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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%