REVISTA BRASILEIRA DE ENERGIAS RENOVÁVEIS

PRESSURIZED WATER PRETREATMENT TO INCREASE SUGAR PRODUCTION FROM GREEN COCONUT[1]

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Abstract

Pretreatment of lignocellulosic industrial waste is necessary to promote the cellulose accessibility. Thus, this study evaluated the production of green coconut sugars during pressurized hydrothermal pre-treatment and enzymatic hydrolysis of the pretreated biomass. Pretreatment of the green coconut shell was carried out at 70 °C, 150 bar and water flow rate of 1 mL/min for 4 h. Samples from the system output stream were analyzed by HPLC and Folin Ciocalteu method. The solid fraction was characterized (chemical composition, XRD and FTIR) and subjected to enzymatic hydrolysis. The liquid fractions of pretreatment provided a defined profile of released glucose, xylose, phenolic components and acetic acid over time. After 40 min, most of fermentable sugars were released, forming a liquor with a sugar content above 10 g/L. Post-treated biomass showed a reduction of cellulose (26 % to 17 %) and hemicellulose (23 % to 18 %) and an increase in lignin content (32 % to 44 %).

Despite this new chemical composition of the biomass, the pretreated material had lower crystallinity indexes and modifications in its chemical groups. This favored cellulosic conversion from 15 % to 55 %, producing 63.5 % more glucose than the untreated green coconut shell during enzymatic hydrolysis. Thus, the pressurized pre-treatment favored the fermentable sugar production from the green coconut shell, and there is also the possibility of utilizing the sugars present in the pre-treatment liquor.

Keywords: lignocellulosic waste, pretreatment, enzymatic hydrolysis.

PRÉ-TRATAMENTO HIDROTÉRMICO PRESSURIZADO PARA MELHORAR A PRODUÇÃO DE AÇÚCAR APARTIR DO COCO VERDE

Resumo

O pré-tratamento dos resíduos industriais lignocelulósicos é necessário para promover a acessibilidade da celulose. Assim, esse trabalho avaliou a produção de açúcares do coco verde durante o pré-tratamento hidrotérmico pressurizado e hidrólise enzimática da biomassa pré-tratada. O pré-tratamento da casca do coco verde foi realizado a 70 °C, 150 bar e vazão de água de 1 mL/min, durante 4 h. Amostras da corrente de saída do sistema foram analisadas em CLAE e pelo método de Folin Ciocalteu. A fração sólida foi caracterizada (composição química, DRX e FTIR) e submetida à hidrólise enzimática. As frações líquidas do pré-tratamento apresentaram um perfil bem definido de liberação de glicose, xilose, componentes fenólicos e ácido acético ao longo do tempo. Após 40 min, a maioria dos açúcares fermentescíveis foram liberados, formando um licor com teor de açúcares acima de 10 g/L. A biomassa pós-tratada mostrou redução de celulose (26 % para 17 %) e hemicelulose (23 % para 18 %) e um aumento no teor de lignina (32 % para 44 %). Apesar dessa nova composição química da biomassa, o material pré-tratado apresentou menores índices de cristalinidade e modificações em seus grupos químicos. Isso favoreceu a conversão celulósica de 15 % para 55 %, produzindo 63,5 % de glicose a mais do que a casca do coco verde não tratada durante a hidrólise enzimática. Portanto, o pré-tratamento pressurizado favoreceu a produção de açúcares fermentescíveis oriundos da casca do coco verde e, ainda, existe a possibilidade de aproveitamento dos açúcares presentes no licor do pré-tratamento.

Palavras-chave: resíduos lignocelulósicos, pré-tratamento, hidrólise enzimática.

