
Xylan	19.7±0.5	17.7±0.5
Arabinan groups	1.7±0.1	2.0±0.1
Acetyl groups	2.5±0.1	2.4±0.1
Lignin	25.8±1.6	29.3±1.6
Extractives	2.3±0.1	2.3±0.1
Ash	3.8±0.1	5.3±0.1

concentration were evaluated during the experiments. The pretreatment solution of alkaline peroxide was prepared by dissolving H_2O_2 in distilled water and adjusting the pH to 11.5 with sodium hydroxide, and the lime pretreatment solution was prepared by dissolving $Ca(OH)_2$ in distilled water. In the lime pretreatment, in all the assays, a certain amount of lime remained insoluble, although this continued dissolving during pretreatment. Nonscreened bagasse (4 g) and screened bagasse (4 g) were treated with 100 ml of the pretreatment solution in 500-ml flasks in an orbital shaker (Marconi MA-832) agitated at 150 rpm.

Enzymatic Hydrolysis

After pretreatment, the substrate was washed to remove insoluble matter, dried, and weighted to measure mass loss. The present market offers many cellulase preparations (including those obtained from *Trichoderma reesei*) containing low levels of β-glucosidase, which leads to an increased accumulation of cellobiose in the enzymatic hydrolyzates of the cellulose. The inability of industrial glucose-fermenting yeasts to ferment cellobiose results in incomplete conversion of cellulose hydrolyzate to ethanol, significantly diminishing its final yield. These drawbacks may be overcome by supplementation of the cellulase complex with a β-glucosidase from other sources [34]. One gram of the pretreated bagasse was hydrolyzed with 300 ml of solution containing cellulase and β-glucosidase with the pH adjusted to 4.8. Cellulases from *T. reesei* (Sigma) loading was 3.42 filter paper units (FPU)/g dry pretreated biomass. β-glucosidase from *Aspergillus niger* (Sigma) was added to completely convert cellobiose to glucose, with loading of 1.00 IU/g dry pretreated biomass. Cellulase activity was determined as FPU per milliliter, as recommended by the International Union of Pure and Applied Chemistry [35, 36]. β-Glucosidase activity was determined through a solution of cellobiose 15 mmol/l and express in units per milliliter [37]. Enzyme activity was 47.44 FPU/ml for cellulases and 343.63 IU/ml for β-glucosidase.

Hydrolysis experiments were carried out in 500-ml flasks in an orbital shaker (Marconi MA-832) agitated at 100 rpm at 50 °C. Aliquots were taken periodically, boiled to deactivate the enzymes, and analyzed for glucose and reducing sugars. The values of glucose and reducing sugars yields used for the statistical analysis were picked at the reaction time after which no significant changes in these variables were detected.

Analytical Methods

Glucose yield was measured using a kit based on the glucose oxidase reaction (GOD-PAP, Laborlab), and TRS yield was determined by the dinitrosalycilic acid (DNS) method [38].

For glucose quantification, 10 μl of the sample and 1.0 ml of the mono-reagent glucose oxidase were added in assays pipes and put in a thermostatic bath (Marconi MA-184) at 37 °C per 10 min. At the end of the reaction, the absorbance was read in spectrophotometer (Femto 600S) at 540 nm.

For the TRS quantification, 0.5 ml of the samples and 1.5 ml of DNS were added in assay pipes and put in a thermostatic bath (Marconi MA-184) at 95 °C per 5 min. After, the samples were cooled immediately by the immersion in an ice bath. The absorbance was read in a spectrophotometer (Femto 600S) at 540 nm. In both methods, the standard glucose (Merck) was used for the preparation of the standard curve.

Results and Discussion

A 2 × 2 × 2 full factorial design with three replicates in the central point was performed for each pretreatment considered. The objective was to evaluate the influence of pretreatment time, temperature, and pretreatment agent concentration on the subsequent enzymatic hydrolysis performance.

