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Full Length Article

Ethanol production from hazelnut shells through enzymatic saccharification and fermentation by low-temperature alkali pretreatment

Emir Zafer Hoşgün [a], Derya Berikten [b], Merih Kıvanç [b], Berrin Bozan [a,*]

[a] Anadolu University, Engineering Faculty, Chemical Engineering Department, Eskisehir, Turkey
[b] Anadolu University, Faculty of Science, Biology Department, Eskisehir, Turkey

HIGHLIGHTS

- Hazelnut shells pretreated with NaOH at low temperature for long pretreatment time.
- Effect of pretreatment conditions on the glucose recovery and ethanol production is studied.
- An energy balance is established and thermal energy consumption and pretreatment energy efficiency are determined.

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ABSTRACT

Low-temperature sodium hydroxide (NaOH) pretreatment, enzymatic saccharification and ethanol fermentation were evaluated for hazelnut shell cellulose to fermentable sugars and ethanol. Maximum glucose recovery (48.33 g/100 g cellulose) was achieved with 6% NaOH for 72 h and at 1/10 solid/liquid ratio. At these conditions, 41.18% of lignin was removed. The theoretical ethanol yield (ethanol produced/potential glucose in biomass) was 40.71%, overall process efficiency was 37.73 g/kg biomass, ethanol productivity and fermentation efficiency were 0.115 g/(L h) and 96.7%, respectively. Total energy consumed for the low-temperature–long-residence-time alkali process was 34.8 MJ/kg untreated hazelnut shells. Pretreatment time has a significant effect on thermal energy consumption for the low-temperature alkali process.

1. Introduction

Because of the possible short-term shortage of nonrenewable fossil fuels like oil, coal and natural gas, the search for new alternative and sustainable resources becomes more important each day [1]. Biomass-based ethanol is one of the best alternative fuels for the transportation sector. Ethanol has a higher octane rating than gasoline and produces fewer emissions, therefore being widely recognized as a substitute and/or additive to gasoline [2]. Most of the fuel ethanol produced in the world is currently sourced from starchy biomass or sucrose (molasses or cane juice). However, because starchy biomass is also a food source, its conversion for energy purposes will result in food versus fuel competition. Furthermore, agricultural production of starchy biomass is too small in comparison with the anticipated levels of production required for total conversion of transportation fuel markets from gasoline to ethanol [3]. Therefore, ethanol from lignocellulosic biomass as an alternative fuel has the potential to become a viable energy source in the near future [4].

Lignocellulosic biomass, such as wood, energy crops and agro-industrial residues is the most abundant renewable resource on the Earth and requires less agricultural inputs for its production [4]. Lignocellulose is a heterogeneous polymeric material comprised of cellulose, hemicellulose and lignin. Cellulose and hemicellulose are polysaccharides that can be used for ethanol production. Lignin is a complex aromatic polymer that leads to a protective barrier against plant cell destruction by fungi and bacteria for conversion to fuel [5]. The basic process steps in producing bioethanol from lignocellulosic materials are pretreatment of

* Corresponding author.
E-mail addresses: ezhosgun@anadolu.edu.tr (E.Z. Hoşgün), derya.berikten@gmail.com (D. Berikten), mkivanc@anadolu.edu.tr (M. Kıvanç), bbozan@anadolu.edu.tr (B. Bozan).

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feedstock, hydrolysis of cellulose and hemicellulose, sugar fermentation and product separation [6,7]. The fermentation process for ethanol production requires the hydrolysis of cellulose of lignocellulosic biomass into their corresponding monomeric carbohydrates. Hydrolysis is usually catalyzed by cellulase. However, the presence of lignin and hemicellulose makes the access of cellulase to cellulose difficult, thus reducing the efficiency of the hydrolysis. Therefore, an effective pretreatment method is required to increase accessibility to cellulose and hemicellulose and to remove lignin from the biomass [6,7].

Although a range of chemical, physical and biological pretreatment processes have been used to release constituent sugars from lignocellulose, these processes suffer from several shortcomings, such as high energy consumption, low conversion efficiency and technological impasses [8]. The selection of an appropriate pretreatment process plays an important role in increasing the efficiency of enzymatic saccharification of lignocellulose, as well as ethanol production. The factors that are most relevant in this selection include chemicals used in the pretreatment, pretreatment temperature and energy balance, pretreatment time and solid loading [9]. Many pretreatment methods have been studied and used in the literature to release fermentable sugars. The most studied pretreatment methods include dilute acid, dilute alkali, steam, liquid hot water, ammonia fiber expansion and wet oxidation pretreatment [8,9]. Among these pretreatment methods, alkaline pretreatment is one of the most extensively investigated methods because it is relatively inexpensive and less energy intensive. The major effect of alkaline pretreatment is the delignification of lignocellulosic biomass, thus enhancing the reactivity of the remaining carbohydrates [10]. Another effect of alkali pretreatment is the disruption of the intermolecular hydrogen bonding between xylan and lignin, resulting in increased porosity of the lignocellulosic biomass. Alkaline pretreatments are carried out under milder conditions by soaking biomass in sodium hydroxide (NaOH), or in other alkalies such as lime or ammonium hydroxide. NaOH is reported to be the most effective alkali at much lower temperatures (room temperature to 50 °C) with extended pretreatment times because NaOH is a stronger base than lime or ammonia [9,10].