INTRODUCTION

The large disposal of agroindustrial residues combined with the need for renewable energy sources has encouraged the production of cellulosic ethanol. Among these residues, the green coconut shell represents problematic environmental issues for the coastal region of Brazil, considering the great annual production of green coconut (3 million tons) and the difficulty in properly allocating its residues (GONÇALVES *et al*., 2014). Thus, the green coconut shell has been studied as raw material to produce ethanol (GONÇALVES *et al*., 2014, ARAÚJO *et al.*, 2017, NOGUEIRA *et al.*, 2018). However, to pretreat this material it is necessary to disassemble its lignocellulosic structure in order to promote the enzymatic accessibility to cellulose. Recently, the advantages of pressurized water pretreatment in relation to traditional acid and alkaline pretreatments have been reported, such as lower sugar degradation, non-use of chemical additives, less corrosive problems in equipment, processing of higher amounts of biomass and possibility of recovering the bioactive components (PRADO *et al*., 2016). Thus, the present study assessed the sugar productions and other components (organic acids and phenolic compounds) released during the pressurized water pretreatment of the green coconut shell. In addition, the modifications suffered by the pretreated material were verified and characterized physically and chemically, and the material potential was also evaluated in terms of cellulosic conversion and glucose production in the subsequent enzymatic hydrolysis.

MATERIALS AND METHODS

The green coconut shell was collected in urban locations in Natal - Brazil, cut, washed with tap water and dried at 50 ° C for 72 hours in oven with controlled air circulation (TE-394/l, TECNAL-Brazil). Then, the material was milled and sieved to a 48-mesh sieve (Hammer Mill Willye, TE-680, TECNAL-Brazil). Next, 40 g of untreated material was pretreated in a stainless steel jacketed extraction vessel (total volume of 145 cm^3, length of 30.7 cm and inner diameter of 2.45 cm) at 70 °C (Thermostatic bath, Marconi, MA-184, Brazil), 150 bar and water flow rate of 1mL/min (HPLC pump, Series III LabAlliance, USA). The pretreatment liquid fractions were collected in the system output stream for 4h (Figure 1).

The pretreatment solid fraction and the untreated material were characterized in terms of polysaccharides, lignin klason (SLUITER *et al*., 2008) and by X-ray diffraction (model XRD-6000, Shimadzu, Japan) and Fourier transform infrared spectroscopy (FTLA 2000 series, ABB Bomem Inc., Canada) according to Nogueira *et al.* (2018). The crystallinity indexes of the materials were calculated according to Equation 1.

$$\boldsymbol{I_{Cr}}(\%) = \left(\frac{I_{002} - I_{am}}{I_{002}}\right) x\ 100 \qquad (1)$$

Where:

I_{Cr} (%) = crystallinity index percentage;

I_{002} = peak intensity in the crystallographic plane002 (2θ, ~22,6°);

I_{am} = valley intensity between the peaks of the crystallographic planes002 e 001 (2θ, ~18,7°).

The pretreated and untreated green coconut were hydrolysed in shaker (150 rpm, 50 °C for 96 h) using enzymatic loads of 20.0 FPU/g of fiber , 20.0 CBU/g of fiber and 10.0 FXU/g of fiber (*Trichoderma reesei* ATCC 26921, Sigma-Aldrich Co., Missouri, USA; NS-22118 DCN00218 and NS-22036 CDN01015, Novozaymes A/S, Bagsvaerd, Denmark) with 5.0% (w/v) of solid load, sodium citrate buffer (50 mM, pH 4.8) and 0.01% (w/v) of sodium azide. At time zero and after 96 h, the hydrolysis samples were collected to determine the sugar production from each material under those conditions.

The sugars and organic acids present in the pretreated liquid fractions and in the enzymatic hydrolysis samples were analyzed by High-Performance Liquid Chromatography (HPLC) using a chromatograph (Acela, Thermo Scientific) and Shim-Pack SCR-101H column (Shimadzu Co., Japan) at 65 °C. The samples (20 µL) were previously filtered on 0.22 µm (Millipore) membranes and injected with a sulfuric acid solution 5.0mM as mobile phase into the column at a flow rate of 0.6 mL/min. Furthermore, the phenolic compounds were determined by Folin Ciocalteu method (HATAMI *et al.* 2014), using known concentrations of gallic acid to determine the calibration curve. The cellulosic conversion was calculated according to Equation 2.

$$\textbf{\textit{Cellulosic conversion}}\ (\%) = \left(\frac{glucose\ \ (g)\ x\ 0.9}{cellulose\ \ (g)}\right)\ x\ 100 \qquad (2)$$

Where:

Glucose (g) = volume used in enzymatic hydrolysis (L) *versus* quantified glucose concentration (g/L);

Cellulose (g) = mass of the dry basis material versus initial amount of cellulose present in the material.