Table 2 shows the design matrix and glucose and TRS yields after hydrolysis of pretreated bagasse for the screened and nonscreened samples for the pretreatment with alkaline hydrogen peroxide. Table 3 shows the design matrix for the pretreatment with lime. In both tables, the glucose and TRS yields were expressed as milligrams per gram of dry raw bagasse (not pretreated). The maximum TRS and glucose yield obtained are marked in italics, and the mean TRS and glucose yield obtained for the screened and nonscreened bagasse samples in all the assays are also shown. The ranges of the factors for the two pretreatments were chosen based on literature [12–27].

It can be seen from Tables 2 and 3 that under the operational conditions used in this work, maximum TRS yield was obtained with lime pretreatment of nonscreened bagasse (554.2 mg/g dry nonscreened bagasse). Lime pretreatment of screened bagasse also resulted in high TRS yield (550.6 mg/g dry screened bagasse); thus, when all the reducing sugars

Table 2 Design matrix presenting TRS and glucose yields after hydrolysis of pretreated bagasse: alkaline hydrogen peroxide (screened—S and nonscreened—NS).

Assay	Time (h)	Temperature (°C)	$[H_2O_2]$ (%)	TRS (NS), (mg/g)	Glucose (NS), (mg/g)	Glucose (NS), yield %	TRS (S) (mg/g)	Glucose (S) (mg/g)	Glucose (S), yield %
1	6	20	1	206.2	64.6	14.5	259.0	103.3	26.9
2	24	20	1	211.5	79.7	17.9	253.5	98.1	25.6
3	6	60	1	433.0	215.3	48.3	342.4	166.7	43.5
4	24	60	1	280.7	121.4	27.3	340.3	181.9	47.4
5	6	20	5	347.0	241.9	54.3	368.0	*239.3*	*62.4*
6	24	20	5	*494.7*	*309.3*	*69.4*	*452.1*	228.1	59.5
7	6	60	5	364.9	252.5	56.7	288.9	188.8	49.2
8	24	60	5	407.0	287.7	64.6	285.5	163.4	42.6
9	15	40	3	359.0	229.6	51.5	309.4	167.6	43.7
10	15	40	3	323.5	209.2	47.0	346.5	195.2	50.9
11	15	40	3	323.9	204.6	45.9	307.1	164.9	43.0
			Mean	341.0	201.4	45.2	323.0	172.5	45.0

Table 3 Design matrix presenting TRS and glucose yields after hydrolysis of pretreated bagasse: lime (screened—S and nonscreened—NS).

Assay	Time (h)	Temp (°C)	lime loading. (g/g)	TRS (NS), (mg/g)	Glucose (NS), (mg/g)	Glucose (NS), yield %	TRS (S), (mg/g)	Glucose (S), (mg/g)	Glucose (S), yield %
1	12	60	0.10	306.3	128.2	28.8	422.7	208.4	54.3
2	36	60	0.10	433.9	232.2	52.1	481.8	235.6	61.4
3	12	70	0.10	351.5	161.0	36.1	474.8	329.0	85.8
4	36	70	0.10	427.7	228.1	51.2	549.3	*335.5*	*87.5*
5	12	60	0.40	379.6	108.2	24.3	516.3	212.3	55.4
6	36	60	0.40	307.1	106.1	23.8	*550.6*	331.5	86.4
7	12	70	0.40	268.6	110.0	24.7	456.3	177.6	46.3
8	36	70	0.40	*554.2*	*296.9*	*66.7*	528.4	171.9	44.8
9	24	65	0.25	535.0	224.9	50.5	484.0	265.9	69.3
10	24	65	0.25	530.0	213.4	47.9	490.1	265.6	69.2
11	24	65	0.25	531.2	223.2	50.1	502.4	260.9	68.0
			Mean	420.5	184.7	41.5	496.1	254.0	66.2

(hexoses and pentoses) are of interest, lime pretreatment is a better choice of pretreatment agent than alkaline peroxide pretreatment. In addition, bagasse screening is not necessary, which reduces substantially the costs of the process.

Table 4 shows the effects of pretreatment time, temperature, and lime loading on TRS yield after hydrolysis for lime pretreatment of nonscreened bagasse. The statistical analysis was performed using the software Statistica (Statsoft, v. 7.0) and the confidence level considered was 90%. Significant effects are marked in italics. A statistical model is not presented because a linear model is not able to represent experimental behavior in this case.

