# Kinetics of Lime Pretreatment of Sugarcane Bagasse to Enhance Enzymatic Hydrolysis

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**Abstract** The objective of this work was to determine the optimum conditions of sugarcane bagasse pretreatment with lime to increase the enzymatic hydrolysis of the polysaccharide component and to study the delignification kinetics. The first stage was an evaluation of the influence of temperature, reaction time, and lime concentration in the pretreatment performance measured as glucose release after hydrolysis using a 2<sup>3</sup> central composite design and response surface methodology. The maximum glucose yield was 228.45 mg/g raw biomass, corresponding to 409.9 mg/g raw biomass of total reducing sugars, with the pretreatment performed at 90°C, for 90 h, and with a lime loading of 0.4 g/g dry biomass. The enzymes loading was 5.0 FPU/dry pretreated biomass of cellulase and 1.0 CBU/dry pretreated biomass of β-glucosidase. Kinetic data of the pretreatment were evaluated at different temperatures (60°C, 70°C, 80°C, and 90°C), and a kinetic model for bagasse delignification with lime as a function of temperature was determined. Bagasse composition (cellulose, hemicellulose, and lignin) was measured, and the study has shown that 50% of the original material was solubilized, lignin and hemicellulose were selectively removed, but cellulose was not affected by lime pretreatment in mild temperatures (60-90°C). The delignification was highly dependent on temperature and duration of pretreatment.

**Keywords** Pretreatment of lignocellulosic biomass  $\cdot$  Sugarcane bagasse  $\cdot$  Kinetics of pretreatment  $\cdot$  Lime pretreatment

### Introduction

Bagasse is a product of sugar cane processing and an important potential energy source that can be used as raw material for cellulosic ethanol production. In Brazil, there are 300 sugar industries that

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produced approximately 163 million tons of bagasse in 2010/2011 [1]. Approximately 90% of this bagasse is used as fuel for boilers, to generate heat for steam production, or for mechanical and electrical power generation. The remaining corresponds to 9 million tons of bagasse [2].

Lignocellulosic materials are resistant to enzymatic hydrolysis. There are major limitations for efficient ethanol production from lignocellulosic biomass [3], among them the presence of lignin surrounding the cellulose, as a physical barrier reducing the available sites for enzymatic attacks [4]. Pretreatment is an important tool for lignocelluloses conversion processes and is required to alter the biomass structure to make cellulose more accessible to the enzymatic complex [5].

Alkaline pretreatment with lime has received a lot of attention in the last years because it removes lignin from biomass, thus improving the reactivity of the remaining polysaccharides, and removes acetyl groups and the various uronic acid substitutions on hemicellulose [6]. In addition, lime pretreatment uses lower temperatures and pressures compared with other pretreatments, uses safe reactives, is inexpensive, and can be recovered by carbonating with CO<sub>2</sub> [7].

The aim of the present study was to evaluate the performance of sugarcane bagasse pretreatment with lime to enhance enzymatic hydrolysis and posterior ethanol production and to determine a kinetic model for bagasse delignification with lime as a function of the temperature. The influence of different pretreatment conditions (temperature, residence time, and lime loading) on sugar yield and the effect of delignification during the pretreatment were assessed. The ranges considered for pretreatment conditions were determined based on results of a previous work [8] that assessed the pretreatment of bagasse manually harvested after burning. The sugarcane bagasse considered in the present work was mechanically harvested without burning. As bagasse is a complex raw material and its handling previous to pretreatment is expected to influence not only its composition but also its recalcitrance, in the present work another experimental design was performed to evaluate the optimal values of the variables affecting pretreatment.

## Materials and Methods

#### Substrate

Fresh sugarcane bagasse was supplied from the sugar plant "Usina da Pedra" (São Paulo, Brazil). It was dried for 3 days at normal environment temperature and ground in knife mill (Wiley model 3) and hammer mill (General Electronic) for 10 min at each mill. It was subsequently sieved using Tyler 35 sieve (around 0.5 mm) and stored in the freezer in sealed plastic bags.

