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Comparison of delignified coconuts waste and cactus for fuel-ethanol production by the simultaneous and semi-simultaneous saccharification and fermentation strategies



Fabiano Avelino Gonçalves^{a,b}, Héctor A. Ruiz^{b,c}, Cleitiane da Costa Nogueira^a, Everaldo Silvino dos Santos^a, José A. Teixeira^b, Gorete Ribeiro de Macedo^{a,*}

^a Laboratory of Biochemical Engineering, Chemical Engineering Department, Federal University of Rio Grande do Norte, 59078-970 Natal, Brazil

^b CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057 Braga, Portugal

^c Biorefinery and Food Engineering Laboratory, Food Research Department/School of Chemistry, Autonomous University of Coahuila, Blvd. V. Carranza e Ing. José Cárdenas Valdés. 25280 Saltillo. Coah.. Mexico

Valdes, 25280 Saltillo, Coah., Mexi

HIGHLIGHTS

- Sequential Alk-H₂O₂/NaOH pretreatment was developed.
- FTIR, SEM, X-ray and crystallinity indexes have evidenced modifications in solids.
- Delignified MCF was more susceptible the enzymatic action.
- SSSF strategy allowed to obtain higher ethanol production than SSF.
- Step of presaccharification had a positive effect on the overall ethanol yield.

G R A P H I C A L A B S T R A C T



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It is of the highest importance to study different alternatives/strategies as simultaneous (SSF) and semisimultaneous (SSF) saccharification and fermentation process, as well as the prospects of the utilization of lignocellulosic residues as raw materials for fuel-ethanol production. In the first part of this work, different raw materials (cactus (CAC), green coconut shell (GCS), mature coconut fibre (MCF) and mature coconut shell (MCS)) were pretreated by sequential alkaline hydrogen peroxide (Alk-H₂O₂)-sodium hydroxide (NaOH) process. The characterization of the obtained solids by FTIR, SEM, X-ray and crystallinity indexes confirmed the higher susceptibility of these pretreated materials to enzymatic action. These results were further confirmed by the corresponding glucose conversion yields – 68.44%, 70.20%, 76.21% and 74.50% for CAC, GCS, MCF and MCS, respectively. Subsequently, the comparison between SSF and SSSF using *Saccharomyces cerevisiae*, *Pichia stipitis*, *Zymomonas mobilis* and pretreated MCF (selected in the enzymatic hydrolysis step) was done, being shown that a short presaccharification step at 50 °C for 8 h in the SSSF had a positive effect on the overall ethanol yield, with an increase from 79.27–84.64% to 85.04–89.15%. In all the cases, the SSSF strategy allowed the obtention of higher ethanol concentrations than SSF.

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* Corresponding author. Tel.: +55 84 3215 3757/3759x229; fax: +55 84 3215 3770. *E-mail address:* gomacedo@eq.ufrn.br (G.R.d Macedo).

1. Introduction

The use of biofuels, with emphasis on fuel-ethanol is an alternative to mitigate the pressure exerted by fossil fuels and their derivatives. However, fuel-ethanol production from corn, sugarcane and beet may be a problem in the near future due to the food competition in the use of these materials for bioenergy production [1]. One option is the production of cellulosic ethanol from coconut trees, as these crops are distributed in over 200 countries. According to FAO (http://www.www.faostat.org.br), the world production of coconut in 2009 was about 55 million tons, mainly in the Philippines (36%), Indonesia (28%) and India (20%). Brazil is the fourth largest producer of coconut, with a production of about 3 million tons (5.5%) (http://www.ibge.gov.br). Associated with the large volume coconut production, large amounts of not used agroindustrial waste, suitable to be applied in fuel-ethanol production, are also obtained. Just in Brazil, the production of CAC was 60,000 tons in 2009, mainly concentrated in the Northeast Region and was fully used in animal feed (http:// www.ibge.gov.br).

Fuel-ethanol production from lignocellulosic materials (LCMs) is complicated due to the recalcitrant nature of the molecules present in these LCMs. In order to make cellulose and hemicellulose more accessible to the attack of cellulases and hemicellulases, a pretreatment is required [1,2]. Pretreatment processes can be physical, chemical, biological or a combination of these methods. The chemical pretreatments used in the delignification of LCMs provide a reduction of the degree of polymerization and crystallinity of cellulose, associated with the swelling of the sample and increase the internal area of LCMs [3]. The application of combined or sequential pretreatments strategies has been shown to be a good way to improve enzymatic hydrolysis and subsequently fuel-ethanol production [3].

