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Assessment of alkaline pretreatment for the production of bioethanol from eucalyptus, sugarcane bagasse and sugarcane straw

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ABSTRACT

The impact of alkaline pretreatment at different alkaline charges (5%, 10% and 15% NaOH w/w, on dry basis) on the chemical composition of eucalyptus, sugarcane bagasse and straw were compared with the subsequent bioconversion into ethanol, using semi-simultaneous saccharification and fermentation (SSSF). By increasing alkaline charge in pretreatments, substantial amount of lignin, hemicelluloses and cellulose were fractionated. The chemical composition of biomasses was expressed based on the complete mass balance. The chemical transformation of biomasses during pretreatments was assessed by comparing the chemical composition of pretreated biomasses and their untreated counterparts. Pretreatments promoted delignification in the range of 11%–51% for eucalyptus, 22%–90% for bagasse, and 60%–99% for straw for the alkaline charges in the range of 5%–15% (NaOH w/w). The removal of lignin from bagasse and straw was higher than that from eucalyptus, which was due to the combined effect of higher frequency of both, the free phenolic groups and the ester bonds in grass lignin that made the lignin solubility escalate in alkaline conditions. It was also observed that bagasse had a removal by 37%–45% hemicelluloses and 0.8%–11% cellulose. For straw, higher amount of carbohydrates was removed, in the range of 55%–66% hemicelluloses and 19%–36% cellulose. Fragments of lignin and carbohydrates were converted into new structures called "pseudo-extractives" in eucalyptus during pretreatments. Pseudo-extractives and native extractives were quantified together, thus increasing the total extractives contents by 3.3 (5% NaOH), 3.5 (10% NaOH) and 2.9 (15% NaOH) times, in line with the original raw materials. Maximum ethanol yield and maximum volumetric productivity of ethanol were achieved for eucalyptus pretreated using 10% NaOH, bagasse pretreated using 15% NaOH and straw pretreated using 5% NaOH. At the optimal pretreatment condition, just about 51% glucose was released from pretreated bagasse. Sugarcane bagasse presented the highest values for the respective parameters, namely: 8.8 g L^{-1} ethanol concentration, 0.101 $g_{ethanol}/g_{biomass}$ ethanol yield and 0.88 g L^{-1} h^{-1} volumetric productivity of ethanol. Alkaline charge proved to be an important control variable for alkaline pretreatments, with determinant effect on chemical transformations of biomasses and result on ethanol production.

1. Introduction

The production of bioethanol from lignocellulosic biomasses consists of the sugar release from the cell wall by hydrolysis (pretreatments and saccharification) and subsequent fermentation of sugars released into ethanol (Rubin, 2008). The pretreatments are considered key steps for bioethanol production with the increase of the cellulose accessibility being one of the main goals. The most appropriate condition for pretreatment, however, varies according to the raw material.

A number of lignocellulosic biomasses have been studied for bioethanol production. These include the following: agricultural residues (Asakawa et al., 2015; Murciano Martínez et al., 2015; Rocha et al., 2015; Oliveira et al., 2013; de Souza et al., 2012), energy crops (Cotana et al., 2015; De Bari et al., 2013) and woods (Nitsos et al., 2013; Romaní et al., 2010; Ballesteros et al., 2004). In the case of Brazil, sugarcane bagasse and sugarcane straw (both residues from sugarcane industries), appear as potential feedstock for bioethanol production, with an estimate of 92 million tons generation for each one annually (Conab, 2015; Oliveira et al., 2013). In addition, the eucalyptus stands out as a potential woody feed-

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stock because of its high productivity and adaptability to Brazilian climate compared to other species of wood.

Lignocellulosic biomasses comprise lignin, cellulose, hemicelluloses, extractives and ash. The first three components are arranged in cell wall forming a complex network in which lignin and hemicelluloses build a physical protection around the cellulose. The objective of several pretreatments is to improve the cellulose accessibility by the removal of lignin and/or hemicelluloses (Rocha et al., 2015; Swain and Krishnan, 2015). Moreover, such removal is usually partial and the effects of residual lignin and of residual hemicelluloses on enzymatic hydrolysis may be different. Cardoso et al. (2013) observed that the effect of residual hemicelluloses on enzymatic hydrolysis was irrelevant compared to the effect of residual lignin on sorghum straw pretreated by acid (H_2SO_4), alkaline (NaOH) and acid (H_2SO_4) followed by alkaline (NaOH) conditions, due to the physical barrier built by lignin. The lignin also participates in non-productive binding with enzymes, and this contributes to the decrease in performance of enzymatic hydrolysis (Berlin et al., 2005).

Alkaline pretreatments are common for the biomass delignification, with additional effects on the removal of silica (acid-insoluble component from ash), or on the partial removal of hemicelluloses (including acetyl groups and uronic acids) and on cellulose swelling, resulting in a substantial increase of the fiber surface area (Mendes et al., 2015; Stoklosa and Hodge, 2015; Asgher et al., 2013; Alvira et al., 2010; Cardona et al., 2010). The alkaline source more often used for alkaline pretreatments, is the sodium hydroxide (NaOH). This pretreatment has been carried out at a room temperature or at a higher temperature (20–121 °C), during the time ranging from minutes to hours (15 min–12 h), and alkaline concentration from 0.5% to 8% w/v (Menezes et al., 2014; Alvarez et al., 2013; Sheikh et al., 2013; de Souza et al., 2012; Alvira et al., 2010; Cardona et al., 2010; Xu et al., 2010). Additionally, alkaline pretreatment has proved to be effective to prepare biomasses for their subsequent use in ethanol production *via* bioconversion processes (Mirahmadi et al., 2010).