#### Lime Pretreatment

Four grams of dry bagasse was added in 500 mL Erlenmeyer flasks with 100 mL of distilled water and stirred in shaker at 150 rpm. Time and temperature of pretreatment, as well as lime loading for each assay, were determined by the experimental design. After completion of the shaker cycle, the reaction was stopped with cold water, and the pretreated biomass was neutralized with hydrochloric acid 5.0 mol/L to pH 7.0, washed with distilled water, and oven-dried at 40°C for 48 h. The dry weight obtained was used to determine mass loss during pretreatment.

## Enzymatic Hydrolysis

One gram of pretreated bagasse was added to 300 mL of distilled water in Erlenmeyer flasks, and the pH was adjusted to 4.8 [9]. The enzyme loadings were 1.0 CBU/g dry



biomass of  $\beta$ -glucosidase and 5.0 FPU/g dry biomass of cellulase. The flasks were sealed and stirred in shaker at 100 rpm at a temperature of 50°C. During hydrolysis, samples of 2.0 mL were collected at times of 1, 3, 6, 12, 24, 36, 48, 60, and 72 h and inactivated by increasing the temperature to 80°C for 15 min. Finally, the concentration of glucose and total reducing sugars (TRS) were determined using a kit based on the glucose oxidase reaction (reagent GOD PAD) and the dinitrosalicylic acid method (DNS) [10], respectively. Cellulase activity (*Trichoderma reesei*, Sigma) was measured by the filter paper method as described by Ghose [11] and was of 64.106 FPU/mL.  $\beta$ -Glucosidase activity (Novozyme 188) was measured as described by Wood and Bhat [12] and was of 309.92 CBU/mL.

After the enzymatic hydrolysis experiments, the residues were washed and dried at 105°C to constant weight for quantification of the hydrolyzed mass.

#### Chemical Characterization

Four grams of a milled sample of bagasse was extracted with 99% ethanol in a Soxhlet apparatus until the liquid around the cartridge remained colorless [13]. Ash content was determined after burning of the samples in a muffle at 550°C for 6 h [14]; 0.3 g of extracted bagasse was hydrolyzed with 72% (w/w) sulfuric acid at 30°C for 1 h. The acid was diluted to a final concentration of 4% (with the addition of 84 mL of water), and the mixture was heated at 121°C for 1 h in autoclave. The residual material was cooled and filtered through paper filter [15]. 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 the absorbance at 205 nm and using the value of 105 Lg<sup>-1</sup>cm<sup>-1</sup> as the absorptivity of soluble lignin [16]. The concentrations of monomeric sugars in the soluble fraction were determined by high-performance liquid chromatography using a SUGAR-PAK (Waters) column at 70°C and eluted with deionized water at 0.5 mL/min using a temperature-controlled refractive index detector (Model 410 waters, pump model 515) at 40°C. Before injection, samples were neutralized with calcium carbonate and filtered using cellulose ester membranes with 0.22 µm pores (Millipore). Under these conditions, glucose, xylose, and arabinose were quantified. 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. These concentrations were used to determine the contents of cellulose, hemicellulose, holocellulose, glucan, and xylan.

## Experimental Design

A central composite design with three replicas at the central point (17 runs) was performed for the optimization of the pretreatment with lime. The variables were coded according to Eq. 1. The real and coded values of the three factors considered are shown in Table 1.

$$x_i = \frac{(X_i - X_0)}{\Delta X_i} i = 1, 2, \dots, k$$
 (1)

where  $x_i$  and  $X_i$  are the dimensionless and the actual values of the independent variable i,  $X_0$  is the actual value of the independent variable at the center point, and  $\Delta X_i$  is the step change of  $X_i$  corresponding to a unit variation of the dimensionless value.



Factors	Range and levels					
	-1.41	-1	0	+1	+1.41	
Reaction time (h)	53.07	65	82.5	100	111.93	
Temperature (°C)	83.27	86	90	94	96.73	
Lime loading $(w/w)$	0.15	0.25	0.4	0.55	0.65	

