I COBBIND
I CONGRESSO BRASILEIRO DE BIOTECNOLOGIA INDUSTRIAL
XXIV SINAFERM ·· XV SHEB ·· XV ENZITEC FLORIANÓPOLIS ·· 2024
Creating connections between biotechnology and industrial sustainability
August 25 to 28, 2024
Costão do Santinho Resort, Florianópolis, SC, Brazil
BIORREFINERY, BIOECONOMY AND CIRCULARITY

EFFECT OF PH, SOLID CONCENTRATION, AND ENZYME DOSAGE ON ENZYMATIC HYDROLYSIS OF STEAM-EXPLOSION PRETREATED *EUCALYPTUS GRANDIS* WOOD

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ABSTRACT

Lignocellulosic materials can be transformed into value-added products such as biofuels (bioethanol, biobutanol), organic acids (lactic, succinic acid), acetone, etc. For its transformation into these products, a first pretreatment step is required to disrupt the lignocellulosic matrix, followed by a hydrolysis step to obtain fermentable sugars. The main aim of this work was to evaluate the effect of pH, solid content, and enzyme dosage on the enzymatic hydrolysis of steam-exploded *Eucalyptus grandis* wood, previously impregnated with sulfuric acid at 0.5% (w/w). The studied factors were solid content (14.6-25.4) % (w/w), enzyme dosage (15-25) FPU/g_{glucan} and pH (4.6-6.3). Results showed that the conditions which maximized both glucose concentration and hydrolysis efficiency corresponded to pH 6, 24% (w/w) solid content, and 25 FPU/g_{glucan} enzyme dosage.

Keywords: Eucalyptus. Enzymatic hydrolysis. High-solid processing. Steam explosion.

1 INTRODUCTION

Eucalyptus grandis is the predominant forest species in Uruguay, which is widely used in local pulp industries. Along with other lignocellulosic materials, *E. grandis* wood can be transformed into value-added products such as biofuels (bioethanol, biobutanol), organic acids (lactic, succinic acid), acetone, etc. Since sugars are part of a complex matrix in lignocellulosic materials, it is necessary to introduce a pretreatment step that deconstructs the internal structure of the lignocellulosic material and make carbohydrates accessible for their processing. The pretreatment of biomass aims to disrupt the lignocellulosic matrix, leaving cellulose exposed and susceptible to the hydrolytic process. There are several types of pretreatments such as chemicals, biological (using fungi or bacteria), physical, and physicochemical[1]. Steam explosion is a physicochemical pretreatment in which biomass is heated quickly by introducing saturated high-pressure steam in a reactor at operating temperatures in the range of 160 to 260ºC, and kept for short periods of time (seconds to minutes). The process concludes with a rapid decrease in pressure, causing a mechanical disruption of the lignocellulosic matrix and a decrease in particle size. The steam condenses and permeates the biomass, initiating an autohydrolysis reaction due to organic acids generated from the acetyl groups of biomass hemicellulose[2]. In some cases, the addition of some acids, primarily SO_2, H_2SO_4 and CO_2 are used as catalyst, which could decrease the pretreatment processing time and temperature and further improve the enzymatic hydrolysis yield[3].

After pretreatment, enzymatic hydrolysis is carried out to obtain fermentable sugars. To achieve an adequate hydrolysis performance, it is necessary to use a cellulase complex enzymes that act synergistically. These complexes typically include endoglucanases, exoglucanases, and β-glucosidase. As a result, disaccharides are generally produced (cellobiose) which are converted into glucose by the action of β-glucosidase[4]. Several parameters such as temperature, agitation, enzyme dosage, pH, and solid content of the samples are critical conditions during enzymatic hydrolysis that must be optimized. The objective of this work was to evaluate the effect of pH, solid content, and enzymatic dosage on pretreated *E. grandis* wood enzymatic hydrolysis to maximize hydrolysis efficiency and glucose concentration.

