Bioresource Technology 129 (2013) 182–190

Contents lists available at SciVerse ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Evaluating pretreatment techniques for converting hazelnut husks to bioethanol

Yalçın Çöpür [a,*], Ayhan Tozluoglu [a], Melek Özkan [b]

[a] Düzce Üniversitesi Orman Fakültesi, Orman Endüstri Mühendisliği Bölümü, Konuralp Yerleşkesi, 81000 Düzce, Turkey
[b] Gebze Yüksek Teknoloji Enstitüsü, Çevre Mühendisliği Bölümü, Muallimköy Kampüsü, 41400 Gebze, Turkey

HIGHLIGHTS

- $NaBH_4$ delignified the highest amount of lignin (49.1%) from the husk structure.
- NaOH treated husk resulted in the highest xylan solubility (77.9%).
- NaOH treated husk had the highest glucan to glucose conversion (74.4%).
- NaOH treated husk the highest ethanol yield (52.6 g/kg husks).

ARTICLE INFO

Article history:
Received 10 September 2012
Received in revised form 9 November 2012
Accepted 10 November 2012
Available online 24 November 2012

Keywords:
Bioethanol
Enzyme
Hazelnut husk
Hydrolysis
Pretreatment

ABSTRACT

This study examined the suitability of husk waste for bioethanol production and compared pretreatment techniques with regard to their efficiencies. Results showed that 4% $NaBH_4$ (90 min) delignified the highest amount of lignin (49.1%) from the structure. The highest xylan solubility (77.9%) was observed when samples were treated with 4% NaOH for 90 min. Pretreatment with NaOH and $NaBH_4$, compared to H_2O_2 and H_2SO_4, resulted in selective delignification. The highest glucan to glucose conversion (74.4%) and the highest ethanol yield (52.6 g/kg husks) were observed for samples treated with 2% NaOH for 90 min.

1. Introduction

Almost 70% of the world's hazelnuts are grown in Turkey, which makes it a significant hazelnut producer. Based on this production, the amount of husk waste is estimated to be 200,000 ton/year (Midilli et al., 2000). This abundant agricultural waste has had no economic value to date and is usually burned in the fields, causing air pollution and soil erosion. In addition, the burning decreases the biological activity of the soil (Arslan and Saracoglu, 2010). Any possible industrial usage of hazelnut husks can be expected to yield economic as well as environmental dividends. The literature on using husk waste in industrial applications has been very limited. In earlier studies, the possible usage of husk waste in particleboard (Copur et al., 2007) and medium-density fiberboard (Copur et al., 2008) productions was examined. The usage of several agricultural residues in bioethanol production has been studied (Balat et al., 2008). On the other hand, no known effort has been made to utilize hazelnut husks as a resource for bioethanol production.

Like woody biomass, husk structure consists mainly of cellulose, hemicelluloses and lignin. Separation of individual lignocellulosic biomass components, such as cellulose or lignin, can increase their value dramatically. Cellulose, if not combined with lignin, can be converted into the biofuel ethanol through hydrolysis and subsequent fermentation (Lynam et al., 2012).

Therefore, several pretreatment techniques have been used in the process for efficient conversion of the structural carbohydrates to fermentable sugars. However, these applications remove some carbohydrates from the structure. Physical, physico-chemical, chemical and biological pretreatment methods have been utilized (Olsson and Hahn-Hägerdal, 1996), and all these techniques are expected to improve the efficiency of cellulose accessibility of hydrolytic enzymes. In addition, the ideal technique has to minimize the formation of degradation products because of their inhibitory effects on subsequent hydrolysis and fermentation processes. Ongoing studies seek to improve the efficiency of pretreatments by increasing the conversion rate for more economical ethanol production.

Several chemicals, including acids, alkalis, organic solvents, etc., are utilized in the pretreatment step of bioethanol production

* Corresponding author. Tel./fax: +90 380 5421137.
E-mail address: yalcincopur@duzce.edu.tr (Y. Çöpür).

0960-8524/$ - see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2012.11.058

(Hendriks and Zeeman, 2009). On the other hand, literature is very limited on the use of sodium borohydride ($NaBH_4$) as a pretreatment chemical. It is known that $NaBH_4$ degrades lignin, while preventing peeling reactions and thus carbohydrate degradation, which improves the pulping selectivity (Copur et al., 2012). In an earlier study, the selective capability of $NaBH_4$ as a pretreatment chemical was examined, and results showed that it is as effective as sodium hydroxide (NaOH) for wheat straw (Copur et al., 2012). In this study, $NaBH_4$ was also tested in the chemical pretreatment step for husks, which have higher lignin and lower carbohydrate compared to woody biomass. The efficiency of the conventional chemicals NaOH, sulfuric acid (H_2SO_4) and hydrogen peroxide (H_2O_2) was also compared with $NaBH_4$ in this study.

Using hazelnut shells as a renewable and low-cost lignocellulosic material for bioethanol production was investigated by Arslan and Saracoglu (2010). They were able to achieve a good fermentability when the lignin content of the shells was removed by treatment with 3% NaOH before the hydrolysis step. The ethanol yield was 84 g ethanol/kg of hazelnut shells. The objective of this present work was to examine the usage of hazelnut husks for bioethanol production and the effect of $NaBH_4$ use in the chemical pretreatment step.

2. Methods

2.1. Biomass

Hazelnut husk samples (10–20 cm) were collected locally (Duzce Province, Turkey) directly after harvest. The moisture content was determined and the samples were stored in plastic bags at −5 °C.