Table 1 Factors and experimental design levels

# **Delignification Parameters**

Kinetic data were collected around the optimum point of pretreatment; 4.0 g of dried and milled bagasse was treated with 0.4 g of lime/g raw bagasse in different conditions of temperature: 60°C, 70°C, 80°C, and 90°C. For each of these isothermals, the composition of pretreated bagasse was determined at the reaction times of 24, 48, 60, 72, 84, 90, and 108 h. The pretreated biomass was neutralized with hydrochloric acid 5 mol/L to pH 7.0, washed with distilled water, oven-dried at 40°C for 48 h, and weighed to determined mass loss. The contents of lignin and carbohydrates were determinate by National Renewable Energy Laboratory procedures [15,16]. The calculation of the holocellulose mass loss and Klason lignin removed were performed using Eq. 2:

$$M_{\rm p} = M_{\rm o} - M_t \tag{2}$$

where  $M_p$  is the mass loss of holocellulose or Klason lignin removed (grams per 100 g of pretreated biomass),  $M_o$  is the initial mass of holocellulose or Klason lignin at t=0 (grams per 100 g pretreated biomass), and  $M_t$  is the mass of holocellulose or Klason lignin at time t (grams per 100 g pretreated biomass).

The removal of lignin was calculated by Eq. 3, reported by Kim and Lee [4].

$$L_{\rm c} = \frac{L_{\rm To} - L_{\rm T}}{L_{\rm To}} \tag{3}$$

where  $L_c$  is the removal of total lignin at time t (grams of lignin removed at time t per grams of initial lignin t=0),  $L_{To}$  is the total lignin content at time t=0 (grams of total lignin at time t=0 per grams of pretreated biomass), and  $L_T$  is total lignin content at time t (grams of total lignin at time t per grams of pretreated biomass).

#### Results and Discussion

# Optimization of the Reaction Conditions

The effects of temperature, lime loading, and reaction time on sugars release after enzymatic hydrolysis were evaluated trough a 2<sup>3</sup> central composite design. The ranges of the factors were chosen based on the literature [8,17]. The response of the statistical analysis was the concentration of glucose at the final time of the hydrolysis reaction, but TRS concentrations were also measured and are shown in the design matrix (Table 2). In this table, it can be observed that the maximum glucose release was obtained in the central points, with a maximum average glucose yield of 0.233 g/g raw biomass, corresponding to



Assay	Time (h)	Temperature (°C)	Lime loading (g/g)	Glucose (g/g)	TRS (g/g)
1	65	86	0.25	0.192	0.336
2	65	86	0.55	0.177	0.333
3	65	94	0.25	0.190	0.344
4	65	94	0.55	0.175	0.349
5	100	86	0.25	0.203	0.379
6	100	86	0.55	0.207	0.367
7	100	94	0.25	0.209	0.385
8	100	94	0.55	0.192	0.348
9	53.07	90	0.4	0.191	0.326
10	111.93	90	0.4	0.202	0.354
11	82.5	83.27	0.4	0.199	0.381
12	82.5	96.73	0.4	0.199	0.337
13	82.5	90	0.15	0.178	0.360
14	82.5	90	0.65	0.200	0.350
15 (1)	82.5	90	0.4	0.239	0.366
15 (2)	82.5	90	0.4	0.230	0.367
15 (3)	82.5	90	0.4	0.228	0.365

Table 2 Design matrix presenting TRS and glucose concentration after hydrolysis of pretreated bagasse with lime

0.366 g/g raw biomass of TRS. The experiments performed in the lower conditions of time and temperature showed lower production of sugars.

The statistical significance of the effects of temperature, reaction time, and lime loading on enzymatic hydrolysis yield was determined using the software Statistic 7.0 and considering a 90% confidence interval. The results can be seen in the standardized Pareto chart (Fig. 1). The significant effects were the quadratic effects of lime loading, time, and temperature, as well as the linear effect of time.

A quadratic model was proposed to describe the experimental data, as expressed by Eq. 4. This model was used to plot the response surfaces and to determine the optimal values of the factors.

$$Glucose(g/g) = 0.2321 + 0.00704t - 0.01252t^2 - 0.00095T - 0.01153T^2 - 0.00038m - 0.01524m^2 - 0.00063Tt + 0.00224tm - 0.00257Tm$$
 (4)

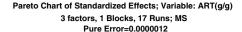
where t, T, and m are the codified values of pretreatment time, temperature, and lime loading, respectively.