It can be seen from Table 4 that the major effect is that of pretreatment time, followed by the three-way interaction. The interaction between pretreatment time and temperature (1 × 2) and the main effect of temperature are also significant. The main effect of lime loading has no influence on TRS yield, but its two-way interaction with temperature (2 × 3) is significant. It can be observed that all the significant effects are positive, which means that maximum TRS yield for the nonscreened bagasse is for high pretreatment time, temperature, and lime loading (see assay 8 in Table 3).

Table 4 Effects on TRS yield after hydrolysis of nonscreened bagasse pretreated with lime.

Factor	TRS (NS)	
	Effect	*p* value
Mean	*420.46*	3.4377×10^{-6}
Pretreatment time (1)	*104.23*	0.0003
Temperature (2)	*43.78*	0.0017
$Ca(OH)_2$ loading (3)	−2.48	0.3119
1×2	*76.68*	0.0006
1×3	2.33	0.3349
2×3	*24.28*	0.0057
1×2×3	*102.38*	0.0003

Significant effects marked in italics.

As the industrial fermenting microorganisms used nowadays for industrial ethanol production do not ferment pentoses, in many practical applications, the product of interest may be glucose. From Tables 2 and 3, it can be noticed that the maximum glucose yield in the range of operational conditions used in this work is for lime pretreatment of screened bagasse (335.5 mg/g dry screened bagasse). For nonscreened bagasse, alkaline hydrogen peroxide seems to be the pretreatment agent of choice, although lime pretreatment leads to just a little lower glucose yield (309.3 mg/g dry nonscreened bagasse with alkaline peroxide versus 296.9 mg/g dry nonscreened bagasse when the pretreatment agent is lime). This is not a statistically significant difference; however, the process based on alkaline hydrogen peroxide requires lower temperature and process time. Overall, it seems to be the more suitable one.

As screening is an expensive unit operation, we investigated not only the best result (lime pretreatment of screened bagasse) but also the two options for pretreatment with nonscreened bagasse: alkaline peroxide and lime pretreatments.

Table 5 shows the scaled regression coefficients of the regression model of glucose yield after hydrolysis for alkaline hydrogen peroxide pretreatment of nonscreened bagasse. It can be seen that at the 90% confidence level, pretreatment time is not significant for glucose yield. However, the interactions between pretreatment time and temperature (1 × 2) and between pretreatment time and H_2O_2 concentration (1 × 3) are significant. The concentration of H_2O_2 is the most important factor affecting this response; temperature is significant, and the interaction between temperature and peroxide concentration (2 × 3) also significantly influences glucose yield.

Table 6 depicts the scaled regression coefficients of the regression models of glucose yield after hydrolysis for lime pretreatment of nonscreened and screened bagasse. For screened bagasse, only the interaction between pretreatment time and lime loading (1 × 3) is not significant. All the other main effects and interactions are significant, and the main effect of pretreatment time is the most important. For screened bagasse, all the factors considered and all their interactions are significant. The major effect is the interaction between temperature and lime loading (2 × 3), followed by the main effect of lime loading.

Table 7 depicts the analysis of variance (ANOVA) for the model of glucose yield after hydrolysis for alkaline peroxide pretreatment of nonscreened bagasse when only the significant coefficients are taken into account. It can be seen that the model presents a high correlation coefficient and can be considered statistically significant with 90% of confidence according to the *F* test, as it presented a calculated value greater than the listed

Table 5 Scaled regression coefficients of the regression model of glucose yield for nonscreened bagasse pretreated with alkaline hydrogen peroxide.

Factor	Glu(NS)	
	Coefficient	*p* value
Mean	*201.42*	3.964×10^{-4}
Pretreatment time (1)	5.96	0.5918
Temperature (2)	*45.36*	0.0405
H_2O_2 concentration (3)	*152.58*	0.0038
1×2	*−35.29*	0.0643
1×3	*45.30*	0.0405
2×3	*−50.86*	0.0326
1×2×3	19.18	0.1781

Significant effects marked in italics.

Table 6 Scaled regression coefficients of the regression models of glucose yield.

