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Bioethanol production from coconuts and cactus pretreated by autohydrolysis

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ABSTRACT

The use of coconut fiber mature, green coconut shell, mature coconut shell and cactus is an important alternative as substrates for bioethanol production, since these lignocellulosic materials (LCMs) are abundant in Brazil, mainly in the Northeast Region. The first objective of this work was to evaluate the autohydrolysis pretreatment (AP) on these LCMs and the susceptibility of the treated materials to enzymatic hydrolysis (EH). The second part of the work deals with the application of semi-simultaneous saccharification and fermentation (SSSF) and simultaneous saccharification and fermentation (SSF) using *Zymomonas mobilis*, *Pichia* stipitis, *Saccharomyces cerevisiae* and as substrate the green coconut shell (selected according to the results obtained in the first part of the work). The LCMs after AP using the highest severity factor (4.64) showed changes in the chemical composition in comparison to the untreated LCMs: between the LCMs the cellulose increase was 48.55%, the hemicellulose decrease was 76.77% and an increase of 62.26% was observed for lignin. The green coconut shell was characterized by SEM, X-ray and FTIR after AP and its EH conversion into glucose was 92.52%. The best results on ethanol yield (90.09%) and ethanol productivity (0.21 g/(L h)) from green coconut shell were obtained by *S. cerevisiae* using SSSF. Overall, an efficient process for the bioethanol production from green coconut shell was developed.

1. Introduction

In search to mitigate climate change and fossil fuel dependence of some countries, arise as an alternative biofuel production, especially ethanol produced from sugarcane sucrose and cornstarch. However, both processes have economical and environmental limitations for its productive expansion. Thus, a promising option to increase the ethanol supply is the use of lignocellulosic materials (LCMs) as substrate, which are initially converted in fermentable sugars from pretreatment and EH processes and subsequent fermentation. Attempts to produce ethanol from LCMs are old and originated in Germany and Russia over 80 years ago from the saccharification of LCM by acid hydrolysis (Bastos, 2007).

The LCMs used for ethanol production should be from forests (extracted from vegetable or cultivated), agroindustrial and urban wastes, vegetables grown in inhospitable environments and photosynthetic aquatic biomass (Gonçalves et al., 2014). In this context, this work uses agroindustrial waste (coconut fiber mature, green coconut shell and mature coconut shell), urban waste (green coconut shell) and vegetable cultivated in inhospitable environments (cactus) aiming at the bioethanol production. According to FAO (2012), the global production of coconut in 2009 was approximately 55 million tons, mainly in the Philippines, Indonesia and India. The fourth largest producer of coconut is Brazil (IBGE, 2012). Besides, in 2009, the production of cactus in Brazil was 60,000 tons (IBGE, 2012).

The recalcitrance of these LCMs demands a pretreatment to facilitate enzymatic action (Romaní et al., 2010). Several methods for vegetable biomass pretreatment have been studied, e.g., biological, physical, chemical or its combination. The autohydrolysis pretreatment (also called liquid hot water or hydrothermal processing – AP) was initially used by Bobleter et al. (1976) to increase susceptibility to EH of LCMs. Normally, the advantages in the use of AP are the simple operation, absence of corrosion in the equipment, unnecessary use of chemical solvent, except water, addition to low operating costs (Cybulska et al., 2010; Romaní

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et al., 2010). In this way, the AP has important features in the preservation of the environment (Garrote et al., 2003).

The AP is carried out by the heating the aqueous suspension in the presence of the LCMs and resulting in the depolymerization and solubilization of the hemicellulose in the liquid phase (oligosaccharides and monosaccharides) (Romaní et al., 2012; Ruiz et al., 2013), as well as products of sugar degradation (furfural and hydroxymethylfurfural – HMF) and acetic acid (Ruiz et al., 2013). Surface area and porosity of LCM increases (Cybulska et al., 2010) and re-location of the lignin on the surface of the LCMs (Ruiz et al., 2013) occurs that, together with an increase in the fraction of the cellulose in the pretreated LCMs, contributes to the improvement of the susceptibility of these solids to EH (Ruiz et al., 2013). The solubilization of hemicellulose by AP may be considered as the first stage in the implementation of the biorefinery concept (Romaní et al., 2011) that makes possible the selective separation of the most important components contained in the LCMs (hemicellulose, cellulose and lignin) including the recovery of lignin after distillation of the fermented broth.

This biorefinery concept consists of an industrial installation that unites equipment and conversion processes to produce power, fuels and other chemicals derived of LCMs (NREL, 2012), use the resources in a sustainable way, without producing waste and other environmental pollutants, based on the association of cleaner processes (Luo et al., 2010). The biorefinery based on lignocellulose is a promising strategy to the implementation of this concept (Uihlein and Schebek, 2009).

