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Production of cellulosic ethanol from cotton processing residues after pretreatment with dilute sodium hydroxide and enzymatic hydrolysis

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HIGHLIGHTS

- A mild alkaline pretreatment enhanced the ethanol production from cotton gin residues.
- Total ethanol productions as high as 232 L ton^{-1} were achieved from cotton gin dust.
- More lignified cotton gin wastes produced 129 L ton^{-1} of ethanol under similar conditions.
- Substrate hydrolysates had no inhibitory effects on ethanol fermentation.

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ABSTRACT

In this study, production of cellulosic ethanol from two cotton processing residues was investigated after pretreatment with dilute sodium hydroxide. Pretreatment performance was investigated using a 2^2 factorial design and the highest glucan conversion was achieved at the most severe alkaline conditions (0.4 g NaOH g^{-1} of dry biomass and 120 °C), reaching 51.6% and 38.8% for cotton gin waste (CGW) and cotton gin dust (CGD), respectively. The susceptibility of pretreated substrates to enzymatic hydrolysis was also investigated and the best condition was achieved at the lowest total solids (5 wt%) and the highest enzyme loading (85 mg of Cellic CTec2 g^{-1} of dry substrate). However, the highest concentration of fermentable sugars – 47.8 and 42.5 g L^{-1} for CGD and CGW, respectively – was obtained at 15 wt% total solids using this same enzyme loading. Substrate hydrolysates had no inhibitory effects on the fermenting microorganism.

1. Introduction

Cotton is the main supply of natural fibers for textile industries and one of the largest sources of agro-industrial residues that can be utilized to produce fuels and chemicals (Howard et al., 2004; Ojumu et al., 2003). In 2014, the total world production of refined cotton fibers was 25.9 million ton, with Brazil and India, the largest world producers, contributing with 1.5 and 6.7 million ton, respectively. The world total area destined to cotton production was 34.4 million hectares across 80 countries, being China, India, United States, Pakistan and Brazil the most important contributors to these very impressive numbers (USDA, 2014).

The high total world production capacity of cotton fibers also equates to the production of high amounts of cotton gin residues (McIntosh et al., 2014). In Brazil, 900 thousand hectares are destined to cotton production (USDA, 2014). The residues from cotton processing are of two types: the cotton gin dust (CGD) and the cotton gin waste (CGW). CGW arises from ginning process and is composed of cottonseed residues, hulls, sticks, leaves, and dirt, whereas CGD contains short fiber residues that are recovered from filter screens during the spinning/weaving processes. Slight differences in their composition are usually found among various mechanical harvesting methods. The major advantage of these cotton residues compared to other lignocellulosic materials is their high cellulose content. Hence, these renewable feedstocks have been tentatively used in a number of bioenergy applications to avoid its disposal by incineration or landfilling, such as in the case of cellulosic ethanol (Agblevor et al., 2003; McIntosh et al., 2014), pyrolysis (Zabaniotou et al., 2000), gasification (Sadaka, 2013), anaerobic fermentation (Isci and Demirer, 2007) and catalytic conversion to value-added chemicals (Grilc et al., 2015). However, the supporting evidence for the application of cotton gin residues for ethanol production is relatively scarce in comparison to other notable

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agricultural residues such as corn stover, sugarcane bagasse and wheat straw (McIntosh et al., 2014).

For conversion of cotton gin residues to cellulosic ethanol, a pretreatment method is needed to increase the accessibility of cellulose to enzymatic hydrolysis (Mosier et al., 2005). Among the various chemical and physical pretreatments used for cotton gin residues, acid pretreatments including steam explosion have been more often used (Agblevor et al., 2003; Ibrahim et al., 2010; Jeoh and Agblevor, 2001; Shen and Agblevor, 2008, 2011) while alkali pretreatments seems to be more effective for other cellulosic materials (Balat et al., 2008; Hendriks and Zeeman, 2009) and has not been studied inasmuch depth. The effectiveness of alkaline pretreatment depends on the biomass chemical composition and the conditions used for pretreatment (Singh et al., 2015). Deejing and Ketkorn (2009) have shown that this pretreatment technology is usually more effective for agricultural residues and herbaceous crops rather than for wood materials.

This study was developed to evaluate the cellulosic ethanol production from CGD and CGW after pretreatment with dilute sodium hydroxide and enzymatic hydrolysis. The alkaline pretreatment performance was investigated using a 2^2 factorial design with variations in the catalyst amount (NaOH) and the pretreatment temperature. The enzymatic hydrolysis was also tentatively improved by using the same type of factorial design in which the process variables were the substrate total solids (TS) and the enzyme loading. Finally, the fermentability of sugar hydrolysates was evaluated using an industrial strain of *Saccharomyces cerevisiae*.

2. Methods

2.1. Material

Cotton gin dust (CGD) and cotton gin waste (CGW) were provided by Hantex Textile Residues Ltd. (Gaspar, Brazil) with a 7 wt% moisture content (wet basis). The commercial enzyme preparation Cellic CTec2® was obtained from Novozymes Latin America (Araucária, Brazil) and the microorganism used in the fermentation experiments was the Thermosacc® Dry *S. cerevisiae* strain from Lallemand (Milwaukee, USA).