Factor	Glu (NS)		Factor	Glu (S)	
	Coefficient	*p* value		Coefficient	*p* value
Mean	*184.75*	0.0001	Mean	*254.02*	1.100×10^{-5}
Pretreatment time (1)	*88.98*	0.0024	Pretreatment time (1)	*36.80*	0.0029
Temperature (2)	*55.33*	0.0062	Temperature (2)	*6.55*	0.0799
Lime loading (3)	*−32.08*	0.0182	Lime loading (3)	*−53.80*	0.0013
1×2	*38.03*	0.0131	1×2	*−36.40*	0.0029
1×3	3.43	0.5169	1×3	*19.95*	0.0097
2×3	*40.98*	0.0113	2×3	*−103.70*	0.0004
1×2×3	*56.48*	0.0060	1×2×3	*−26.05*	0.0057

Nonscreened and screened bagasse pretreated with lime

one [39]. Furthermore, it does not present evidence of lack of fit, as the calculated value for the *F* test for lack of fit is much smaller than the listed value.

Table 8 shows the ANOVA for the models of glucose yield after hydrolysis for lime pretreatment of nonscreened and screened bagasse when only the significant coefficients are taken into account. Both models present high correlation coefficients, and the *F* value for statistical significance of the regression are higher than the listed ones. Nevertheless, both models presented evidence of lack of fit, as they presented high lack of fit calculated *F* values. A model with evidence of lack of fit cannot be used for prediction or optimization purposes. However, it can be used to plot qualitative response surfaces that can aid in determining the best experimental region.

The response surface for glucose yield from nonscreened bagasse pretreated with alkaline hydrogen peroxide is depicted in Fig. 2. Figure 2a shows glucose yield versus peroxide concentration and temperature when pretreatment time is 6 h, and Fig. 2b shows the same response surface when pretreatment time is 24 h. From this figure, it can be seen

Table 7 ANOVA for the model describing glucose yield for nonscreened bagasse (NS) pretreated with alkaline peroxide.

Source of variation	Sum of squares (SQ) Glu	Degrees of freedom (*df*) Glu	Mean square (MS) Glu	*F* value Glu
Regression (*R*)	62,444.2	5	12,488.8	33.5[a]
Residual (*r*)	1,861.7	5	372.3	
Lack of fit (Lf)	1,508.2	3	502.7	2.8[b]
Pure error (Pe)	353.5	2	176.8	
Total (*T*)	64,305.9	10		
R^2	0.971			
F listed values (90% of confidence)				$F_{5,\ 5}=3.45$[a] $F_{3,\ 2}=9.16$[b]

[a] F test for statistical significance of the regression $=MS_R/MS_r$

[b] F test for lack of fit $=MS_{Lf}/MS_{Pe}$

Table 8 ANOVA for the models describing glucose yield for lime pretreatment of nonscreened (NS) and screened (S) bagasse.

Source of variation	Sum of squares (SQ)		Degrees of freedom (*df*)		Mean square (MS)		*F* value	
	Glu (NS)	Glu (S)	Glu (NS)	Glu (S)	Glu (NS)	Glu (S)	Glu (NS)	Glu (S)
Regression (*R*)	36,647.8	34,907.4	6	7	6,108.0	4,986.8	4.55[a]	34.32[a]
Residual (*r*)	5,371.0	435.9	4	3	1,342.8	145.3		
Lack of fit (Lf)	5,293.2	420.3	2	1	2,646.6	420.3	67.93[b]	53.85[b]
Pure error (Pe)	77.9	15.6	2	2	39.0	7.8		
Total (*T*)	42,018.9	35,343.3	10	10				
R^2	0.872	0.988						
F listed values (90% of confidence)							$F_{6,\ 4}=4.01$[a] $F_{2,\ 2}=9.00$[b]	$F_{7,\ 3}=5.27$[a] $F_{1,\ 2}=8.53$[b]

[a] *F* test for statistical significance of the regression=MS_R/MS_r

[b] *F* test for lack of fit=MS_{Lf}/MS_{Pe}

that high glucose yields can be obtained with both high and low pretreatment time. When pretreatment time is low, the highest glucose yields are in the region of high peroxide concentration and high temperature. For high pretreatment time, temperature has low influence, and the highest glucose yields are in the region of high peroxide concentration and low temperature. As the influence of temperature is low, pretreatment with alkaline peroxide can be performed at ambient temperature for high pretreatment time.

