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Bioethanol production by *Saccharomyces cerevisiae*, *Pichia stipitis* and *Zymomonas mobilis* from delignified coconut fibre mature and lignin extraction according to biorefinery concept

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ABSTRACT

In search to increase the offer of liquid, clean, renewable and sustainable energy in the world energy matrix, the use of lignocellulosic materials (LCMs) for bioethanol production arises as a valuable alternative. The objective of this work was to analyze and compare the performance of *Saccharomyces cerevisiae*, *Pichia stipitis* and *Zymomonas mobilis* in the production of bioethanol from coconut fibre mature (CFM) using different strategies: simultaneous saccharification and fermentation (SSF) and semi-simultaneous saccharification and fermentation (SSSF). The CFM was pretreated by hydrothermal pretreatment catalyzed with sodium hydroxide (HPCSH). The pretreated CFM was characterized by X-ray diffractometry and SEM, and the lignin recovered in the liquid phase by FTIR and TGA. After the HPCSH pretreatment (2.5% (v/v) sodium hydroxide at 180 °C for 30 min), the cellulose content was 56.44%, while the hemicellulose and lignin were reduced 69.04% and 89.13%, respectively. Following pretreatment, the obtained cellulosic fraction was submitted to SSF and SSSF. *Pichia stipitis* allowed for the highest ethanol yield – 90.18% – in SSSF, 91.17% and 91.03% were obtained with *Saccharomyces cerevisiae* and *Zymomonas mobilis*, respectively. It may be concluded that the selection of the most efficient microorganism for the obtention of high bioethanol production yields from cellulose pretreated by HPCSH depends on the operational strategy used and this pretreatment is an interesting alternative for add value of coconut fibre mature compounds (lignin, phenolics) being in accordance with the biorefinery concept.

1. Introduction

The emerging need to obtain clean, low cost and renewable energy in support of a sustainable energy matrix, demands for the development of biotechnology processes, as is the case of the production of biofuels, that will contribute to the energetic self-sufficiency worldwide. USA and Brazil have achieved a situation close to ideal as from the bioethanol production "food source" with innumerable economic gains. Being a step toward energetic sustainability, the matrix becomes more "green", but does not fully satisfy the socio-environmental issues and global geographic distribution. In this regard, an alternative solution is the bioethanol production from LCMs and other non-food source of carbohydrates, available according to the location, making possible a global production of this biofuel. However, some barriers are present in the production of cellulosic ethanol at commercial scale, especially the stages of pretreatment, enzymatic hydrolysis processing, fermentation (hexose and pentose) and the integral use of the raw material. There is also a need to select raw materials, according to the regions, that can be employed as substrate to produce bioethanol.

Brazil makes use of high amounts of sugarcane for the production of bioethanol and generates as waste straw and bagasse. However, in the current conjuncture of the Brazilian sugar and bioethanol production model, these residues are generally intended for electricity production [1], making uncertain its use for ethanol. As an alternative to mitigate this possible absence of

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residues from sugarcane, Brazil possesses raw materials from several other crops as it the case of the coconut residue (known as CFM). The cultivation of coconut is distributed in more than 200 countries and global production in 2009 was about 55 million tons [2]. Brazil is the fourth largest producer, with a production of about 3 million tonnes (5%), the Northeast Region accounting for 82.28% of the total cultivated area [3]. Gonçalves et al. [4] reported that the estimated content of bagasse (fibre) in the coconut in 50%.

For the production of second generation bioethanol within the biorefinery concept, a pretreatment stage for the fractionation of the material is necessary, with the finality of increasing the susceptibility of the material to enzymatic attack. Yang and Wyman [5] reported that the pretreatment stage is the most expensive step in bioethanol production. Pretreatment of LCMs are based on physical, chemical, biological, physicochemical and combined processes and are a key factor in the economic efficiency and one of the main challenges in large-scale production of ethanol [6].

According to Park et al. [7], the thermochemical pretreatment process is now regarded as the most effective one, being pH and temperature the main parameters to be considered. The alkaline pretreatment is used with the intention to remove mainly the lignin. Sodium hydroxide, calcium hydroxide and ammonia are highlighted in this pretreatment due to the low cost and the need of smaller enzymatic loads to convert cellulose into glucose [7]. The use of sodium hydroxide as catalyst pretreatment by steam explosion was carried out by Park et al. [7] and resulted in an elevated delignification and effective enzymatic hydrolysis on *Eucalyptus grandis*. Because a minimal reduction of the lignin on the solid phase in the LCM pretreated by steam explosion occurs [7], similar facts take place with the LCM pretreated by autohydrolysis [8]. Therefore, the use of sodium hydroxide in hydrothermal pretreatment consists an alternative to reduce the lignin content of the LCM, as used by Rawat et al. [9] in poplar (*Populus deltoides*) and Kim and Han [10] on rice straw.