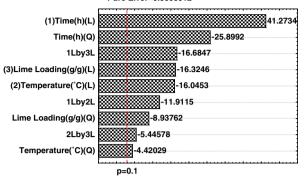
Table 3 shows the analysis of variance (ANOVA) for the full quadratic model (Eq. 4). The F value for the regression was calculated as  $MS_R/MS_r$  and compared to the listed F value ( $F_{9,7}$ ) [18]. The F value for lack of fit was calculated as  $MS_{Lf}/MS_{Pe}$  and compared to the listed F value ( $F_{5,2}$ ). As the calculated F value for the regression is higher than the listed value and the calculated F value for lack of fit is lower than the listed value, the model is statistically significant and can be used to predict the glucose yields of the enzymatic hydrolysis and to plot the response surfaces.

The response surfaces plotted using Eq. 4 can be seen in Fig. 2a-c. It can be observed that the highest yields were obtained for intermediate temperature, lime loading, and



Fig. 1 Pareto chart of standardized effects for the glucose yield (grams per gram of raw bagasse)





Standardized Effect Estimate (Absolute Value)

reaction time, with higher and lower values of the factors resulting in a decrease of yield. Optimization performed using the model equation points to a maximum glucose yield of 0.22 (grams per gram of raw bagasse) when bagasse is pretreated with a lime loading of 0.4 g/g raw biomass for 90 h at 90°C.

Figure 3 shows the experimental hydrolysis profiles for bagasse pretreated with lime in the optimal conditions (90°C, 90 h and 0.4 g lime/g raw bagasse) and the hydrolysis profile for bagasse without pretreatment. The maximum glucose yield was 228.45 mg/g raw biomass, corresponding to 409.9 mg/g raw biomass of TRS. The importance of the stage of pretreatment in the process can be observed, as glucose release after hydrolysis of untreated bagasse is very low.

Table 4 shows the compositions of raw bagasse and bagasse pretreated in the optimal conditions. For the raw bagasse, carbohydrates account for 61% of the dry weight, which makes this biomass an adequate substrate for ethanol production. The hemicellulose fraction comprises 23.66% of the raw material, xylose being the main sugar (80%). Cellulose and lignin contents are 37.35% and 25.10%, respectively. After the pretreatment stage, approximately 4% of the cellulose, 27% of hemicellulose, and 39% of lignin were solubilized. These data demonstrate that the cellulose portion of the biomass is virtually unaffected by the pretreatment, which is desirable in an effective pretreatment. The

Table 3 ANOVA of the model described by Eq. 4

Source of variation	Sum of squares	Degrees of freedom	Mean square	F calc	F listed (90%)
Regression (R)	$4.55 \times 10^{-3}$	9	5.06×10 <sup>-4</sup>	4.72	2.74 (F <sub>9, 7</sub> )
Residual (r)	$7.51 \times 10^{-3}$	7	$1.07 \times 10^{-4}$		
Lack of fit (Lf)	$6.89 \times 10^{-3}$	5	$1.38 \times 10^{-4}$	4.40	$9.29 (F_{5, 2})$
Pure error (Pe)	$6.30 \times 10^{-3}$	2	$3.10 \times 10^{-5}$		
Total (T)	$5.31 \times 10^{-3}$	16			
$R^2$	85.85				
Maximum accountable variation (%)	98.82				



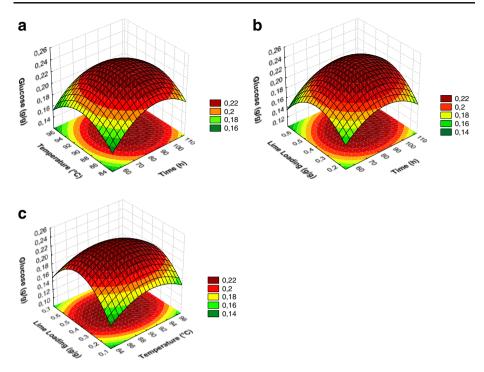
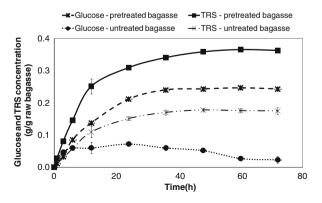


Fig. 2 Response surface of glucose yield a temperature versus time, b lime loading versus time, and c lime loading versus temperature. The factors not shown in each figure were maintained in the center point value

pretreatment with lime has a major effect in delignification and in increasing the accessible surface area, but its effect in removing hemicellulose is lower than in removing lignin [6].

