Fuel 139 (2015) 102–106

Contents lists available at ScienceDirect

Fuel

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

Enhanced enzyme saccharification of *Sawtooth Oak* shell using dilute alkali pretreatment

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HIGHLIGHTS

- *Sawtooth Oak* shells were pretreated for ethanol production for the first time.
- The efficiency of temperature, time, and alkalinity was compared.
- 2% NaOH at 121 °C/15 psi for 60 min had the highest delignification (39.34%).
- 2% NaOH at 121 °C/15 psi for 60 min results the highest sugar release.
- The optimal enzyme loading was 20 FPU cellulase, 20 CBU β-glucosidase and 1.5 FXU xylanase.

ARTICLE INFO

Article history:
Received 20 May 2014
Received in revised form 14 August 2014
Accepted 15 August 2014
Available online 26 August 2014

Keywords:
Glucose
Oak shell
Hydrolysis
Dilute alkali pretreatment

ABSTRACT

This study examined the suitability of *Sawtooth Oak* (*Quercus acutissima*) shell waste as feedstock for bioethanol production for the first time. The impacts of varying parameters of dilute alkali pretreatment (temperature, time, and alkalinity) on enzymatic saccharification of oak shell were investigated. Results showed that the combination of 2% NaOH/121 °C (15 psi)/60 min achieved the highest delignification (39.34%) as well as the highest sugar release (426.36 mg/g pretreated material). In addition, optimal combination of 20 FPU cellulase, 20 CBU β-glucosidase and 1.5 FXU xylanase released total sugar yield that peaked at 653 mg/g pretreated material (613 mg glucose and 40 mg xylose). The maximum sugar recovered from the holocellulose fraction was 494.5 mg/g oak shell. This represents a conversion efficiency approaching 78.8% based on monomeric sugar recovery.

1. Introduction

The depletion of conventional fossil fuels reserves with increasing energy consumption and greenhouse gas emissions have led to a move towards renewable and sustainable energy sources. The lignocellulosic biomass will become one of the most credible alternatives for bioethanol production because of the local availability of the raw material, and usually at reduced prices [1,2]. These lignocellulosic biomass include agricultural and forest residues, food waste and other municipal wastes, waste paper, and so on [3,4].

Acorn, the seeds of *Quercus* species, is an important wildlife forestry starch source and abundant in North-America, European and Asian countries [5]. There are about 900 known oak tree species throughout the world, of which, about 300 species are found in China. It is estimated that there are about 1.33×10^7–1.67×10^7 h m^2 of oak forest in China, which can produce 6–7 billion kilograms acorn [6]. The amount of shell waste from the tannin or acorn production is estimated to be 1.5–1.75 billion kilograms (the weight ratio of shell and acorn is about 1:4) [7]. In earlier studies, the possible usage of oak shell for activated carbon [8,9], composites [10] or ellagic acid production [11] was examined. However, no known effort has been made to utilize oak shells as a resource for bioethanol production.

Like woody biomass, oak shell consists mainly of cellulose, hemicelluloses and lignin. Cellulose, if not combined with lignin, can be converted into bioethanol through hydrolysis and subsequent fermentation [12]. Alkali pretreatment involves the application of alkaline solutions like NaOH or KOH to remove lignin and a part of the hemicelluloses, and efficiently increase the accessibility of enzyme to the cellulose [13]. Compared with acid reagents, alkali treatment appears to be the most effective method in breaking the ester bonds between lignin, hemicellulose and cellulose, and avoiding fragmentation of the hemicellulose polymers, which

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http://dx.doi.org/10.1016/j.fuel.2014.08.037
0016-2361/

is suitable for pretreatment of high-lignin materials. Arslan and Saracoglu [14] studied the effect of acid pretreatment for hazelnut shell hydrolysate, in which NaOH was just used for delignification. Uzuner and Ekmecelioglu [15] discussed a rapid screening approach to factors affecting dilute acid hydrolysis of Hazelnut shells. Therefore, no studies have investigated the effect of alkali pretreatment for hydrolysis of high-lignin materials, especially for oak shell. Furthermore, it is the first time that oak shell was investigated for energy production as a renewable and low cost lignocellulosic material. This study studied the possibility of enhancing enzymatic saccrification of *Sawtooth Oak* shell. Optimum pretreatment conditions as well as dosage of enzymes were identified in order to promote production.

2. Materials and methods

2.1. Materials

Acorns used in this research were harvested from *Sawtooth Oak* trees at Haozhou, Anhui Province, China. Oak shells were stripped from acorns and dried at 50 °C for 48 h, ground in a rotary mill (FW177, Sikete, Tianjin, China) and passed through a 1.5 mm screen. And the moisture content was about 4.24%. All chemicals used in the experiments were of analytical-reagent grade and purchased from Sinopharm Chemical Reagent Co. Ltd., China.