Turkey is a country that has rich agricultural potential with 8.1 million hectares of agricultural cultivable land [11]. The total amount of agricultural solid waste is estimated as 12 million tonnes with an energy potential of 5.5 million tonnes of oil equivalent [12]. Turkey dominates the world's hazelnut crop, producing about 70–75% of the world's total. Hazelnut is mainly consumed as a major raw material in chocolate, confectionery and baking industries and as an ingredient in edible nut mixes. Hazelnut shells are generally used as a fuel in hazelnut-growing areas [13]. Hazelnut production in Turkey is reported to be 660,000 tonnes in shell for the year 2012. If an average of 50% of hazelnut is hazelnut shell, the total shell yield from hazelnut can be estimated to be more than 300,000 ton/year. Therefore, hazelnut shells, as lignocellulosic biomass, provide a renewable and low-cost raw material to produce fermentable sugars. To date, no study has been reported on the alkali pretreatment for enzymatic saccharification and ethanol production of hazelnut shells.

Therefore, the objectives of this study were to find a combination of pretreatment time, solid to liquid (S/L) ratio and NaOH concentration that would result in an increase in enzymatic saccharification and ethanol fermentation of hazelnut shells with low-temperature NaOH pretreatment. In addition, because the energy efficiency of sugar production directly affects overall energy production economics, energy consumption and energy efficiency (η) (the amount of sugar produced per MJ of energy consumed) for pretreatment were estimated.

2. Materials and methods

2.1. Materials

Hazelnut shells used in this study were purchased from Beşikdüzü, Trabzon, Turkey. The dried materials (9% moisture) were ground by a grinder and screened with a sieve shaker to obtain particle sizes between 0.224 and 0.850 mm. Celluclast 1.5L® and Novozym 188 were purchased from Sigma-Aldrich (St. Louis, USA). Aminex HPX 87H column was purchased from Bio-Rad Laboratories (California, USA). All chemicals used were standard analytical grade.

2.2. Low-temperature alkali (LTA) pretreatment

Pretreatments were performed in one-factor-at-a-time experiments, which vary only one factor or variable at a time while keeping others fixed. First, the effect of pretreatment time (24 h, 48 h, 72 h and 96 h) on the composition of the pretreated solid and enzymatic hydrolysis was investigated using 2.0% NaOH and 1/10 for the S/L ratio. Further, hazelnut shells treated with various concentrations of NaOH (2.0%, 4.0%, 6.0%, 8.0% and 10.0% (w/v)) with best pretreatment time and 1/10 for S/L. Finally, S/L ratios (w/v) of 1/5, 1/10, 1/20 and 1/40 was investigated using the best NaOH and pretreatment time. Treatments were performed at 30 °C in a water bath. After pretreatment, the pretreated material was filtered and the solid phase was washed thoroughly with water until the pH was 7.0. The remaining solid was air-dried until 50% moisture and then stored at 4 °C for enzymatic hydrolysis.

2.3. 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 the 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. Supernatants were used for ethanol fermentation. Glucose content in the enzymatic hydrolysates was determined using high-performance liquid chromatography (HPLC).

2.4. Fermentation process

The *Saccharomyces cerevisiae* strain was used as the yeast for this purpose. *S. cerevisiae* was used at the inoculum concentration of around 10% (v/v) for the fermentation studies. The fermentation medium consisted of substrate hydrolysate as a carbon source and 1 g/L yeast extract, 5.0 g/L $(NH_4)_2SO_4$, 0.025 g/L $MgSO_4{\cdot}7H_2O$ The culture was incubated at 30 °C and 150 rpm for 24 h. The test tubes were closed with lids. The cells were collected by centrifugation for 15 min. Supernatants were analyzed for ethanol using HPLC.

2.5. Analytical methods

The chemical compositions of raw and pretreated hazelnut shells were determined according to National Renewable Energy Laboratory (NREL) [14–16] methods. First, 0.3 g of solid stalk was hydrolyzed by 3 mL of 72% (w/w) H_2SO_4 at 30 °C for 60 min and then the reaction mixture was diluted to 4% (w/w) and autoclaved at 121 °C for 60 min. Lignin was determined by solid residue, and cellulose and hemicellulose amounts were determined from the filtrate using HPLC. Total reducing sugars determination was performed by the dinitro salicylic acid (DNS). In this assay, each sample of 0.025 mL diluted in 1.475 mL citrate buffer (0.05 M) was