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Acid, alkali and peroxide pretreatments increase the cellulose accessibility and glucose yield of banana pseudostem

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ABSTRACT

Lignocellulosic biomasses such as banana pseudostem are attractive cellulose sources for bioenergy production, and for the use in biorefinery processes. However, pretreatment of lignocellulosic material is required to remove hemicellulose and lignin, while increasing cellulose accessibility to enzymatic hydrolysis (i.e., decreasing biomass recalcitrance). The effect of different concentrations of acid (H_2SO_4), alkaline (NaOH) and peroxide (H_2O_2) pretreatments on the chemical composition, cellulose accessibility, and enzymatic digestibility of banana pseudostem were studied. The water insoluble solids (WIS) recovery was low (~30%) for the severe pretreatment conditions applied, indicating high material solubilization. Acid pretreatment completely removed the hemicellulose content, whereas alkaline and peroxide pretreatments reduced its amount to 4.38 and 8.68%, respectively. In contrast, the lignin content increased (from 17.26 to 39.99%) after severe acid pretreatment, while alkaline and peroxide pretreatments reduced the lignin content to 7.65% and 7.17%, respectively. In line with hemicellulose and lignin removal, the cellulose content increased from 60.84 to 75.48 and 74.37%, respectively for alkaline and peroxide pretreatments, with no alteration for acid. Dye adsorption assays showed that alkaline and acid pretreatments resulted in high internal and external specific surface areas – indicative of high cellulose accessibility – when compared with peroxide pretreatments. Overall, alkaline and acid pretreatments resulted in the highest glucose yields from enzymatic hydrolysis of banana pseudostem, compared with peroxide pretreatment. In conclusion, concentrations of each pretreatment that led to the highest glucose yields was identified, confirming that the banana pseudostem is a great source of fermentable sugars, with high potential for biofuel production.

1. Introduction

China, the Portuguese Madeira Islands, India and Brazil, among other countries, have large-scale banana production (Li et al., 2010; Cordeiro et al., 2004; Chittibabu et al., 2011; Souza et al., 2012), which generates approximately 4 tons of residue – in the form of unused banana pseudostem – per ton of harvested fruit (Souza et al., 2010). Therefore, banana pseudostem and fruit-bunch stem are available in large scale in numerous tropical and subtropical countries, and represent a major income source in some communities/countries (Cordeiro et al., 2004). Due to its high cellulose content, the pseudostem from some banana species has been used in paper making and in the pulping industry since the 60's (Guha, 1960), and the cellulose of banana biomass waste has potential for use in the production of sodium carboxymethyl cellulose (Adinugraha and Marseno, 2005) and polyphenol oxidase (Wuyts et al., 2006).

Banana pseudostem residue may also have industrial applications, as feedstock for high-value products such as biofuels, which could replace non-renewable fuel sources, to mitigate the ever-growing CO_2 emissions (Quintero et al., 2008). While the use of banana pseudostem for second-generation (2G) ethanol production (bioethanol) is particularly attractive, this residue is difficult to convert into bioethanol or other high-value molecules, due to its chemical composition and physicochemical properties. While the pseudostem from banana species such as *Musa cavendishii* have higher cellulose content than grasses and wheat straw (44% of cellulose and only 8% of lignin in dry mass; Souza

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Table 1
Solid recovery and chemical composition of untreated and pretreated banana pseudostem.

Pretreatment condition (% m/m)		Composition (% dry base)			Water-insoluble solids
		Glucan/Cellulose	Hemicellulose	Lignin	(WIS) (%)
	Untreated	60.84 ± 1.34	19.62 ± 0.53	17.26 ± 0.31	–
H_2SO_4	5	59.68 ± 1.14	12.57 ± 0.53	26.28 ± 0.37	60.57 ± 1.59
	10	61.62 ± 1.26	11.66 ± 1.99	25.66 ± 2.06	58.20 ± 2.01
	15	63.76 ± 1.23	8.29 ± 2.45	29.16 ± 1.54	53.30 ± 1.95
	20	63.37 ± 1.71	4.13 ± 0.10	30.08 ± 0.63	46.90 ± 1.05
	25	66.28 ± 0.68	3.97 ± 0.50	31.15 ± 2.99	44.90 ± 1.56
	30	65.11 ± 1.68	–	36.22 ± 1.15	30.30 ± 0.98
	35	62.26 ± 0.89	–	36.47 ± 2.07	31.20 ± 1.09
	40	60.76 ± 1.32	–	39.99 ± 2.35	32.10 ± 2.05
NaOH	5	59.65 ± 2.76	14.02 ± 1.02	17.11 ± 2.36	46.00 ± 2.25
	10	64.32 ± 1.59	12.31 ± 0.49	13.10 ± 1.18	43.50 ± 2.19
	15	61.63 ± 3.94	11.47 ± 1.05	11.09 ± 1.08	33.80 ± 1.97
	20	70.01 ± 3.83	9.68 ± 2.55	10.78 ± 1.67	29.20 ± 2.13
	25	76.52 ± 1.61	5.35 ± 0.56	6.24 ± 0.83	27.60 ± 1.75
	30	75.48 ± 1.89	4.38 ± 0.36	7.65 ± 0.97	25.00 ± 1.14
H_2O_2	2	61.77 ± 3.02	15.93 ± 1.03	14.19 ± 2.17	48.60 ± 2.30
	4	66.91 ± 2.39	14.17 ± 1.68	10.49 ± 0.70	42.70 ± 1.39
	6	70.99 ± 1.99	11.31 ± 1.54	9.29 ± 1.24	39.90 ± 1.89
	8	74.37 ± 2.57	8.68 ± 0.77	7.17 ± 0.48	32.40 ± 2.21