There are different arrangements for the bioconversion of sugars from pretreated biomasses into bioethanol. The semi-simultaneous saccharification and fermentation (SSSF) has been recently studied and has proved to be a promising strategy for bioconversion. SSSF is separated in two sequential steps: pure enzymatic pre-hydrolysis, also called presaccharification, and simultaneous saccharification and fermentation (SSF) (Cotana et al., 2015; Gonçalves et al., 2014). Presaccharification is used for releasing sugars from the biomasses and providing these sugars for yeasts in the beginning of SSF (Santos et al., 2012; de Souza et al., 2012). SSF is a combined process in which enzymes for saccharification and yeasts for fermentation are added and performed together in the same reactor. As a result, the SSSF process allows improvements in ethanol yield, reduction in the content of enzymes inhibition and reduction in fermentation time (Baeyens et al., 2015; Cotana et al., 2015; Gonçalves et al., 2014).

However, temperature remains one of the limitations of saccharification and fermentation in SSSF process. The optimal temperature for enzymes (for saccharification) is usually higher than that for yeasts (for fermentation). To overcome this limitation, thermotolerant yeast strains were investigated, which had demonstrated improvements in ethanol production (Ballesteros et al., 2004).

The production of bioethanol by SSSF from biomasses pretreated by alkaline processes formed the basis of the present study. The novelty of this study was to identify the effect of alkaline charge used in alkaline pretreatments on the chemical transformation of biomasses and their subsequent performance in enzymatic hydrolysis, by focusing on the bioethanol production. The objectives of this study were: (i) to evaluate alkaline pretreatments (5%, 10% and 15% NaOH w/w) with respect to their impact on the chemical composition of eucalyptus, sugarcane bagasse and straw and (ii) to evaluate the performance of pretreated biomasses during bioethanol production *via* SSSF.

2. Experimental

2.1. Materials

The 7 years old clonal hybrid of eucalyptus (*Eucalyptus urophylla* x *Eucalyptus grandis*), was supplied by a Brazilian pulp company as wood chips. The chips were sieved and those with 0.5 cm × 3 cm × 3 cm lesser dimensions were collected for chemical analyses and pretreatments. Sugarcane bagasse and straw of five-months old (cultivar RB867515) were supplied by the Center Sugarcane Experimentation (Oratórios, Minas Gerais State, Brazil) at the Federal University of Viçosa after chipping (bagasse and straw) and juice removal (bagasse). Bagasse and straw used in the study were less than 10 mm diameter. The biomasses were dried up to nearly 85% dryness and stored in polyethylene bags at room temperature before use. Moisture content was determined according to TAPPI T 264 cm-07 (TAPPI, 2011). The chemicals used were sodium hydroxide of lentils analytical grade (Merck Milipore, Germany), commercial cellulase Celluclast 1.5 L (Sigma-Aldrich, Brazil) (from *Trichoderma reesei* ATCC 26921) and *Saccharomyces cerevisiae* LBM-1 isolated from fermentation vats in Brazil.

2.2. Alkaline pretreatments

Milder conditions for alkaline pretreatment than that previous investigated (Carvalho et al., 2015) were performed in the present study for eucalyptus wood, sugarcane bagasse and sugarcane straw. One-hundred grams o.d. (oven-dried equivalent) of biomasses were used for alkaline pretreatment in three alkaline charges as follows: 5%, 10% and 15% NaOH (on a dry biomass basis). Duplicate pretreatments were performed in a Regmed reactor, with a rotating pressure vessel (2 L capacity), using the following parameters: liquor; biomass ratio = 2:1 L kg^{-1} (eucalyptus) and 7:1 L kg^{-1} (bagasse and straw, which are more porous than eucalyptus and absorb more liquor), maximum temperature = 175 °C; time to maximum temperature = 90 min, and time at maximum temperature = 15 min (H Factor = 628). After the pretreatment, the reactor was cooled, and the pretreated biomasses were washed with enough water and then, dewatered. The pretreated biomasses were dried for 24 h at 23 ± 1 °C and 50 ± 2% relative humidity to constant weight and then stored at room temperature in polyethylene bags.

2.3. Presaccharification tests

Before presaccharification, pretreated eucalyptus chips were converted to sawdust at 20/80 mesh, using a Wiley mill bench model, in order to improve enzymes accessibility since the aforementioned biomass presents low porosity. Bagasse and straw, which are naturally more porous and already had smaller dimensions, were used without additional grinding. In a 125 mL erlenmeyer flask, 4 g of pretreated biomass was suspended in 50.0 mL of citrate buffer (50 mM, pH 4.8) and supplemented with the commercial cellulase preparation Celluclast 1.5 L in the ratio of 15 Filter Paper Units (FPU) per gram of substrate (1.4 mL) (de Souza et al., 2012). The erlenmeyer flask was capped and incubated in a shaker at 50 °C and 180 rpm agitation. Samples were collected at 0, 12, 24, 36, 48, 60 and 72 h of enzymatic hydrolysis, centrifuged for 10 min at 10,000 x *g*, and the supernatants were used for glucose determination. Glucose concentration in hydrolysates,