Other authors have evaluated the pretreatment of lignocellulosic biomass with lime. Rabelo [9] results have shown that the highest glucose yields were for pretreatment performed for 65.6 h, at 70°C, and with 0.4 g lime/g biomass. The highest glucose yields were obtained in the extremities of the operational range studied, so in the present work the ranges of pretreatment time and temperature were increased. The optimal conditions

**Fig. 3** Comparison of the hydrolysis profile of untreated bagasse and pretreated in optimal conditions





Composition	Raw bagasse 100 (g)	Pretreated bagasse 58.73 (g)		
Ash	1.79±0.48	_		
Cellulose	37.35±0.51	$35.50\pm0.52$		
Hemicellulose	$23.66 \pm 0.88$	$12.53 \pm 0.34$		
Lignin	$25.10\pm0.48$	$9.16\pm0.14$		
Extractives	$3.25 \pm 0.20$	-		

Table 4 Composition of raw material and pretreated bagasse

were obtained for higher temperature and pretreatment time and the same lime loading. Chang et al. [7] determined optimal conditions for lime pretreatment of switchgrass, which were 2 h of pretreatment at 100°C and 120°C and a lime loading of 0.1 g/g biomass. After pretreatment, approximately 10% of the cellulose, 26% of hemicellulose, and 29% of lignin were solubilized. The conditions determined in the present work for bagasse are of lower temperature, higher pretreatment time, and lime loading and lead to less cellulose and more lignin removal, which is a desired characteristic. Chang et al. [19] performed a systematic study of pretreatment conditions to enhance the enzymatic digestibility of bagasse and wheat straw. They concluded that for short pretreatment times (1–3 h), high temperatures (85–135°C) were required, whereas for long pretreatment times (e.g., 24 h), low temperatures (50–65°C) were effective. They recommended a lime loading of 0.1 g lime/g biomass. They have shown that after pretreatment of bagasse for 1 h at 120°C and 0.1 g lime/g biomass, no glucan or xylan was removed, whereas 14% of lignin was solubilized. The results obtained in the present work show higher hemicellulose and lignin removal with higher pretreatment times and lime loading.

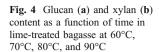
## Delignification of Sugarcane Bagasse with Lime Pretreatment

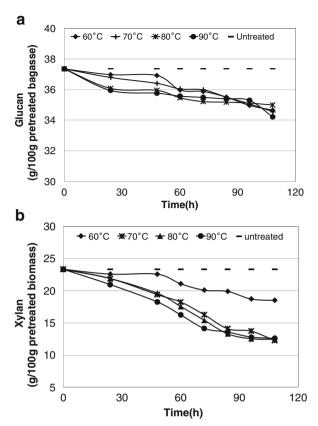
Figure 4a, b shows the contents of glucan and xylan in the bagasse during delignification with lime at different temperatures. The highest glucan loss occurred at 90°C and the highest solubilization of xylan occurred for temperatures higher than 70°C, both at the maximum time of pretreatment considered. On the other hand, the lowest sugar loss occurred at 60°C and for pretreatment times lower than 48 h in both cases. Nonetheless, these were the conditions where there was less lignin degradation, as can be observed in Fig. 5a, b, which shows the Klason and soluble lignin contents in the bagasse. It can be observed from these figures that lignin solubilization is enhanced significantly with increased time and temperature. The tendency of reduction in the content of acid-soluble lignin was similar to that of Klason lignin. The lignin content in the bagasse pretreated at 90°C for 108 h decreased from 25.10 down to 9.10 g Klason lignin/100 g pretreated biomass and from 1.72 g down to 0.66 g soluble lignin/100 g pretreated biomass.

Equation 3 was used to calculate the removal of lignin during the stage of delignification for each isotherm. Figure 6 shows the profiles of total removal of lignin as a function of time. The highest removals obtained were 52% and 62% for temperatures of 80°C and 90°C, respectively. The lower lignin removal attained was 30% for the delignification performed at 60°C. In this figure, it can also be observed that the highest delignification occurred at the maximum considered pretreatment time in all isotherms.

The holocellulose-to-lignin selectivity (grams of holocellulose lost per grams of lignin removed) is a parameter of vital importance because it describes the effectiveness of the







process. Ideally, a good delignification process should remove lignin without a significant loss of holocellulose; therefore, small values are desired for this parameter [20].