2.2. Pretreatment

The dilute sodium hydroxide (NaOH) aqueous solution at concentration of 0.75%, 1.0% and 2.0% (w/v) was used to soak milled oak shells, with the solid loading of 10% (w/v). Treatments were performed at either 60 °C in a water bath or in an autoclave at 121 °C (15 psi) and residence times were 30, 60 and 90 min. After pretreatment, the pretreated material was filtrated by a vacuum pump. The solid phase was washed thoroughly with water until the pH was 7. A portion was stored at −20 °C for enzymatic hydrolysis study and the remaining solids were dried at 45 °C in an air-forced oven for chemical composition analysis.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis experiments were conducted to select the best pretreatment condition to yield maximum enzymatic digestibility for further research. Enzymatic saccharification at a solid loading of 5% (w/v) was performed in shaking water bath at 50 °C, 150 rpm for up to 72 h in 250 mL screw-capped Erlenmeyer flasks with 50 mM citrate buffer (pH 4.8). Sodium azide (10 mM) was used to prevent microbial contamination. The enzymes used in this study included cellulase (Aladdin industrial Co. Lot No. K1220003, Shanghai, China) with an activity of 19.6 filter paper unit (FPU)/g (measured using filter paper as substrate), β-glucosidase with an activity of 9.3 Cellobiase Units (CBU)/mg (Sigma–Aldrich, Lot No. BCBH2676V, USA,) and xylanase with an activity of 1.5 Farvet Xylan Unit (FXU)/mg (Sigma–Aldrich, X2753-10G, USA) (given by the manufacturer). The samples taken were first put into boiling water for 5 min to stop the enzymatic activity, centrifuged at 8000*g* for 5 min, filtered and stored at −20 °C for determination.

2.4. Analytical methods

Moisture content of the biomass was measured by drying the sample at 105 °C in an oven to constant weight [16]. The composition of oak shell was analyzed according to the NREL protocols [17]. After the reaction, samples were analyzed for sugar concentration analysis by high performance liquid chromatography (HPLC) using an Aminex HPX-87H ion Exclusion Column (Bio-Rad, Hercules, CA, USA) at 55 °C with 0.005 mol/L H_2SO_4 as the mobile phase at a flow of 0.6 mL/min.

3. Result and discussion

3.1. Characterization of oak shells

Like woody biomass, shell structure consists mainly of cellulose, hemicelluloses and lignin [12,14]. Table 1 summarizes the composition of various lignocellulosic materials. It was found that the holocellulose fraction of oak shell totaled 56.8% of the dry biomass. Cellulose was the major constituent at 36.4% with the remaining 20.4% derived from hemicellulose. Lignin will limit enzymatic hydrolysis of lignocellulosic biomass by cross-linking with cellulose and hemicellulose fibers [25]. In general, the chemical composition of oak shells used in this study is similar to that of hazelnut shell. The measured high lignin content of oak shell (36.6%) was lower than 43.1% of hazelnut husks reported by Arslan and Saracoglu [14], but higher than most other lignocellulose feedstock such as wheat straw, cotton stalk, and even softwoods and hardwoods (Table 1), thus making the accessibility of cellulose polymers a challenge.

3.2. Solids recovery after pretreatment

The solids recovered after pretreatment are presented in Fig. 1. Lower yields after pretreatment could be explained mainly by the removal of lignin, hemicellulose and other soluble substances from the structure. Solid recovery ranged from 48.06% (w/w) under severe conditions (2% NaOH/121 °C/90 min) up to 82.09% when pretreated in 0.75% NaOH for 30 min at 60 °C. Temperature had the greatest impact on the loss of solid, followed by residence time and then alkalinity. At the combination of 1% NaOH/ 121 °C/ 60 min, a solid recovery of 68.9% was achieved for oak shell. It is within the reported values of 43.8–71.5% [26–28] for different materials.

3.3. Delignification during alkaline pretreatment

By determining the reduction of acid-insoluble lignin in pretreated samples as a function of temperature, alkali concentration and residence time, the effect of pretreatment on the delignification of oak shells was quantified. Results of a percent reduction compared to original starting material are shown in Fig. 2. All parameters contributed towards delignification, in which temperature had the most significant effect. At 121 °C, delignification ranged from 10.24% (0.75% NaOH/30 min) to a maximum of 39.34% (2% NaOH/60 min). In our previous work, 2% NaOH (at room temperature for 48 h) coupled with steam explosion (at 118 °C/ 1.0 MPa for 480 s) resulted in a delignification level of 31.5%, which was not so effective as that of 121 °C/2% NaOH/60 min, one reason may be that sodium hydroxide can effectively break the ester bonds crosslinking lignin and xylan at severer conditions, thereby resulting in biomass porosity [29]. At the lower temperature (60 °C), delignification ranged from 5.08% (0.75%/30 min) to 23.35% (2%/90 min).

Mcintosh and Vancov [26] reported with the increase of residence time, delignification of sorghum straw was improved at both 60 °C and 121 °C. However, in this study increasing residence time did not always improved delignification. On the contrary, the maximum delignification occurred at 2% NaOH/60 min/121 °C, instead of 2% NaOH/90 min/121 °C. Combined with Fig. 1, the higher solid loss of 2% NaOH/90 min/121 °C (51.94%) may largely stem from the