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ORIGINAL PAPER

A study on the pretreatment of a sugarcane bagasse sample with dilute sulfuric acid

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Abstract Experiments based on a 2^3 central composite full factorial design were carried out in 200-ml stainless-steel containers to study the pretreatment, with dilute sulfuric acid, of a sugarcane bagasse sample obtained from a local sugar–alcohol mill. The independent variables selected for study were temperature, varied from 112.5°C to 157.5°C, residence time, varied from 5.0 to 35.0 min, and sulfuric acid concentration, varied from 0.0% to 3.0% (w/v). Bagasse loading of 15% (w/w) was used in all experiments. Statistical analysis of the experimental results showed that all three independent variables significantly influenced the response variables, namely the bagasse solubilization, efficiency of xylose recovery in the hemicellulosic hydrolysate, efficiency of cellulose enzymatic saccharification, and percentages of cellulose, hemicellulose, and lignin in the pretreated solids. Temperature was the factor that influenced the response variables the most, followed by acid concentration and residence time, in that order. Although harsher pretreatment conditions promoted almost complete removal of the hemicellulosic fraction, the amount of xylose recovered in the hemicellulosic hydrolysate did not exceed 61.8% of the maximum theoretical value. Cellulose enzymatic saccharification was favored by more efficient removal of hemicellulose during the pretreatment. However, detoxification of the hemicellulosic hydrolysate was necessary for better bioconversion of the sugars to ethanol.

Keywords Lignocellulose · Compositional analysis · Acid hydrolysis · Enzyme hydrolysis

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Introduction

Brazil is the biggest producer of sugarcane in the world. In 2009, for example, more than 604 million tons of sugarcane was processed by the Brazilian sugar–alcohol mills, leading to the production of roughly 33 million tons of sugar and 26 billion liters of ethanol [11].

Adequate climate conditions, ample rainfall at the right times, and abundant productive land, among other factors, make Brazil the only major producing country that can significantly increase its ethanol production and play an important role in satisfying the future global demand without jeopardizing food production [5].

Today, most Brazilian sugarcane processing facilities produce both sugar (sucrose) and alcohol (ethanol) from sugarcane juice. The bagasse, i.e., the fibrous material left after crushing the cane to extract the juice, is burned to supply all the energy required in the process, including electricity, whose surplus is sold to the national distribution grid [12]. If, instead, the bagasse were used for ethanol production and the leaves and tops, currently left in the field, were burned for energy generation, much more ethanol would be produced from each hectare of sugarcane processed [10].

A number of different strategies have long been envisioned to convert the polysaccharides contained in low-value lignocellulosic "wastes" into fermentable sugars. One of them, claimed to be close to commercialization, involves hemicellulose hydrolysis with dilute acids followed by cellulose hydrolysis with enzymes [19]. Such pretreatment with dilute acids at high temperatures promotes intense hemicellulose solubilization. Although removed only to a limited extent, the lignin is widely redistributed on the fiber surfaces, which helps to enhance the susceptibility of the cellulose to enzymatic hydrolysis [21, 23, 34].

In the present manuscript, we report the results of a study intended to quantify the effects caused by the operational conditions of pretreatment with dilute sulfuric acid (acid concentration, temperature, and residence time) on a sugarcane bagasse sample acquired from a local mill. Both the liquid and the solid fractions, generated after the pretreatments, were quantitatively recovered and had their compositions determined, which provided a comprehensive picture of the system under study. We also evaluated the enzymatic digestibility of all the pretreated solids as well as the fermentability of the hemicellulosic hydrolysate obtained under defined conditions.

Materials and methods

Sugarcane bagasse obtained from a local sugar–alcohol mill (Usina Vale do Rosario, Morro Agudo, SP, Brazil), milled to pass through a 20 mesh sieve, was used in this study. All other reagents were of analytical grade.