Figure 3 shows the response surface for glucose yield from nonscreened bagasse pretreated with lime. Figure 3a shows glucose yield versus lime loading and temperature when pretreatment time is 12 h, and Fig. 3b shows the same response surface when pretreatment time is 36 h. It can be seen that for nonscreened bagasse pretreated with lime, pretreatment time has a strong influence on glucose yield after hydrolysis, with high pretreatment time resulting in higher glucose yields. For high pretreatment time, lime loading had a weak influence and temperature a strong influence, with high temperature leading to high glucose yield. The maximum glucose yield was for high lime loadings and high temperature, but high yields are obtained even for low/moderate loadings if temperature is high.

The response surface for glucose yield from screened bagasse pretreated with lime is shown in Fig. 4. Figure 4a shows glucose yield versus lime loading and temperature when pretreatment time is 12 h, and Fig. 4b shows the same response surface when pretreatment time is 36 h. For low pretreatment time, maximum glucose yield was obtained for high temperature and low lime loading. For long pretreatment time, there was high glucose yield in two regions: low temperature and high lime loading or high temperature and low lime loading.

Conclusions

The effectiveness of alkaline hydrogen peroxide and lime pretreatment in improving sugar cane bagasse susceptibility to enzymatic hydrolysis was evaluated. Two complete 2 × 2 × 2

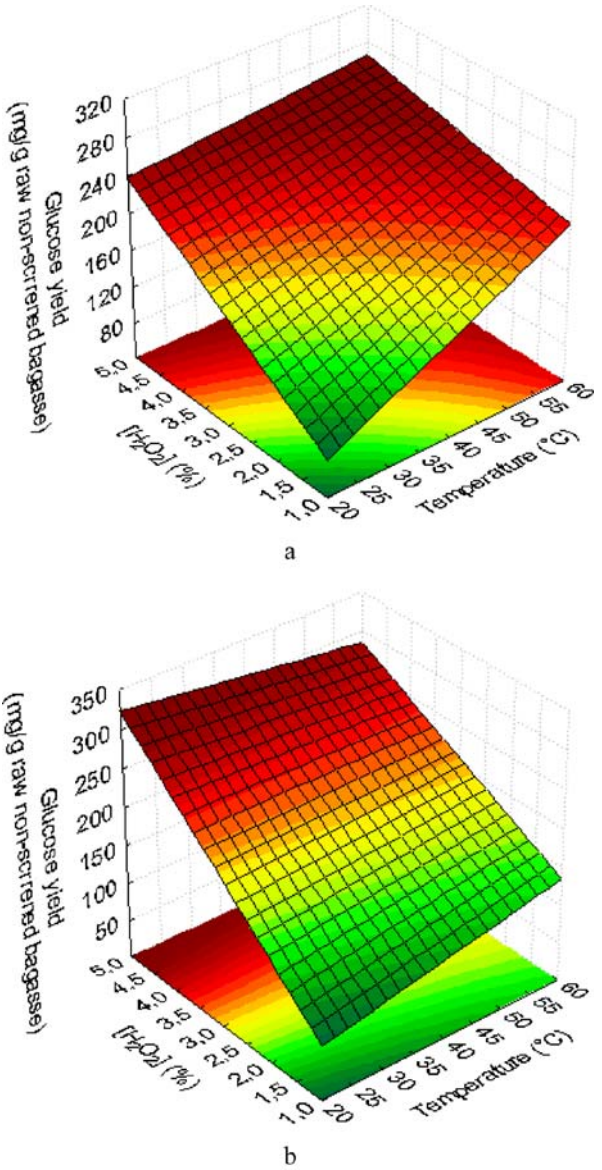

Fig. 2 Glucose yield from non-screened bagasse pretreated with alkaline hydrogen peroxide. **a** Pretreatment time of 6 h. **b** Pretreatment time of 24 h

factorial designs were carried out to determine the influence of pretreatment time, temperature, and H_2O_2 concentration or lime loading on the performance of enzymatic hydrolysis. The performance was evaluated by glucose and TRS yield after hydrolysis of the pretreated bagasse.

