

Effect of Temperature and Time on the Steam Pretreatment of Hazelnut Shells for the Enzymatic Saccharification

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Saturated steam pretreatment of hazelnut shells was studied for fermentable sugars after enzymatic hydrolysis. The elevation of temperature (from 160 to 200 °C) and time (0–30 min) of steam pretreatment were investigated. Hemicellulose removal (%) gradually increased with increasing pretreatment temperature and time. 83 % of initial hemicellulose was removed when the biomass was treated at 200 °C for 30 min. Maximum cellulose digestibility (46%) was obtained at the conditions of 180 °C for 30 min (46 %). Glucose recovery ranged from 337 to 456 mg/ g initial solid.

1. Introduction

Lignocellulosic biomass is a potential raw material for ethanol production. Fermentation process for ethanol production requires the hydrolysis of cellulose and hemicelluloses of lignocellulosic biomass into their corresponding monomeric carbohydrates. The hydrolysis is usually catalyzed by cellulase enzymes (Kravanja and Friedl, 2010). However, the presence of lignin and hemicellulose makes the access of cellulase enzymes to cellulose difficult, thus reducing the efficiency of the hydrolysis. Therefore, an effective pretreatment step is required to disrupt the structure of lignocellulosic structure, to increase accessible surface area and porosity (Kumar and Wyman, 2009). A large number of pretreatment processes have been proposed, such as dilute-acid hydrolysis (Dagnino et al., 2013), alkaline hydrolysis (Chen et al., 2009, Wu et al., 2009), liquid hot water pretreatment (Hongdan et al., 2013), and biological process (Sanchez, 2009).

Pretreatment of biomass with hot water or saturated steam, which is known as autohydrolysis, is a process that treats lignocellulosic materials with water as a chemical free media. This method is a simple, low cost environmental friendly technology for generation of sugars from agricultural and municipal wastes (Yoon 1998). Pretreatment with hot water or saturated steam has been shown to remove up most of hemicellulose and to provide the alterations in the structure of the lignocellulose, which make the cellulose more accessible for enzyme (Mosier et al., 2005). Previous research by autohydrolysis mainly concentrated on bamboo (Xiao et al., 2013), Bermuda grass (Lee et al., 2009), and sugarcane bagasse (Hongdan et al., 2013).

Hazelnut shell is an important biomass of Turkey because two-third of World hazelnut production is from Black-Sea region of Turkey. Nowadays, hazelnut shells have been directly combusted in the boilers for heat recovery. They have been studied such as adsorbent (Dogan et al., 2009), hydrogen production (Midillia et al, 2001), and pyrolysis (Caglar and Aydinli, 2009). Sugar production from hazelnut hemicellulose were also investigated by acidic hydrolysis at different acid concentrations and temperatures (Arslan et al., 2012). However, to our knowledge, there are no reports on enzymatic hydrolysis of hazelnut shells.

The aim of this study was to investigate the effect of saturated steam parameters on the hemicellulose removal and enzymatic hydrolysis of hazelnut shells.

2. Material and Methods

2.1 Material

Hazelnut shells used in this study were purchased from Beşikdüzü, Trabzon, Turkey. Raw materials were air dried until 9% moisture. The dried materials were grounded by grinder and screened with a sieve shaker to obtain particle sizes between 0.224-0.850 mm. Samples were stored in plastic bags at +4 °C for future use. Celluclast 1.5 L and Novozyme 188 were purchased from Sigma Aldrich (St. Louis, USA). Aminex HPX 87P column was purchased from Bio-Rad Laboratories (California, USA). All chemicals used were standard analytical grades.

2.2 Pretreatment and enzymatic hydrolysis of hazelnut shells

Saturated steam treatment of hazelnut shells was performed in a PARR stainless steel reactor at 160°C, 180 °C and 200 °C, for 15 min and 30 min. Approximately 7 g of dry hazelnut shells were mixed with 70 mL of water in a Teflon liner. The vessel was heated until desired temperature and pretreatment time was initiated. After treatment the reactor vessel was moved from heating jacket. The content of the reactor was cooled down to 80 °C. The pretreated solid was used as the substrate for enzymatic hydrolysis.

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 for 48 h by shaking at 150 rpm. The reactions were stopped in a boiling water bath for 15 min and hydrolysates were clarified by centrifuging at 5,000 rpm for 5 min. The supernatants were analyzed for glucose and xylose using HPLC. Concentration of reducing sugar was determined by DNS method (Miller, 1959).