Treatments with H_2SO_4 and NaOH were performed at 121 °C/1atm for 30 min (in an autoclave), and treatment with H_2O_2 was performed at 25 °C, for 4 h. Concentrations were measured in the solid/soluble fraction of untreated and pretreated banana pseudostem samples. The hemicellulose content represents the sum of xylose, arabinose and acetyl group (as anhydromers) in the soluble/insoluble fraction. The lignin content represents the sum of soluble and insoluble lignin fractions. Data are displayed as mean values, with standard deviation values in parentheses (experiments were performed in triplicates). (–) not detected.

et al., 2012), representing a rich source of cellulose, the lignocellulosic nature of the banana pseudostem means that its cellulose fraction is not easily accessible to the enzyme digestion required for ethanol production.

The inherent resistance of lignocellulosic material to enzymatic digestion, termed biomass 'recalcitrance', can be overcome by pretreatments that remove or modify lignin, leaving cellulose more accessible to enzymes (Sant'Anna et al., 2014; Meng et al., 2013; Brienzo et al., 2017). Pretreatments disrupt the biomass structure, to enhance the effectiveness of enzymatic hydrolysis (Meng et al., 2013). The most commonly used pretreatment to remove hemicelluloses is the exposure of biomass to alkaline (NaOH) or acid (H_2SO_4) solutions (Hu and Wen, 2008; Idrees et al., 2013; Souza et al., 2012). Also, previous studies from our group showed that peroxidase pretreatment of lignocellulosic materials (sugarcane bagasse) removes lignin efficiently, producing reusable solubilized hemicellulose (Brienzo et al., 2009; Monte et al., 2010). The results reported by Souza and co-workers (2012) indicate that is possible to produce approximately 187 L of ethanol for each ton of banana pseudostem using a low severity acid pretreatment. However, the effects of pretreatment on the banana pseudostem have not been analyzed systematically, to identify the ideal pretreatment conditions for maximal glucose yield from this biomass source.

Given the abundance of banana pseudostem as raw material, and the importance of using lignocellulosic waste for 2G ethanol production, the effect of NaOH, H_2SO_4 and H_2O_2 pretreatments on the banana pseudostem were studied. As well as comparing the chemical characteristics of pretreated and untreated samples, the exposure of inner and outer surfaces of cellulose using dye adsorption were studied (Direct Orange and Direct Blue), and visualized directly the changes in surface morphology induced by pretreatments, using scanning electron microscopy (SEM). Finally, it was assessed whether pretreatments improved the susceptibility of banana pseudostem to enzymatic hydrolysis, raising the glucose yield to levels compatible with efficient biofuel production.

2. Methodology

2.1. Banana pseudostem

Banana pseudostems were collected from mature plants at a local area in Duque de Caxias (Rio de Janeiro, RJ, Brazil). After collection, pseudostems were sliced and dried under sunlight for four days, cut into small pieces, ground by knife mill and selected using 20-mesh sieves. Dried and milled samples were stored at room temperature, in plastic bags.

2.2. Alkaline pretreatment

For alkaline pretreatment, 5 g of dried and milled samples were transferred to 100 mL glass bottles and mixed with solutions of 5, 10, 15, 20, 25 or 30% NaOH (m/m) in a total reaction volume of 50 mL. After homogenization, samples were autoclaved at 121 °C/1atm for 30 min, allowed to cool down at room temperature, and the soluble and solid (insoluble) fractions were separated by filtration using a paper filter. The solid fraction was washed with deionized water to reach pH 7, dried in an oven at 45 °C and stored in plastic bags until further analysis (Brienzo et al., 2016). Pretreatments were performed in duplicates and the solid recovery average was shown.

