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Detailed study of efficient ethanol production from elmwood by alkali pretreatment

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ABSTRACT

An alkaline pretreatment was performed on hardwood elm to improve enzymatic hydrolysis and ethanol production. The pretreatment was conducted with 8% (w/v) NaOH solution at 0, 25, and 80 °C for 2 h, and the best results were obtained by the pretreatment at 0 °C. The glucose yield from untreated wood was only 8.0% and improved to 71.5% after the pretreatment at 0 °C, whereas the corresponding ethanol yield was improved from 11.1% to 45.7%. In order to decrease ineffective adsorption of cellulase enzyme on lignin and enhance the enzymatic hydrolysis and fermentation yields, a non-ionic surfactant, Tween-20, was used in the hydrolysis process. The addition of 2.5 g L^{-1} Tween-20 further favorably modified the yields of enzymatic hydrolysis and ethanol production to 79.8% and 57.3%, respectively. Changes in the wood's structural properties by the pretreatment were followed in detail by swelling and buffering capacity measurements as well as SEM and FTIR analyses. Furthermore, the adsorption and desorption of cellulase during the enzymatic hydrolysis were investigated, and a consistent relation was observed between adsorbed and desorbed enzymes and enzymatic hydrolysis yield.

1. Introduction

Recently, higher energy demand and depletion of oil sources have drawn attention toward bioethanol production as a renewable and environmentally friendly fuel [1]. Ethanol is typically produced through fermentation of starch- and sugar-based materials [2]. However, the limitations of current feedstocks restricted their application for ethanol production [3]. Accordingly, lignocellulosic materials have drawn worldwide attention as an alternative feedstock due to their availability and low cost [1,4]. Wood waste is a promising lignocellulosic substrate since a large amount of waste is produced in wood processing industries, which typically subject to burning for heating purposes [5]. However, lignocelluloses carbohydrates cannot be directly fermented to ethanol by microorganisms. Therefore, a hydrolysis process is necessary to convert them to fermentable sugars [6]. Furthermore, as a result of their recalcitrant structures, the hydrolysis of native lignocelluloses is inefficient; thus, a pretreatment process is necessary for efficient hydrolysis [7]. The pretreatment process is typically aimed at reduction of cellulose crystallinity, increasing accessible surface area, and removal of lignin and hemicelluloses [8].

Several pretreatment methods have been developed to improve the yield of enzymatic hydrolysis of lignocelluloses [7]. Alkaline pretreatment of lignocelluloses by sodium hydroxide is one of the most efficient methods for enhancement of ethanol production. This low cost and less energy consuming technique has a high impact on many lignocellulosic substrates, such as hardwoods and agricultural residues [9–11]. Acetyl groups and lignin are considered as the main factors preventing the hydrolysis of hemicellulosic polysaccharides which can be removed by an alkaline pretreatment. In fact, the pretreatment can break down the acetyl groups cross-linking between hemicelluloses and lignin resulted in delignification [5,12]. The lignin removed by the alkaline pretreatment can be converted to high value-added products improving economic and environmental impacts in a biorefinery [13–15]. Sodium hydroxide can be used at either low concentration and high temperature or high concentration and low temperature. Pretreatment at high NaOH concentration (6–20%), low temperatures, and ambient pressure can dissolve and regenerate the cellulose part of lignocelluloses. High concentration alkali pretreatment provides an opportunity to reuse the solution, which can significantly improve the economic and environmental impacts of the process [16]. Alkali pretreatment at high concentration was successfully used

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to improve ethanol production from different substrates. Mirahmadi et al. [16] showed a significant reduction in hemicellulose content and crystallinity of cellulose in softwood spruce and hardwood birch by alkali pretreatment with 7% (w/w) NaOH solution at different temperatures for 2 h. They [16] showed that pretreatment at 5 °C improved the yield of enzymatic hydrolysis of spruce from 14.1% to 35.7%. Cabrera-Rodríquez et al. [17] investigated the effect of temperature and pretreatment time on the chemical composition and enzymatic hydrolysis of spruce in cold alkaline pretreatment. The pretreatment using 7% (w/w) sodium hydroxide solution at 0 °C showed 40% enhancement in the glucose yield. Salehian and Karimi [5] applied 8% NaOH pretreatment to different parts of pinewood and demonstrated that enzymatic hydrolysis and ethanol yields were significantly enhanced by the pretreatment. In the case of pine needle leaves, the pretreatment at 0 °C for 1 h increased the glucose yield from 27.2 to 60.2% and ethanol yield from 26.4 to 51.1% [5].

Enzyme loading level has a significant effect on the overall economy of ethanol production from lignocelluloses. The addition of non-ionic surfactants to the enzymatic hydrolysis stage is suggested to reduce the enzyme requirement and increase the cellulose conversion rate [18,19]. Tween-20 and Tween-80 are the most effective surfactants for improving the hydrolysis yield, where Tween-20 produces better results [20]. Tween-20 can accelerate the fermentation process [21] and increase the final ethanol yield [22]. Alkasrawi et al. [18] investigated the effect of Tween-20 concentration on the ethanol yield of steam-pretreated softwood. They showed that the addition of 2.5 g L^{-1} Tween-20 leads to a faster fermentation process, higher ethanol yield, lower enzyme requirement, and higher enzyme activity [18].

