Improving enzymatic hydrolysis of hazelnut shells by alkaline peroxide (APO) pretreatment

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Abstract—Monomeric sugars from lignocellulosic biomass are generally produced by enzymatic hydrolysis. However, an effective pretreatment method is required to remove lignin from biomass and to increase accessibility of the enzymes to cellulose and hemicellulose. The efficiency of alkaline peroxide (APO) pretreatment method on digestibility of hazelnut shells was investigated. The biomass was pretreated with H₂O₂ (0.2%, 2.0% and 4.0%, v/v) in 2% NaOH solution at different temperatures (30-60-90 °C) and pretreatment times (6, 24, 48 h). Considering the percent glucose recovery (81.86%), saccharification (85%) and cellulose digestion (98.74%), the optimum conditions for pretreatment were selected as an alkaline solution containing 2% H₂O₂, at 30°C and 6 h-processing time.

Keywords—Hazelnut shells, pretreatment, alkaline peroxide, enzymatic hydrolysis

I. INTRODUCTION

Lignocellulosic biomasses are richly present on the earth, and used for producing fuel and chemicals [1]. Monomeric sugars from lignocellulosic biomass are generally produced by enzymatic hydrolysis. However, the presence of lignin makes the access of enzymes to cellulose or hemicellulose difficult, thus reducing the efficiency of the hydrolysis. An effective pretreatment method is required to selectively remove lignin from biomass and to increase accessibility of cellulose and hemicellulose for catalytic conversion. Among the various chemical pretreatments, exposing biomass to the combination of NaOH and hydrogen peroxide enhances the depolymerization of lignin by reacting with lignin and related phenolics [2]. Alkaline peroxide (APO) pretreatment was found to be more effective in lignin solubilization and improving enzymatic digestibility of biomass when it is compared to alkali pretreatment. Also it was reported that pretreatment liquid do not include inhibitory sugar degradation products such as furfural or HMF (hydroxy methyl furfural). This aspect is important for production of ethanol via microbial fermentation of pretreated biomass [3].

In this study the effect of alkaline peroxide pretreatment on enzymatic hydrolysis of hazelnut shells was investigated. Different pretreatment parameters (peroxide loading, temperature, and time) were evaluated and optimum conditions were obtained according to the highest glucose recovery, which was based on the initial cellulose content.

II. MATERIAL AND METHOD

A. Material

Hazelnut shells used in this study were purchased from Trabzon, Turkey. The dried materials (9% moisture) were grounded by grinder and screened with a sieve shaker to obtain particle sizes between 0.224-0.850 mm. The Aminex HPX 87P column was purchased from Bio-Rad Laboratories (California, USA). All used chemicals were standard analytical grades. The standard reagents of xylose, glucose, and enzymes were purchased from Sigma-Aldrich.

B. Pretreatment and Enzymatic Digestion of Hazelnut Shells

Dried and milled hazelnut shells were pretreated with H₂O₂ (0%, 2.0% and 4.0%, v/v) in 2% (w/w) NaOH at different temperatures (30-60-90 °C) and pretreatment times (6, 24, 48 h). The solid to liquid ratio was kept at 1/10.

Enzymatic hydrolysis was carried out in stoppered conical flasks (50 mL). The pH was adjusted to 4.8 with acetate buffer, and a mixture of Cellulase (60 FPU/g dry biomass) and β-Glucosidase (40 CBU/g dry biomass) was added to pretreated substrate in a total working volume of 20 ml. The hydrolysis reactions were carried out at 50°C in an incubator for 48 h by shaking at 150 rpm. The reactions were stopped by boiling the flasks in a water bath for 15 minutes and hydrolysates were clarified by centrifuging at 5000 rpm for 5 min. The supernatants were analyzed for glucose and xylose contents using HPLC.

C. Analytical Methods

The chemical composition of raw and pretreated hazelnut shells was determined according to NREL methods [4,5]. 0.3 g solid was hydrolyzed in 3 mL 72% (w/w) H₂SO₄ solution at 30°C for 60 min; then, the reaction mixture was diluted to 4% (w/w) and autoclaved at 121°C for 60 minutes. Upon filtration of the resulting slurry, lignin content was calculated from the remaining solid residue, while cellulose and hemicellulose amounts were determined from filtrate by using High Performance Liquid Chromatography (Agilent 1100). The HPLC system was equipped with a Bio-Rad Aminex HPX-87P
column (300 mm × 7.8 mm) and a refractive index detector. The analytical column was operated at 80°C with 0.2 µm filtered HPLC grade water as the mobile phase. Mobile phase flow rate was 0.6 mL/min [6].

Total reducing sugars (TRS) were determined by the dinitrosalicylic acid (DNS) method [7]. Enzyme activity of Celluclast 1.5L® was determined by NREL protocols and reported as Filter Paper Unit (FPU) [8]. One unit of FPU is defined as the amount of enzyme required to liberate 1 µmol of glucose from Whatman no:1 filter paper per minute at 50°C. One cellobiose unit (CBU) is the amount of enzyme that converts 1 µmol of cellobiose to 2 µmol of glucose per minute.