2.3 Analytical methods

The chemical composition of raw and pretreated hazelnut shells were determined according to NREL (Sluiter et al., 2008a., 2008b) methods. 0.3 g solid was hydrolyzed by 3 mL of 72 % (w/w) H₂SO₄ at 30 °C for 60 min then, the reaction mixture was diluted to 4 % (w/w) and autoclaved at 121 °C for 60 min. Lignin was determined by solid residue, cellulose and hemicellulose amount were determined from filtrate by using High Performance Liquid Chromatography (Agilent 1100). The HPLC system was mainly 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.

Enzyme activity of Celluclast 1.5L® was determined by NREL protocols and reported as Filter Paper Unit (FPU) (Adney and Baker, 2008). One unit of FPU is defined as the amount of enzyme required to liberate 1μmol of glucose from Whatman no:1 filter paper per minute at 50 °C. One cellobiose unit (CBU) is the amount of enzyme that converts 1mmol of cellobiose to 2 mmol of glucose per minute. Hemicellulose removal (%) (Eq.1), Cellulose digestion (%) (Eq.2), saccharification yield (%) (Eq.3) are calculated as follows;

$$\text{Hemicellulose removal (\%)} = \left(100 - \frac{\text{Amount hemicellulose in pretreated solid}}{\text{Amount of hemicellulose in initial solid}}\right) \times 100 \quad (1)$$

$$\text{Cellulose Digestion (\%)} = \frac{\text{Amount of glucose produced} \times 0.9}{\text{Amount of cellulose in pretreated solid}} \times 100 \quad (2)$$

$$\text{Glucose Recovery (mg/g)} = \frac{\text{Amount of glucose produced} \times 0.9}{\text{Amount of cellulose in unpretreated solid}} \times 100 \quad (3)$$

3. Results and discussions

3.1 Composition of hazelnut shell

Figure 1 showed to composition of dried hazelnut shells. Lignin fraction was the main content (51.3 %) of total raw material. Hazelnut shells were consisted 16.7 % cellulose and 13.3 % hemicellulose.

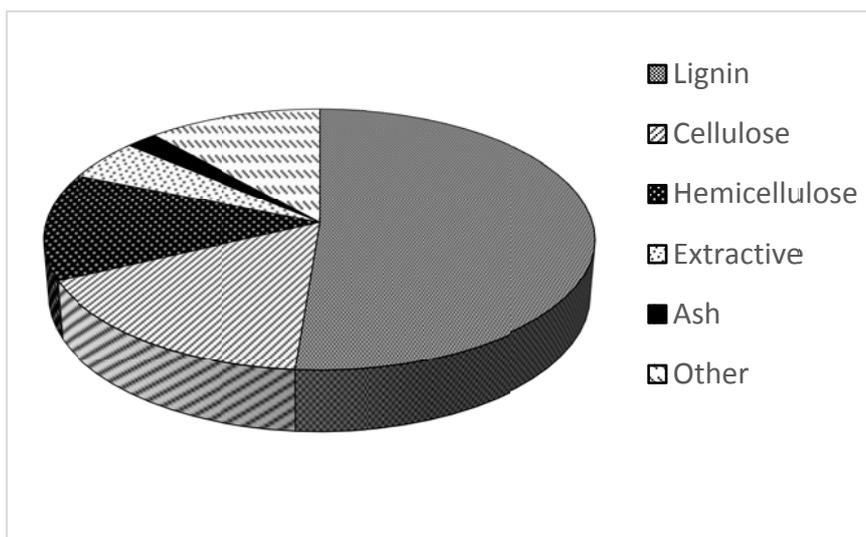


Figure 1. Composition of hazelnut shells

3.2 Effect of pretreatment parameters on hemicellulose removal.

The effect of temperature (160–200 °C) and pretreatment time (0–30 min) of saturated steam pretreatment were investigated. Effect of pretreatment parameters on hemicellulose removal was showed in Figure 2. Hemicellulose removal ranged from 37 % to 83 %. Removal gradually increased with increasing temperature and pretreatment time. When temperature increased from 160 °C to 200 °C for 30 min, hemicellulose removal rate was increased almost 1.5 fold. However differences was only 7 % on the hemicellulose removal values between 180 °C and 200 °C for same pretreatment time. At 100°C effect of pretreatment time was higher than that obtained from 200 °C. These results are consistent with those of previous studies and suggest that autohydrolysis predominantly affects hemicellulose (Fernandez-Bolanos et al., 2001; Kabel et al., 2007). During autohydrolysis, hemicelluloses are depolymerized and converted into oligomers (Lee et al., 2009).