2.2. Methods

The raw samples were first steam exploded and then pretreated with NaOH, H_2SO_4, H_2O_2 and $NaBH_4$. The treated samples were enzymatically hydrolyzed and fermented to produce ethanol.

2.2.1. Pretreatments

2.2.1.1. Steam explosion. The steam explosion (SE) was carried out in a 20 L reaction vessel. Samples of 1000 g (oven dry-o.d.) were treated for 5 min at 198–200 °C (15 bar). The steam-exploded samples were filtrated using 200-mesh wire screen, and the liquid and solid parts were separately collected from each. Extractives, holocellulose, ash, acid soluble/insoluble lignin and sugar contents were determined in the solid material prior to chemical treatment.

2.2.1.2. Chemical pretreatments. The steam-exploded solid samples of 40 g (o.d.) each were chemically treated using NaOH, H_2SO_4, H_2O_2 and $NaBH_4$. The treatments were made at 0.5, 2 and 4% (w/v) concentrations. The solid loading applied was 10% (w/v). Duplicate samples were treated at 121 °C (15 psi) for residence times of 30, 60 and 90 min with each raw material. After treatment, the liquid part was filtrated and the solid part was stored in sealed plastic bags at 4 °C for enzymatic hydrolysis. Treatment yield, acid soluble/insoluble lignin and sugar contents were determined in each of the solid samples. The optimum chemical pretreatments for further enzymatic hydrolysis were determined based on the highest ratio of glucan and lignin.

2.2.2. Enzymatic hydrolysis

The enzymatic hydrolysis was accomplished on 5 g (o.d.) chemically-treated samples using a mixture (50% v/v) of *Celluclast 1.5 L* (700 U/g) and *Cellobiase* (*Novozym 188*) (250 U/g). Hydrolysis was carried out at 5% solid loading in 100 ml of 50 mM sodium acetate buffer at pH 5.0. In addition, sodium azide (NaN_3, 0.0001 M) was used in this study to prevent microbial contamination. The enzyme reaction was performed in a rotary shaker at 42 °C for 100 rpm. Samples of 1.5 ml were taken at 0, 6, 24, 48, and 72 h. The samples taken were first put into boiling water for 10 min to stop the enzymatic activity. Then, the samples were centrifuged at 10,000 rpm for 5 min. The glucose and xylose contents of the samples were determined.

2.2.3. Fermentation of hydrolyzates

Hydrolyzates were centrifuged at 5000 rpm for 10 min. Twenty milliliters samples of the supernatant were transferred to 100 ml serum bottles for fermentation and 5 g/L of yeast extract, 3.75 g/L of $(NH_4)_2SO_4$, 2.1 g/L of K_2HPO_4, 0.375 g/L of $MgSO_4\ 7H_2O$, and 0.5 g/L of $CaCl_2\ 2H_2O$ were added to the mixture. For fermentation, 5% (v/v) *Saccharomyces cerevisiae ATCC 26602* from overnight cultures was also added and the samples were incubated in an orbital shaker at 100 rpm for 72 h at 30 °C. Periodically taken samples (0, 6, 24, 48, and 72 h) were centrifuged at 10,000 rpm for 10 min and then the supernatants were filtered through 0.45 μm pore-sized filters and stored at −20 °C until HPLC analysis.

2.2.4. Analytical methods

The yield of the samples was determined by gravimetric measurements. The chemical composition of the samples was obtained using appropriate methods: hot and cold water (Tappi T 207 om-88), 1% NaOH (Tappi T 212 om-88) solubility, extractives content (Tappi T 204 om-88), ash (Tappi T 211 om-85) and holocellulose (Wise, 1952).

Laboratory Analytical Procedures (LAP) from the NREL (Sluiter et al., 2004) was used to determine sugar and lignin contents of the samples. The sugar contents were determined by utilizing HPLC (Agilent 1200 system) equipped with Shodex 1011 column (mobile phase: 5 mM H_2SO_4, flow rate: 0.5 ml/min, column temperature: 60 °C) and the refractive index detector. The acid-insoluble lignin was obtained by weighing the solid samples. The acid-soluble lignin was analyzed at the adsorption of 320 nm against blank deionized water.

The percentage of solids recovered was calculated on an oven-dry basis as follows:

$$\text{The percentage of solids recovered} = \left(\frac{W_2}{W_1}\right) \times 100$$

W_1 is the dry weight of the sample before pretreatment (g); W_2 is the dry weight of the treated sample (g).

The reduction in lignin was calculated regarding the initial dry weight of lignin in the untreated material (LU) and the dry weight of lignin in the remaining solids after treatment (LP). The percentage of lignin reduction was calculated with the following equation:

$$\text{The percentage of lignin reduction} = \left(\frac{\text{LU} - \text{LP}}{\text{LU}}\right) \times 100$$

In addition, the solubilization of glucan and xylan in the pretreated samples was calculated in the same manner by substituting the appropriate percentage for glucan and xylan.

The percentage of glucan conversion in enzymatically hydrolyzed samples was calculated as follows:

$$\text{The \% of glucan conversion} = \left(\frac{\%\ \text{GH}}{\%\ \text{GP}}\right) \times 100$$

The %GH is the dry weight percentage of glucose in the enzyme hydrolysis supernatants and the %GP is the dry weight percentage of glucose in the treated samples. The conversion of xylan during enzymatic hydrolysis was also calculated in the same manner by substituting the appropriate percentage for xylan.