



Enhancing enzymatic hydrolysis of green coconut fiber—Pretreatment assisted by tween 80 and water effect on the post-washing



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ABSTRACT

This study evaluated the hydrothermal, alkaline (NaOH) and acid (H₂SO₄) pretreatment all combined with Tween 80 of the green coconut fiber aiming to improve the fermentable sugars production during the enzymatic hydrolysis. In addition, the water use during the post-washing of the pretreated materials was also assayed, seeking to ration its use without compromising the enzymatic hydrolysis and the removal of inhibitors. Untreated and pretreated biomass were assayed in terms of total solids, extractables, polysaccharides, lignin and total ashes according to the protocols proposed by NREL. Enzymatic hydrolysis was carried out using the preparation of cellulases from *Trichoderma reesei* ATCC 26921 and β-glucosidases (NS-22118 DCN00218) and xylanases (NS-22036 CDN01015). Additionally, the mass loss during pretreatment and washing was estimated. Results showed that the use of 3.0% (w/w) Tween 80 in the dilute alkaline pretreatment (2.0% (w/v) NaOH, 121 °C, 10 min) increased the conversion into glucose during the enzymatic hydrolysis of the pretreated materials from 48.7% to 56.1%. However, it did not show any influence on the pretreated materials in acid and hydrothermal conditions. The evaluation of the washing of the pretreated materials reduced the volume of water initially used in up to 75%, without affecting the final sugar production and the reduction of inhibitory compounds in the residual water, contributing to the viability of the production of cellulosic ethanol from green coconut fiber.

1. Introduction

Cellulosic ethanol is a viable and sustainable alternative as an energy source, using the conversion of agroindustrial waste (Machado et al., 2010) or low cost lignocellulosic materials into fermentable sugars for the production of ethanol. The green coconut industry is present in more than 200 countries, producing about 55 million tons of green coconut a year and discarding large amounts of waste. In order to reuse and valorize this biomass, the green coconut fiber has been used as a raw material for the production of ethanol (Gonçalves et al., 2014; Gonçalves et al., 2015a,b,c; De Araújo et al., 2017).

In order to facilitate the enzymatic hydrolysis of the biomass, it is necessary to disassemble the lignocellulosic matrix of the plant tissues and reduce the lignin and hemicelluloses content (Gonçalves et al., 2015). Thus, pretreatments such as hydrothermal, dilute acid and alkali are widely used in the removal of these constituents (Qi et al., 2010; Galbe and Zacchi, 2012; Pandey and Negi, 2015). However, lignin forms hydrophobic compounds that deposit on the substrate surface when the temperature of the pretreatment is reduced to the

initial condition, preventing the access of the enzymes to cellulose and hemicelluloses (Donohoe et al., 2008). Several studies have indicated that non-ionic surfactants can maintain hydrophobic compounds in the aqueous phase, making it difficult to place them on the substrate surface during the pretreatment stage (Kurakake et al., 1994; Pandey and Negi, 2015; Li et al., 2016). Therefore, the addition of Tween 80 during pretreatment stage could improve delignification and thus allowing greater enzymatic digestibility of pretreated materials on saccharification.

Moreover, the production of phenolic compounds and organic acids that inhibit the action of enzymes, bacteria and yeasts in the following stages of enzymatic hydrolysis and fermentation are common during physical-chemical pretreatments (Sousa Filho et al., 2016). Inhibitors present in the biomass after pretreatment should be removed, thus avoiding conversions in sugars and ethanol being impaired (Gonçalves et al., 2015). There are several methods to remove these inhibitors such as the use of acids and bases, ion exchange chromatography, evaporation, among others (Larsson, 2000). However, the simplest and most used is water washing. This washing affects the process, since the

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saccharification step is impaired when the pretreated solids are not washed previously (Chundawat et al., 2007). However, the emerging technology of producing ethanol from lignocellulosic biomass is constantly questioned by the high water consumption, making its management a critical factor for the viability and sustainability of this industry (Ramchandran et al., 2013). To the best of our knowledge, there are few reports about the water rationing in the pretreatment stage, in particular for green coconut fiber. Additionally the effect of using Tween 80 during pretreatment stage on the saccharification of coconut fiber has not been performed.

Thus, the objective of this work was to study the use of the Tween 80 surfactant in the hydrothermal, acid and alkaline pretreated treatments of the green coconut fiber in terms of the production of fermentable sugars in the saccharification and structural modifications of the pretreated biomass. In addition, the washing step of the pretreated materials has been evaluated, by analyzing the conditions of disposal (pH and color), mass yield and fermentable sugars production with the purpose of reducing the use of water in the process.

2. Materials and methods

2.1. Chemicals and biomass

All grade chemicals such as glucose, xylose, arabinose, acetic acid, formic acid, furfural, hydroxymethylfurfural and the cellulases preparation from *Trichoderma reesei* ATCC 26921 were acquired from Sigma-Aldrich Co. (St. Louis, Missouri, USA). A β -glucosidase (NS-22118 DCN00218) and xylanase (NS-22036 DCN01015) preparation were from Novozymes A/S (Bagsvaerd, Denmark).

Green coconuts were harvested at urban locations in Brazil (Northeast Region). After selection, the epicarp and mesocarp of the coconuts were cut approximately 3 cm and washed twice with distilled water (10 L to 5 kg biomass) then dried at 50 °C for 72 h in oven with controlled air circulation (Model: TE-394/I, TECNAL-Brazil), which was followed by milling (Hammer Mill Willye, TE - 680, TECNAL-Brazil). The milled biomass was then sifted through a 48-mesh sieve. Therefore, the biomass was stocked in plastic bags at room temperature.

