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Original article

Ammonium sulfite pretreatment of wheat straw for efficient enzymatic saccharification

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ABSTRACT

Pretreatment is an important upstream process that affects the economics of biofuels production from lignocellulose. This study established a novel pretreatment process for efficient bioconversion of wheat straw using ammonium sulfite as pretreatment option. Effects of temperature, time, ammonium sulfite concentration and sodium carbonate supplementation level on pretreatment were evaluated. 99.9% of glucan and 88.0% of xylan were hydrolyzed with 35 FPU cellulase and 70 FXU xylanase (per gram of dry biomass) in 24 h after pretreatment by 20% (w/w) ammonium sulfite supplemented with 4% (based on ammonium sulfite dosage) sodium carbonate at 180 °C for 1 h. Total monosaccharide yield of 0.413 g/g native wheat straw was achieved with 82.6% acid insoluble lignin removal. Characterization results of flourier translation infrared spectrum revealed that lignin can be removed by sulfonation and ammonolysis. The results in the research provide an efficient method for lignocellulose pretreatment.

Introduction

Alternative fuels production from renewable feedstocks is badly needed to reduce our heavy dependence on fossil fuels and to reduce the greenhouse gas emissions. Lignocellulosic biomass has been recognized as a potential sustainable source for fermentation to biofuels [16]. The polysaccharides of cellulose and hemicellulose contained in the biomass can be hydrolyzed to sugars and then fermented to biofuels such ethanol and butanol [2,25]. In order to harness the polysaccharides in the lignocellulosic biomass, we must overcome the following problems caused by the biomass recalcitrance (i) the relatively slow kinetics of the transformation from polysaccharides to fermentable sugars, (ii) the low yields of fermentable sugars, and (iii) the removal of lignin, a protective barrier that prevents plant cell destruction [7]. Various pretreatment techniques have been developed to make the polysaccharides more accessible to enzymes or directly convert the polysaccharides to fermentable sugars [13]. Most of the pretreatment methods are focused on removing hemicellulose and lignin as much as possible because they are considered to be the key limiting factors [24]. However, the present pretreatment methods are far from cost-effective, highly efficient and environmentally friendly.

A traditional pulping method-ammonium sulfite (AS) was used in the present study to treat wheat straw for improving the enzymatic hydrolysis. AS, a pulping option for papermaking, has been investigated for a long time [9]. But there was no report about AS pretreatment for efficient enzymatic saccharification of lignocellulosic biomass. Sulfite pretreatment has been developed to treat woody biomass and more than 90% of cellulose conversion was achieved [27,32]. Sulfur dioxide pretreatment on agricultural residue sugarcane bagasse was conducted by Verardi et al., and 100% of theoretical glucose from pretreated biomass was obtained by enzymatic hydrolysis [26]. However, the method presented in this study differs from the reported method in some aspects. The pretreatment option AS was not reported before, and the active reagents in the AS pretreatment liquor can be sulfite, bisulfite, sulfur dioxide, ammonium and ammonia, which depends on the pretreatment pH and temperature. Under different pH and temperature condition, lignin can be sulfonated in different ways [5]. During AS pretreatment process, the lignin content can be removed by at least two different ways. On the one hand, lignin can be sulfonated by sulfite which results in the hydrosoluble sulfonated lignin [33]. On the other

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hand, ammonium, an effective swelling reagent for lignocellulosic materials, has high selectivity for reactions with lignin by cleaving the C—O—C bonds in lignin as well as ether and ester bonds in the lignin–carbohydrate complex [11]. The protective barrier of lignin which prevent the polysaccharides digestion can be disrupted by AS treatment, which will increase the accessibility of enzyme to polysaccharides and reduce the unproductive binding of enzyme to the lignin. More importantly, the hemicellulose and cellulose content in the pulp remain stable [1] due to the neutral pH which decreased the peeling at high pH value or degrading at low pH value. Therefore, high monosaccharides yield is obtained from the native biomass after AS pretreatment and enzymatic hydrolysis.

To the best of our knowledge, this is the first report on AS pretreatment for improving the enzymatic hydrolysis. Wheat straw can serve as a low cost attractive feedstock for biofuels production [20]. In the present work, pretreatment conditions for obtaining high polysaccharides digestibility and fermentable sugars yield from wheat straw using AS as pretreatment option were examined. The native and pretreated wheat straw samples were characterized by scanning electron microscope (SEM), flourier translation infrared spectrum (FT-IR) and X-ray diffraction (XRD), and the extractives from the spent liquor after pretreatment was characterized by FT-IR.

Materials and methods

Materials and reagents

Wheat straw (purchased from Hénan Province, China) was thoroughly washed two times with tap water to remove the dust and then oven-dried at 80 °C for 12 h. The wheat straw was ground and homogenized with a sieve of 40–60 mesh (particle size 0.3–0.4 mm). The native wheat straw composed of (% dry weight basis) glucan 37.2, xylan 28.8, acid insoluble lignin (AIL) 18.9, acid soluble lignin (ASL) 3.2 and ash 2.4. The chemical composition was analyzed as described below under the Section 2.5. Cellulase and xylanase were kindly provided by Imperial Jade Bio-Technology Co., Ltd. (Yinchuan, China). The activities of cellulase and xylanase were 205 FPU/mL and 487 FXU/mL, respectively. The unit of xylanase was expressed as the amount of enzyme required to produce 1 μmol of xylose from 1% (wt) birchwood xylan solution under the given conditions (pH 5, 50 °C). AS with 90% purity was purchased from Macklin (Shanghai, China). All other reagents with analytical grade were purchased from local company and used without further purification.