The alkaline hydrogen peroxide (Alk-H₂O₂) process is based on the pretreatment of LCMs using hydrogen peroxide at alkaline conditions. This process is operated at low temperature and pressure and the peroxide decomposes into oxygen and water and so can be considered a process with a low environmental impact [4,5]. According to Gould [6], the use of hydrogen peroxide improves the subsequent delignification of LCMs, because hydrogen peroxide at alkaline conditions promotes the oxidative depolymerization of lignin, due to the break of carbon–carbon linkages in the lignin [7]. Xiang and Lee [8] reported two important factors in the oxidation process: pH of the reaction and decomposition of hydrogen peroxide. Additionally, the use of sodium hydroxide (NaOH) allows the delignification of LCMs by breaking the ester bonds crosslinking lignin and xylan, increasing the internal surface area [9].

On the other hand, there are different alternatives or strategies in the fermentation process for fuel-ethanol production [10]. During the last years, simultaneous saccharification and fermentation (SSF) has shown to have several advantages compared with separate hydrolysis and fermentation (SHF) in terms of overall ethanol yield and volumetric productivity of ethanol. Moreover, SSF reduces processing time as a consequence of the fast glucose conversion to ethanol by the fermenting microorganisms that reduce the enzyme inhibition due to the presence of sugars. Reduction in equipment costs is also obtained by carrying the hydrolysis and fermentation in a single reactor [11]. However, the difference between the optimal temperature for the enzyme action and microorganism growth is an issue that needs to be solved for an efficient SSF [12]. The operational strategy of semi-simultaneous saccharification and fermentation (SSSF) is a good alternative that includes a short presaccharification period before the SSF process and that has been shown to produce higher ethanol concentration, yield and productivity than SSF and SHF [10]. In this context, the objective of this work was to compare and evaluate the SSSF and SSF strategies for fuel-ethanol production by *S. cerevisiae* PE2, *P. stipitis* Y7124 and *Z. mobilis* B14023 using a selected raw material as the MCF pretreated by the Alk-H₂O₂/NaOH process.

2. Materials and methods

2.1. Raw materials and chemical characterization

CAC, GCS, MCF and MCS were obtained from the agroindustries and urban locations in the Northeast of Brazil. The composition of the raw materials was obtained according to Gouveia et al. [13] and Sluiter et al. [14].

2.2. Pretreatment process

2.2.1. Preparation of raw materials before the pretreatment

The raw materials were washed five times with distilled water at 70 °C for removal of residual compounds. After this procedure, the LCMs were dried in an oven with air circulation at 40 °C for 24 h. The LCMs were milled to a particle size of 48 mesh (0.3 mm).

2.2.2. Alkaline hydrogen peroxide (Alk-H₂O₂) pretreatment

0.4 g of LCM were mixed with 31.75 mL of hydrogen peroxide in a flask with a concentration of 7.35% (v/v) at 25 °C for 1 h with agitation at 150 rpm. The pH of hydrogen peroxide solution was adjusted to 11.5 with NaOH. The LCM residual solid was separated via vacuum filtration and washed with distilled water [15].

2.2.3. Delignification process with sodium hydroxide (NaOH)

The Alk- H_2O_2 pretreated solids from each LCM were transferred to flasks with a 4% (w/v) solution of NaOH. The mixture remained at 100 °C under agitation at 100 rpm for 1 h. After delignification, the solids were separated from the liquor by filtration. The solids underwent seven washes with distilled water [16].

2.3. Characterization of delignified pretreated solids

2.3.1. Chemical composition after delignification

The chemical composition was performed as described above (see Section 2.1).

2.3.2. Fourier-transform infrared (FTIR)

The FTIR spectra of delignified pretreated solids and untreated LCMs were measured on an FTIR spectrometer (FTLA 2000 series, ABB Bomem Inc., Quebec, Canada). The conditions of analysis were: resolution of 4 cm^{-1} using 20 scans and frequency range of $400-4000 \text{ cm}^{-1}$. The samples were ground with spectroscopic grade potassium bromide (KBr).

FTIR analysis was conducted to examine the cellulose structure of delignified pretreated solids and untreated LCMs. Two infrared ratios related to cellulose structure were calculated: (1) 1426 cm⁻¹/896 cm⁻¹, the ratio of peak areas at 1426 and 896 cm⁻¹, which is referred to as crystallinity index [17] or lateral order index (LOI) [18]; (2) 1373 cm⁻¹/2917 cm⁻¹, the ratio of peak areas at 1373 and 2917 cm⁻¹, which is known as total crystallinity index (TCI) [19].

2.3.3. X-ray diffraction analysis and crystallinity

Cellulose crystallinity of delignified pretreated solids and untreated LCMs was analyzed in an X-ray diffractometer (Bruker D8 Discover, USA). The operating voltage and current were 40 kV and 40 mA, respectively. The crystallinity index (CI) was defined using the Eq. (1) [3]. Download English Version:

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