Characterization of the sugarcane bagasse

The sugarcane bagasse composition was characterized, according to a method validated for this particular raw material [13], as follows: Initially, 2 g milled bagasse (moisture content ~10%), previously extracted with water and ethanol, was mixed with 15 ml 72% (w/w) sulfuric acid at 45°C for 7 min. Afterwards, 275 ml distilled water was added to the mixture, which was then autoclaved at 121°C for 30 min. After cooling, the mixture was filtered through a quantitative filter paper previously weighed. The filtrate was collected and amounted to 500 ml, in a volumetric flask, with the water used to wash the insoluble solids and distilled water. The solids (acid-insoluble lignin + acid-insoluble ash) were dried in an oven at 105°C to constant weight. After recording the dry weight, the material was transferred to a previously weighed crucible, which was then heated in a muffle furnace at 800°C for 2 h. The difference of weights at 105°C and 800°C was used to calculate the percentage of acid-insoluble lignin in the bagasse. In turn, the filtrate was analyzed by high-performance liquid chromatography (HPLC) to quantify the concentrations of cellobiose, glucose, xylose, arabinose, acetic acid, furfural, and hydroxymethylfurfural, which were then used to calculate the percentages of cellulose and hemicellulose present in the bagasse according to Eqs. 1 and 2. Acid-soluble lignin was also determined in the filtrate. To this determination, a sample was alkalinized to pH 12 with 6 M NaOH and, after appropriate dilution with distilled water, had its absorbance at 280 nm recorded. The concentration of acid-soluble lignin was then calculated according to Eqs. 3 and 4, which allowed the percentage of acid-soluble lignin in the bagasse to be determined. The total amount of lignin in the bagasse was calculated as the sum of acid-insoluble and acid-soluble fractions. For the determination of structural ash, a sample of the extracted bagasse was transferred to a previously weighed crucible and heated in a muffle furnace at 800°C for 2 h, which allowed the amount of inorganic materials in the extracted bagasse to be determined by gravimetry.

$$\text{Cellulose} = 0.95C_{\%} + 0.90G_{\%} + 1.29HMF_{\%} \quad (1)$$

$$\text{Hemicellulose} = 0.88X_{\%} + 0.88A_{\%} + 1.38F_{\%} + 0.70AA_{\%} \quad (2)$$

$$\text{ASL} = \left[4.187 \times 10^{-2}(A_{READ280} - A_{CALC280}) - 3.279 \times 10^{-4}\right] \quad (3)$$

$$A_{CALC280} = [(C_F \times \varepsilon_F) + (C_{HMF} \times \varepsilon_{HMF})]. \quad (4)$$

In Eqs. 1 and 2, $C_{\%}$, $G_{\%}$, $HMF_{\%}$, $X_{\%}$, $A_{\%}$, $F_{\%}$, and $AA_{\%}$ stand for the percentages (w/w) of cellobiose, glucose, hydroxymethylfurfural, xylose, arabinose, furfural, and acetic acid in the sugarcane bagasse, calculated from the respective concentrations determined in the filtrate. In Eqs. 3 and 4, ASL stands for the acid-soluble lignin concentration, $A_{READ280}$ for the absorbance reading at 280 nm after dilution correction, C_F and C_{HMF} for the concentrations of furfural and HMF, respectively, determined by HPLC, and ε_F and ε_{HMF} for the extinction coefficients of furfural and HMF at 280 nm, 146.85 and 114.00 l/g cm, respectively.

Cellobiose, glucose, xylose, arabinose, and acetic acid concentrations were determined by HPLC (Waters) using a refraction index detector (2414) and a Biorad Aminex HPX-87H column at 45°C. Sulfuric acid (0.01 N) at flow rate of 0.6 ml/min was used as eluent, and the injection volume was 20 µl. Furfural and hydroxymethylfurfural concentrations were also determined by HPLC, using a UV–Vis detector (2489) at 280 nm and a Hewlett-Packard RP18 column at 25°C. Acetonitrile:water (1:8) supplemented with 1% acetic acid was used as eluent at flow rate of 0.8 ml/min. The injection volume was 20 µl.

The sugarcane bagasse sample was also submitted to a second compositional analysis, following the methods

recommended by the National Renewable Energy Laboratory (NREL) [37].

Pretreatment of the sugarcane bagasse with dilute sulfuric acid

The pretreatment of the sugarcane bagasse with dilute sulfuric acid in each of the experimental conditions was performed as follows: Initially, the bagasse and the aqueous acid solution were loaded into a 200-ml stainless-steel container (19 × 7 cm), which was tightly sealed and immersed in a silicone bath provided with electrical heating. The heater was turned on, and when the temperature reached the programmed value, the residence time started to be counted. At the due time, the hydrolysis was stopped by immersing the container into an ice bath, which quenched the reaction. Both the heating and the cooling times were negligible. After removing the screw cap from the container, the hemicellulosic hydrolysate was quantitatively separated from the pretreated solids, hereinafter referred to as cellulignin, by filtration. The cellulignin was toroughly washed with deionized water and dried in an oven at 105°C. The filtrate (hydrolysate plus washing water) was amounted to 125 ml, in a volumetric flask, with distilled water. The compositions of both fractions were determined as described in the previous section.