Elm tree, *Ulmus minor*, is among the fast-growing hardwood trees that can resist various conditions. Elms are widely cultivated and used in many urban areas throughout the Northern Hemisphere [23], and especially in northern Iran around paddy fields [24]. Worldwide, since prehistoric times, elmwood has been extensively used for many different purposes, e.g., food, medicine, fiber, cattle fodder, timber for construction, and firewood [23]. To our knowledge, no previous work has reported on using elmwood for ethanol production.

In this study, the goal is efficient ethanol production from elmwood. Alkaline pretreatment using sodium hydroxide at different temperatures was applied to improve ethanol production. The reasons for the improvements were investigated by adsorption and desorption of cellulase, swelling and buffering capacities, and SEM and FTIR analyses.

2. Materials and methods

2.1. Materials and microorganism

Elmwood was obtained from Dorcheh region (32°36′N, 51°33′E; Isfahan, Iran). The wood was debarked, cut into small pieces (about 2–3 cm), ball-milled, and sieved using 20 and 80 mesh to achieve appropriate particle sizes of 0.840–0.177 mm. The wood was then stored in resealable bags at room temperature until use. The dry weight of the materials was measured by oven drying at 105 °C until constant weight [25].

Two commercial enzymes, cellulase (Celluclast 1.5 L, Novozymes, Denmark) and β-glucosidase (Novozyme 188, Novozymes, Denmark), were used in enzymatic hydrolysis. The activity of cellulase was 52.5 FPU mL^{-1}, measured according to the standard method presented by Adney and Baker [26], while the β-glucosidase activity was 240 IU mL^{-1} measured by the Ximenes et al. [27] method. The protein content of the cellulase enzyme, as measured by Bradford assay [28], was 42 mg mL^{-1}. A flocculating strain of *Saccharomyces cerevisiae* (CCUG 53310, Culture Collection, University of Gothenburg, Sweden) was used for fermentation of glucose to ethanol. The yeast maintenance and propagation were performed according to the method presented by Karimi et al. [29].

2.2. Pretreatment

The ground elmwood, 5% (w/w, based on dry weight), was thoroughly mixed with 8% (w/v) NaOH solution for 10 min at room temperature. Then, the suspension was treated for 2 h at 0, 25, or 80 °C in a laboratory water bath (Model WNE 14, Memmert, Germany) while being manually mixed every 10 min using a glass rod. The mixture was then centrifuged at 4000 × rpm and room temperature for 6 min. Afterwards, the sediments were washed several times with distilled water until pH 7 was achieved. Beside neutralization, the dissolved and separated materials, which may have inhibitory effects on the hydrolytic enzymes, were removed during the washing. At the end, the collected solids were freeze dried (Christ Alpha 1-2/ LD plus freeze dryer, Osterode am Harz, Germany) for 72 h and kept in resealable bags at room temperature until use [25].

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was performed on the pretreated and untreated elmwood in 118 mL glass bottles (717561, Pajuhesh Setayesh Sepahan, Isfahan, Iran). A suspension of 50 g L^{-1} substrate (based on dry weight) was prepared in a 50 mM citrate buffer (pH 4.8). The suspension was then autoclaved at 121 °C for 20 min. After cooling to room temperature, 30 FPU cellulase and 60 IU β-glucosidase per gram of dry substrate were added. To avoid any microbial growth during the enzymatic hydrolysis, 0.5 g L^{-1} sodium azide was added to the hydrolysis mixture. Hydrolysis was performed at 45 °C and 130 rpm for 72 h. Liquid samples were periodically taken and analyzed for sugar content. The yield of glucose formation was calculated according to Eq. (1) [25]:

$$\text{Glucose yield}(\%) = \frac{\text{Produced glucose}(\text{g}L^{-1})}{\text{Glucan in sample}(\text{g}L^{-1}) \times 1.111} \times 100 \quad (1)$$

where 1.111 is a factor for hydration of glucan to glucose. Furthermore, the effect of surfactant on the hydrolysis was studied by repeating the hydrolysis experiments with the addition of 2.5 g L^{-1} Tween-20.

2.4. Simultaneous saccharification and fermentation (SSF)

Simultaneous saccharification and fermentation (SSF) for ethanol production from the treated and untreated wood was carried out at 36 °C and 130 rpm under anaerobic conditions for 72 h. A fermentation medium containing 5 g L^{-1} yeast extract, 7.5 g L^{-1} $(NH_4)_2SO_4$, 3.5 g L^{-1} K_2HPO_4, 0.75 g L^{-1} $MgSO_4{\cdot}7H_2O$, 1 g L^{-1} $CaCl_2{\cdot}2H_2O$, and 50 g L^{-1} of wood sample was prepared in 50 mM citrate buffer in a 118 mL glass bottle, and initial pH was adjusted to 5 using 2.5 M sodium hydroxide and 0.5 M sulfuric acid [30]. The mixture was then autoclaved at 121 °C for 20 min. After cooling to room temperature, 15 FPU g^{-1} cellulose, 30 IU g^{-1} β-glucosidase (based on the substrate dry weight), and 1 g L^{-1} microorganism were loaded. This process was repeated in the presence of 2.5 g L^{-1} Tween-20 to measure the effect of the surfactant on the ethanol yield.

It should be considered that in enzymatic hydrolysis, the hydrolysis accompanies the enzyme inhibition by glucose, while in SSF the produced glucose is consumed upon production; thus, there is no product inhibition and it is possible to reduce the enzyme loading in SSF, but not in enzymatic hydrolysis [31,32]. This is the reason