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EFFECT OF HEMICELLULOSE LIQUID PHASE ON THE ENZYMATIC HYDROLYSIS OF AUTOHYDROLYZED *EUCALYPTUS GLOBULUS* WOOD

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Abstract

In this work, *Eucalyptus globulus* wood (EGW) was pretreated under autohydrolysis process at 210 and 220 ºC, obtaining a pretreated solid with high cellulose content. Moreover, the effect of the hemicellulosic liquid phase (HLP) addition on the enzymatic hydrolysis was studied. When the enzymatic hydrolysis was performed without addition of HLP, 87.38 and 107.46 g/L of glucose was obtained for 210 ºC and 220 ºC, respectively, showing that the unwashed pretreated solids are susceptible to the enzymatic hydrolysis contributing to reduce operational cost. Additionally, the impact of the inhibitory compounds in the HLP was shown to affect the enzymatic hydrolysis. However, a positive effect was shown on the enzymatic hydrolysis when the xylanases were added using 100 % of HLP, increasing to 35 % and 29 % in the glucose production respect to whole-slurry without addition of xylanases.

Keywords: Enzymatic hydrolysis, Autohydrolysis, Lignocellulosic material, Hemicellulosic liquid phase

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INTRODUCTION

The biorefinery concept is often considered for the production of fuels (i.e. bioethanol) and chemicals from lignocellulosic materials (Romaní et al., 2011). One promising technology is to convert these lignocellulosic materials to fermentable sugars using enzymes that are applied after a pretreatment. The pretreatment is required to alter the structural and chemical composition, improving the accessibility of the cellulose component to the action of hydrolytic enzymes. Autohydrolysis pretreatment is an eco-friendly process in which the lignocellulosic material is pretreated with water at high temperatures (Romaní et al., 2010). When the lignocellulosic material is pretreated under harsh conditions, the biomass chemical components will degrade into by-products that become inhibitors for the subsequent processing (enzymatic hydrolysis and fermentation). On the other hand, enzymatic hydrolysis that converts lignocellulosic materials to fermentable sugars may be the most complex step in this process due to the effects and interactions between enzyme-substrate. Nowadays, it is relevant to find alternatives in the conversion of these materials, which can significantly reduce capital-operational cost. One way is the use of the whole-slurry or slurries after pretreatment process without washing or detoxification, eliminating the solid-liquid separation step in the pretreatment area and reducing the operational costs. For the all mentioned above, the objective of the present work was to investigate the effect of whole-slurry (100 % HLP plus unwashed pretreated solid), slurries (50 % HLP plus unwashed pretreated solid), unwashed pretreated solid and the addition of xylanases (100 % HLP plus unwashed pretreated solid) on the enzymatic hydrolysis using autohydrolyzed EGW as substrate.

METHODOLOGY

Raw Material

EGW used in this study was kindly provided by a local pulp factory (ENCE, Pontevedra, Spain). The material composition was previously analyzed by Romaní et al. (2012), containing 44.7 % cellulose, 16 % xylan, 1.1 % arabinan, 3 % acetyl groups, 24.7 % Klason lignin, 2.9 % extractives and 0.23 % ash.

Autohydrolysis process

Milled EGW with a particle size of 8 mm and water were mixed in order to obtain a ratio 8:1 liquid/solid and treated in a 3.75 l total volume stainless steel reactor. The reactor was operated in non-isothermal heating regimen at 210 and 220 ºC. At the end of autohydrolysis process, the liquid and solid phases were separated by filtration. The autohydrolysis pretreated solids were not washed and these were used as substrate for enzymatic hydrolysis. The HLP was added at different proportions (Figure 1).

Enzyme

Commercially available enzyme solutions, cellulase (Celluclast 1.5 L), β-glucosidase (Novozyme 188) and endo-1, 4-β-xylanase (Shearzyme 500L), were kindly supplied by Novozymes. The enzyme activities of commercial concentrates were 44.7 FPU/mL for Celluclast 1.5 L, 611.2 UI/mL for Novozyme 188 and 171 UI/mL for Shearzyme 500L. The enzymatic activities were analyzed according to the standard analytical methods (Bailey et al., 1992; Adney and Baker, 1996; Ruiz et al., 2012).

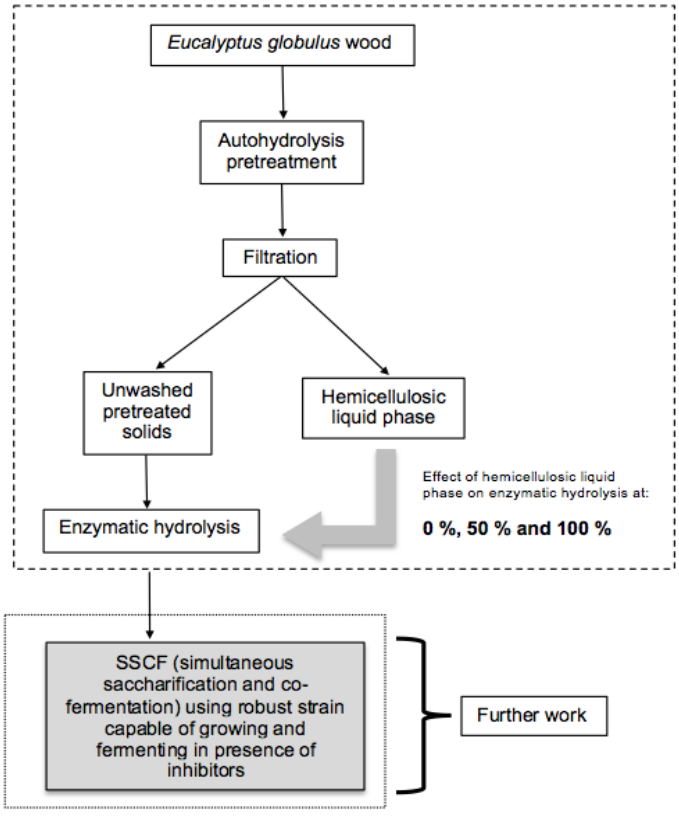

Figure 1. Schematic representation of the *Eucalyptus globulus* wood processing.