2.3. Acid pretreatment

Acid pretreatment was performed by adding 5 g of dried and milled samples to 250 mL glass bottles containing 100 mL of 5, 10, 15, 20, 25, 30, 35 or 40% H_2SO_4 (m/m). Samples were autoclaved at 121 °C/1 atm for 30 min, allowed to cool down at room temperature and vacuum filtered. The solid fraction was washed with deionized water to reach pH 5 (Brienzo et al., 2014), dried at 45 °C in an oven and stored in plastic bags until further analysis. Pretreatments were performed in duplicates and the solid recovery average was shown.

2.4. Peroxide pretreatment

Peroxide pretreatment was performed by adding ~5 g of dried and milled samples to 250 mL bottles containing 100 mL of 2, 4, 6 or 8%

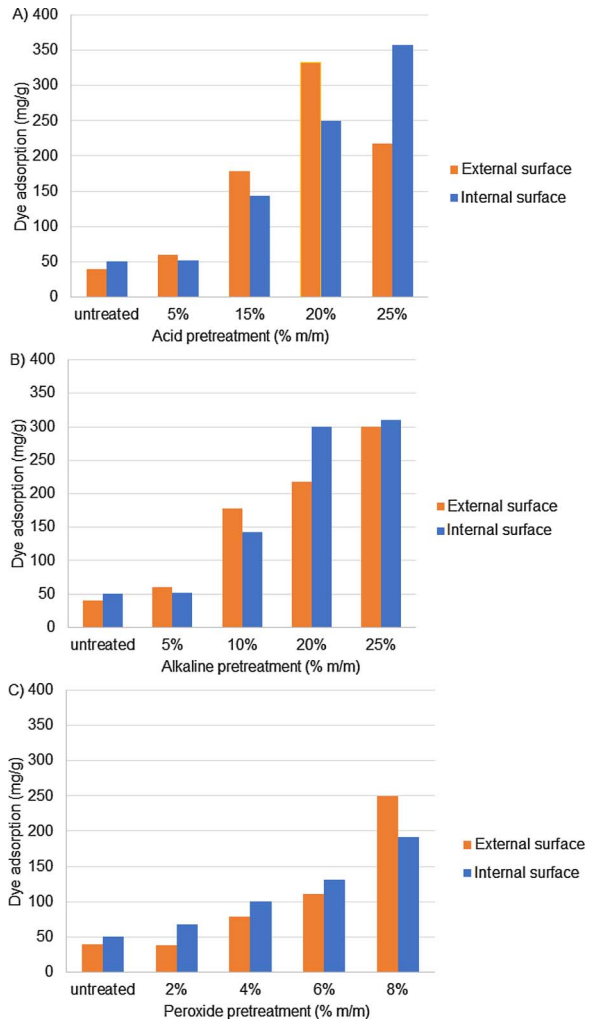

Fig. 1. Direct Orange (external specific surface area, in orange) and Direct Blue (internal specific surface area, in blue) dye adsorption analysis of banana pseudostem left untreated or pretreated with sulfuric acid (H_2SO_4, A), sodium hydroxide (NaOH, B) or hydrogen peroxide (H_2O_2, C). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

H_2O_2 (m/m). After homogenization, samples were heated in a thermostatic water bath at 25 °C for 4 h, and the soluble and insoluble (solid) fractions were separated by filtration using a paper filter. The solid fraction was washed with deionized water until neutral pH was reached (Monte et al., 2010), dried at 45 °C in an oven and stored in plastic bags until further analysis. Pretreatments were performed in duplicates and the solid recovery average was shown.

2.5. Chemical characterization

The chemical composition of untreated and pretreated samples was determined according to the National Renewable Laboratory Analytical Procedure (NREL, USA) (Sluiter et al., 2010). The concentrations of the monomeric sugars glucose, arabinose and xylose in the soluble fraction were determined by HPLC using a BIORAD HPX87H column, eluted at 0.6 mL/min with 5 mM sulfuric acid, at 60 °C. Mannose and galactose peaks overlapped and, thus, these sugars were not quantified. The following monomer-to-anhydromonomer conversion factors were used: 0.90 for glucose, 0.88 for arabinose and xylose, and 0.72 for acetyl content. After correction by a hydration factor, the glucose content was reported as glucan (total glucose), and the contents of other sugars added to that of acetic acid were used to calculate the hemicellulose content. Lignin was reported as the sum of insoluble (gravimetrically determined) and soluble (determined by spectrophotometry at 240 nm) lignin.

