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Enhancement of enzymatic hydrolysis of sugarcane bagasse by pretreatment combined green liquor and sulfite

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HIGHLIGHTS

- Sugarcane bagasse is pretreated by combination with green liquor (GL) and sulfite.
- GL-sulfite pretreatment could protect carbohydrates and selectively remove lignin.
- Enzymatic hydrolysis efficiency is improved by GL-sulfite pretreatment.
- Pretreatment using Kraft-GL allows higher glucose yield than that using Soda-GL.
- GL-sulfite pretreatment could couple with a pulp mill for biorefinery.

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ABSTRACT

Green liquor (GL) is readily available in any pulp mills which could be recycled. Alkaline sulfite pretreatment process was developed for non-woody biomass biorefinery. In this study, GL combined with sulfite was effectively utilized to improve the enzymatic hydrolysis of sugarcane bagasse by selectively removing lignin. Compare to conventional alkaline sulfite pretreatment, the solid yield could be higher with low degradation of polysaccharide. With the increment of the pretreatment temperature from 100 to 140 °C, the enzymatic hydrolysis efficiency was improved. And a higher glucose yield was achieved under pretreatment condition of 0.4 g/g-DS Na_2SO_3 and 1.5 mL/g-DS GL due to the higher degradation of xylan. The highest glucose yield of 96.8% could be reached with 3.36 g/(L·h) of initial rate after Kraft GL-sulfite pretreatment at 140 °C. The superior performance of Kraft GL allows conversion of cellulose to glucose more significantly than Soda GL. In addition, surface tension test indicated that a part of lignin was dissolved in the pretreatment liquor as lignosulfonate. The results showed that GL combined with sulfite is a promising way for sugarcane bagasse pretreatment.

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1. Introduction

Considering the environmental problems associated with the use of fossil fuel, biofuel production from lignocellulosic biomass has received great attention all over the world [1]. Among these biomass feedstocks, sugarcane bagasse is a fibrous residue that remains after sucrose extraction, which has been used to produce second generation ethanol in many countries [2–4]. In general, 280 kg of bagasse could be generated from 1 ton of sugar cane [5], and about 100 million dry tons of sugarcane bagasse are produced globally every year [6]. Sugarcane bagasse mainly consists of approximately 40–50% cellulose, which can be used as a source of cellulose [7]. One of the main bottlenecks to produce ethanol from lignocellulosic biomass is the complex structure of biomass. A pretreatment process is typically employed to disrupt the cellulose-hemicellulose-lignin complexes in order to enhance the enzymatic hydrolysis efficiency [8,9]. The major key factors for pretreatment are producing highly digestible solids for further enzymatic hydrolysis and avoiding the degradation of sugars [10]. In addition, it also should minimize the formation of inhibitors and be cost effective.

Alkaline sulfite pretreatment has been reported to improve cellulose enzymatic hydrolysis efficiency significantly owing to the

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change of chemical composition, biomass ultrastructure and porosity during pretreatment, with the consequent increase in enzyme accessibility to cellulose [11–15]. This pretreatment method is preferable for non-woody lignocellulosic biomass with loosen structure. The pretreatment liquor consists of lignosulfonate and monomeric sugars which can be converted to biofuel and chemicals after further extraction. Up to 85% cellulose conversion to glucose after 96 h of hydrolysis was obtained by enzymatic hydrolysis of sugarcane bagasse pretreated with alkaline sulfite under a pretreatment condition which yielded 53% and 29% removal of lignin and hemicellulose, respectively [12]. Different from alkaline pretreatment, sulfonation of lignin is also observed, which increases fiber swelling and improves lignin dissolution. The topochemical characterization of alkaline sulfite pretreated sugarcane indicated that surface lignin was dissolved during the pretreatment while surface hemicellulose was exposed or relocated. The surface coverage of carbohydrates increased after alkaline sulfite pretreatment as shown by X-ray photoelectron spectroscopy and Time-of-Flight secondary ion mass spectrometry [13].

GL is a type of alkaline liquor which is completely recoverable after combustion of the black liquor in the recovery boiler. There are two main types of GL. GL from the Soda pulping process (Soda-GL) is mainly composed of sodium hydroxide and sodium carbonate. Whereas, GL from the Kraft pulp mills (Kraft-GL) generally contains sodium carbonate and sodium sulfide. In recent years, alkaline pretreatment process based on GL has been well developed [10,16,17]. This moderate alkaline pretreatment could selectively remove lignin and keep as much polysaccharides as possible. In the meantime, no toxic byproducts are produced to affect the further fermentation step. In our previous study, both furfural residues produced from corncobs during the process of furfural production and sugarcane bagasse were pretreated with a combination of GL and ethanol to enhance the enzymatic hydrolysis [18–20]. And the results showed that the lignin in these two biomasses could be effectively removed and enzymatic hydrolysis efficiency was improved significantly. In addition, GL coupled with hydrogen peroxide could also remove lignin in furfural residues effectively [21]. Utilizing GL in biomass pretreatment can be used to repurpose a pulp mill for bioethanol production [16].