2 MATERIAL & METHODS

The Eucalyptus grandis wood was provided by a local industrial plywood plant (LUMIN, Tacuarembó). *E. grandis* wood was chipped and milled and then subjected to steam explosion pretreatment, which was performed in an equipment (Advance Bio Systems LLC, model S1401-D2011) installed at the Pilot Plant of Latitud in the Technological Laboratory of Uruguay (LATU, Montevideo). Steam explosion was performed at 180°C with sulfuric acid impregnation at 0.5% (w/w), for a residence time of 10 min. After pretreatment, the solid and liquid fractions were separated by filter pressing (20 MPa). The chemical composition of the raw material and both liquid and solid fractions after pretreatment was determined following NREL protocols[5,6].

Steam-exploded solids were employed as substrates for enzymatic hydrolysis experiments. A Box-Behnken experimental design with five center points and eight additional points was followed to evaluate the effect of pH, solid content, and enzyme dosage on enzymatic hydrolysis efficiency. The studied factors were solid content (14.6-25.4)% (w/w), enzyme dosage (15-25) FPU/g_{glucan} and pH (4.6-6.3). Enzymatic hydrolysis was carried out in 250 mL Erlenmeyer flasks containing 100 mL of suspension at 50°C with orbital agitation (150 rpm). The cellulase complex Cellic CTec2 was used for the assays, which was purchased from Sigma

Aldrich® (cellulase activity 174 FPU/mL). The pH was fixed with acetic acid-sodium acetate buffer at the desired values. Hydrolysis experiments were stopped at 72 h by inactivating the enzyme at 95°C for 10 min. Then, samples were centrifuged to remove the solid wastes. Glucose, xylose, arabinose and cellobiose analysis in the supernatants was carried out by HPLC.

3 RESULTS & DISCUSSION

Chemical characterization of untreated and steam-pretreated *E. grandis* wood are showed in Table 1. Pretreatment led to the complete removal of extractives and acetyl groups from the solid. The other components were partially removed: xylan 92.9%, glucan 25.3%, and lignin 17.3%.

Table 1: Chemical composition of untreated and steam-exploded *E. grandis* wood.

Sample	Solid yield (%)	Glucan (%)	Xylan (%)	Arabinan (%)	Total lignin (%)	Acetyl groups (%)	Extractives (%)	Ash (%)
Untreated wood	-	48.9 ± 1.3	14.5 ± 1.5	ND*	29.1 ± 0.1	1.8 ± 0.4	3.4 ± 0.0	0.3 ± 0.0
Steam-exploded wood	61.0 ± 0.5	60.3 ± 1.7	1.7 ± 0.1	ND*	39.5 ± 1.9	ND*	-	ND*

**Not detected: concentrations less than 0.1%.*

Table 2: Experimental results obtained for enzymatic hydrolysis at 72 h of steam-explosion pretreated *E. grandis* wood.