The influence of screening the bagasse before pretreatment in hydrolysis performance was assessed. All the tests were performed using bagasse as it comes from a sugar/alcohol factory and bagasse with screened size of 0.248 to 1.397 mm (12–60 mesh).

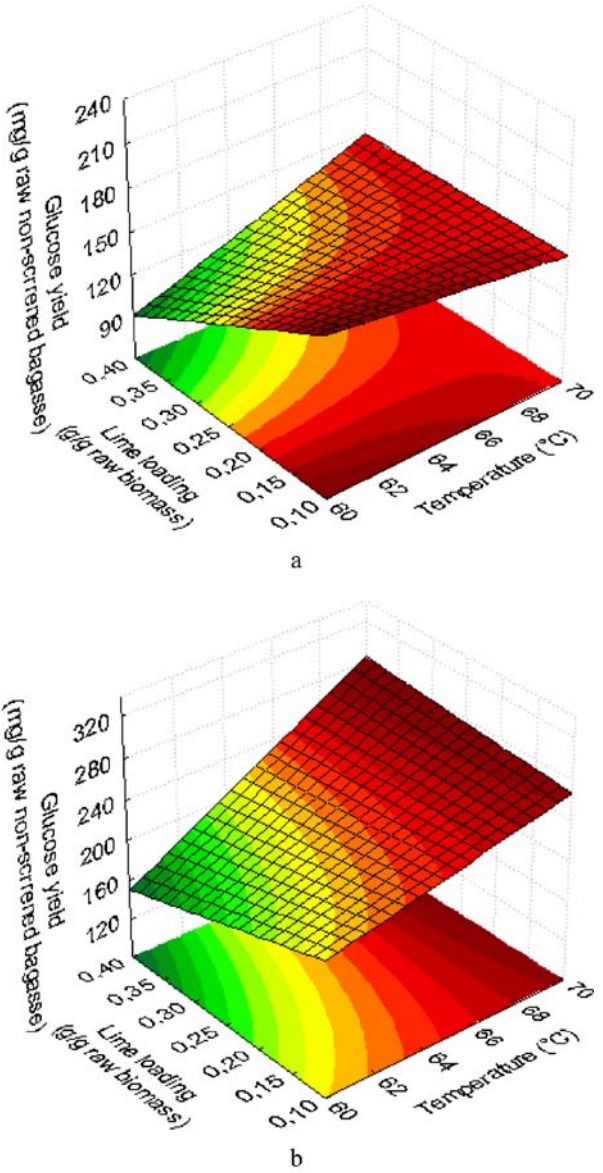

Fig. 3 Glucose yield from non-screened bagasse pretreated with lime. **a** Pretreatment time of 12 h. **b** Pretreatment time of 36 h

The results show that when hexoses and pentoses are of interest, lime should be the pretreatment agent chosen, as high TRS yields are obtained for nonscreened bagasse using 0.40 g lime/g dry biomass at 70 °C for 36 h.

When the product of interest is glucose, the best results were obtained with lime pretreatment of screened bagasse. However, the results for alkaline peroxide and lime pretreatments of nonscreened bagasse are not very different. As screening is an expensive unit operation, the use of nonscreened bagasse is preferred.