The experiments, summing up to 22 pretreatments, were based on a 2^3 central composite full factorial design [4]. The independent variables were temperature (A), varied from 112.5°C to 157.5°C, residence time (B), varied from 5.0 to 35.0 min, and sulfuric acid concentration (C), varied from 0.0 to 3.0% (w/v). A bagasse loading of 15% (w/w) was used in all experiments. The bagasse solubilization (S), the percentages of cellulose ($\%C$), hemicellulose ($\%H$), and lignin ($\%L$) in the cellulignin, and the efficiencies of xylose recovery in the hemicellulosic hydrolysate (η_X) and of cellulose enzymatic saccharification (η_G) were considered as the response variables. Quadratic models of the type described by Eq. 5, in which Y_i represents the response variable, b_0, b_i, b_{ii}, and b_{ij} represent the regression coefficients, and X_i and X_j represent the coded levels of the independent variables, were generated by regression analysis to quantify the influence of the independent variables on S, $\%C$, $\%H$, $\%L$, η_X, and η_G.

$$Y_i = b_0 + \sum_{i=1}^{n} b_i X_i + \sum_{i=1}^{n} b_{ii} X_i^2 + \sum_{i=1}^{n-1} \sum_{j=i+1}^{n} b_{ij} X_i X_j. \qquad (5)$$

The bagasse solubilization was calculated as the percent difference between the weights of insoluble solids before and after hydrolysis (dry basis). The percentages of cellulose, hemicellulose, and lignin in the cellulignin were calculated as described in the previous section. The efficiency of xylose recovery in the hemicellulosic hydrolysate was calculated as the percent ratio among the weights of monomeric xylose recovered in the hemicellulosic hydrolysate and potentially available from the bagasse submitted to pretreatment (dry basis). The efficiency of cellulose enzymatic saccharification, performed under conditions established previously [32] (cellulase II; 10% solids; 10 FPU + 0.05 g Tween 20 per gram pretreated bagasse), was calculated as the percent ratio among the weights of monomeric glucose recovered in the cellulosic hydrolysate and potentially available from the bagasse submitted to saccharification (dry basis).

Results and discussion

The chemical composition of the sugarcane bagasse sample used in the present study, determined according to the method validated by Gouveia et al. [13], was as follows: 45.0% cellulose, 25.8% hemicellulose, 19.1% lignin, 1.0% structural ash, 9.1% extractives. According to the method recommended by the National Renewable Energy Laboratory of the USA [37], the chemical composition of this same sample was as follows: 44.9% glucan, 22.2% xylan, 1.1% arabinan, 2.6% acetyl groups, 14.1% acid-insoluble lignin, 5.2% acid-soluble lignin, 1.4% structural ash, 8.5% extractives.

As can be seen in Table 1, cellulose content between 35.0% and 45.0%, hemicellulose content between 26.2% and 35.8%, and lignin content between 11.4% and 25.2% have been reported by other researchers for different samples of sugarcane bagasse. Interpretation of such diversity in compositional data is not feasible since the chemical composition of lignocellulosic materials depends, among other factors, on the variety, location, and agricultural practices used to grow the crop [18] as well as on the methods employed for the compositional analyses [3, 16]. Moreover, the sugarcane bagasse, like many other biomass feedstocks, is a byproduct of an industrial process, which introduces an additional source of compositional variance [15].

Pretreatment with dilute sulfuric acid has become a state-of-the-art technology for pretreating different lignocellulosic materials. It promotes conversion of hemicellulose into fermentable monomeric sugars and makes cellulose more accessible to hydrolytic enzymes, exo- and endoglucanases. The conditions leading to optimal hemicellulose hydrolysis efficiency and cellulose digestibility, however, depend on the composition of the raw material [17].