Figure 7a–d shows the holocellulose loss versus lignin removed during delignification at the different temperatures studied. These correlations do not include the initial point for the holocellulose loss and lignin removal at time 0 (0 g holocellulose lost or lignin removed per grams of pretreated bagasse) [20]. Linear regression was employed to obtain the slope of the curve, which is the selectivity. The best holocellulose-to-lignin selectivity was obtained for the temperature of 90°C, where more lignin solubilization occurs for lower holocellulose loss.

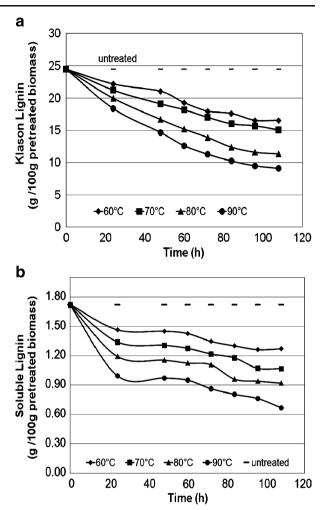
# Kinetics of Delignification

Lignin degradation is known to occur in three separate phases: initial (rapid) phase, bulk (dominant) phase, and the final residual (slow) phase [21–23]. From the kinetic viewpoint, lignin can be classified into three different classes according to the phase where it degrades (Eq. 5):

$$\frac{L_{k}}{L_{ko}} = a_{i} \exp(-k_{i} \times t) + a_{b} \exp(-k_{b} \times t) + a_{r} \exp(-k_{r} \times t)$$
(5)



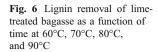
Fig. 5 Klason (a) and soluble (b) lignin content as a function of time in lime-treated bagasse at 60°C, 70°C, 80°C, and 90°C

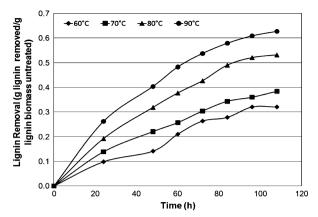


where t is the time period during which data were collected;  $L_{\rm k}$  is the mass of Klason lignin at time t;  $L_{\rm ko}$  is the initial mass of Klason lignin at t=0;  $a_1$  is the maximum fraction of lignin fragments released in the initial stage;  $a_2$  is the maximum fraction of lignin fragments released in the bulk stage;  $a_3$  is the maximum fraction of lignin fragments released in the residual stage; and  $k_{\rm i}$ ,  $k_{\rm b}$ ,  $k_{\rm r}$  are the reaction rate constants for the initial, bulk, and residual delignification stages, respectively. This equation is subjected to the constraint that  $a_{\rm i} + a_{\rm b} + a_{\rm r} = 1$  when  $L_{\rm k}/L_{\rm ko} = 1$  at t=0.

In the selectivity and removal of lignin results, it was observed that bagasse delignification in short times (≤108 h) only presented one phase (dominant or bulk). The main feature of the residual stage is that lignin removal proceeds very slowly, while the carbohydrates are degraded [24]. This fact was not observed for the pretreatment conditions considered in this work; in all the cases, the degradation of lignin is significant during all the pretreatment duration. So it was concluded that, under these conditions, the delignification of sugarcane bagasse pretreated with lime lacks the residual phase for low







pretreatment times, which is in accordance to the results obtained by Cotlear [20], which showed that this stage came only after the first week of pretreatment.

As for the initial phase, De Groot et al. [25] have shown that in the case of the soda pulping of bagasse the delignification lacks an initial phase. They support their claim by showing that the alkaline delignification of bagasse proceeds from the very beginning with

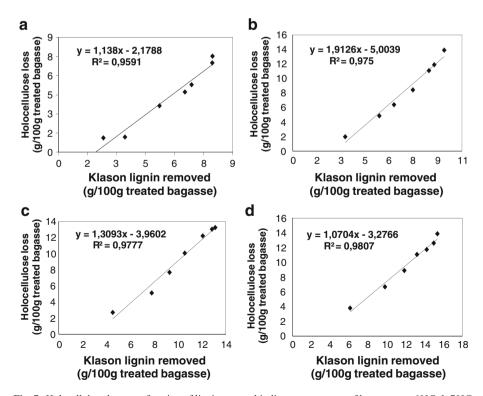


Fig. 7 Holocellulose loss as a function of lignin removal in lime pretreatment of bagasse at a 60°C, b 70°C, c 80°C, and d 90°C



