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ENZYMATIC HYDROLYSIS OF RAW AND PRE-TREATED EUCALYPTUS

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Abstract

Ethanol from lignocellulosic biomass (LCB), such as agricultural and forest residues, is known as the second generation (2G) ethanol. Carbohydrate fractions in LCB (cellulose and hemicellulose) are not readily available for microbial fermentation and they need to be released by additional pre-treatment and hydrolysis operations. This process is intrinsically more difficult and costly. In this study the efficiency of enzymatic hydrolysis of the species *Eucalyptus urophylla* and the hybrid *E. urophylla* x *E. grandis*, submitted or not to alkaline pre-treatment with green liquor, has been evaluated. The hydrolysis was carried out with a enzymatic cocktail containing aggressive cellulases, high level of β-glucosidases and hemicellulase at 6% (g enzyme/g solid), pH 5.0, 50 $g.L^{-1}$ solids for 72 h, 45 °C and 250 rpm. The pre-treatment promoted disruption of the cellular structure of the samples, allowing easier accessibility for the enzymes and demonstrating higher effectiveness of enzymatic hydrolysis. The maximum efficiency of hydrolysis (81.3%) was observed for pretreated *E. urophylla*.

Keywords

Second generation ethanol; pre-treatment; enzymatic hydrolysis; eucalyptus.

INTRODUCTION

Second generation (2G) ethanol, based on lignocellulosic raw materials of lower cost, is an option to reduce environmental and energy problems caused by pollution, scarcity and high prices of fossil fuels. It is an environmentally viable fuel, because much of the greenhouse gases emitted into the atmosphere in this production is absorbed by the plant during photosynthesis. Therefore, its use will contribute to the mitigation of global warming.

Cell wall of lignocellulosic materials is formed by cellulose, hemicellulose and lignin fractions. The difference is that the classic processes for the production of ethanol involve the direct fermentation by the yeast action and in the 2G ethanol bioprocess, the lignin has to be broken first. After that, hemicellulose and cellulose are hydrolyzed in pentose and hexose sugars (RADOMSKI et al., 2012).

The first step for converting lignocellulosic materials into ethanol is the pre-treatment, whose objective is to change, physically or chemically the lignocellulosic composition, to improve the rate of hydrolysis by enzymes and also increase the production of fermentable sugars (MOSIER et al., 2005). There are many types of pre-treatments proposed in the literature, including the pre-treatment with green liquor based on adaptations of the Kraft pulping process in pulp and paper mills. These factories could be turned into bio-refineries, reducing initial investments and operating costs (MAGALHÃES et al., 2011).

This study evaluates the efficiency of enzymatic hydrolysis of two species of eucalyptus, with and without alkaline pre-treatment and effects of pre-treatment in morphology was observed by Scanning Electron Microscopy (SEM).

METHODS AND MATERIAL

Two 5-years-old species of eucalyptus, *E. urophylla* and *E. urophylla* x *E. grandis*, in raw and pre-treated (PT) form, grounded using a Willey mill and sifted between 45 and 60 mesh, were used. Pre-treatment was carried out with industrial green liquor into a high pressure bench reactor with a maximum capacity of 50 mL. The eucalyptus mass and the green liquor volume were inserted in a ratio of 4:16 (g:mL) and the process conditions used were 180 °C for 40 min.

Enzymatic hydrolysis was performed by incubating the biomass with the enzymatic cocktail containing aggressive cellulases, high level of β-glucosidases and hemicellulase, and citrate buffer pH 5.0, on a rotary shaker for 72 h, 45 °C and 250 rpm. The solid concentration was 50 $g.L^{-1}$ and enzyme concentration was 6% (g enzyme/g solid). The total reducing sugars after hydrolysis were determined by the DNS colorimetric method (MILLER, 1959). The hydrolysis yield was calculated by comparing the amount of sugar generated in the process and the maximum possible quantity (theoretical) of sugar in the raw biomass.

The SEM analysis using the *Veja 3* model equipment of Tescan, was performed in *E. urophylla* samples, before and after pre-treatment.

The results were evaluated by analysis of variance and the means were compared by Tukey test at 5% significance level, using the *Statistica 7.0 software*.

RESULTS AND DISCUSSION

The results of enzymatic hydrolysis are expressed as concentration of total reducing sugars TRS ($g.L^{-1}$) and yield (%), being represented in Figure 1A and Figure 1B, respectively. The lowest values were found for the raw eucalyptus samples and among the pre-treated samples, the highest was related to the species *E. urophylla*.