In the present study, we submitted the aforementioned sample of sugarcane bagasse to a series of hydrolyses in 200-ml stainless-steel containers. The concentration of

Table 1 Composition (% w/w, dry basis) of sugarcane bagasse samples previously reported in the literature

	Zhao et al. [41]	Martin et al. [24]	Sasaki et al. [33]	Neureiter et al. [27]	Aguilar et al. [2]	Teixeira et al. [38]
Cellulose	45.0	43.1	35.0	40.2	38.9	39.6
Hemicellulose	31.8	31.1	35.8	26.4	26.2	29.7
Lignin	20.3	11.4	16.1	25.2	23.9	24.7
Others	2.9	14.4	13.1	8.2	11.0	6.0

Table 2 Efficiencies of xylose recovery in the hemicellulosic hydrolysate (η_X) and of cellulose enzymatic saccharification (η_G), bagasse solubilization (S), and percentages of cellulose ($\%C$), hemicellulose ($\%H$), and lignin ($\%L$) in the cellulignin, as a function of temperature (A), residence time (B), and sulfuric acid concentration (C) used in the pretreatment

Assay	A (°C)	B (min)	C (% w/v)	S (% w/w)	$\%C$ (% w/w)	$\%H$ (% w/w)	$\%L$ (% w/w)	η_X (% w/w)	η_G (% w/w)
01	120	10	0.5	14.8	44.4	25.4	25.8	2.6	16.5
02	150	10	0.5	26.1	53.8	19.0	26.6	16.6	24.1
03	120	30	0.5	22.9	49.7	21.7	25.9	12.2	20.9
04	150	30	0.5	33.8	53.7	11.4	29.1	43.6	30.0
05	120	10	2.5	17.1	49.1	24.9	25.1	3.6	16.7
06	150	10	2.5	35.6	59.8	10.2	29.8	51.2	29.8
07	120	30	2.5	30.4	52.1	17.5	26.9	22.8	24.1
08	150	30	2.5	41.7	59.3	3.7	33.8	57.6	33.0
09	135	20	1.5	37.1	60.4	8.8	30.9	61.8	31.1
10	135	20	1.5	34.4	53.4	10.3	30.2	50.4	32.6
11	135	20	1.5	37.4	58.6	8.3	31.9	57.4	33.2
12	112.5	20	1.5	14.9	45.4	25.3	23.9	2.0	16.7
13	157.5	20	1.5	34.7	54.2	11.2	28.6	50.2	29.0
14	135	5	1.5	25.9	52.7	20.7	28.0	17.6	24.0
15	135	35	1.5	36.4	59.4	10.1	30.8	50.2	30.2
16	135	20	0	12.6	44.8	27.3	24.2	0.9	18.5
17	135	20	3.0	34.0	55.3	8.8	31.1	54.6	33.9
18	135	20	1.5	34.3	53.7	11.7	28.9	47.6	32.1
19	135	20	1.5	34.7	54.9	11.5	30.3	48.2	31.1
20	135	20	1.5	32.7	51.7	12.1	30.1	43.0	25.5
21[a]	150	30	2.0	37.8	56.5	8.8	29.9	57.3	33.7
22[a]	150	30	2.0	37.4	56.7	8.4	30.7	55.5	30.7

[a] Assays performed at the selected experimental conditions

sulfuric acid, the temperature, and the residence time, variables reported to influence the hydrolysis of different lignocellulosic materials [6, 7, 9, 25, 31, 36], were varied according to a central composite full factorial design.

As can be seen in Table 2, the highest bagasse solubilization (41.7% w/w) was observed when the pretreatment was performed with 2.5% (w/v) acid at 150°C for 30 min. Such conditions led to extensive hemicellulose removal from the bagasse. Cellulose and lignin, on the other hand, were less solubilized and, thus, became the only major constituents of the pretreated material. Such findings were expected, because sugarcane bagasse amorphous hemicellulose, composed of acetylated glucuronoarabinoxylan, can be easily hydrolyzed by dilute acids at high temperatures [1, 20, 27]. Cellulose, on the other hand, is known to be much more recalcitrant towards dilute acid hydrolysis and, because of the surface-governed reaction mechanism, is expected to be substantially less hydrolyzed than hemicellulose [39, 40]. In turn, at temperatures exceeding its phase-transition point, lignin can become fluid, coalesce, and move throughout the cell wall matrix; it can even exit the biomass into the liquid phase during the pretreatment, but redeposits on the residual surfaces upon cooling [35]. Moreover, although lignin also breaks down and forms soluble compounds at high temperatures, many of these compounds react with themselves and form longer chains that precipitate on the fiber surfaces during batch pretreatment operations [22].