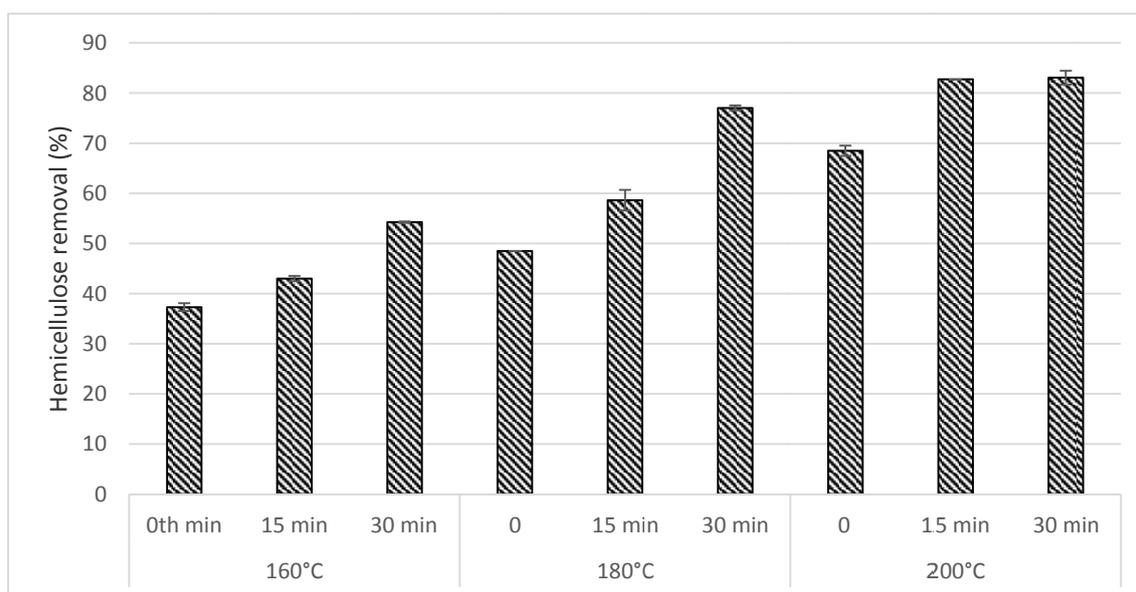


Figure 2 Effect of pretreatment conditions on hemicellulose removal

3.3 Effect of pretreatment parameters on the cellulose digestibility

The saturated steam pretreated hazelnut shells were enzymatic hydrolyzed to generate monosaccharides used for fermentation processes. The application of autohydrolysis can significantly reduce nonspecific binding of cellulase by lignin to enhance enzymatic hydrolysis of lignocellulosic substrates. Results of

cellulose digestion (%) was presented in Figure 3. Maximum cellulose digestibility was obtained at the conditions of 180 °C for 30 min (46 %).