2.2. Pretreatment factorial design

Pretreatment of the cotton gin residues CGD and CGW was investigated through a 2^2 factorial design in which the following conditions were used: catalyst concentration (NaOH) of 2 and 4 wt% (0.2 and 0.4 g g^{-1} of dry biomass, respectively) and temperature (100 and 120 °C) (Table 1). Also, three replicates were performed at 110 °C and 3 wt% NaOH, which corresponds to the center point of the factorial design. The pretreatment TS (or the solid-to-liquid ratio in relation to the dry biomass) and the residence time at the desired temperature were established at 10 wt% and 60 min as recommended by Silverstein et al. (2007).

After pretreatment the materials were separated by filtration in a Büchner funnel: the alkali-soluble (mostly lignin and alkali-soluble polysaccharides) and the alkali-insoluble (mostly cellulosic fibers) fractions. The fibers were washed with water until neutral pH and drained in a Büchner funnel to the lowest water retention level. These materials were stored in vacuum-sealed plastic bags at 4 °C with a moisture content around 70 wt%. Both glucans and lignin recoveries in the alkali-treated materials were obtained according to the following equation:

$$\text{Recovery}\ (\%) = \frac{DM_f \times C_f}{DM_i \times C_i} \times 100 \quad (1)$$

Table 1
Factorial designs (2^2) used for the alkaline pretreatment of cotton gin residues and for the enzymatic hydrolysis of the best alkali-treated substrates derived from pretreatment (4% NaOH at 120 °C).

Experiment[a]	Alkaline pretreatment		Enzymatic hydrolysis	
	NaOH (%)[b]	Temperature (°C)	Total solids (%)	Enzyme (mg g^{-1})[c]
CGD-1	4 (+1)	120 (+1)	15 (+1)	85 (+1)
CGD-2	2 (−1)	120 (+1)	5 (−1)	85 (+1)
CGD-3	4 (+1)	100 (−1)	15 (+1)	55 (−1)
CGD-4	2 (−1)	100 (−1)	5 (−1)	55 (−1)
CGD-5	3 (0)	110 (0)	10 (0)	70 (0)
CGW-6	4 (+1)	120 (+1)	15 (+1)	85 (+1)
CGW-7	2 (−1)	120 (+1)	5 (−1)	85 (+1)
CGW-8	4 (+1)	100 (−1)	15 (+1)	55 (−1)
CGW-9	2 (−1)	100 (−1)	5 (−1)	55 (−1)
CGW-10	3 (0)	110 (0)	10 (0)	70 (0)

[a] CGD – cotton gin dust; CGW – cotton gin waste.
[b] Concentrations of 2%, 3%, and 4% correspond of a NaOH loading of 0.2, 0.3, and 0.4 g g^{-1} dry substrate.
[c] Enzyme loadings of 55, 70, and 85 mg g^{-1} correspond to 7.5, 9.5, and 11.5 FPU g^{-1}, both in relation to the substrate dry mass.

where DM_f is the dry mass after pretreatment, DM_i is the dry mass before pretreatment, C_f is the glucan or lignin content after pretreatment and C_i is the glucan or lignin content before pretreatment. Delignification was estimated by subtracting the lignin recovery from the maximum attainable yield which is set at 100%.

The cellulosic materials were characterized before and after pretreatment with regard to their total moisture, ash and total extractives content as recommended by the NREL/TP-510-42621 (Sluiter et al., 2008a), NREL/TP-510-42622 (Sluiter et al., 2008b) and NREL/TP-510-42619 (Sluiter et al., 2008c) methods, respectively. Acid-insoluble lignin and carbohydrates were determined after a two-stage sulfuric acid hydrolysis to its component sugars as recommended by NREL/TP-510-42618 method (Sluiter et al., 2012), while acid-soluble lignin was quantified by UV spectrophotometry according to NREL/TP-510-42617 method (Hyman et al., 2008). Total carbohydrate content was analyzed in acid hydrolysates by high performance liquid chromatography (HPLC) using an Aminex HPX-87H column (Bio-Rad) that was preceded by a cation-H pre-column. Analyses were performed at 65 °C with 5 mmol L^{-1} H_2SO_4 as the mobile phase at a flow rate of 0.6 mL min^{-1}. Quantitative analyses were performed by external calibration using primary standard solutions of cellobiose, glucose, xylose and arabinose, as well as acetic acid and furfural and hydroxymethylfurfural as carbohydrate dehydration by-products.

The standard procedure for enzymatic hydrolysis was carried out in two replicates for 96 h at 150 rpm using the substrate at 5 wt% TS and the enzyme loading at 55 mg of Cellic CTec2 g^{-1} of dry substrate, which corresponded to a total of 7.5 FPU g^{-1} dry substrate as determined by the I.U.P.A.C. method (Ghose, 1987) with adaptations (Schwald et al., 1988). Erlenmeyer flasks containing the reaction mixture in 50 mmol L^{-1} acetate buffer pH 4.8 were incubated at 50 °C in a rotary shaker incubator. Aliquots of approximately 1 mL were collected in several reaction times, heated for 5 min in a boiling water bath, centrifuged at 10,000*g* and subjected to analysis in the same HPLC system mentioned above for determining the substrate chemical composition. In this case, the glucose was the only component monitored by external calibration, which was then converted to anhydroglucose. Hydrolysis yields were always calculated in relation to the amount of glucan (cellulose) present in the original pretreated material.

2.3. Enzymatic hydrolysis factorial design

After selecting the best pretreatment condition, the enzymatic hydrolysis of the corresponding cellulosic substrates was