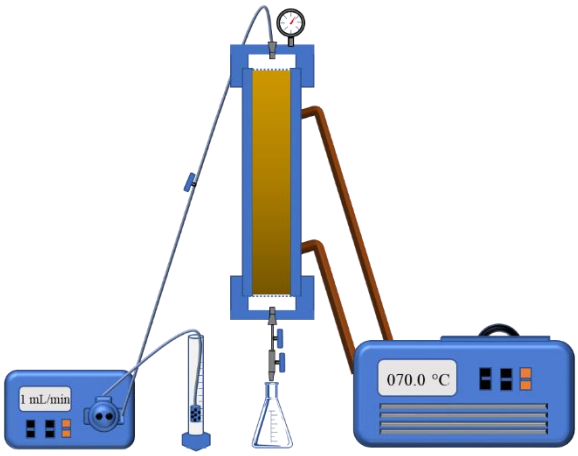


Figure 1 – Schematic of the apparatus used to the pressurized water pretreatment

RESULTS AND DISCUSSION

The liquid fractions collected over 4 h of pretreatment showed significant concentrations of glucose, xylose, acetic acid and phenolic components as shown in Figure 2. These components are derived from degradation of cellulose, hemicellulose, lignin and also extractives present in untreated green coconut fiber. It was found that no hydroxymethylfurfural or furfural were detected, suggesting that the pretreatment operating conditions minimized the sugar monomers degradation. In addition to the low temperature, the use of a semi-batch mode may have prevented the degradation of sugar monomers since they were withdrawn from the system constantly. In addition to reducing the degradation of sugars, the use of milder temperatures during pretreatment can maintain the bioactive properties of the released phenolic components, allowing the generation of other products in

the cellulosic ethanol industry. Furthermore, it can be seen in Figure 2 that the liquid fractions exhibited minimum concentrations of sugars from 40 min of operation. Similar periods were reported when using pretreatment on subcritical conditions (PRADO *et al*, 2014).

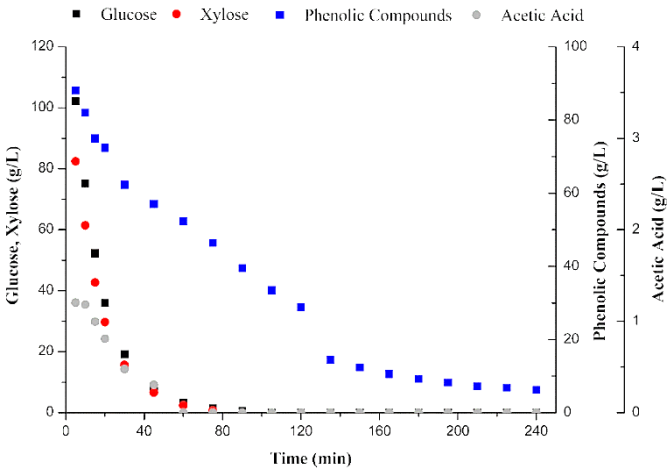


Figure 2 – Glucose, xylose, phenolic compounds and acetic acid released in the liquid fractions of the green coconut pretreatment over time.