2.2. Pretreatments

For the diluted alkaline pretreatment, the coconut fiber was mixed with a solution of 2% (w/v) NaOH and (0–3% w/w dry fiber) Tween 80 in the ratio 1:10 (solid/liquid) in a 1 L beaker. The mixture was then autoclaved at 121 °C for 10, 20 and 30 min. The diluted acid pretreatment was performed with a solution of 3% (w/v) H₂SO₄ and (0–3% w/w dry fiber) Tween 80 and green coconut fiber to obtain a ratio 15: 100 (solid/liquid). This mixture was autoclaved at 121 °C for 10, 35 or 60 min. The hydrothermal pretreatment was performed with a solution of (0–3% w/w dry fiber) Tween 80 and green coconut fiber to obtain a ratio 15:100 or 1:10 (solid/liquid). Then, the mixture was pretreated in autoclave at 121 °C for 10 or 60 min. After each pretreatment, 12 washes were performed with distilled water from the pretreated material then the solid fraction that was filtered and stored at –4 °C for use in the enzymatic hydrolysis experiments.

2.3. Washing of pretreated lignocellulosic materials

The biomass washing was carried out after pretreatment for 15 min using a beaker containing distilled water at room temperature stirring at 150 rpm (shaker Tecnal, model TE-139). For each of the 12 washing, a water volume of 200 mL was used for each 15.0 g of material submitted to pretreatment, verifying pH and water coloration by visual observation

For each washing step, samples of the washing water were collected for HPLC analysis in order to quantify the sugars (glucose, xylose, arabinose), acids (acetic and formic) and other inhibitory compounds

(furfural and HMF) released. At the end of every three washing, the material was filtered and the solid fraction was stored in a refrigerator at –4 °C for use in enzymatic hydrolysis experiments. Additionally, the mass loss during pretreatment and washing were calculated in relation to the initial mass of the material before pretreatment, according to Eq. (1).

$$Yield(\%) = \left(\frac{M_{fp}}{M_{ip}} \right) \times 100 \quad (1)$$

where *Yield* (%) is the percentage of the mass pretreatment yield, *M_{ip}* (g) is the initial mass on dry basis before pretreatment and *M_{fp}* (g) is the final mass on dry basis after pretreatment and washing step.

2.4. Characterization of green coconut fiber

The chemical characterizations of untreated and pretreated coconut fiber were assayed in terms of total solids (Sluiter et al., 2008b), extractables (Sluiter et al., 2005a), polysaccharides (Sluiter et al., 2008a), lignin (Sluiter et al., 2008a) and total ashes (Sluiter et al., 2005b) according to the protocols proposed by NREL.

Pretreated and untreated biomasses were selected to carry out the characterization according to the production of sugars in the enzymatic hydrolysis experiments. The four biomasses were named as I (coconut fiber pretreated by 2% (w/v) NaOH at 10% solid loading and 121 °C for 10 min), II (2% (w/v) NaOH assisted by 3% (w/w) Tween 80 at 10% solid loading and 121 °C, for 10 min), III (autohydrolysis pretreatment with 15% solid loading at 121 °C for 60 min) and IV (autohydrolysis assisted by 3% (w/w) Tween 80 pretreated at 15% solid loading and 121 °C for 60 min).

2.4.1. Crystallinity

X-ray diffraction analysis was performed on untreated and pretreated lignocellulosic materials using the X-ray diffractometer (model XRD-6000, Shimadzu, Japan). The assays were performed with copper K α radiation, voltage of 40 kV and electric current of 30 mA. The crystallinity index (ICr) was determined according to Eq. (2):

$$ICr(\%) = \left(\frac{I_{002} - I_{am}}{I_{002}} \right) \times 100 \quad (2)$$

where *ICr* (%) is the percentage of crystallinity index, *I₀₀₂* is the peak intensity in the crystallographic plane 002 (2 θ , ~22.6°) and *I_{am}* is the valley intensity between the peaks of the crystallographic planes 002 and 001 (2 θ , ~18.7°).

2.4.2. Scanning electron microscopy (SEM)

The surface of the untreated and pretreated lignocellulosic materials was analyzed by the scanning electron microscope (Hitachi, model TM3000). The assays were performed using CeB6 filament, 15 kV voltage and 10 mm working distance. The images were recorded with amplitude of 200 times.

2.4.3. Fourier transform infrared spectroscopy (FTIR)

FTIR spectra of untreated and pretreated lignocellulosic materials were measured on a spectrometer (FTLA 2000 series, ABB Bomem Inc., Canada). The analysis conditions were in 4 cm⁻¹ resolution, in the region of 400–4000 cm⁻¹. The crystallinity of the materials was also evaluated according to the methodology proposed by Nelson and O'Connor (1964), using the ratio between the areas of the peaks 1426/896 cm⁻¹/cm⁻¹ for the lateral crystallinity index (LOI) and 1373/2917 cm⁻¹/cm⁻¹ for the total crystallinity index (TCI).

2.5. Enzymatic hydrolysis

The preparation of cellulases from *Trichoderma reesei* ATCC 26921 and β -glucosidases (NS-22118 DCN00218) and xylanases (NS-22036

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