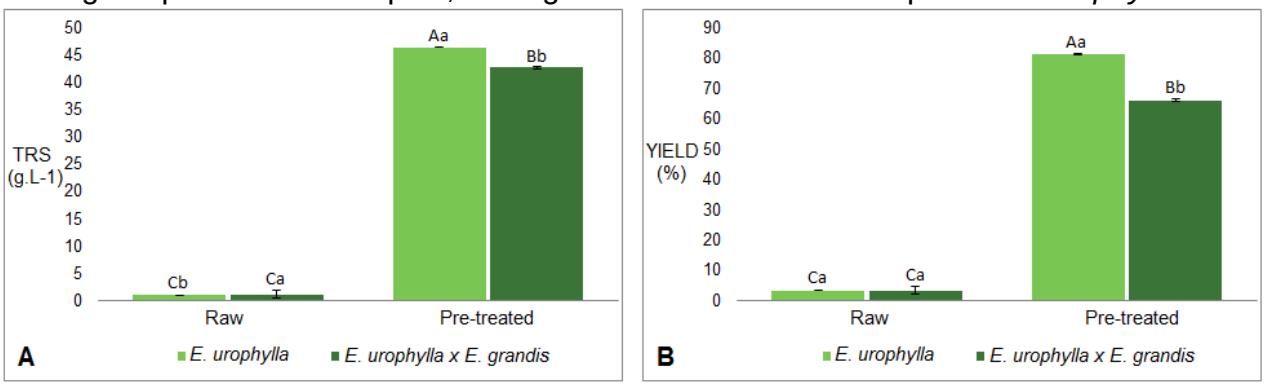

Figure 1. (A) TRS concentration ($g.L^{-1}$) and (B) yield (%) of enzymatic hydrolysis.
Equal characters are not statistically different at 5%. Capital characters indicate the comparison among tests and small characters the comparison between the two species separately.

Lima et al. (2013) evaluated the effect of the alkaline pre-treatment with NaOH at different concentrations in the enzymatic digestibility of *E. urophylla* x *E. grandis*, and the yield, using a 2% NaOH concentration, was lower than the one found in the presente study. However, with higher concentrations the yield has increased to 85%.

This fact is explained by a small loss of cellulose and higher solubilization of lignin in pre-treatment with the higher pH.

Lavorente et al. (2011) also examined the efficiency of enzymatic hydrolysis of *E. urophylla* x *E. grandis* barks, put on seven different sequential pre-treatments with water, hydrochloric acid and sodium hydroxide at different concentrations. The authors verified that the pre-treatment with water, 1% HCl and 4% NaOH improved the glucose concentration, generating a more active hydrolysis process with 66% efficiency. Equivalent result was found in this study for the *E. urophylla* x *E. grandis*.

Similar results to this study were also found by Sun et al. (2013) who used pre-treated eucalyptus in mild alkaline process followed by treatment with various ionic liquids. The maximum yield of TRS was 80% at 50 °C, 180 rpm, after 72 hours. The authors similarly analyzed the hydrolysis of untreated samples whose glucose concentration was 0.14 $g.L^{-1}$ approximately.

Weiqi et al. (2013) performed hydrolysis of pre-treated eucalyptus in hot water, at a solid concentration of 2%, 50 °C for 60 h. Yields observed were 88.12% for glucose and 91.6% for xylose. Higher yields (97.2%) were observed in the work of Yu et al. (2010) who used pre-treatment with hot water in two sequential steps, 180 °C and 240 °C for *E. grandis*. The hydrolysis was performed with solid concentration of 5% during 72 h, being similar to this experiment.

SEM images for *E. urophylla* samples (Figure 2) showed that cellulose fibers seemed to be more opened, desestrutured, and porous after the pre-treatment process. Similar effect was described by Castoldi et al. (2014) in biological pre-treatment of *E. grandis*. The authors observed that the untreated samples showed rigid, aligned, and uniform fibrils, while treated samples had fibers detachment and pores formation. Pores formation may be indicative of lignin removal (CASTOLDI et al., 2014).

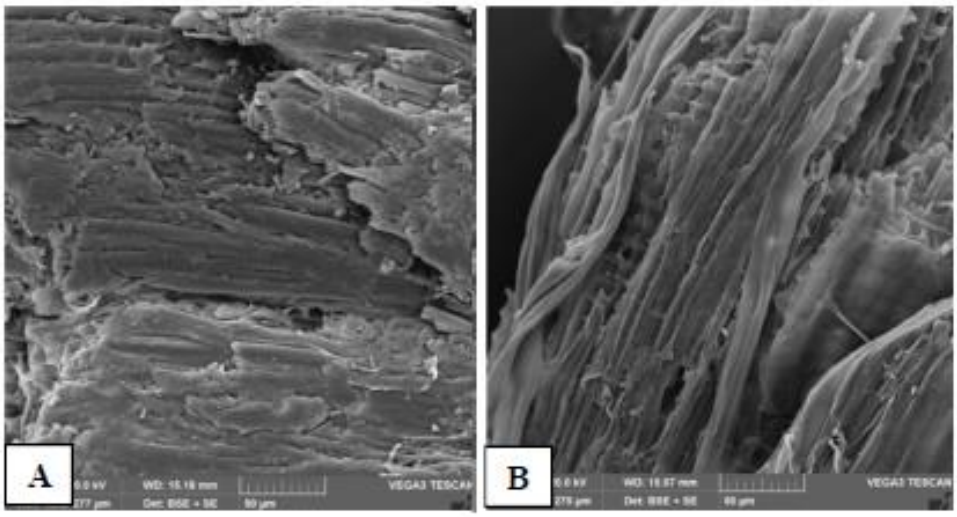

Figure 2. *E. urophylla* sample (A) before and (B) after alkali pre-treatment (Magnification 500x).

CONCLUSIONS

A significant increase in reducing sugar concentration and yield of enzymatic hydrolysis for both eucalyptus species was noted when the alkaline pretreatment was performed. Pre-treated *E. urophylla* was the biomass that has allowed the more efficient hydrolysis, reaching a maximum yield of 81.3%. This result was corroborated by the SEM images, in which a disruption of the lignocellulosic structure after pre-

treatment with green liquor could be observed, suggesting the increase of accessibility for the enzymes.

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