For the determination of percent glucose digestion, saccharification and glucose recovery the following calculations were performed:

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\text{Cellulose Digestion} \% = \frac{\text{Amount of glucose produced} \times 0.9}{\text{Amount of cellulose in pretreated solid}} \times 100
\]

\[
\text{Saccharification} \% = \frac{\text{Amount of TRS produced} \times 0.9}{\text{Amount of cellulose and hemicellulose in pretreated solid}} \times 100
\]

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\text{Glucose Recovery} \% = \frac{\text{Amount of glucose produced} \times 0.9}{\text{Amount of cellulose in unpretreated solid}} \times 100
\]

**D. Statistical Analysis**

Sampling and analyses were performed in duplicate or triplicate, and the data were presented as mean ± standard deviation. Analysis of variance and comparison of treatment means (LSD, 5% level) were performed using Statgraphics plus 3.1.

**III. RESULTS AND DISCUSSION**

The holocellulose fraction of unpretreated hazelnut shells was 30 % of the dry biomass; this fraction was composed of 16.70% cellulose and 13.30% hemicellulose. Lignin constituted a significant part (51.3%) of the raw material.

**A. Effect of Hydrogen Peroxide Addition**

The impact of hydrogen peroxide addition during the alkaline pretreatment on the digestibility of the biomass was investigated. Upon enzymatic hydrolysis of the solid residue obtained from the pretreatment held at 30°C for 24h, the percent cellulose digestion, saccharification and glucose recoveries were evaluated (Figure 1.) Addition of hydrogen peroxide resulted in a significant improvement of biomass digestibility, when compared to the alkaline pretreatment in the absence of hydrogen peroxide (2% (w/w) NaOH solution).Cellulose digestion increased from 38.46% to 98.74% with addition of 2% H₂O₂. Saccharification, which is related to the release of total reducing sugars from the hydrolysed biomass, was also enhanced. Overall process efficiency (%Glucose recovery) was improved to 81.87 % from 31.96%. Increasing the amount of hydrogen peroxide further to 4 % had no statistically enhancing effect on the enzymatic hydrolysis.

Fig. 1. Effect of H₂O₂ concentration (in 2% (w/w) NaOH solution) on the enzymatic hydrolysis of APO pretreated hazelnut shells at 30 °C for 24 hr. (Values are means ± SD on the basis of dry seed. Different letters a–f within the column show significant differences at p < 0.05).

**B. Effect of pretreatment time at low temperature**

The APO pretreatment with 2% H₂O₂ was conducted at 30°C for 6, 24 and 48h. Extending the pretreatment time at the tested conditions had no statically enhancing effect on % glucose recovery and saccharification of the biomass upon enzymatic hydrolysis (Figure 2). Cellulose digestion was slightly improved by increasing the pretreatment time from 6 h to 24 h, but exposing the biomass to further pretreatment of 48h did not result in a significant enhancement. Therefore, the 6h-processing was selected as a sufficient pretreatment time.

Fig. 2. Effect of pretreatment time on the enzymatic hydrolysis of APO pretreated hazelnut shells with 2%H₂O₂ in 2%NaOH at 30°C. (Values are means ± SD on the basis of dry seed. Different letters a–f within the column show significant differences at p < 0.05)
C. Effect of pretreatment temperature

APO pretreatment with 2% H₂O₂ for 6h was conducted at 30, 60 and 90°C. Increasing pretreatment temperature had no significant effect on cellulose digestion and saccharification of the biomass (Figure 3). However, glucose recovery decreased from 81.25% at 30°C to 67.61% at 90°C. Since the solid residues from the pretreatment slurry were subjected to the enzymatic hydrolysis, this decrease in glucose recovery was possibly due to the increase of solubility of cellulose during the pretreatment operation. Because of the dissolution of cellulose, the solid residue recovered after the pretreatment was poor in terms of cellulose content, which in turn resulted in a significant decrease of glucose recovery from the biomass. Considering the results and the energy requirements of the process, pretreating the biomass at 30°C was the optimal condition for maximum recovery of monomeric sugars from hazelnut shells.

Comparing the total reducing sugars, glucose and xylose amounts obtained upon enzymatic hydrolysis of unpretreated and APO-pretreated hazelnut shells, the improving effect of the pretreatment method was clearly seen. As it is illustrated on Figure 4, the release of monomeric sugars was enhanced significantly by prior processing of the biomass.

CONCLUSION

The complex composition and structure of lignocellulosic biomass requires an effective pretreatment method prior to hydrolysis of this rich raw material for production of monomeric sugars. From the various pretreatment techniques, alkaline pretreatment was found to be effective in removal of lignin; thus facilitating the access of enzymes to the hydrolysable holocellulose component of the biomass. In the present study, the efficiency of alkaline pretreatment was enhanced by addition of hydrogen peroxide, which increased the monomeric sugar recovery from hazelnut shells. The effects of concentration of peroxide, pretreatment time and temperature were assessed. The optimum operation conditions were selected as APO pretreatment with 2% H₂O₂ at 30°C for 6 h, where 81.25 % glucose recovery, 94.54% saccharification and 90.36% cellulose digestion were achieved. This relatively low-temperature, low-H₂O₂-loading and short pretreatment method is energetically feasible and provides a cheap and an efficient pretreatment choice for effective conversion of biodegradable biomass to valuable monomeric sugars for further usage.

REFERENCES