During this pretreatment, the hemicellulose is degraded to low molecular weight oligomers, monomers and organic acids that form a black liquor [11]. Additionally, the black liquor contains a high lignin concentration and phenolic compounds, the recovery of lignin from the liquor being done by the acid precipitation method and converted into synthesis gas or as used as raw material for several aromatic substances of low molecular weight [11,12]. The phenolic compounds are natural sources of antioxidants and may be applied as food additives and cosmetic applications [8,13].

Several strategies for the production of cellulosic ethanol may be applied at the fermentation stage, for example, separate hydrolysis and fermentation (SHF) and simultaneous saccharification and fermentation (SSF). Attributes of SSF are less inhibition of enzyme and longer time of EH compared to SHF; SHF possesses a greater rate of hydrolysis [14]. As an alternative to merge the advantages provided by SHF and SSF, arises the use of semi-simultaneous saccharification and fermentation (SSSF) which includes a presaccharification stage before SSF [15]. SSSF is expected to have higher productivity and yield than SSF and SHF on their own, if the presaccharification time is appropriate [14,16].

Therefore, this work aims to evaluate a strategy for the application of CFM as a raw material for bioethanol production by carrying a HPCSH step followed by two alternative fermentation strategies – SSF and SSSF – using three fermenting microorganisms - *Saccharomyces cerevisiae*, *Pichia stipitis* and *Zymomonas mobilis* – and characterization of lignin obtained in the liquid phase.

2. Materials and methods

2.1. Raw material and chemical characterization

CFM was obtained from the agroindustries locations in the Northeast of Brazil. Chemical characterization was performed by quantitative acid hydrolysis with 5 mL of 72% (w/w) sulfuric acid for 1 h and quantitative post-hydrolysis with 4% sulfuric acid (adding water until 148.67 g) at 121 °C during 1 h. Before HPLC analysis, the solid residue from post-hydrolysis process was recovered by filtration and considered as Klason lignin. Monosaccharides sugars and acetic acid contained in the hydrolysates were determined by HPLC in order to estimate the contents of samples of cellulose and hemicellulose [15]. Composition of LCMs was determined according to the protocols of the a National Renewable Energy Laboratory [17]. Residual ash content was estimated from 2 g sample maintained at 550 °C for 5 h and weighed to measure the residual ash content [18]. The moisture was estimated from a 2 g sample maintained at 105 °C for 24 h and weighed to calculate the residual content [17].

2.2. Pretreatment process

2.2.1. Preparation of raw material before the pretreatment

The CFM was washed for the removal of non-lignocellulosic residues. After five washes with water at 70 °C, the material was dried in an oven with air circulation at 40 °C for 24 h. After this procedure, the CFM was milled into a particle size of 48 mesh (0.3 mm), with the purpose of standardizing the granulometry of the CFM [15].

2.2.2. Hydrothermal pretreatment catalyzed with sodium hydroxide (HPCSH)

The CFM, sodium hydroxide and water were mixed in order to obtain a 10:1 liquid/solid ratio, the conditions of HPCSH are shown in Table 1. These experiments were carried out in 50 mL total volume stainless steel cylinders reactors. The reactor was closed and mounted vertically and then submerged in a Julabo oil bath open heating circulator (Julabo, Germany) with PID temperature control, previously heated to the desired reaction temperature (Table 1). At

Table 1
Operational conditions of pretreatment.

Assay	Operational conditions					
	Normalized variables			Real value		
	T (°C)	t (min)	NaOH (%)	(°C)	(min)	(%)
	X_1	X_2	X_3	Temperature	Time	NaOH
Lineal						
1	−1	−1	−1	160	10	1.0
2	−1	−1	1	160	10	4.0
3	−1	1	1	160	50	4.0
4	−1	1	−1	160	50	1.0
5	1	−1	−1	200	10	1.0
6	1	−1	1	200	10	4.0
7	1	1	1	200	50	4.0
8	1	1	−1	200	50	1.0
Central point						
9	0	0	0	180	30	2.5
10	0	0	0	180	30	2.5
Quadratics						
11	−1	0	0	160	30	2.5
12	1	0	0	200	30	2.5
13	0	−1	0	180	10	2.5
14	0	1	0	180	50	2.5
15	0	0	−1	180	30	1.0
16	0	0	1	180	30	4.0