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Diluted phosphoric acid pretreatment for production of fermentable sugars in a sugarcane-based biorefinery

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HIGHLIGHTS

- Diluted phosphoric acid was employed as catalyst in sugarcane bagasse pretreatment.
- Factorial design showed the effects of temperature, time and acid concentration.
- Very effective hemicellulose solubilization was achieved at 186 °C, reaching 98%.
- Relatively low amounts of fermentation inhibitors were released in the hydrolysate.
- Phosphoric acid in the hydrolysate can be a P-source for subsequent fermentation.

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ABSTRACT

The influence of time (8–24 min), temperature (144–186 °C) and phosphoric acid concentration (0.05–0.20%, w/v) on the pretreatment of sugarcane bagasse in a 20 L batch rotary reactor was investigated. The efficiency of the pretreatment was verified by chemical characterization of the solid fraction of the pretreated bagasse and the conversion of cellulose to glucose by enzymatic hydrolysis. Models representing the percentage of cellulose, hemicelluloses, lignin, solubilized hemicellulose and the enzymatic conversion of cellulose to glucose were predictive and significant. Phosphoric acid concentration of 0.20% at temperature of 186 °C, during 8 and 24 min, was shown to be very effective in solubilizing hemicellulose from sugarcane bagasse, reaching solubilization of 96% and 98%, respectively. Relatively low amounts of inhibitors were produced, and the phosphoric acid remaining in the hemicellulosic hydrolysate is at adequate levels for supplying phosphorous requirement during subsequent fermentation.

1. Introduction

Social and environmental concerns, such as the world's dependence on nonrenewable fuels derived of petroleum, use of food for fuel production and the reduction the negative impacts to the environment, particularly due to the emission of greenhouse gasses, are the driving forces that propel the use of renewable raw materials for the production of alternative energy. For these reasons, the concept of biorefinery is emerging as a promising alternative because it is based on the integration of processes for the production of energy, fuel – especially second generation ethanol – and a wide variety of high value-added chemical products from the full use of renewable biomass (Ragauskas et al., 2006).

In this way, Brazil is in a privileged position to assume the leadership in the full use of biomass because it is one of the largest producers of renewable raw materials in the world (CGEE, 2010). Sugarcane plantations are a notable source of these materials, of which Brazil itself represents the largest producer in the world, reaching a production capacity exceeding 623 million tons in the 2010/2011 harvest (MAPA, 2011). However, only sucrose, which corresponds to one-third of the sugarcane biomass, is used to produce sugar and ethanol. The remaining two-thirds represent the bagasse, by-product obtained after crushing the sugarcane for stripping the juice to be used for sugar and/or ethanol production, and the leaves and tips, which constitute sugarcane straw (BNDES, 2008; DEAGRO, 2011). Approximately 250 kg of bagasse are generated per ton of sugarcane, thus representing the main lignocellulosic material generated in Brazil. The bagasse is largely used as the main source of energy for the production of steam used in the mill, and electricity (CGEE, 2010; Pandey et al., 2000; Stambuk

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et al., 2008). However, if technological improvements are made to the boilers it is possible to satisfy the energy requirements of the plants with only half of the produced bagasse. If residual bagasse from the sugar mills in Brazil were converted to ethanol, then the amount of ethanol produced per unit area of sugarcane crop land in Brazil would improve significantly.

Lignocellulosic materials consist of three main organic fractions: cellulose (35–50%), hemicellulose (20–35%) and lignin (12–20%). They also contain small amounts of minerals (ash) and various other compounds, which are called extractives. The cellulose chains are packed by hydrogen bonds in so-called 'microfibrils', which are attached to each other by hemicellulose, and covered by lignin. These microfibrils are often associated in the form of macrofibrils. Because of this complexity, pretreatment of these lignocellulosic materials is normally necessary before enzymatic hydrolysis (Himmel, 2008).

Pretreatment is mainly aimed at disorganizing the complex lignocellulose by reducing the lignin and hemicellulose content, reducing the cellulose crystallinity, and increasing the porosity, causing the opening of the lignocelllulose cell wall structure for enzymatic degradation (Hendriks and Zeeman, 2009). The pretreatment can be accomplished by appropriate physical, physicochemical, chemical or biological means (Alvira et al., 2010; Mosier et al., 2005). Dilute acid hydrolysis is one of the most commonly used method of chemical pretreatment, which main objective is to solubilize the hemicellulosic fraction of the biomass and to render the cellulose more accessible for enzymatic hydrolysis. It can be performed during a short retention time at a high temperature (above 160 °C) or over a relatively long retention time at a lower temperature. In general, pretreatments performed at higher temperatures and shorter residence times generate higher recovery yields of soluble xylose and improved enzymatic cellulose digestibility. Depending on the substrate and the conditions applied, 80–95% of the hemicellulosic sugars can be recovered from the lignocellulosic feedstock by dilute acid pretreatment (Balat et al., 2008).