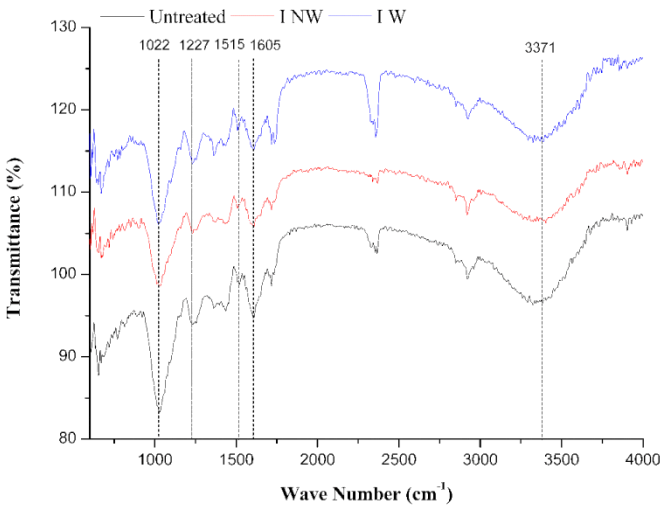
In Table 1, the chemical composition (cellulose, hemicellulose and lignin) and crystallinity index of the untreated and washed (W) and unwashed (NW) post-treatment materials are shown. It was observed that the pressurized water pretreatment considerably reduced the cellulose and hemicellulose contents of the green coconut shell and consequently raised the lignin content of the pretreated material. This result can be explained by the release of sugars and acetic acid during the pretreatment (Figure 2). In addition, Gonçalves *et al*. 2015 also reported an increase in the percentage of lignin after autohydrolysis pretreatment of the green coconut shell. The crystallinity index of the pretreated material was reduced when compared to the untreated material, which may be related to the increase in the commonly amorphous lignin content.

Table 1 – Chemical composition and crystallinity indexes of untreated and pretreated green coconut

Pretreatment		Cellulose (%, w/w)	Hemicellulose (%, w/w)	Klason Lignin (%, w/w)	Crystallinity index (%)
Untreated		25.61±0.43	23.48±0.20	32.22±2.39	30.94
H_2O	NW	15.53±0.17	17.50±0.19	43.81±1.50	28.24
	W	17.26±0.18	18.38±0.33	43.83±0.98	29.76

Through the FTIR analysis, some modifications were detected in the transmittance peaks after pressurized hydrothermal pretreatment and these peaks are highlighted in Figure 2. The axial deformation of the group (O-H) occurs at 3371 cm^{-1} and represents the stretching cellulose and lignin vibration in the fiber. The intensity of this peak for the pretreated material was reduced in relation to the untreated, indicating that the interaction between cellulose and lignin was altered after pretreatment. At the band peak around 1022 cm^{-1} the (C-O) stretching is identified in cellulose and hemicellulose, which was reduced after pretreatment and that can be due the less interaction among those compounds or due to the lower content of them in the pretreated material. The peaks around 1227 cm^{-1} and 1515 cm^{-1}, 1605 cm^{-1} are related to aromatic ring vibration of lignin, esters, ethers and phenolic compounds (XIAO *et al*., 2011, GONÇALVES *et* al., 2014, NOGUEIRA *et al*., 2018). As it can be seen in Figure 3, those peaks were modified when compared to the untreated material, probably because of the phenolic compound released during pretreatment (Figure 2) and the higher lignin content in the pretreated green coconut fiber.

Despite the increase in lignin content and reduction of cellulose and hemicellulose, the pretreated materials underwent physico-chemical changes that promoted the cellulose conversion from 15.0% (untreated) to 54.8% (pretreated and not post washed) and 49.5% (pretreated and post washed), increasing glucose production by 63.5% relative to the untreated green coconut shell during enzymatic hydrolysis. These cellulose conversion values



were higher than those reported by Nogueira *et al*. (2018) who also used the green coconut shell, but performed batch hydrothermal pretreatment at 121 ° C and 1 bar.

Figure 3 – FTIR spectra of untreated and washed and not washed pressurized water pretreatment.

CONCLUSION

The green coconut shell was subjected to a pressurized water pretreatment which produced liquid fractions with high sugar and phenolics compounds concentration until 40 min of operation. The degradation of those components was minimized probably due to the mild temperature and/or the semi-batch mode operation, providing sugars and phenolics compounds recovery. After the pretreatment, the biomass presented physico-chemical changes that enhanced cellulosic conversion in enzymatic hydrolysis. Thus, the pretreatment with pressurized water favors the release of sugars from the green coconut shell and also allowed the by-products products. Such fact could contribute to economic and environmental viability in the ethanol production chain, avoiding chemical additives and waste generation.

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