In this study, aiming at improving enzymatic hydrolysis of sugarcane bagasse, GL and sulfite were combined for the pretreatment of sugarcane bagasse. The influence of GL type and pretreatment temperature on enzymatic hydrolysis efficiency was investigated under two series conditions (low alkali concentration with high Na_2SO_3 loading and high alkali concentration with low Na_2SO_3 loading).

2. Materials and methods

2.1. Materials

The sugarcane bagasses were kindly provided by Guitang Corporation (Guangxi, China). The dry sugarcane bagasses were ground and screened with 40 meshes before the experiments. Two kinds of GL were used in this study: Soda GL from soda pulping process and Kraft GL from Kraft pulping process. Both two GL were precipitated 12 h before utilization. The main components of Soda GL and Kraft GL are shown in the Table 1.

2.2. GL combined with sulfite pretreatment

In general pretreatment, approximately 5 g of crude material (dry weight basis) was impregnated with the GL-sulfite liquor at a sample/liquor ratio of 1:20 (w/v). The impregnated sugarcane bagasses were cooked at desired temperature for 3 h. Then, the pretreated sugarcane bagasses were filtered through filter paper and washed with de-ion water to neutral pH. Pretreated materials were stored at 4 °C for further enzymatic hydrolysis. The reaction parameters in detail were given in the Table 2.

Table 1
Chemical composition of two different green liquors.

Component	Content, g/L	
Na_2CO_3	75.20	Soda GL [19]
NaOH	23.04	
Na_2CO_3	99.58	Kraft GL [20]
NaOH	18.02	
Na_2S	28.05	

The solid yield was calculated by using the following equation:

$$\text{Solid yield } (\%) = M_1 \times 100/M_0 \quad (1)$$

where M_1 is mass of pretreated dry solid (g), M_0 is mass of untreated dry solid (g).

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis of untreated and pretreated sugarcane bagasses was carried out at 50 °C using a shaking incubator at 150 rpm. The substrate consistency was 5% (w/v). The filter paper activity of cellulase (Celluclast 1.5 L, Sigma Co., St. Louis, MO, USA) was 67 FPU/mL, and the cellobiase activity of Novozyme 188 (Sigma Co.) was 175 CBU/mL. The enzyme loading for substrate was 18 FPU/g-cellulose for cellulase and 27 CBU/g-cellulose for β-glucosidase. The hydrolysis of sugarcane bagasse without pretreatment was performed as a control. Samples after enzymatic hydrolysis were collected at 0, 6, 9, 12, 24, 36, 48 and 72 h followed by centrifuged at 10,000*g* for 5 min. Then, the supernatants were filtered through 0.22 μm filters and diluted properly for further sugar analysis. HPLC analysis of the sugars was conducted using a Waters 2695 HPLC system equipped with Refractive Index Detector and a Bio-Rad Aminex HPX-87P column at 85 °C using water as the mobile phase and a flow rate of 0.6 mL/min. The glucose yield was calculated by assuming that 1 g of cellulose present in the liquid theoretically renders 1.11 g of glucose [22].

2.4. Analysis of substrate composition

The compositional analysis of sugarcane bagasse and substrates after pretreatment followed a standard NREL method [23]. The dried pretreated substrate (300 mg) was loaded into pressure tube with the addition of 3.0 mL of 72 wt% H_2SO_4 solution. The mixture was left at 30 °C for 1 h and stirred with a glass rod every 10 min. Afterwards, 84 mL purified water was added to reach a H_2SO_4 concentration of 4 wt%. Then the samples were heated to 120 °C for 1 h in an autoclave. The resultant solution was filtered and neutralized by calcium carbonate. Then the filtrate was used for sugar analysis by HPLC as described in enzymatic hydrolysis section. The precipitate was washed with de-ion water until neutral pH, then dried at 105 °C and weighed to determine Klason lignin.

2.5. Contact angle analysis

Sodium acetate buffer (pH 4.8) contact angles on samples (untreated and pretreated sugarcane bagasse) surfaces were determined according to Yu's method [19] on a contact angle goniometer OCA15Pro instrument. The samples were dried at 50 °C for 12 h followed by pressed into 15 mm diameter disks under 20 t/m^2 - pressure. About 5 μL sodium acetate buffer were dropped carefully on the sample surfaces and the pictures in 1 s were captured to

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