2.6. Dye adsorption

The dye adsorption of pretreated solid samples was measured by an adaptation of the Simons' staining method (Chandra et al., 2008). First, 50 mg of dry pretreated samples were placed in six 15 mL centrifuge tubes containing 0.5 mL phosphate buffered saline solution (PBS, pH 6, 0.3 M PO_4, 1.4 mM NaCl). The amount of dye adsorbed onto each sample was determined by the difference between the initial and final dye concentrations in the supernatant. The Direct Orange (DO) and Direct Blue (DB) dye concentrations were determined using the following formulas (Eqs. (1) and (2), respectively):

$$A_{455nm} = E_{O/455}LC_O + E_{B/455}LC_B \quad (1)$$

$$A_{624nm} = E_{O/624}LC_O + E_{B/624}LC_B \quad (2)$$

Where A is the adsorption of the mixture at 450 or 624 nm, E is the extinction coefficient of each component at the respective wavelength, and L is the path length (width of the cuvette, 1 cm). The extinction coefficients were calculated using standard curves for each dye. The values calculated and used in this study were as follows: EO/455 = 25; EB/455 = 0.9; EO/624 = 3.2; and EB/624 = 15.5 $L\,g^{-1}\,cm^{-1}$.

2.7. Enzymatic hydrolysis

Pretreated and untreated samples (0.1 g) were digested in 15 mL flasks containing 5 mL of 0.05 M citrate buffer (pH 4.8) supplemented with 15 FPU/g cellulase (Celluclast 1.5 L, Novozymes) and 15 U/g cellobiase (β-glucosidase; Novozyme 188), to provide enough enzymatic activity and prevent product inhibition, respectively. Reactions were incubated for 3–72 h at 50 °C, in an orbital shaker at 170 rpm. The glucose yield (determined by HPLC) was calculated from samples taken at different reaction times (3, 6, 9, 16, 24, 48 and 72 h). Enzymatic hydrolysis assays were performed in experimental duplicates, and averaged results were reported. The glucan conversion was calculated according to Eq. (3):

$$\text{Glucan conversion (\%)} = [(\text{glucose}) + 1.053*(\text{cellobiose})]/[1.111*f*(\text{biomass})]*100 \quad (3)$$

Where:[Glucose] Concentration of glucose released during enzymatic hydrolysis (g/L)[Cellobiose] Concentration of cellobiose released during enzymatic hydrolysis (g/L)[Biomass] Dry biomass concentration at the beginning of the enzymatic hydrolysis (g/L)f Glucan fraction in dry biomass (g/g)1.053 Correction factor of cellobiose to glucose equivalents1.111 Conversion factor of glucan to glucose equivalents

2.8. Scanning electron microscopy (SEM)

For SEM analysis, untreated and pretreated banana pseudostem samples were washed with deionized water and dried at 45 °C for 24 h. Samples were mounted onto stubs using carbon double-sided tape, coated with 5 nm platinum, and examined in a FEI Quanta FEG 450 scanning electron microscope, operating at an accelerating voltage of 1 kV.

3. Results

3.1. Severe alkaline and peroxide pretreatments are more efficient than severe acid pretreatment at increasing the cellulose content of banana pseudostem

Banana pseudostem is an abundant agricultural waste product with potential as a raw material for biofuel production, due to its high cellulose content. It was examined the effects of different pretreatments