Although hemicellulose was extensively removed from the bagasse during hydrolysis with 2.5% (w/v) sulfuric acid at 150°C for 30 min, the amount of xylose recovered in the hemicellulosic hydrolysate did not exceed 57.6% of the maximum theoretical value (Table 2). In this context, Martin et al. [24] also observed that wet oxidation of sugarcane bagasse also led to hemicellulose removal without a corresponding increase in the amount of monomeric sugars recovered in the liquid fraction. According to those authors, mild alkaline pretreatment conditions were unable to promote complete saccharification of the soluble oligomers, while harsh acidic conditions caused degradation of pentose sugars into furfural and other undesirable compounds. In the present study, neither post-hydrolysis of the hemicellulosic hydrolysate with 4% (w/v) sulfuric acid nor pre-impregnation of the bagasse with the acid solution before the hydrolysis operation increased the xylose recovery in the hydrolysate, which also did not present a significant amount of furfural. Therefore, it is believed that sugar degradation reactions leading to compounds others than furfural [29] and condensation reactions among hemicellulose and lignin derivatives leading to "pseudo-lignin" insoluble compounds [26] occurred in the system under study and, thus, limited the maximum achievable hydrolysis yield. In spite of this, it is worth mentioning that efficiencies of xylose recovery in the hemicellulosic hydrolysate above 80% have already been demonstrated for the same types of raw material and pretreatment [2, 20, 27].

The lowest bagasse solubilization (12.6%, w/w) was observed when hydrolysis was performed in the absence of sulfuric acid at 135°C for 20 min. This result confirms the importance of adding an acid catalyst during batch pretreatment of lignocellulosic materials to improve hemicellulose removal at moderate temperatures [17].

As shown in Table 2, the efficiency of cellulose enzymatic saccharification after 24 h varied from 16.5% to 33.9% (w/w), being 33.0% for bagasse pretreated with 2.5% (w/v) acid at 150°C for 30 min, and 18.5% for

Table 3 Analysis of variance of the proposed models

Source	*S*					*%C*				
	SS[a]	*DF*[b]	*MS*[c]	*F*	*p*	*SS*[a]	*DF*[b]	*MS*[c]	*F*	*p*
Block	7.21	1	7.21			7.72	1	7.72		
Model	1,352.37	5	270.47	43.01	<0.0001	359.65	5	71.93	11.34	0.0002
Residual	81.76	13	6.29			82.47	13	6.34		
Lack of fit	74.06	9	8.23	4.27	0.0877	30.24	9	3.36	0.26	0.9578
Pure error	7.70	4	1.92			52.23	4	13.06		
Total	1,441.34	19				449.84	19			
R^2	0.9430					0.8135				
	%H					*%L*				
Block	9.03	1	9.03			1.90	1	1.90		
Model	879.61	7	125.66	26.25	<0.0001	125.15	6	20.86	19.79	<0.0001
Residual	52.66	11	4.79			12.65	12	1.05		
Lack of fit	43.87	7	6.27	2.85	0.1640	9.61	8	1.20	1.58	0.3462
Pure error	8.79	4	2.20			3.04	4	0.76		
Total	941.30	19				139.69	19			
R^2	0.9435					0.9082				
	η_X					η_G				
Block	0.77	1	0.77			1.10	1	1.10		
Model	8,377.30	6	1,396.22	21.89	<0.0001	589.28	5	117.86	13.77	<0.0001
Residual	765.28	12	63.77			111.25	13	8.56		
Lack of fit	682.99	8	85.37	4.15	0.0927	73.73	9	8.19	0.87	0.6046
Pure error	82.29	4	20.57			37.52	4	9.38		
Total	9,143.35	19				701.63	19			
R^2	0.9163					0.8412				

[a] *SS* sum of squares

[b] *DF* degrees of freedom

[c] *MS* mean square

bagasse pretreated in the absence of sulfuric acid at 135°C for 20 min. These data are in accordance with the literature [14] and show that more efficient removal of hemicellulose during pretreatment with dilute sulfuric acid favors subsequent enzymatic saccharification of the cellulose. In spite of this, even though the digestions were performed under conditions optimized previously [32], the efficiency of cellulose saccharification did not exceed 45.4% after 72 h of hydrolysis. Considering this, an alternative to improve the saccharification could be use of harsher pretreatment conditions, which certainly would also lead to greater degradation of the sugars solubilized in the hemicellulosic hydrolysate [30]. Otherwise, the cellulases could be supplemented with hemicellulases to remove residual hemicellulose remaining in solids pretreated under milder conditions, increasing the yield of recovery of total sugars [28].

