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Effect of freeze storage on hemicellulose degradation and enzymatic hydrolysis by dilute-acid pretreatment of Mongolian oak

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ABSTRACT

The objective of this research was to investigate the effect of freeze storage on the efficiency of hemicellulose degradation and enzymatic hydrolysis by dilute-acid pretreatment of Mongolian oak. The freeze storage of Mongolian oak was conducted depending on several factors (freezing time, solid to freezing solution ratio, and freezing solution type) before dilute-acid pretreatment process of constant condition. The results showed that the major contents of reducing sugars and their degradation products in the liquid hydrolysates were slightly increased until a freezing time of 120 min. The xylose content was the highest (18.22 g/100 g initial input) at a specific solid to freezing solution ratio of 5:3 (w/v), while the sugar degradation product contents were not remarkably increased at these points. Based on these conditions, when distilled water was used as the freezing solution, glucose yield was maximized (36.20% of initial input (conversion rate of 87.92%)) after enzymatic hydrolysis to a greater degree than control.

1. Introduction

Numerous studies of biofuels and chemicals derived from biomass have been performed due to the increasing severity of worldwide problems such as fossil resource exhaustion and environmental pollution. Bioethanol is one of the most common bioenergies, and fermentable sugars are also potent materials for the production of comprehensive targets in wide areas of industry [1,2]. In addition, the building block chemicals from carbohydrates and lignin extracted from biomass have attracted attention due to their high economic value after the biorefinery process was introduced [3,4]. For example, various organic acids (succinic acid, fumaric acid, and levulinic acid) mainly from C6 sugars (glucan) have been more versatile chemicals because they can be used as raw materials/additives in the fields of cosmetic, food, and medical industries as well as fuel industry [3].

Therefore, various types of resources have been used in academic and industrial fields for the improvement of bioenergy productivity and process efficiency [5,6]. Among several generations of biomass, woody biomass (lignocellulosic biomass) is mainly composed of cellulose, hemicellulose, and lignin and has been actively studied in recent years because it might be relatively free from concerns about price competition with food markets [7]. However, it has certain resistances to external force called biomass recalcitrance, such as complex structures. Thus, pretreatment of lignocellulosic biomass is necessary to overcome this problem [8,9].

Pretreatment methods have been developed in many different forms and can be classified as physical (milling, extrusion, microwave, and freeze), chemical (acid, alkaline, ionic liquid, and organosolv), physico-chemical (steam explosion, liquid hot water, ammonia fiber explosion, and wet oxidation), and biological [10–12]. Furthermore, combination processes of more than two pretreatment methods have been recently designed to remedy their own drawbacks, improve the efficiency of bioethanol (or chemical) production, and obtain multiple targets [6].

In particular, freeze storage (or freeze treatment) is a promising physical treatment approach that utilizes the fundamental principle of water; the density of water decreases and its volume expands when it freezes [13]. This property has been mainly

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studied in the fields of food preservation and storage [14–16]. Meanwhile, only a few studies about effects of freeze storage on biomass have been reported with the exception of one, which conducted the freeze pretreatment of rice straw to enhance enzymatic conversion [17]. In this previous study, it was reported that freezing process of biomass improved enzymatic hydrolysis yields by increasing the response surface area and enzyme accessibility in the pretreated biomass. In spite of the most crucial advantages of this method, including a more eco-friendly impact and less toxic chemicals, the complex structure of lignocellulosic biomass is not degraded easily using only freeze storage, and it require a huge amount of cooling energies. However, sometimes it is inevitable that the freeze storage is required to prevent critical damage to biomass because of harsh environments (long-distance transportation or severe climate). Therefore, in this study, we combined freeze storage with dilute-acid pretreatment, which is the most commonly used pretreatment method for lignocellulosic biomass and could lead to higher enzyme accessibility, in hope of increasing the pretreatment performance.

The major aims of this study were to investigate the effects of freeze storage on the efficiencies of hemicellulose degradation and enzymatic hydrolysis by dilute-acid pretreatment under mild conditions using Mongolian oak, the main native hardwood in South Korea, and to evaluate optimum conditions of freeze storage factors for these efficiency improvements. First, the Mongolian oak was frozen with freezing solution for different freezing times or/and in different solid to freezing solution ratios, and then dilute sulfuric acid treated. After optimization of the freeze storage condition, the degrees of hemicellulose degradation and enzymatic hydrolysis were compared with controls depending on the freezing solution type. Their efficiencies were evaluated with the chemical compositions in the hydrolysates and the glucose yield after enzymatic hydrolysis.