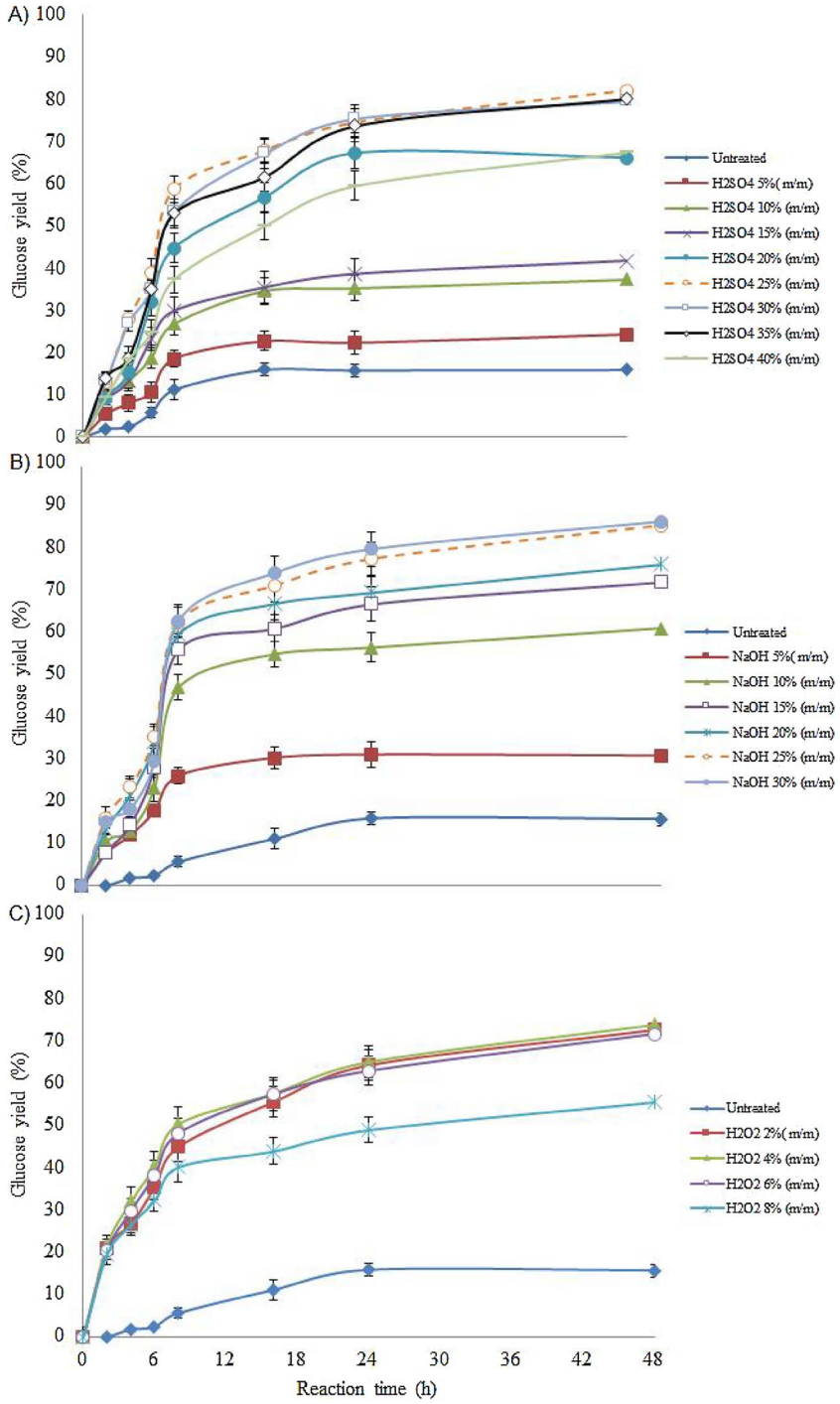

Fig. 2. Glucose yield from enzymatic hydrolysis of banana pseudostem samples left untreated, or pretreated with acid (A), alkali (B) or peroxide (C).
Enzymatic hydrolysis was performed using 15 FPU/g total cellulase and 15 U/g of β-glucosidase. Samples were pretreated with acid (H_2SO_4, at 121 °C/1atm for 30 min; A), alkali (NaOH, at 121 °C/1atm for 30 min; B) or peroxide (H_2O_2, at 25 °C for 4 h; C).

capable of making the cellulose content of banana pseudostem accessible for enzymatic digestion, with a view at using the pseudostem in 2G ethanol production, and in the generation of other high-value molecules.

Pretreatment decreases the recalcitrance of lignocellulosic material, by removing lignin and hemicellulose, thereby exposing cellulose to enzyme action (Meng et al., 2013; Brienzo et al., 2017). The residual pseudostem of banana cultivation was subjected to different pretreatments, including acid pretreatment, which preferentially removes hemicellulose (Souza et al., 2012), and both peroxide and alkaline pretreatments, which remove lignin and hemicellulose (Hu and Wen, 2008; Brienzo et al., 2009). Then, it was evaluated the chemical and physicochemical changes on the biomass exerted by each pretreatment condition, and their effect cellulose enzymatic hydrolysis.

Severe pretreatment conditions (≥25% H_2SO_4, ≥20% NaOH and 8% H_2O_2) effectively removed biomass components (Table 1), and resulted in lower water-insoluble solids (WIS) recovery than lower severity conditions (5% acid, 5% alkaline and 2% of peroxide) (Table 1). The solid recovery values observed for pseudostem were lower than those reported for other lignocellulosic materials, such as sugarcane fractions (Brienzo et al., 2014), bagasse (Benjamin et al., 2013) and wood residues (Cavalaglio et al., 2016)., Depending on the