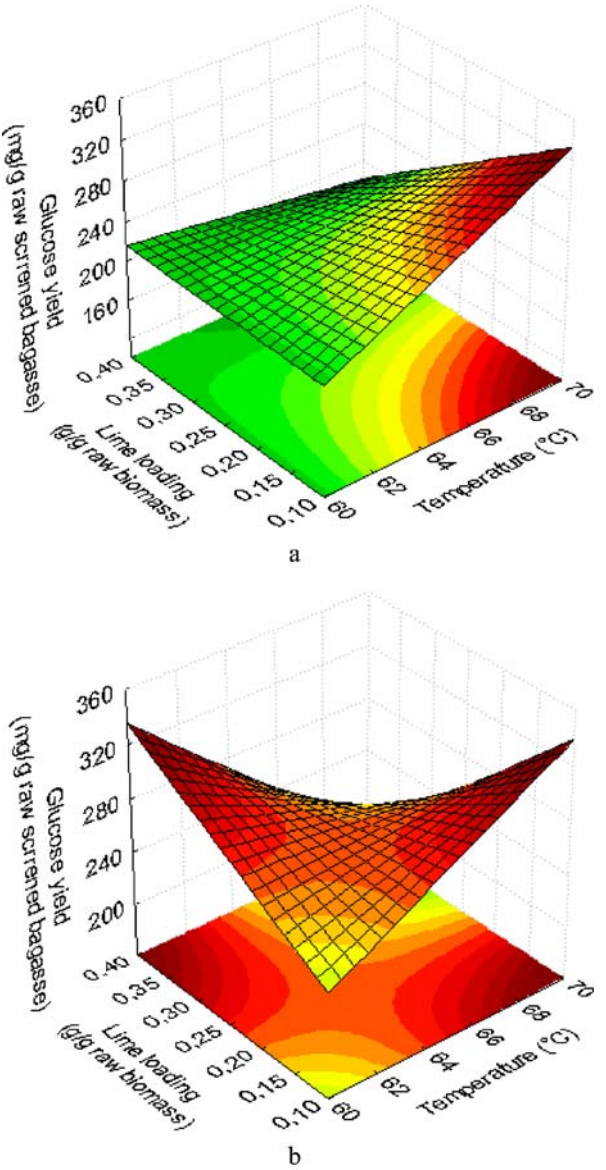

Fig. 4 Glucose yield from screened bagasse pretreated with lime. **a** Pretreatment time of 12 h. **b** Pretreatment time of 36 h

For screened bagasse, lime pretreatment can be performed under three conditions for high glucose yields: 0.10 g lime/g dry biomass at 70 °C for 12 h, 0.10 g lime/g dry biomass at 70 °C for 36 h, or 0.40 g lime/g dry biomass at 60 °C for 36 h.

For nonscreened bagasse, the best results are for alkaline peroxide pretreatment performed with 5% H_2O_2 at ambient temperature for 24 h. Lime pretreatment with 0.40 g lime/g dry biomass at 70 °C for 36 h also leads to high glucose yield. The choice between alkaline peroxide and lime pretreatment in this case is not straightforward, and fermentation of the hydrolysis product to evaluate ethanol yields should help in the

decision. There is no statistically significant difference in the processes; however, the pretreatment with peroxide can be performed at ambient temperatures and it takes less time.

As the maximum TRS and glucose yields were always found at the extremes of the studied intervals, in future work, we will investigate if it is possible to improve glucose and/or TRS yield by redefining the factor levels to cover a bigger area around the optimal conditions determined in this work.

Acknowledgments The authors acknowledge Capes for financial support.

