High Boiling Solvent Pre-treatment of Hazelnut Shells for Enzymatic Hydrolysis

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In this study, effect of different high-boiling-organic solvent (ethanolamine, diethylene glycol and ethylene glycol) pre-treatments on the chemical composition and enzymatic saccharification of hazelnut shells was investigated. Results showed that, ethanolamine was the most effective solvent for lignin removal (72% at 150°C), followed by diethylene glycol (35%) and ethylene glycol (30%). The temperature effect with ethylene glycol and diethylene glycol pre-treatment on lignin removal was higher than that of ethanolamine. Cellulose digestion varied between 32% and 57%. The maximum glucose recovery (378.61 mg glucose/g non-pre-treated cellulose) was obtained when hazelnut shells were pre-treated at 150°C for 60 minutes (min) with ethylene glycol solvent. Thus, ethylene glycol pre-treatment can be applied for efficient enzymatic hydrolysis of hazelnut shells for ethanol production.

Keywords: Hazelnut shells, Organic solvents, Pre-treatment.

1. Introduction

The utilization of lignocellulose materials for bioethanol production requires an effective pre-treatment step to disrupt the lignocellulose structure and increase the accessible surface area and porosity. A range of chemical, physical and biological processes has been configured to release constituent sugars from lignocellulose [1]. Among the pre-treatment technologies, organosolv pre-treatment is a promising method to enhance the recovery of glucose by improving digestibility. Organosolv processes use either low-boiling solvents (e.g., ethanol, methanol) or high-boiling solvents (e.g., ethylene glycol, ethanolamine) [2].

Ethylene glycol (EG) is bulk commodity chemical, which has low toxicity and high boiling points (197°C). EG is a good delignification solvent especially in the presence of acid catalysts. Pre-treatment with EG alone reported to improve glucan digestibility (65%) significantly compared to untreated bagasse. Pre-treatment of bagasse at 90°C by the EC (ethylene carbonate)–EG system recovered the majority of the


glucan component and removed the most of xylan and lignin components \[2\].

Ethanolamin was used as organasolv in the ionic liquid (1-ethyl-3-methylimidazolium acetate) for the pretreatment of corncob and rice straw. Pretreatment with EMIM-AC/ethanolamine was improved the sugar conversion and lignin was removed efficiently. Pretreatment with EMIM-AC/ethanolamine solution Comparing with untreated biomasses increased sugar conversion 3-5 fold \[3\].

The aim of the study was to investigate effect of different high-boiling-organic solvent (ethanolamine, diethylene glycol and ethylene glycol) pre-treatments on the chemical composition and enzymatic saccharification of hazelnut shells.

2. Material and Methods

2.1. Material

The hazelnut shells used in this study were purchased from Beşikdüzü, Trabzon, Turkey. Raw materials were air-dried down to 9% moisture. The dried materials were ground 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 Novozym 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. Pre-treatment and enzymatic digestion of hazelnut shells

High-boiling solvent treatment of hazelnut shells was performed in a PARR stainless steel reactor at 120°C and 150°C for 60 min. Approximately 7 grams of dry hazelnut shells were mixed with 70 mL of solvent (ethanolamine, diethylene glycol and ethylene glycol) in a Teflon liner. The vessel was heated to the desired temperature and pre-treatment time was initiated. After treatment, the reactor vessel was moved from the heating jacket. The content of the reactor was cooled down to 80°C. The pre-treated 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 cellulose (60 FPU/g dry biomass) and β-Glucosidase (40 CBU/g dry biomass) was added to the pre-treated 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 in a boiling water bath for 15 min and hydrolysates were clarified by centrifuging at 5000 rpm for 5 min. The supernatants were analysed for glucose and xylose using HPLC. The concentration of reduced sugar was determined by DNS method \[4\].

2.3. Analytical methods

The chemical composition of raw and pre-treated hazelnut shells were determined according to the NREL \[5,6\] methods. 0.3 g solid was hydrolyzed by 3 mL of 72% (w/w) H\(_2\)SO\(_4\) at 30°C for 60 minute, then the reaction mixture was diluted to 4% (w/w) and autoclaved at 121°C for 60 min. Lignin was determined by solid residue, the cellulose and hemi cellulose amounts 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. The mobile phase flow rate was 0.6 mL/min. The enzyme activity of Celluclast 1.5L® was determined by National Renewable Energy Laboratory (NREL)


This study showed that ethanolamine was the most effective solvent for cellulose recovery from hazelnut shells (Figure 2). Pre-treatment temperature had no effect on cellulose recovery.

Figure 3 shows the effects of the organic solvent type on delignification. The best result for lignin removal was obtained from pre-treatment of hazelnut shells by an ethanolamine solution during which more than a rate of 72% lignin removal was achieved within 60 min at 150°C.

3. Results and discussion

Composition of hazelnut shells

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

![Figure 1. Composition of hazelnut shells](image)

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![Figure 2. Effects of pre-treatment temperature and pre-treatment solvent on cellulose recovery (%) of hazelnut shells pre-treated for 60 min.](image)

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The protocols and reported as Filter Paper Unit (FPU) [7]. 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. Hemicellulose removal (%), cellulose digestion (%), saccharification yield (%).

\[
Lignin \text{ removal} \% = \left( 100 - \frac{\text{Amount of lignin in pre} - \text{treated solid}}{\text{Amount of lignin in initial solid}} \right) \times 100
\]

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

\[
\text{Saccharification} \% = \frac{\text{Amount of total reducing sugar produced} \times 0.9}{\text{Amount of cellulose and hemicellulose in pre} - \text{treated solid}} \times 100
\]

\[
\text{Glucose Recovery} \ (\text{mg/g}) = \frac{\text{Amount of glucose produced} \times 0.9}{\text{Amount of cellulose in unpre} - \text{treated solid}} \times 100
\]

Pre-treatment with an ethanolamine solution resulted in lower cellulose digestion, although ethylene glycol and diethylene glycol produced higher cellulose digestion. Cellulose digestion (Figure 6) increased from a value of 34.72% when the pre-treatment was carried out with ethanolamine (150°C), to 57.81% at 150°C with ethylene glycol.

Glucose recovery which is expressed on produced glucose over non-pre-treated cellulose is illustrated on Figure 7. The pre-treatment temperature for ethanolamine pre-treatment had no effect on glucose recovery. Figure 7 also shows that glucose recovery can be improved by increasing the temperature with ethylene glycol, while diethylene glycol had the opposite effect.
Conclusion

This study showed that ethanolamine was the most effective solvent for lignin removal (72% at 150°C), followed by diethylene glycol (35%) and ethylene glycol (30%). The maximum glucose recovery (378.61 mg glucose/g non-pre-treated cellulose) was obtained when hazelnut shells were pre-treated at 150°C for 60 min with ethylene glycol solvent. Ethylene glycol or diethylene glycol might be used for the pre-treatment of hazelnut shells to improve enzymatic hydrolysis of hazelnut shells for ethanol production.