Diluted phosphoric acid for chemical pretreatment of different biomasses have been investigated (Gámez et al., 2006, 2004; Israilides et al., 1978; Um et al., 2003; Vázquez et al., 2007). These studies have shown that treatments performed at low temperatures and long residence times can be effective using relatively high concentration solutions of phosphoric acid (2–6%). However, phosphoric acid is much more expensive than sulfuric acid, which is the cheapest and most widely investigated acid catalyst. Recently, steam treatment of sugarcane bagasse with more diluted phosphoric acid was investigated at shorter residence times and higher temperatures, and shown to be an effective method to hydrolyse hemicelluloses (Geddes et al., 2010). Phosphoric acid pretreated sugarcane bagasse allowed efficient fermentation of hemicellulose hydrolysate by engineered *Escherichia coli* without the need of detoxification (Nieves et al., 2011).

In this work, dilute phosphoric acid pretreatment of sugarcane bagasse was investigated using concentration levels adequate for the subsequent microbial fermentation of the hemicellulose hydrolysate for fuel, chemical or enzyme production. An experimental design was applied to assess the effects of temperature, time and phosphoric acid concentration on the pretreatment in a 20 L batch rotary reactor.

2. Methods

2.1. Raw material

The sugarcane bagasse used in this study was provided by Olho D'Água sugar plant (www.grupoolhodagua.com.br/BACKUP/site/index.php). The bagasse was collected straight from the mill and left to dry at sunlight for two days.

2.2. Pretreatment

2.2.1. Experimental design

A linear experimental design 2^3 with 3 central points and a total of 11 assays was applied to evaluate the best conditions for pretreatment. Three levels were defined for each independent variable (time: 8, 16 and 24 min; temperature: 144, 165 and 186 °C; and phosphoric concentration: 0.05, 0.13 and 0.20%, w/v). The following response factors were considered: the percentages in the solid fraction of cellulose (%C), hemicellulose (%H) and lignin (%L); the solubilized hemicellulose (%H_S); and the enzymatic conversion of cellulose (%CC).

The software Statistica (Statsoft 7.0) was used to analyze the results, which were subjected to an analysis of variance (ANOVA). The independent variables and responses (coded values) were correlated through the linear model represented by Eq. 1 in which y_i represents the response variable; β_0, β_j, and β_{ij} are the coefficients of the regression model; and X_i and X_j represent the coded levels of the independent variables.

$$y_i = \beta_0 + \sum \beta_j X_i + \sum \beta_{ij} X_i X_j \quad (1)$$

2.2.2. Experimental procedure

Bagasse samples of 500 g each were introduced into a 20 L rotary reactor (Regmed Indústria Técnica Ltda., model AU/E-20) with phosphoric acid solutions at different concentrations, according to the experimental design. The concentration of bagasse in the reactor was maintained at 5% (w/v). The reactor temperature was increased by an electrical resistance heating system, and the counted time was initialized when the process reached the desired temperature. After finishing the reaction, the temperature was reduced to 80 °C, and the reactor was discharged. The pretreated sugarcane bagasse was filtered, and the pulp obtained (solid fraction) was washed three times (10 L per wash) with hot water (70 °C). The solid fractions obtained were separated for chemical and morphological characterization and enzymatic hydrolysis. The liquid fractions were submitted to chromatographic analysis to determine the concentration of fermentation inhibitors.

2.3. Enzymatic hydrolysis

The effects of the pretreatment conditions on cellulose conversion were evaluated by enzymatic hydrolysis of the solid fractions obtained in each pretreatment. For the enzymatic hydrolysis, the commercial enzymes Celluclast® 1.5 L and Novozym® 188 (β-glucosidase) were used. The assays were carried out in 250 mL Erlenmeyer flasks to which the bagasse and the enzyme diluted in 50 mM sodium citrate (pH 4.8) were added. The conditions were as follows: activity of 20 FPU $g^{-1}_{bagasse}$ and 4 IU $g^{-1}_{bagasse}$ of β-glucosidase, maintaining a final ratio of 5:1 (Celluclast:β-glucosidase); 50 °C and 150 rpm in a shaker (New Brunswick Scientific, model C25KC); a final volume of 100 mL, and a 2.0% (w/v) final concentration of bagasse. The sample (1 mL) was taken after 72 h of hydrolysis, subjected to a boiling bath for 5 min, immersed in an ice bath, and then centrifuged and filtered through a 0.22 μm membrane.

2.4. Analytical methodology

2.4.1. Analysis of chemical composition

The raw and pretreated bagasse samples were analyzed according to the methodology described by Rocha et al. (2011). The methodology is based on acid hydrolysis of extractive-free material

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