References

1. Fan, L. T., Lee, Y. H., & Gharpuray, M. M. (1982). *Advances in Biochemical Engineering*, *23*, 157–187.
2. Gharpuray, M. M., Lee, Y. H., & Fan, L. T. (1983). *Biotechnology and Bioengineering*, *26*, 426–433.
3. Lynd, L. R., Elander, R. T., & Wyman, C. E. (1996). *Applied Biochemistry and Biotechnology*, *57–58*, 741–761.
4. Laser, M., Schulman, D., Allen, S. G., Lichwa, J., Antal, M. J., Jr., & Lynd, L. R. (2002). *Bioresource Technology*, *81*, 33–44.
5. Mosier, N., Wyman, C., Dale, B., Elander, R., Lee, Y. Y., Holtzapple, M., & Ladisch, M. (2005). *Bioresource Technology*, *96*, 673–686.
6. Lee, J. (1997). *Journal of Biotechnology*, *56*, 1–24.
7. Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., & Lee, Y. Y. (2005a). *Bioresource Technology*, *96*, 1959–1966.
8. Wyman, C. E., Dale, B. E., Elander, R. T., Holtzapple, M., Ladisch, M. R., & Lee, Y. Y. (2005b). *Bioresource Technology*, *96*, 2026–2032.
9. Hinman, N. D., Schell, D. J., Riley, C. J., Bergeron, P. W., & Walter, P. J. (1992). *Applied Biochemistry and Biotechnology*, *34/35*, 639–649.
10. Parisi, F. (1982). *Advances in Biochemical Engineering*, *38*, 53–87.
11. Kaar, W. E., & Holtzapple, M. T. (2000). *Biomass Bioengineering*, *18*, 189–199.
12. Gould, J. M. (1984). *Biotechnology and Bioengineering*, *26*, 46–52.
13. Gould, J. M. (1985). *Biotechnology and Bioengineering*, *27*, 225–231.
14. Gould, J. M. (1987). Int. CI. C 13K1/02. US, PI 4,649,113.
15. Azzam, A. M. (1989). *Journal of Environmental Science and Health Part B*, *24*(4), 421–433.
16. Amjed, M., Jung, H. G., & Donker, J. D. (1992). *Journal of Animal Science*, *70*, 2877–2884.
17. Krishna, S. H., Prasanthi, K., Chowdary, G. V., et al. (1998). *Process Biochemistry*, *33*, 825–830.
18. Lesoing, G., Klopfenstein, T., Rush, I., & Ward, J. (1981). *Journal of Animal Science*, *51*, 263.
19. Verma, M. L. (1983). In G.R. Pearce (Ed.) (pp. 85–99). Canberra, ACT: Australian Government Publishing Service.
20. Playne, M. J. (1984). *Biotechnology and Bioengineering*, *26*, 426–433.
21. Nagwani, M. (1992). M.S. thesis, Texas A&M University.
22. NREL (National Renewable Energy Laboratory—EUA). (1996)
23. Chang, V. S., Burr, B., & Holtzapple, M. T. (1997). *Applied Biochemistry and Biotechnology*, *63–65*, 3–19.
24. Chang, V. S., Nagwani, M., & Holtzapple, M. T. (1998). *Applied Biochemistry and Biotechnology*, *74*, 135–159.
25. Holtzapple, M. T., & Davison, R. R. (1999). Int. CI. C 13K1/02. US, PI 5,865,898.
26. Kaar, W. E., & Holtzapple, M. T. (2000). *Biomass Bioengineering*, *18*, 189–199.
27. Kim, S., & Holtzapple, M. T. (2005). *Bioresource Technology*, *96*, 1994–2006.
28. Ferraz, A., Baeza, J., Rodriguez, J., & Freer, J. (2002). *Bioresource Technology*, *74*(3), 201–212.
29. Lin, Y. L., & Dence, C. W. (1992). *Methods in lignin chemistry* (pp. 33–62). Berlin: Springer.
30. Irick, T. J., West, K., Brownell, H. H., Schiwald, W., & Saddler, J. N. (1988). *Applied Biochemistry and Biotechnology*, *17*, 137–149.
31. Kaar, W. E., & Brink, D. L. (1991). *Journal of Wood Chemistry and Technology*, *11*, 479–494.
32. Kaar, W. E., Gool, L. G., Merriman, M. M., & Brink, D. L. (1991). *Journal of Wood Chemistry and Technology*, *11*, 447–463.
33. Laver, M. L., & Wilson, K. P. (1993). *Tappi Journal*, *76*(6), 155–159.
34. Szczodrak, J., & Fiedurek, J. (1996). *Biomass & Bioenergy*, *10*(5/6), 367–375.

35. Ghose, T. K. (1987). *Pure and Applied Chemistry*, *59*(2), 257–268.
36. Adney, B., & Baker, J. (1996). Chemical analysis and testing task—Laboratory analytical procedure. LAP-006.
37. Wood, T. M., & Bhat, K. M. (1988). *Methods in enzymology*. San Diego: Academic, 160, 87–116.
38. Miller, G. L. (1959). *Analytical Chemistry*, *31*(3), 426–428.
39. Barros Neto, B., Scarmin, I. S., & Bruns, R. E. (2003). 2nd ed. Campinas, SP: Editora da UNICAMP.