2. Experimental

2.1. Materials

Small diameter thinning logs of Mongolian oak (*Quercus mongolica*) were generously provided by the Kwanak arboretum (Seoul National University Forest, Anyang, South Korea). The logs were chipped using a wood crusher (PRCS-3300ED, PoongrimEMG, Hwaseong, South Korea) and then ground using a Cutting Mill Pulverisette 15 (FRITSCH GmbH, Idar-Oberstein, Germany) to pass through a 0.5 mm screen. These were stored at 4 °C until freezing. The chemical composition (carbohydrate, lignin, fat, and ash) of Mongolian oak was analyzed using the procedure described by National Renewable Energy Laboratory (NREL) (Table 1) [18]. Novozymes' cellulosic ethanol enzyme kit was kindly provided by Novozymes Korea Ltd. (Seoul, South Korea). Among them, two enzymes (NS22086 (Cellulase complex, with a declared activity of 1000 BHU (2)/g) and NS22118 (β-glucosidase, with a declared activity of 250 CBU/g)) were used for enzymatic hydrolysis.

Table 1
Chemical compositions (% on a dry weight basis) of Mongolian oak.

Components	Dry solids (%)
Glucan	41.61 ± 0.22
Xylan	17.15 ± 0.21
Arabinan	1.01 ± 0.10
Galactan	2.07 ± 0.11
Mannan	0.92 ± 0.08
Acid-insoluble lignin	22.63 ± 0.19
Acid-soluble lignin	1.41 ± 0.13
Fat	1.76 ± 0.12
Ash	0.19 ± 0.01

Values are the mean (±the standard deviation).

2.2. Freeze storage

Schematic experimental flowchart of the whole process including the freeze storage is shown in Fig. 1. All of the ground Mongolian oak (10 g) was placed in a 50 mL polypropylene tube (BD, Franklin Lakes, NJ, USA), mixed with distilled water or 1% (w/w) sulfuric acid (solid:liquid = 5:0, 5:1, 5:2, 5:3, 5:4, and 5:5 (w/v)), and then frozen at −10 °C for 0.5 h, 1 h, 2 h, and 4 h.

2.3. Dilute-acid pretreatment

After freezing, the frozen Mongolian oak was directly loaded into a 20 mL pretreatment batch-type reactor (Bolted Closure Vessels, Hanwoul Engineering Inc., Gunpo, South Korea) made of stainless steel (SUS 316). Prepared solution (distilled water or dilute sulfuric acid) was then poured into the reactor until the final solid:liquid ratio was 1:5 (w/v) and the sulfuric acid concentration was 1% (w/w). The reactor was heated to 150 °C in 40 min and maintained for another 10 min. At the end of the pretreatment, the liquid fraction was separated from the pretreated solid fraction by filtration using filter paper (No. 2, Adventec, Kyoto, Japan) and partially filtered through a 0.45 μm membrane filter (Adventec Co., Tokyo, Japan) for sugar analysis. The solid fraction (water-insoluble residue (WIS)) was washed with distilled water and stored at below 4 °C for enzymatic hydrolysis. The yield of WIS was calculated by measuring the moisture content and wet weight of the solid fraction.

2.4. Enzymatic hydrolysis

Enzymatic hydrolysis was conducted in a 250 mL Erlenmeyer flask using 50 mL of 0.05 M sodium acetate buffer (pH 5) and 2% (w/w) dry matter at 50 °C on a shaking incubator (HB-201SL, Hanbaek, Bucheon, South Korea) set to 150 rpm for 72 h. NS22086 (Cellulase) at 15 FPU/g substrate and NS22118 (β-glucosidase) at 1.75 CBU/g substrate were loaded into the flask. After incubation, the liquid fraction was sampled and filtered using a 0.45 μm membrane filter (Adventec Co., Tokyo, Japan) for glucose analysis.

2.5. Sugar analysis

The concentration of reducing sugars (glucose, xylose, arabinose, galactose, and mannose) in the liquid fractions after pretreatment or enzymatic hydrolysis was determined by a bio-liquid chromatograph (ICS-2500, Thermo Dionex, Palo Alto, CA, USA) outfitted with a CarboPac PA-1 column (250 × 4 mm, Dionex, Palo Alto, CA, USA) and a pulsed amperometry detector (HP 1100, Hewlett Packard, USA). The analysis was performed at 40 °C with potassium hydroxide (1–36 min: 2 mM, 35–36 min: 2 → 100 mM, 36–56 min: 100 mM, 56–57 min: 100 → 2 mM, 58–63 min: 2 mM) as the eluent at a flow rate of 1 mL/min and an injection volume of 10 μL. Sugar degradation products (furfural, 5-HMF, levulinic acid, acetic acid, and formic acid) were determined by a high performance liquid chromatograph (Ultimate-3000, Thermo Dionex, Palo Alto, CA, USA) with 0.01 N sulfuric acid as an eluent at 40 °C with a 0.5 mL/min flow rate and an injection volume of 10 μL. Standard solutions of glucose, xylose, arabinose, galactose, mannose, furfural, 5-HMF, levulinic acid, acetic acid, and formic acid were prepared from Sigma–Aldrich Co. (St. Louis, MO, USA) to make the calibration curves. Peaks were identified by comparing the peak retention times and assessing the concentrations corresponding to the different peaks. All analyses were performed in triplicate.