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Pretreatment combining ultrasound and sodium percarbonate under mild conditions for efficient degradation of corn stover

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ABSTRACT

Ultrasound (US) can be used to disrupt microcrystalline cellulose to give nanofibers via ultrasonic cavitation. Sodium percarbonate (SP), consisting of sodium carbonate and hydrogen peroxide, generates highly reactive radicals, which cause oxidative delignification. Here, we describe a novel pretreatment technique using a combination of US and SP (US–SP) for the efficient saccharification of cellulose and hemicellulose in lignocellulosic corn stover. Although US–SP pretreatment was conducted under mild condition (i.e., at room temperature and atmospheric pressure), the pretreatment greatly increased lignin removal and cellulose digestibility. We also determined the optimum US–SP treatment conditions, such as ultrasonic power output, pretreatment time, pretreatment temperature, and SP concentration for an efficient cellulose saccharification. Moreover, xylose could be effectively recovered from US–SP pretreated biomass without the formation of microbial inhibitor furfural.

1. Introduction

Lignocellulosic biomass, which is composed mainly of cellulose, hemicellulose, and lignin, is an abundant and renewable resource, and is therefore considered to be a promising carbon source for biofuels or bio-based chemicals [1,2]. However, the use of lignocellulosic biomass is limited by its low digestibility, which is mainly ascribed to the high crystallinity of cellulose, and the lignin covering [3,4]. Cellulose, which is a highly crystalline polymer with units linked via multivalent hydrogen bonding, is recalcitrant to biodegradation by the cellulolytic enzyme cellulase. Lignin, which is an amorphous three-dimensional macromolecule composed of phenylpropanoid units, covers the cellulose microfibrils and hinders enzyme access to cellulose. Pretreatment to disrupt the cellulose crystalline structure and remove the lignin covering is therefore essential for efficient saccharification of cellulose in lignocellulosic biomass. Many strategies have been proposed for efficient pretreatment, including steam explosion, liquid hot water, dilute acid, lime, and ammonia fiber explosion [5–8]. However, pretreatment processes usually require specialized equipment and have high energy consumptions, because they are performed at high temperatures and pressures. Furthermore, conventional acid pretreatment at high temperature (160 °C∼) often causes excessive degradation of hemicellulose, resulting in the formation of furfural that strongly inhibits fermentative microbes [9,10]. Since xylose, a constituent of hemicellulose, can be utilized as a carbon source to produce ethanol and organic acids by genetically-engineered microbes [11], hemicellulose fraction should also be effectively degraded and recovered without the formation of inhibitors by excessive degradation. Therefore, the pretreatment conditions are required to be mild, ideally room temperature and atmospheric pressure, but sufficiently effective.

Ultrasonic irradiation in a solution has physical and chemical effects primarily derived from acoustic cavitation. Ultrasound (US) has been used to disintegrate microcrystalline cellulose to cellulose nanofibers using the high local energy provided by ultrasonic cavitation [12–14]. US has also been used to assist the extraction of lignin in lignocellulosic biomass in pulping process [15]. Because of its desirable effects, US has recently been used to improve the digestibility of lignocellulosic biomass [16–24]. However, the enhancement achieved by the introduction of US was generally moderate in most systems, except for some cases such as US-assisted enzymatic degradation [23] and US pretreatment combined with ionic liquids [18,21] or *N*-methylmorpholine-*N*-oxide [20], which are known to dissolve crystalline cellulose. Although these systems are effective, expensive solvents need to be recovered and reused after the pretreatment.

Here, we describe a novel pretreatment technique that combines US and sodium percarbonate (SP) for the efficient degradation

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of corn stover. SP consists of Na_2CO_3 and H_2O_2, and is commonly used as an environmentally-benign oxidative bleaching reagent. In alkaline solution, H_2O_2 dissociates into highly reactive hydroxyl (·OH) and superoxide (O_2^{-}·) radicals. These species contribute to oxidative delignification by attacking lignin side chains [25,26]. Ultrasonic irradiation would facilitate the degradation of H_2O_2 to hydroxyl radicals [27,28]. A combination of US and SP (US–SP pretreatment) has great potential as a simple and effective pretreatment of lignocellulosic biomass under mild conditions. We evaluated the efficacy of this novel pretreatment technique in the degradation of corn stover, based on lignin removal and cellulose crystallinity.

2. Materials and methods

2.1. Biomass pretreatment by ultrasonication

Corn stover was used as the lignocellulosic biomass in this study. All chemicals were purchased from Wako Pure Chemicals Industries, Ltd., Japan. Corn stover was crushed with a grinder (Wonder Blender, Osaka Chemical Co., Ltd., Osaka, Japan), screened to obtain biomass particles of size less than 2 mm, and dried at 50 °C for 24 h before use. The crushed biomass (2.0 g) was suspended in 50 mL of SP solution (Na_2CO_3: 0.4 mol/L; H_2O_2: 0.6 mol/L) in a glass beaker of inner diameter 4.7 cm and height 9.0 cm. Basic conditions of US–SP pretreatment are as follows. Ultrasonication was carried out using a probe-type ultrasonic generator (Sonifier 250, Branson Ultrasonics, Danbury, USA) with a vibration tip (1/2″ extension) fixed at a height of 15 mm from the bottom. It was operated at 20 kHz and an ultrasonic power output of 50 W; the temperature of the reaction mixture was kept at 30 °C in a thermo-controlled water bath during irradiation. After ultrasonication for 3 h, the biomass was washed with 50 mL of water, followed by washing twice with 50 mL of acetate buffer (50 mmol/L, pH 5) to neutralize the alkali and replace it with buffer. This process is defined as US–SP pretreatment. In a similar manner, US pretreatment was conducted by ultrasonication of the biomass in deionized water while SP pretreatment was performed by immersing the biomass in SP solution.

