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Effects of different pretreatment methods on chemical composition of sugarcane bagasse and enzymatic hydrolysis

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HIGHLIGHTS

- The two combinative pretreatments of LHW–NaOH and NaOH–LHW, were compared.
- The first step for combinative pretreatment had a more significant impact.
- LHW–NaOH pretreated SCB achieved the highest total sugar recovery of 73%.
- NaOH–LHW pretreated SCB showed the highest enzymatic hydrolysis efficiency.

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ABSTRACT

Different pretreatment processes, including liquid hot water (LHW) pretreatment, sodium hydroxide (NaOH) pretreatment, and their combinative pretreatments, were conducted to improve the enzymatic digestibility and sugar recovery from sugarcane bagasse (SCB). LHW pretreatment solubilized over 82% of xylan and 42% of lignin, after which the SCB presented the poorest enzymatic digestibility. NaOH pretreatment could remove 78% of lignin and retain most of glucan. For combinative pretreatments, the sequence of two procedures had a significant effect on the chemical composition, substrate characteristic and the subsequent enzymatic hydrolysis process. LHW–NaOH pretreatment could solubilize over 92% of xylan and remove 76% of lignin, and the highest total sugar recovery of 73% was achieved after 72 h enzymatic hydrolysis. While NaOH–LHW pretreatment, which could remove nearly 84% of lignin, but only solubilize 71% of xylan, showed the highest enzymatic digestibility. The pretreatment efficiency was: NaOH–LHW > NaOH > LHW–NaOH > LHW.

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1. Introduction

Generally, the enzymatic degradation process of lignocellulose consists of two steps: firstly, the lignocellulosic material is pretreated to destroy its complicated natural composite; and secondly, the material is enzymatically converted to fermentable sugars (Jørgensen et al., 2007). Because of the complex chemical structure of the lignocellulosic materials, the direct enzymatic digestion of cellulose is hindered. The objectives of pretreatment are to modify or remove lignin and/or hemicellulose, decrease the crystallinity of cellulose, and increase biomass surface area to benefit lignocellulosic enzymes accessing the recalcitrant structure of cellulose for maximum recovery of sugars (Balat et al., 2008; Jorgensen et al., 2007).

Over the past several decades, a variety of physical, biological, chemical, and thermal pretreatments have been conducted, including the use of dilute acid (Schell et al., 2003), aqueous ammonia (Kim et al., 2003) and liquid hot water pretreatment (Yu et al., 2011). Several combined pretreatment technologies have been also reported, such as a combination of sulfuric acid-free EtOH heat treatment followed by mechanochemical pulverization, and H_2O_2 followed by biological pretreatment (Kim and Lee, 2005; Teramoto et al., 2008; Yu et al., 2009). Among the many conventional processes, liquid hot water treatment and alkali treatment are well known for providing successful fractionation of lignocellulosic biomass. Liquid hot water (LHW) pretreatment, where uses pressure to maintain the water in the liquid state at elevated temperatures, is an attractive approach since no additional chemicals are used. An effective LHW pretreatment generates reactive cellulose fiber, allowing the recovery of most of the pentosan, and only a few of the degradation products that can hinder subsequent hydrolysis and fermentation (Yu et al., 2012b, 2011). Alkali pretreatments could increase cellulose digestibility and they are more effective for lignin solubilization, exhibiting minor cellulose and hemicellulose solubilization than acid or hydrothermal process (Carvalheiro et al., 2008). Sodium hydroxide causes swelling, increasing the internal surface of cellulose and decreasing the degree of

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polymerization and crystallinity, which provokes lignin structure disruption (Taherzadeh and Karimi, 2008). Alkaline pretreatments do not require specialized equipment, as the alkaline reagents typically used do not cause corrosion like dilute acids, and high pressures like those used in AFEX are not utilized.

A combinative pretreatment of LHW and aqueous ammonia (LHW–AA) had been reported by Kim and Lee (2006), which was suggested for further improvement of enzymatic hydrolysis, while the combinative pretreatment of LHW and NaOH has been rarely reported. This paper aimed at investigating the effects of LHW pretreatment, NaOH pretreatment, and especially the two combinative pretreatments of LHW and NaOH, on the chemical composition, structure and enzymatic digestibility of the sugarcane bagasse (SCB).

2. Methods

2.1. Material

SCB was provided by Guangxi FengHao Group Co. Ltd. (Pingxiang, China). It was premilled and screened, and the fraction between 20 and 80 meshes was used for experiments. A cellulase mixture namely Cellic CTec2, was provided from Novozymes A/S (Bagsaevrd, Denmark). The cellulase activity was 310 FPU/mL (FPU is the activity unit of cellulase when filter paper is used as the enzymatic substrate), assayed by the description of IUPAC (Ghose, 1987). The β-glucosidase activities was 704 U/mL, which was determined by the method of Sylwia, W (Wołosowska and Synowiecki, 2004).