Run	Solid content (%)	Enzymatic dosage (FPU/g_{glucan})	pH	Glucose (g/L)	Hydrolysis efficiency* (%)	Run	Solid content (%)	Enzymatic dosage (FPU/g_{glucan})	pH	Glucose (g/L)	Hydrolysis efficiency* (%)
1	20	25	6.0	104.9 ± 1.5	71.0 ± 1.2	14	24	20	6.0	116.3 ± 1.7	66.6 ± 1.1
2	20	20	5.4	89.9 ± 1.3	61.2 ± 1.0	15	24	20	4.8	93.0 ± 1.4	52.0 ± 0.9
3	16	20	6.0	78.4 ± 1.1	67.4 ± 1.1	16	20	20	5.4	88.9 ± 1.3	60.5 ± 1.0
4	20	20	5.4	89.9 ± 1.3	61.3 ± 1.0	17	20	25	4.8	90.0 ± 1.3	59.8 ± 1.0
5	24	15	5.4	94.7 ± 1.4	54.6 ± 0.9	18	15	25	4.8	70.8 ± 1.0	65.0 ± 1.1
6	24	25	5.4	112.5 ± 1.6	62.7 ± 1.0	19	25	25	4.8	112.9 ± 1.6	59.0 ± 1.0
7	20	15	6.0	87.3 ± 1.3	60.8 ± 1.0	20	20	20	6.3	102.1 ± 1.5	70.5 ± 1.2
8	16	20	4.8	65.9 ± 1.0	58.7 ± 1.0	21	20	20	4.6	75.2 ± 1.1	50.3 ± 0.8
9	20	20	5.4	88.2 ± 1.3	59.9 ± 1.0	22	16	15	4.8	60.5 ± 0.9	52.1 ± 0.9
10	16	15	5.4	66.2 ± 1.0	60.6 ± 1.0	23	16	15	6.0	72.5 ± 1.1	63.3 ± 1.0
11	20	15	4.8	75.5 ± 1.1	52.0 ± 0.9	24	24	25	6.0	121.7 ± 1.8	68.4 ± 1.1
12	20	20	5.4	91.6 ± 1.3	62.6 ± 1.0	25	16	15	6.0	71.5 ± 1.0	62.5 ± 1.0
13	16	25	5.4	76.0 ± 1.1	63.5 ± 1.1						

**Hydrolysis efficiency calculated as the ratio between final glucose concentration and theorical glucose concentration obtained from pretreated material.*

The studied factors, conditions, and their respective enzymatic hydrolysis parameters are presented in Table 2. The data obtained were statistically evaluated by multiple (linear) regression analysis. Analysis of variance (ANOVA) at significance level of p = 0.05 was used. Different interactions between pH, solid content and enzyme dosage resulted not significant. In terms of final glucose concentration, all studied factors had a positive impact. Solid content was the most influential factor, followed by enzymatic dosage. Regarding hydrolysis efficiency, solid content had a negative impact while pH and enzyme dosage had a positive effect. Solid content impact could be explained by means of possible inhibition effects when working with high solid concentrations due to limitations in mass transfer and product inhibition. The greatest influence on hydrolysis efficiency was given by pH followed by the enzyme dosage. Considering the high cost of cellulases, an economic analysis should be carried out to evaluate the convenience of obtaining high hydrolysis efficiency at the expense of increasing the enzyme dosage.

The lowest hydrolysis efficiency obtained was 50.3% when working at pH 4.6, 20% solid content and 20 FPU/g_{glucan} enzyme dosage. According to final glucose concentration, the lowest concentration was 60.5 g/L, which was obtained at pH 4.8, 16% solid content and 15 FPU/g_{glucan} enzyme dosage. The highest glucose concentration (116 g/L) was obtained when working at pH 6, 24% solid content and 20 FPU/g_{glucan} enzyme dosage, whereas the highest hydrolysis efficiency (71%) was achieved at pH 6, solid content 20% and enzymatic dosage 25 FPU/g_{glucan}. For both final glucose concentration and hydrolysis efficiency maximization, the optimal factor values resulted of pH 6, 24% solid content and 25 FPU/g_{glucan} enzyme dosage.

4 CONCLUSION

This work allowed to evaluate the effect of pH, solid content, and enzyme dosage on the enzymatic hydrolysis performance of steam-exploded *E. grandis* wood. All studied factors had a significant impact on enzymatic hydrolysis efficiency. Regarding hydrolysis efficiency, pH resulted the most influential variable, followed by enzyme dosage. In contrast, regarding final glucose concentration, solid content resulted the most critical factor, allowing to achieve a maximum concentration of 116 g/L. In terms of maximizing both final glucose concentration and hydrolysis efficiency, the optimal factor values resulted of pH 6, 24% solid content and 25 FPU/g_{glucan} enzyme dosage.

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ACKNOWLEDGEMENTS

Financial support was provided by ANCAP-CIDEB of Uruguay. The authors thank LUMIN Company for providing the *Eucalyptus grandis* wood.