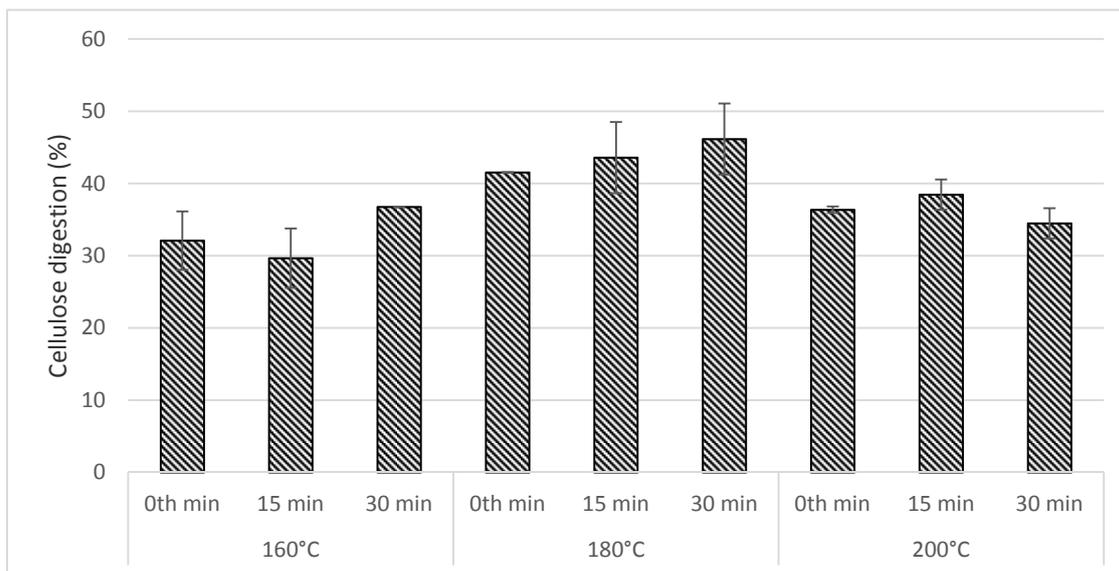


Figure 3. Effect of processing parameters on the cellulose digestion of pretreated hazelnut shells

Total reducing sugar, glucose and xylose produced by enzymatic hydrolysis of raw material was only 45, 15 and 1 mg/g untreated solid, respectively (Figure 4). Fermentable sugar concentration was increased with increase in temperature as well as pretreatment time. Total reducing sugar from enzymatic hydrolysis was ranged between 119 to 177 mg/g substrate. Maximum reducing sugar was obtained at 200 °C for 30 min. Produced glucose was varied between 63 and 91 mg/g substrate. Higher glucose content was obtained at 200°C. Glucose recovery ranged from 337 to 456 mg/ g initial solid (Figure 5).



Figure 4. Fermentable sugar from enzymatic hydrolysis of pretreated hazelnut shells

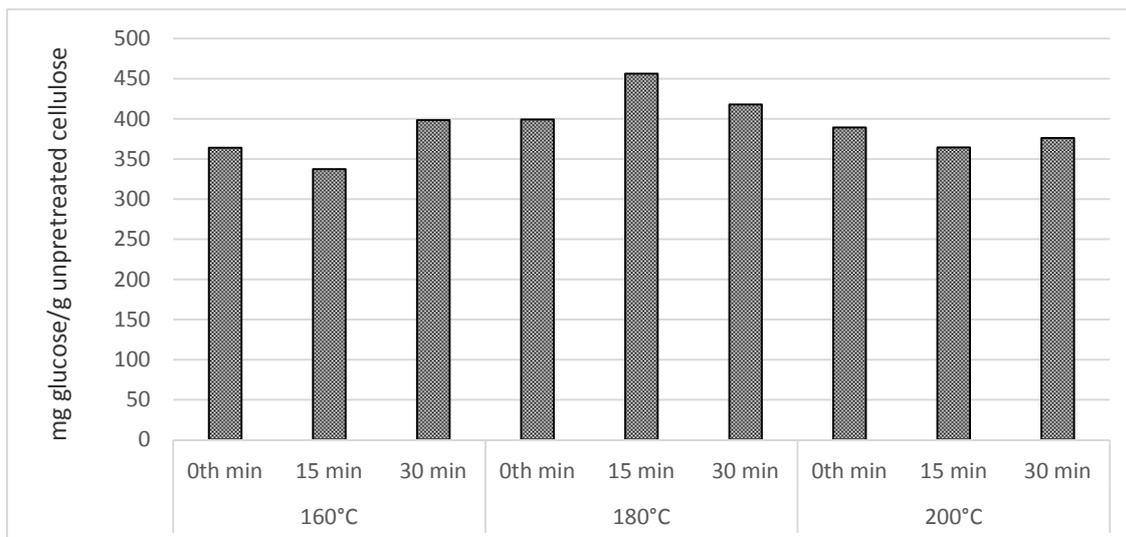


Figure 5. Glucose recovery enzymatic hydrolysis of pretreated hazelnut shells

4. Conclusion

In response saturated steam pretreatment of hazelnut shells, the maximum hemicellulose removal (83%) observed at the pretreatment conditions of 200°C for 30 min. Maximum cellulose digestibility (46%) is found at the conditions of 180°C for 30 min. Saturated steam pretreatment allowed to produce around 177 mg/ g substrate of fermentable sugar. After 15 min pretreatment at 180°C, around 46% of glucose recovered. The results obtained in this study was promising for the production of fermentable sugar after the saturated steam pretreatment of hazelnut shells. However, further investigations are needed to increase sugar yield, and separation lignin and hemicellulose rate.

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