Enzymatic hydrolysis

The enzymatic hydrolyses were carried out in 100 mL Erlenmeyer flaks with a working volume of 40 mL at 48.5 ºC in an orbital shaker at 150 rpm (see Table 1). A 50 mM citrated buffer was used to maintain the pH at 4.8 and thymol as microbial preservative. All determinations were performed in duplicate. Samples of 800 µl were withdrawn from the flaks at 0, 2, 5, 9, 24, 48, 72 and 96 h. Samples were immediately centrifuged at 6000 rpm for 6 min. Glucose concentration was determined by HPLC (Ruiz et al., 2012).

RESULTS

Autohydrolysis process

Autohydrolysis caused a substantial fractionation of components including monosaccharides, oligosaccharides, acetyl groups and degradation of sugars as furfural and hydroxymethylfurfural (HMF). The concentrations (g/l) of the liquid phase components derived principally from *Eucalyptus globulus* hemicellulose fractions by autohydrolysis at 210 ºC were 8.97, 8.85, 0.64, 3.91, 0.26, 1.66 and 2.55 for xylooligosaccharides, xylose, glucose, acetic acid, HMF, furfural and acetyl groups, respectively. Meanwhile, at 220 ºC the concentration of the main components were 1.46, 7.14, 1.94, 5.8, 0.95, 4.97 and 0.5 for xylooligosaccharides, xylose, glucose, acetic acid, HMF, furfural and acetyl groups, respectively. The glucan content in the solid residue after of autohydrolysis pretreatment was 58.27 and 60.4 g/100 g of pretreated solid for 210 and 220 ºC, respectively. This reveals that the glucan was almost not affected by the autohydrolysis process with an average recovery of 94.5 % of the glucan present in the wood. This increase could be correlated to the solubilization in the liquid phase of hemicellulose components. Respect to Klason lignin, the content was 38.04 and 34.04 g/100 g of pretreated solid at 210 and 220 ºC, respectively. The results are in agreement with previous studies reported by Romani et al. (2012).

Enzymatic hydrolysis

Different experiments were performed to evaluate the effect of hemicellulosic liquid phase on enzymatic hydrolysis (Table 1). The enzymatic hydrolysis of EGW gave different results according to the different composition of the pretreated material at 210 and 220 ºC. The highest glucose concentration at 210 ºC was 87.38 g/L on the unwashed pretreated EGW without addition of HLP for an enzyme loading of 25 FPU/ g of substrate and the lowest glucose concentration observed was 45.15 g/L using 100 % of HLP. To explain this behavior, Kumar and Wyman (2009) showed that xylooligosaccharides strongly inhibit cellulase action. Hodge et al., (2008), reported that acetic acid, phenolic compounds and furans inhibited enzymatic hydrolysis. Respect to xylanases supplementation, an enzyme loading of 16 and 25 FPU/g of substrate caused a positive effect in the glucose production, increasing from 45.15 g/L to 60.87 g/L and 53.18 g/L to 68.78 g/L, corresponding a increase 35 % and 29 % respectively, respect to whole-slurry without addition of xylanases on the enzymatic hydrolysis. Moreover, non-significant increase in glucose was observed compared with the unwashed pretreated solid. For 220 ºC, the highest glucose concentration was 107.46 g/L using an enzyme loading of 25 FPU/ g of substrate without addition of HLP. As is mentioned above, the lowest glucose concentration was 61.92 g/L with 100 % of HLP.

Table 1. Glucose production after enzymatic hydrolysis using different proportions of hemicellulosic liquid phase and operational conditions.

Operational conditions	210 ºC			220 ºC		
	RES a	RLS b	Glucose (g/L)	RES a	RLS b	Glucose (g/L)
0 % *	16	6	74.38	25	4	107.46
50 % *	16	6	62.16	25	4	89.63
100 % *	16	6	45.15	25	4	81.78
0 % *	25	6	87.38	25	6	90.18
50 % *	25	6	74.08	25	6	71.64
100 % *	25	6	53.18	25	6	61.92
100 % + xylanases **	16	6	60.87	---	---	---
100 % + xylanases **	25	6	68.78	---	---	---

[a] Ratio enzyme-substrate (FPU/g of substrate)

[b] Ratio Liquid-substrate (g/g)

* Percentage of HLP added to the enzymatic hydrolysis

** Percentage of HLP added to the enzymatic hydrolysis plus xylanases (xylanase/cellulase ratio of 4 IU/FPU).

CONCLUSIONS

In summary, our results indicate that the unwashed solid obtained after autohydrolysis pretreatment are susceptible to the enzymatic hydrolysis contributing to reduce operational cost. Moreover, when 100 % of the HLP is added to the enzymatic hydrolysis a strong inhibition was observed. However, a positive effect was shown on the enzymatic hydrolysis when the xylanases were added. Nevertheless additional research is needed to determine the main operational effects on enzymatic hydrolysis using whole-slurry after autohydrolysis pretreatment and in a further work, the use of a robust strain capable of growing and fermenting in the presence of inhibitors.

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