Pretreatment of wheat straw

An aqueous solution of AS with known concentration was prepared, and sodium carbonate (SC) with known concentration was added to maintain the pH. For each test, 20 g of oven-dried wheat straw was mixed with 200 g solution with the solid-to-liquid (S/L) ratio of 1:10 (w/w). The suspension was subjected to pretreatment in a 500 mL high pressure reactor. The pretreatment was performed on the scheduled program (40 ± 3) min of heating and 4 levels of dwell temperature levels from 120 to 180 °C with 4 dwell time levels from 0.5 to 2 h. During pretreatment, the reactor was rotated at 50 rpm. After the pretreatment, the reactor was cooled to 85 °C within (10 ± 3) min, and then the suspension was taken out and filtered with a 300-mesh nylon bag. The spent liquor was collected for analysis and the solid fraction was washed with tap water to remove the adhering AS. After washing, the pretreated wheat straw was transferred to a pre-weighed plastic bag. The moisture content of the pretreated wheat straw was measured and the biomass recovery was calculated. The washed pretreated wheat straw were stored at 4 °C for enzymatic hydrolysis. A series of experiments were conducted to investigate the effect of temperature, time, AS concentration, and SC supplementation levels on pretreatment.

Characterization of extractives, native and pretreated samples

The spent liquor after pretreatment was centrifuged at 6142*g* RCF for 10 min, and the extractives were obtained from the supernatant by oven drying at 80 °C. The native and pretreated wheat straw was freeze dried. The extractives were characterized by FT-IR, and the native and pretreated wheat straw was characterized by SEM, FT-IR and XRD according to our previous study [18,19].

Enzymatic hydrolysis

Enzymatic hydrolysis tests were conducted in 100 mL conical flasks by suspending 1 g of pretreated samples in 50 mL buffer (100 mM sodium acetate with pH adjusted to 5 by acetic acid). 0.02% (w/v) sodium azide was used in the hydrolysis system to inhibit the growth of microorganisms. The enzymes cocktail used in this research included cellulase with an activity of 35 filter paper unit (FPU)/g biomass and xylanase with an activity of 70 Farvet xylan unit (FXU)/g biomass. The bottles were incubated at 50 °C for 24 h using an incubator shaker. After enzymatic hydrolysis, the samples were first put into boiling water for 5 min to stop the enzyme activity, and then centrifuged at 6142*g* RCF for 10 min, filtered and stored at room temperature for analysis. The digestibilities of glucan and xylan were calculated as the following Eqs. (1) and (2), and the yields of glucose and xlyose were calculated as the following Eqs. (3) and (4), Glucan recovery, xylan recovery and biomass recovery were calculated as the following equation (5, 6 and 7).

$$\text{Glucan digestibility} = \frac{\left[c(Glucose) + {}^{20}/_{19} \times c(Cellobiose)\right] \times 0.90}{Csubstrate \times Glucan\ content} \times 100\% \quad (1)$$

$$\text{Xylan digestibility} = \frac{c(Xylose) \times 0.88}{Csubstrate \times Xylan\ content} \times 100\% \quad (2)$$

$$\text{Glucose yield} = \frac{c(Glucose) \times 0.9 \times Biomass\ recovery}{Csubstrate \times Glucan\ content} \quad (3)$$

$$\text{Xylose yield} = \frac{c(xylose) \times 0.88 \times Biomass\ recovery}{Csubstrate \times Xylan\ content} \quad (4)$$

$$\text{Glucan recovery} = \frac{M(Pretreated\ biomass) \times Glucan\ content}{M(Native\ biomass)} \quad (5)$$

$$\text{Xylan recovery} = \frac{M(Pretreated\ biomass) \times Xylan\ content}{M(Native\ biomass)} \quad (6)$$

$$\text{Biomass recovery} = \frac{M(Pretreated\ biomass)}{M(Native\ biomass)} \quad (7)$$

where c(Glucose), c(Cellobiose) and c(Xylose) means the concentration of glucose, cellobiose and xylose in the enzymatic hydrolysis system, g/L. 20/19, multiplication factor that converts cellobiose to equivalent glucose. Glucan content and xylan content means the glucan and xylan content in the substrate, g/g. Csubstrate means the substrate loading, g/L. 0.90 and 0.88 were the conversion factors used to convert glucan and xylan into glucose and xylose, respectively. Glucan recovery was calculated as the weight of glucan in the pretreated biomass divided by the weight of glucan in the native biomass, g/g. Xylan recovery was calculated as the weight of xylan in the pretreated biomass divided by the weight of xylan in the native biomass, g/g. Biomass recovery was calculated as the dry weight of the pretreated biomass divided by the dry weight of the native biomass, g/g.

Analytical methods

The chemical composition of native and pretreated wheat straw was analyzed according the standard procedure developed by NREL [23]. Sugars (cellobiose, glucose, xylose and arabinose) and acetate were