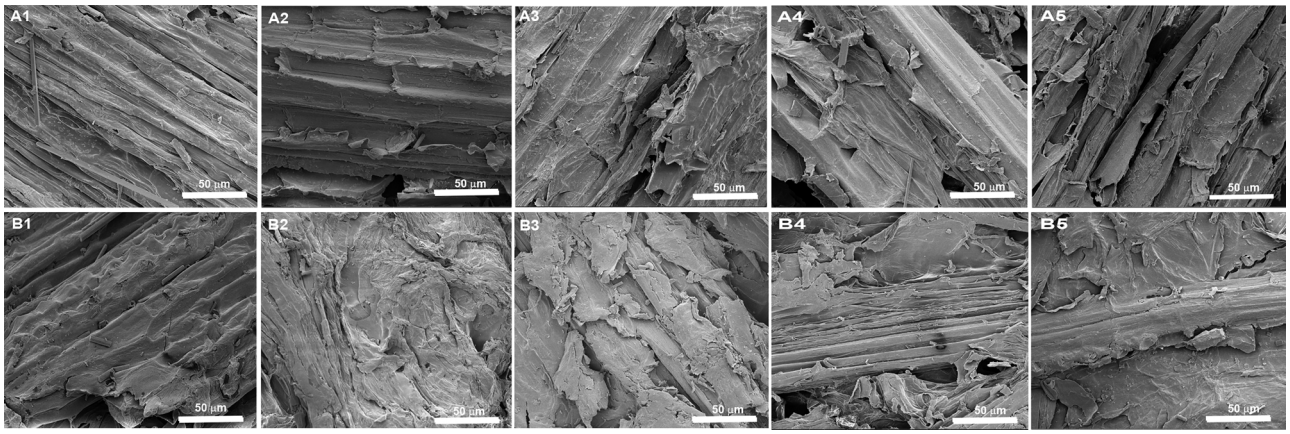

Fig. 3. Scanning electron microscopy (SEM) images of banana pseudostem samples left untreated (A1 and B1) or pretreated with different concentrations of sulfuric acid or hydrogen peroxide.
Samples were pretreated with acid (A2, 5%; A3, 10%; A4, 15%; and A5, 20% m/m, at 121 °C/1atm for 30 min) or hydrogen peroxide (H_2O_2; B2, 2%; B3, 4%; B4, 6%; and B5, 8% m/m, at 25 °C for 4 h).

pretreatment type and condition, the removal of hemicellulose and lignin contributed to mass solubilization and to the low WIS recovery. Moreover, the banana pseudostem extractives, which correspond to ~10% of the total mass (Cordeiro et al., 2004), are solubilized, contributing to decrease the WIS.

The chemical characterization of the untreated pseudostem showed that this fraction is composed of 60.84% glucan (representing the cellulose fraction), 19.62% hemicellulose, 19.26% lignin, and 10.5% extractives (Table 1). Previous studies reported similar values, of 64.58% glucan, 24.84% hemicellulose, and 10.78% lignin (Li et al., 2010), and 66.6% glucan (of which 13% were ashes and 14.1% were extractives) (Cordeiro et al., 2004) for banana pseudostem. The chemical characterization reported in this study confirms the high glucan content of the banana pseudostem, reinforcing the notion that this residue represents a good source of fermentable sugars (Li et al., 2010; Idrees et al., 2013), with potential for the production of ethanol after pretreatment (Souza et al., 2012). Moreover, the low lignin content observed in this and in other studies indicates that the banana pseudostem is likely to be less recalcitrant than other lignocellulosic materials.

The mass loss (represented by low WIS recovery) observed after pretreatment with high catalyst concentrations (25.0–32.10%; Table 1) can be attributed to the depolymerization of carbohydrates/hemicellulose and to the solubilization of extractives (Brienzo et al., 2014). Acid pretreatment breaks glycosidic bonds randomly, removing hemicellulose while increasing the cellulose content of biomass (Brienzo et al., 2017), and improving the accessibility of cellulose to hydrolytic enzymes (Brienzo et al., 2017; Meng et al., 2013; Chandra et al., 2008). On the other hand, alkaline pretreatment causes nucleophilic attacks that break the lignin structure, solubilizing lignin fragments or hemicellulose from α-O-4 linkages (Fengel and Wegener, 1984). Similarly to alkaline pretreatment, peroxide pretreatment contributes to lignin structure breakdown.

Under the most extreme conditions used (i.e., at the highest concentrations of catalysts), hemicellulose was completely removed upon acid pretreatment, and its percentage decreased to approximately 4.38 and 8.68% under the most severe alkaline and peroxide pretreatments, respectively, compared with 19.62% in the untreated control (Table 1).

Acid pretreatment led to an increase in the glucan and lignin content, as result of hemicellulose removal (Table 1). However, the increase in the percentage of cellulose was observed at acid concentration up to 25%, which led to contents of 66.28% of glucan, 3.97% of hemicellulose, and 31.15% of lignin. However, the glucan content decreased to 60.76%, (compared with 66.28% after pretreatment with 25% acid) under higher acid concentrations (40%), despite the complete removal of hemicellulose (Table 1). These results suggest that the most aggressive acidic conditions used caused significant cellulose degradation, resulting in a decrease in the cellulose content of the banana pseudostem biomass and, therefore, leaving less substrate for enzymatic hydrolysis. Severe alkaline and peroxide pretreatments increased the glucan content by a further 10 percentage points (to 76.52% and 74.37%, after pretreatment with 25% NaOH and 8% H_2O_2, respectively) compared with 25% acid, despite incomplete hemicellulose removal (5.35% and 8.68%, respectively). Therefore, severe alkaline (25%) and peroxide (8%) pretreatments were more efficient at increasing the glucan content in banana pseudostem biomass than severe acid pretreatment (at 25%), with efficient removal of both hemicellulose and lignin.