The effects of the US–SP pretreatment parameters on biomass degradation were examined by varying the pretreatment conditions, i.e., the ultrasonic power output, pretreatment time, pretreatment temperature, and SP concentration.

For comparison with US–SP pretreatment, dilute acid (DA) pretreatment was also examined. According to the procedure reported in the literature [29], the crushed biomass (4.0 g) was suspended in 50 mL of 1 wt% dilute sulfuric acid in PTFE bottle (φ53 mm × 90 mm, Sanplatech Co. Ltd., Osaka, Japan). The bottle containing the biomass was then heated at 121 °C for 1 h in an autoclave. After cooling the bottle to room temperature, the pretreated biomass was washed in a similar manner to US pretreatment.

For the analysis of lignin degradation and furfural formation during pretreatment, the supernatant of the pretreated biomass was analyzed using a high-performance liquid chromatograph (Acquity UPLC H-Class, Waters, Milford, USA) equipped with a BEH C18 column (Waters, particle size: 1.7 μm; φ2.1 mm × 100 mm) and UV detector (280 nm); acetonitrile and 10 mM phosphate buffer were used as the mobile phase.

2.2. Characterization of pretreated biomass

The chemical changes in the pretreated biomass were identified using Fourier-transform infrared (FTIR) spectroscopy with attenuated total reflection (Nicolet6700, Thermo Fisher Scientific, Waltham, MA, USA). FTIR spectra were recorded in the mid-IR region, from 2000 to 1200 cm^{-1} at a resolution of 2 cm^{-1}, by averaging 16 scans.

Powder X-ray diffraction (XRD) was used to determine the crystallinity of the pretreated biomass. Biomass samples were lyophilized and scanned with an X-ray diffractometer (RINT-2200VHF+/PC, Rigaku, Tokyo, Japan) at 2θ = 5–30°, a scan speed of 1°/min, and a step size of 0.02°. The crystallinity index (CrI) was calculated using Eq. (1) [30,31]:

$$\mathrm{CrI} = \frac{I_{200} - I_{\mathrm{AM}}}{I_{200}} \times 100 \quad (1)$$

where I_{200} and I_{AM} are the intensities of the peaks at 2θ = 22° and 2θ = 18°, respectively.

2.3. Enzymatic saccharification of biomass

Lignocellulosic biomass generally contains cellulose with the content between 35% and 50%. However, cellulose content in the corn stover used in this study was not clear. We thus evaluated the effectiveness of pretreatment by comparing glucose concentration generated in degradation.

Enzymatic saccharification of cellulose in biomass was performed using cellulase from *Trichoderma reesei* and β-glucosidase (cellobiase) from *Aspergillus niger*, both of which were obtained from Sigma–Aldrich (St. Louis, MO, USA). The suspension of untreated or pretreated biomass (100 mL, 20 mg/mL) in 50 mmol/L acetate buffer (pH 5) was preheated at 40 °C for 30 min. Enzymatic hydrolysis was started by adding cellulase (0.01 g-protein/g-biomass) and β-glucosidase (0.01 g-protein/g-biomass) to the suspension, with gentle shaking at 100 stroke per minute and 40 °C. A preliminary test confirmed that cellobiose generated by cellulase was completely converted to glucose by β-glucosidase under these conditions. Samples (300 μL) were collected from the reaction mixture at scheduled times and centrifuged to separate the supernatant from solid cellulose. For saccharification of hemicellulose in biomass, hemicellulase (0.2 g/g-biomass, from *A. niger*, Sigma–Aldrich) was used in addition to cellulase (0.01 g-protein/g-biomass) and β-glucosidase (0.01 g-protein/g-biomass).

The concentration of sugars in the supernatant was determined using a high-performance liquid chromatograph (Acquity UPLC H-Class, Waters, Milford, USA) equipped with a BEH amide column (Waters, particle size 1.7 μm; φ2.1 mm × 50 mm) and an evaporative light-scattering detector; acetonitrile and ultrapure water containing 0.2 vol% triethylamine were used as the mobile phase.

3. Results and discussion

3.1. Characterization of pretreated biomass

Photographs of the solution of the untreated and pretreated biomass are shown in Fig. 1. Each pretreatment was conducted at 30 °C for 3 h. The US-pretreated biomass became swollen but the color did not change. The swelling is ascribed to the following factors. Ultrasonic waves generate cavities (microbubbles) in a solution, and some of the microbubbles collapse during compression of the wave, leading to a locally generated extreme state with a temperature higher than 5000 K and pressure of around 50 MPa, commonly called a hot spot [32,33]. This local high energy disrupts the hydrogen bonds in the cellulose microfibrils, resulting in disassociation of the bundles and biomass swelling. Furthermore, asymmetric bubble collapse near a solid surface induces a microjet, which hits the solid surface at high speed (>100 m/s) [34]. A microjet generated at the biomass surface would have a large impact on