Statistical analysis of the experimental data showed that all three independent variables significantly influenced the pretreatment and enzymatic saccharification of the sugarcane bagasse. Such influences could be successfully described by quadratic models (Eqs. 6–11), whose suitability of fit and statistical significance are presented in Tables 3 and 4.

$$S = 34.48 + 6.54A + 4.08B + 4.74C - 3.71A^2 - 4.38C^2 \quad (6)$$

$$\%C = 55.62 + 3.56A + 1.43B + 2.76C - 2.03A^2 - 1.92C^2 \quad (7)$$

$$\%H = 10.82 - 5.32A - 3.29B - 3.91C + 2.78A^2 + 1.52B^2 + 2.69C^2 - 1.48AC \quad (8)$$

$$\%L = 30.10 + 1.82A + 1.01B + 1.48C - 1.59A^2 - 0.99C^2 + 0.95AC \quad (9)$$

$$\eta_X = 51.71 + 16.01A + 8.89B + 11.26C - 10.63A^2 - 7.16B^2 - 9.89C^2 \quad (10)$$

$$\eta_G = 29.94 + 4.57A + 2.42B + 2.82C - 3.38A^2 - 1.89C^2. \quad (11)$$

According to the above equations, temperature (A) was the factor that influenced the response variables the most,

Table 4 Regression coefficients, standard errors, and significance levels of the terms retained in the proposed models

Term	S			$\%C$			$\%H$		
	C^a	SE^b	p	C^a	SE^b	p	C^a	SE^b	p
Intercept	34.48	0.89		55.62	0.89		10.82	0.87	
A	6.54	0.71	<0.0001	3.56	0.71	0.0002	−5.32	0.62	<0.0001
B	4.08	0.71	<0.0001	1.43	0.71	0.0668	−3.29	0.62	0.0002
C	4.74	0.71	<0.0001	2.76	0.71	0.0019	−3.91	0.62	<0.0001
A^2	−3.71	0.79	0.0004	−2.03	0.79	0.0230	2.78	0.68	0.0018
B^2			NS			NS	1.52	0.68	0.0481
C^2	−4.38	0.79	<0.0001	−1.92	0.79	0.0294	2.69	0.68	0.0023
AB			NS[c]			NS[c]			NS[c]
AC			NS[c]			NS[c]	−1.48	0.77	0.0828
BC			NS[c]			NS[c]			NS[c]
	$\%L$			η_X			η_G		
Intercept	30.10	0.36		51.71	3.24		29.94	1.03	
A	1.82	0.29	<0.0001	16.01	2.26	<0.0001	4.57	0.83	<0.0001
B	1.01	0.29	0.0047	8.89	2.26	0.0020	2.42	0.83	0.0119
C	1.48	0.29	0.0003	11.26	2.26	0.0003	2.82	0.83	0.0047
A^2	−1.59	0.32	0.0003	−10.63	2.52	0.0012	−3.38	0.91	0.0027
B^2			NS[c]	−7.16	2.52	0.0148			NS[c]
C^2	−0.99	0.32	0.0092	−9.89	2.52	0.0020	−1.89	0.91	0.0586
AB			NS[c]			NS[c]			NS[c]
AC	0.95	0.36	0.0228			NS[c]			NS[c]
BC			NS[c]			NS[c]			NS[c]

[a] C coefficient

[b] SE standard error

[c] NS not significant ($P > 0.10$)