While lower alkaline conditions (5 and 15%) resulted in little chemical composition modification, treatment with the highest concentration of NaOH (30%) decreased slightly the cellulose content of the biomass (to 75.48%), relative to 25% NaOH (Table 1), suggesting that a small amount of cellulose degradation occurred in the most extreme alkaline conditions tested, similarly to that observed in the most extreme acid conditions. The aggressive conditions of alkali pretreatment possibly removed hemicellulose and lignin to an extent that cleaved hydrogen bonds in amorphous regions of the cellulose molecules. This effect is expected to increase the crystalline cellulose fraction, which has higher recalcitrance compared with the amorphous fraction (Fig. 1C). Notably, severe acid pretreatment did not remove efficiently the lignin content from the biomass, with an increase in the lignin content from 17.26% (in the untreated) to 39.99% after 40% acid pretreatment. In comparison, severe alkaline and peroxide treatments reduced the lignin content to ~7% (Table 1).

3.2. Pretreatments with acid and alkali increase cellulose accessibility

The removal of biomass components such as hemicellulose and lignin increases cellulose accessibility to enzymatic digestion (Brienzo et al., 2017, 2015; Meng et al., 2013; Sant'Anna et al., 2014). To evaluate cellulose accessibility after treatment, the internal and external specific surface areas of banana pseudostem samples were estimated, using blue and orange dye adsorption, respectively (Chandra et al., 2008). Increases in dye adsorption represent increases in cellulose accessibility, due to a direct relationship between the alpha-cellulose content of the biomass and the glucose yield from enzymatic hydrolysis (Brienzo et al., 2015, 2017).

Both the external and the internal surface areas of banana pseudostem samples increased after pretreatment, compared with untreated samples (Fig. 1), in line with the removal of hemicellulose and lignin after treatment (Table 1), which exposes cellulose areas to dye

adsorption. Lower severity pretreatments led to limited increases in the internal and external surface areas (with predominant increases in the external surface area) compared with untreated samples (Fig. 1). The external and internal specific surface areas increased gradually with pretreatment severity, with the exception of 25% acid pretreatment, which decreased the external surface area, relative to pretreatment with 20% acid (Fig. 1A). Pretreatment with acid and alkali resulted in higher levels of dye adsorption than peroxide pretreatment (Fig. 1A and B), and treatment with up to 6% peroxide increased the internal surface area predominantly, while the use of 8% peroxide resulted in a higher external surface area than the internal surface area (Fig. 1C).

For the same amount of lignin removal and glucan content increase, severe peroxide pretreatment (8%) was less efficient than severe alkali pretreatment (30%) at removing hemicellulose from the banana pseudostem biomass (Table 1), which may explain the limited increase in cellulose accessibility after peroxide treatment (Fig. 1C). This pretreatment may have affected the physicochemical properties of the banana pseudostem biomass in a manner that reduced the accessibility of cellulose molecules. The lower temperature used in peroxide pretreatment, compared with that used in alkaline and acid pretreatments (Brienzo et al., 2017), and the increased/decreased swelling of the biomass observed after peroxide treatment (Junior et al., 2013) might explain the increased accessibility obtained after pretreatment with NaOH or H_2SO_4.

3.3. Acid and alkaline pretreatments are more efficient than peroxide pretreatment at increasing the glucose yield from banana pseudostem

Enzymatic hydrolysis of untreated banana pseudostem resulted in 15.9% glucose yield after 48 h of reaction, which is lower than the yield obtained with other untreated lignocellulosic materials such as sugarcane bagasse (Corrales et al., 2012; Brienzo et al., 2015). All pretreatments improved the enzymatic hydrolysis yield of banana pseudostem (Fig. 2), in agreement with the chemical composition and dye adsorption data showing that all pretreatments removed hemicellulose from the biomass (Table 1) and increased cellulose accessibility (Fig. 1).

Lignin and hemicellulose removal from lignocellulosic materials − as a result of pretreatment − probably creates pores in the biomass that allow the entry of cellulolytic enzymes, which hydrolyze cellulose into glucose. This effect is especially potent when the lignin content is less than 10% (Mooney et al., 1998), which was obtained in the present study using strong alkali or peroxide concentrations. Severe pretreatments increased the glucose yield by 4.6–5.3 fold, and the material pretreated with 25% alkali showed the highest glucose yield (85%) after enzymatic hydrolysis, followed by 25% acid pretreatment (82% glucose yield), whereas 4% peroxide pretreatment had the lowest glucose yield (74%) of all pretreated conditions (Fig. 2).