2.2. Pretreatment

2.2.1. Liquid hot water pretreatment

The experimental system consisted of an autoclave reactor, a feeding system and a product collector. Details of the experimental apparatus are described elsewhere (Zhuang et al., 2009). About 30 g of the SCB (5% w/v in water) was put into the reactor, which was sealed and heated to the reaction temperature (180 °C) with the magnetic agitator operating at 500 rpm for 20 min. The reaction pressure was controlled by the addition of nitrogen at 4.0 MPa. After pretreatment, the temperature was cooled down to less than 100 °C by cold water (Yu et al., 2011). When the reactor was cooled down to room temperature, the liquid was collected for composition analysis, and the solid residue was dried in a forced-air oven at 105 °C, and kept at desiccator for the subsequent chemical analysis and enzymatic hydrolysis experiment.

2.2.2. Sodium hydroxide pretreatment

Oven-dried SCB was placed in a laboratory bottle (Pyrex glass) and then mixed with 0.25 M NaOH at a ratio of 1 g original raw material to 20 mL liquid. The solid/liquid slurry was incubated in a water bath at 80 °C for 3 h, with agitation. After pretreatment, the solid residue was separated by filtering and washed with tap water until neutral pH. The solid residue was dried in a forced-air oven at 105 °C, and kept at desiccator for the subsequent chemical analysis and enzymatic hydrolysis experiment.

2.2.3. Liquid hot water–sodium hydroxide combined pretreatment

SCB was treated with hot liquid water described in Section 2.2.1. The solid residue was separated by filtering, and then washed with tap water until neutral and then treated with 1% NaOH described in Section 2.2.2. After treatment, the solid residue was separated and washed as described above. At last, the solid residue was dried in a forced-air oven at 105 °C, and kept at desiccator for the subsequent chemical analysis and enzymatic hydrolysis experiment.

2.2.4. Sodium hydroxide–liquid hot water combined pretreatment

SCB was treated with 1% NaOH described in Section 2.2.2. After the treatment, the solid residue was separated by filtering, washed with tap water until neutral and then treated with hot liquid water described in Section 2.2.1. The solid residue was separated, washed and dried as follow.

2.3. Enzymatic hydrolysis

The enzymatic hydrolysis experiments were conducted at a solid loading of 5% dry mass (DM) (w/v), 50 °C and 150 rpm in 150 mL Erlenmeyer flasks, each containing 50 mL of 0.05 M sodium citrate buffer (pH 5.0) sealed with rubber stoppers. The enzyme loadings were 5, 10, 20, 30, 40, 50 FPU/(g glucan). Samples were taken out at 72 h measured by high performance liquid chromatography (HPLC). All experiments were carried out in duplicate. The enzymatic digestibility was calculated as the ratio of glucose in the enzymatic hydrolysis per 100 g of potential glucose in the substrate.

2.4. Analytical methods and calculations

2.4.1. Components analysis

The components of SCB before and after pretreatment were determined according to the standardized methods of the National Renewable Energy Laboratory (NREL, Golden, CO, USA) (Sluiter et al., 2004). The total sugars in the liquid fraction were calculated after a secondary hydrolysis into monosaccharide with 4% of sulfuric acid. The glucose, xylose, cellubiose were determined by HPLC using a Shodex sugar SH-1011 column coupled with a refractive index detector. The mobile phase was 0.005 mol/L H_2SO_4 at a flow rate of 0.5 mL/min, with a column temperature of 50 °C. The yield of sugar in the hydrolyzate was calculated on the basis of the amount of sugar polymers in the treated solids.

2.4.2. XRD

Crystallinity of different samples was analyzed by wide-angle X-ray diffraction on an X Pert Pro MPD (PW3040/60, Philips, Holand) instrument using Cu kα radiation ($\lambda = 0.154$ nm) at 40 kV and 40 mA, with a step size of 0.017° and a recorded range from 5° to 50°. In order to compare the crystalline changes among different samples, CrI (%) was calculated as follows (Segal et al., 1959):

$$\mathrm{CrI} = (I_{002} - I_{am})/I_{002} \times 100$$

where I_{002} is the intensity of diffraction (002) plane at about $2\theta = 22.5°$ and I_{am} is the intensity of baseline at about $2\theta = 18.4°$.

3. Results and discussion

3.1. Composition change after different pretreatments

The compositions of SCB after different pretreatment methods related to the initial dry mass were summarized in Table 1. Because arabinose, galactose and mannose made up only a small portion of the sugars in SCB, only glucose and xylose were considered in our studies. It was shown that all the pretreatments drastically changed the composition of SCB and caused minor glucan loss, especially after the NaOH pretreatment.

After the LHW pretreatment, a large portion of xylan was degraded and removed. However, about 58% of lignin remained in the residue where lignin still posed 23.07%. When the second step

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