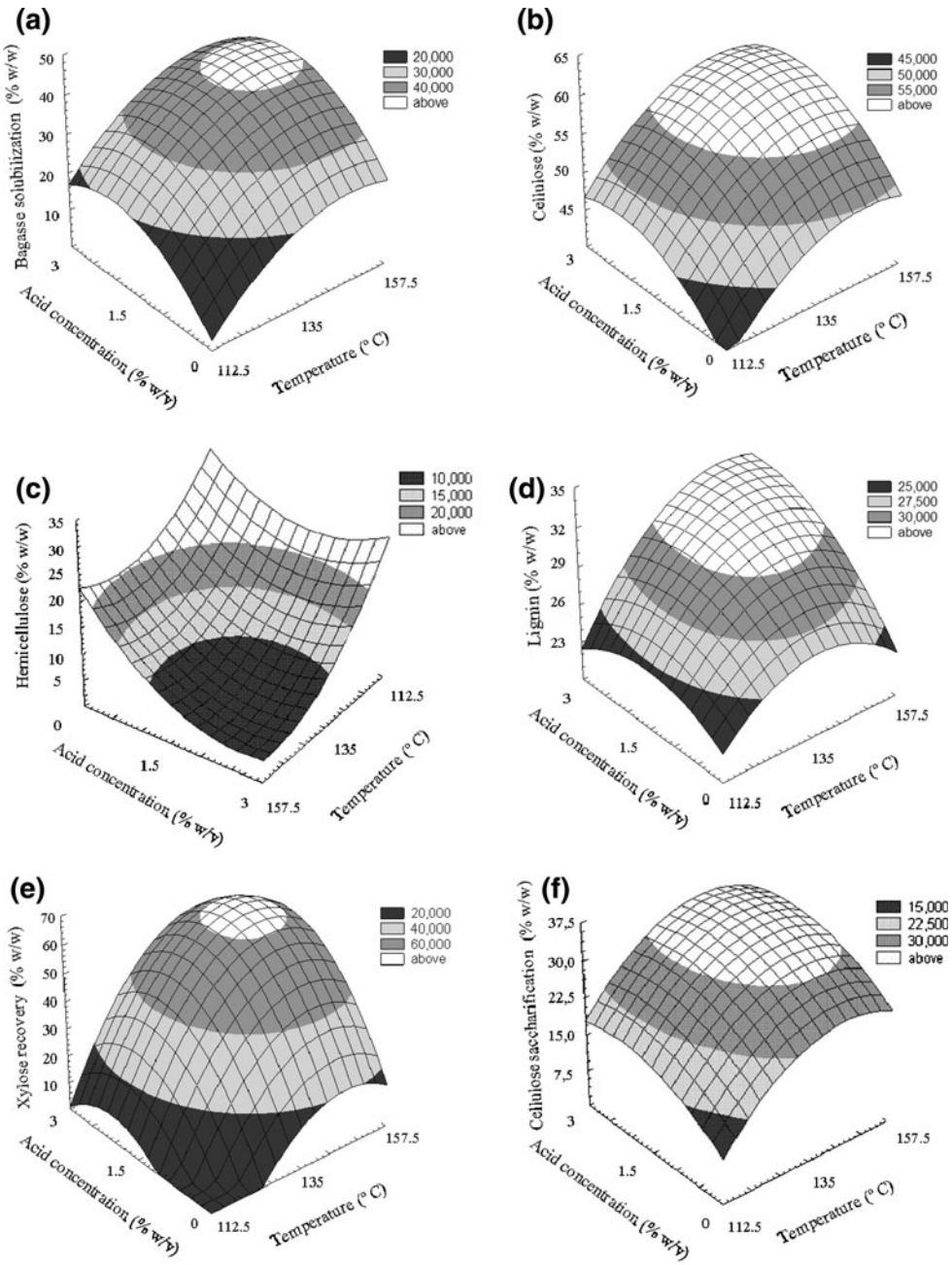

Fig. 1 Response surface plots showing the influence of temperature and sulfuric acid concentration on **a** bagasse solubilization (S), **b** percentage of cellulose in the cellulignin ($\%C$), **c** percentage of hemicellulose in the cellulignin ($\%H$), **d** percentage of lignin in the cellulignin ($\%L$), **e** efficiency of xylose recovery in the hemicellulosic hydrolysate (η_X), and **f** efficiency of cellulose enzymatic saccharification (η_G). Residence time was fixed at 30 min