The enzymatic hydrolysis data show that the use of appropriate concentrations of acid or alkali during pretreatment leads to higher glucose yields for banana pseudostem samples, compared with untreated samples (Fig. 2). While the glucose yield increased progressively with increasing concentrations of NaOH, the most extreme condition of acid pretreatment (40%) used resulted in a lower glucose yield (60%) compared with moderate acid concentrations (20%), which resulted in 82% yield (Fig. 2). Extreme acidity may generate a more recalcitrant biomass, by degrading preferentially the amorphous fraction of cellulose, thereby increasing the proportion of the more recalcitrant crystalline fraction in the biomass. This high severity acid pretreatment condition is not commonly used. Pretreatment with such extreme acidity had not been reported previously for banana pseudostem samples, and our study indicates that extreme acidity is not an ideal pretreatment for this particular biomass type.

While lignin removal increases the glucose yield from enzymatic hydrolysis (Siqueira et al., 2011), the magnitude of this effect may vary depending on the initial lignin content in the raw material (Benjamin et al., 2013), on the distribution of lignin in different biomass fractions (Brienzo et al., 2016), and on the physicochemical properties of the biomass (Zhu et al., 2008; Brienzo et al., 2017). The lignin content was similar after strong alkaline and peroxide pretreatments (7.65 and 7.17, respectively; Table 1); however, the glucose yield was higher for the alkaline pretreated material (Fig. 2), in agreement with the higher glucose accessibility in these samples (Fig. 1). Pretreatment with peroxide did not have a concentration-dependent effect on the glucose yield, with 2–6% peroxide pretreatments leading to similar increases in the glucose yield (relative to the untreated), while pretreatment with 8% peroxide reduced the glucose yield compared with moderate peroxide pretreatment (Fig. 2C). Higher peroxide pretreatment is likely to have attacked mainly the amorphous cellulose fraction, resulting in a more recalcitrant substrate.

3.4. Effective pretreatments alter the surface of banana pseudostem samples

Scanning electron microscopy (SEM) analysis was used to evaluate the changes in the cell wall surface morphology in pretreated banana pseudostem samples. The SEM analysis was performed with 5–20% acid pretreated samples, because these conditions removed hemicellulose effectively, and with 2–8% peroxide pretreated samples, because these conditions removed both hemicellulose and lignin effectively. On the other hand, peroxide pretreated samples resulted in lower accessibility and glucose yield by enzymatic hydrolysis than acid pretreated. Untreated banana pseudostem had a smooth surface with an undamaged fibrous organization (Fig. 3A1, B1), which appeared only slightly modified by pretreatment with a low concentration (5%) of acid (Fig. 3A2). However, alterations in the material surface morphology with increased acid pretreatment severity were observed (Fig. 3). Exposure to 10% acid led to a disorganization of the surface of material (Fig. 3A3, A4 and A5), which was already apparent after 2% peroxide pretreatment (Fig. 3B2). When samples were pretreated with a higher concentration of peroxide (6–8%), a stronger morphological change was observed, compared with untreated samples (Fig. 3B3, B4 and B5). The removal of biomass components, such as hemicellulose and lignin, by the peroxide pretreatment resulted in morphological surface modification of banana pseudostem samples. Damaged surfaces were no longer smooth, showing danification and were clearly fragmented in appearance. In line with the complete hemicellulose removal by strong (30%) acid pretreatment (Table 1), this pretreatment also altered the morphology of banana pseudostem surfaces (Fig. 3).

Pretreatments that resulted in a high ($\geq$65%) glucose yield from enzymatic hydrolysis – such as 20% acid and 2–6% peroxide (Fig. 2) – also damaged the banana pseudostem surface considerably (Fig. 3). Interestingly, the surface damage observed by SEM was similar for 2% peroxide pretreated samples, which had lower dye accessibility (Fig. 1C), and for 20% acid pretreated material, which had considerably higher dye accessibility than 2% peroxide (Fig. 1A).

4. Conclusion

A detailed evaluation of the effects of different pretreatments on the banana pseudostem showed that all severe pretreatments resulted in low solid recovery, in agreement with the high amount of hemicellulose and lignin removal from these samples. Acid pretreatment was particularly effective at removing hemicellulose, while alkaline and peroxide pretreatments removed both hemicellulose and lignin effectively. The removal of biomass components exposed cellulose, increasing its accessibility and, consequently, the glucose yield from enzymatic hydrolysis. However, the most severe pretreatments with acid and peroxide resulted in cellulose degradation, increasing biomass recalcitrance relative to lower severity conditions (possibly due to a reduction in the amorphous cellulose fraction). The banana pseudostem − an abundant waste product of banana production – has considerable potential as a feedstock for the generation of biofuels and other high-value molecules. Acid and alkaline pretreatments in specific conditions can

modify the banana pseudostem accessibility and provide high glucose yields by enzymatic hydrolysis, for fermentation processes.

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