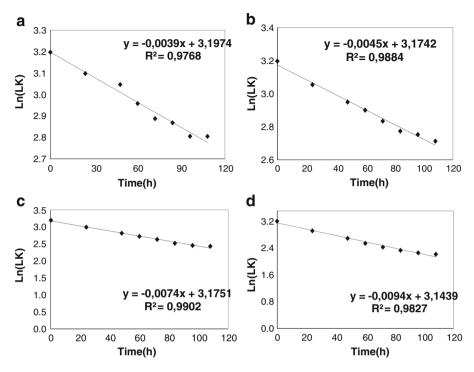
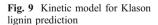


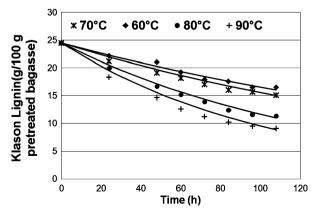
Fig. 8 Graph of natural logarithmic of Klason lignin versus time at a 60°C, b 70°C, c 80°C, and d 90°C

a high degree of lignin removal compared to carbohydrate removal, which is not a characteristic of the initial phase but of the bulk phase [20]. The same occurs in the lime delignification of sugarcane bagasse in the conditions studied in this work, and we considered that there is no initial phase, although there is a possibility that this phase is so small that it would not be detected by simple visual inspection [25].

Considering only the bulk phase, the model presented in the Eq. 5 is reduced to Eq. 6:

$$ln(L_{k}) = -k_{b} \times t + ln L_{ok}$$
(6)







Plotting  $\ln(L_k)$  versus time (Fig. 8), it was possible to calculate the kinetic constants at 60°C, 70°C, 80°C, and 90°C as  $k_{b1}$ =0.0039 h<sup>-1</sup>,  $k_{b2}$ =0.0045 h<sup>-1</sup>,  $k_{b3}$ =0.0074 h<sup>-1</sup>, and  $k_{b4}$ =0.0094 h<sup>-1</sup>, respectively. These results show that the kinetic constant increased as temperature increased. The curves fitted to predict  $L_k$  (continuous line) as a function of time (Eq. 6) compared to the experimental data (points) are shown in Fig. 9 for the temperatures considered. It can be seen that the model represented the experimental data accurately.

Although the model of Eq. 5 is semi-empirical, one of its important characteristics is that the resulting rate constants for each phase can be fitted to the well-known Arrhenius equation [23,26]:

$$\ln(k_{\rm b}) = \ln(A) + \left(-\frac{E_{\rm a}}{RT}\right) \tag{7}$$

where  $k_b$  is the rate constant for the bulk phase (1/h), A is the Arrhenius constant or frequency factor (1/h),  $E_a$  is the activation energy (joules per mole), R is the universal gas constant (8.314 J/mol K), and T is the absolute temperature (kelvin).

The determination of the activation energy was made by plotting the  $Ln(k_b)$  versus 1/T, and the value obtained was 31.47 kJ/mol. This value is much smaller than the activation energy for wood and corn stover (approximately 120-139 and 50 kJ/mol, respectively) [26] in the dominant phase, when these are pretreated with lime in oxidative conditions. For lignin removal in alkaline conditions, sugarcane bagasse has a more favorable structure than wood and corn stover in the conditions studied, as it has lower delignification activation energy.

## **Conclusions**

This study has shown that lime pretreatment is an efficient method to increase the enzymatic accessibility of the water-insoluble, cellulose-rich component in sugarcane bagasse. After pretreatment, the enzymatic conversion from cellulose to glucose increased nearly four times compared to the untreated bagasse. The best pretreatment conditions to obtain high cellulose conversions to glucose were at 90°C for 90 h with lime loading of 0.4 g/g raw bagasse. The holocellulose-to-lignin selectivity (grams of holocellulose lost per gram of lignin removed) was low for the temperature of 90°C, which is desirable in an efficient pretreatment.

The kinetic model of delignification of sugarcane bagasse with lime was empirically established as a first-order reaction corresponding to the bulk phase for pretreatment time up to 108 h. The activation energy for the delignification reaction was estimated as 31.47 kJ/mol, which is much lower than the activation energy of delignification for wood and corn stoyer.

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