followed by acid concentration (C) and residence time (B), in that order. Neureiter et al. [27] found that acid concentration, and not temperature, was the most important variable impacting the xylose yield from sugarcane bagasse, although temperature had a strong influence on furfural generation. Aguilar et al. [2], on the other hand, observed that both temperature and acid concentration influenced the kinetics of xylose generation from xylan and of xylose degradation to furfural. While the time profile for xylose concentrations clearly reached a maximum and then decreased with reaction time, particularly at higher temperatures, the furfural concentrations tended to stabilize through the reaction, thus indicating the occurrence of parallel degradation reactions. Last, but not least, Lavarack et al. [20] demonstrated that, in addition to temperature, acid concentration, and residence time, loading of solids and type of catalyst also influenced the sugarcane bagasse pretreatment rates and yields. Particle size, however, did not significantly influence such responses in that study.

By fixing the residence time at 30 min, the aforementioned models could be simplified to Eqs. 12–17, which allowed the generation of the response surface plots shown in Fig. 1.

$$S = 38.56 + 6.54A + 4.74C - 3.71A^2 - 4.38C^2 \quad (12)$$

$$\%C = 57.05 + 3.56A + 2.76C - 2.03A^2 - 1.92C^2 \quad (13)$$

$$\%H = 9.05 - 5.32A - 3.91C + 2.78A^2 + 2.69C^2 - 1.48AC \quad (14)$$

$$\%L = 31.11 + 1.82A + 1.48C - 1.59A^2 - 0.99C^2 + 0.95AC \quad (15)$$

$$\eta_X = 53.44 + 16.01A + 11.26C - 10.63A^2 - 9.89C^2 \quad (16)$$

$$\eta_G = 32.36 + 4.57A + 2.82C - 3.38A^2 - 1.89C^2. \quad (17)$$

As can be seen in Fig. 1, solubilization of bagasse was favored by harsher pretreatment conditions, which, as already discussed, promoted almost complete removal of the hemicellulosic fraction and improved enzymatic saccharification of cellulose remaining in the insoluble solids.

By using 2% (w/v) sulfuric acid to pretreat the sugarcane bagasse at 150°C for 30 min, the values of S, η_X, η_G, $\%C$, $\%H$, and $\%L$ predicted by the aforementioned models were 42.7, 62.0, 34.5, 59.5, 4.5, and 32.3%, respectively. The prediction intervals (95% confidence level) were as follows: 36.6–48.7% for S, 42.3–81.6% for η_X, 27.4–41.6% for η_G, 53.2–65.2% for $\%C$, 0–10.0% for $\%H$, and 29.6–34.6% for $\%L$. Two additional experiments were then performed at these selected conditions, to validate the models. The results obtained in these supplementary pretreatments (Table 2, assays 21 and 22) were compatible with the expected values, predicted by the empirical models.

The hemicellulosic hydrolysates obtained in the validation experiments, with 2% (w/v) sulfuric acid at 150°C for 30 min, were mixed and used as a source of sugars to produce ethanol with a strain of the naturally pentose-fermenting yeast *Pichia stipitis*. As described by Canilha et al. [8], detoxification of the hydrolysate prior to inoculation strongly improved the bioconversion rates and yields. Versatility of the yeast strain, acquired from a Brazilian Culture Collection, to utilize all the main carbon sources encountered in the hemicellulosic hydrolysate was also demonstrated.

Conclusions

The present manuscript draws attention to the fact that the chemical composition of lignocellulosic materials depends on many factors, including plant genetics, growth environment, and methods of harvesting and storage. In addition, the sugarcane bagasse, like many other feedstocks, is a "waste" generated in an industrial process, leading to varying processing efficiency as an additional source of compositional variance. As the conditions of pretreatment with dilute sulfuric acid leading to optimal results in terms of xylose recovery and cellulose digestibility are expected to depend on the composition of the raw material, a small-scale, composition-sensitive experimental approach would certainly help in defining the most adequate conditions for pretreating each particular sample of raw material before proceeding to larger-scale reactors. We report herein that the statistical methodologies of screening design and response surface analysis could be successfully used to optimize xylose recovery from a sugarcane bagasse sample in 200-ml containers, which also improved the subsequent enzymatic saccharification of the cellulose. Markedly, the pretreatment could be reproduced later in a 100-l reactor located at the Department of Biotechnology of the Engineering College of Lorena (Lorena, S.P., Brazil).

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