Moreover, there are different fermentative strategies for bioethanol production (Shen and Agblevor, 2011). Lately, the separate hydrolysis and fermentation (SHF) have shown to have several disadvantages relative the yield and volumetric productivity of ethanol compared with SSF. Furthermore, the SSF has less processing time, enzymatic inhibition and equipment costs (Shen and Agblevor, 2010). However, for an efficient SSF is necessary approaches the optimal temperature of enzymatic action and the growth of microorganism (De Souza et al., 2012). The fermentative strategy of SSSF consists an interesting alternative that has a small presaccharification period prior to the SSF and shows higher ethanol productivity, yield and concentration compared to SHF and SSF strategies (Shen and Agblevor, 2011).

The objective of this work was to study the AP on cactus, coconut fiber mature, green coconut shell and mature coconut shell followed by EH as a suitable LCMs material for bioethanol production. Moreover, SSF and SSSF strategies for bioethanol production utilizing *Pichia stipitis*, *Saccharomyces cerevisiae* and *Zymomonas mobilis* were developed.

2. Materials and methods

2.1. Raw material, chemical and physical agents in the chemical characterization

The LCMs used in this work were the cactus, coconut fiber mature, green coconut shell and mature coconut shell derivatives of the urban locations and agroindustries in Brazil (Northeast Region).

The chemical characterization was carried out with 0.3 g from LCMs and 5 mL of sulphuric acid (concentration of 72% (w/w)) during 1 h. The post-hydrolysis using 4% sulphuric acid and adding water (until 148.67 g) at 121 °C for 1 h. Before analyzing in high performance liquid chromatography (HPLC), the LCMs solids residues derivatives of post-hydrolysis process were recovered by the filtration and regarded as Klason lignin, based on standard test methods (T-249 and T- 264) of the Technical Association of the Pulp and Paper Industry (TAPPI) (www.tappi.org). Monosaccharides, acetic acid, furfural and HMF contained in the hydrolysates were determined by the HPLC, with the purpose of content estimate of the acetyl groups, arabinan, cellulose and xylan of samples. Compositions of LCMs were based in the protocol analysis of National Renewable Energy Laboratory (NREL) (Sluiter et al., 2008) and its subsequent amendments. Residual ash content was estimated from 2 g sample maintained at 550 °C by 5 h and weighed to measure the residual ash content (Sluiter et al., 2008). Moisture was determined from 2 g sample maintained at 105 °C by 24 h and weighed to calculate the dry weight (Sluiter et al., 2008).

Table 1
Autohydrolysis pretreatment of LCMs. (A) Operational conditions; (B) severity factor.

A

Assay	Operational conditions			
	Real value		Normalized variables[a]	
	T (°C)	t (min)	T (°C)	t (min)
	X_1	X_2	X_1	X_2
1	160	10	−1	−1
2	160	50	−1	1
3	160	30	−1	0
4	200	10	1	−1
5	200	50	1	1
6	200	30	1	0
7	180	10	0	−1
8	180	50	0	1
9	180	30	0	0
10	180	30	0	0

B

Severity factor (R_0)			
T (°C)/t (min)	10	30	50
160	2.76	3.24	3.46
180	3.35	3.83	4.05
200	3.94	4.42	4.64

Note: the mathematical model (Eq. (1)) corresponding to the severity parameter $\log R_0$ is $R_0 = \int_0^t \exp\left(\frac{T-100}{14.75}\right)\ dt$.

[a] X_1: temperature, X_2: time.

2.2. Pretreatment stage

2.2.1. Preparation of lignocellulosic materials before of the pretreatment

The LCMs were initially washed with the purpose of withdrawing the residual compounds existing. Afterwards of five washes utilizing water (70 °C), the LCMs were dried in an oven with air circulation during 24 h at 40 °C. After this procedure, the LCMs were milled to standardize a particle size of 0.3 mm (48 mesh) (Gonçalves et al., 2014).

2.2.2. Autohydrolysis pretreatment (AP)

The LCMs and water were mixed to obtain a ratio 1:10 solid/liquid (v/w), the conditions and severity of pretreatment are shown in Table 1A and B. The stainless steel cylinders reactors with total volume of 50 mL were utilized in the experiments. These reactors were closed and then immersed in oil bath (Julabo, Germany) equipped with a PID temperature control, pre-heated to the desired reaction temperature, based on Table 1. After the end of the desired reaction time established in Table 1, the reactors were removed from the oil bath and submerged in an ice-water bath during 5 min. The solid phase and liquid (liquor) were separated by vacuum filtration (Ruiz et al., 2012), both being characterized according to the methods presented in Sections 2.3 and 2.4. The effects of the temperature and time on pretreated LCMs were analyzed according to severity parameter of the $\log R_0$ (Overend and