



Enhancement of high-solids enzymatic hydrolysis of corncob residues by bisulfite pretreatment for biorefinery



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HIGHLIGHTS

- High-solids enzymatic hydrolysis of FRs was investigated.
- Glucose yield was greatly improved by increasing hydrophobic interactions.
- Hydrolysis residues had a high thermal stability and concentrated high derivatives.
- Bisulfite pretreatment can contribute to fully utilizing the furfural residues.

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ABSTRACT

Co-production of glucose, furfural and other green materials based on a lignocellulosic biorefinery is a promising way to realize the commercial application of corncob residues. An effective process was developed for glucose production using low temperature bisulfite pretreatment and high-solids enzymatic hydrolysis. Corn cob residues from furfural production (FRs) were pretreated with 0.1 g NaHSO₃/g dry substrate at 100 °C for 3 h. Lignin was sulfonated and sulfonic groups were produced during pretreatment, which resulted in decreasing the zeta potential of the samples. Compared with raw material, bisulfite pretreatment of FRs increased the glucose yield from 18.6 to 99.45% after 72 h hydrolysis at a solids loading of 12.5%. The hydrolysis residues showed a relatively high thermal stability and concentrated high derivatives. Direct pretreatment followed by enzymatic hydrolysis is an environmentally-friendly and economically-feasible method for the production of glucose and high-purity lignin, which could be further converted into high-value products.

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1. Introduction

Hazardous dense haze in China in recent years has been increasingly threatening public health. The Chinese government set up a national network to monitor the impact of air pollution on human health (Li and Zhang, 2014). Increased vehicle emission is one major reason behind the widespread haze, therefore driving clean energy development (Jacobson, 2009). As a renewable alternative energy source made from lignocellulosic materials, cellulosic ethanol has received worldwide attention. However, the wide use of ethanol in vehicles was limited by its high production cost. About 20–40% of the costs of ethanol are due to biomass supply and 90% of the costs of supplying biomass are logistics related

costs (Ekşioğlu et al., 2009). Biomass can be processed into useful biomaterials and maximize the economic value in a biorefinery (Kaparaju et al., 2009). There is an urgent need to design integrated biorefineries that are capable of producing bioethanol and chemicals (Demirbas and Demirbas, 2010). Therefore, this study considers the use of corncob residues (FRs) to produce ethanol in a biorefinery concept, which focuses on polysaccharide bioconversion as well as lignin utilization.

FRs are an industrial waste lignocellulosic material generated from furfural industry. China is the dominant supplier in the world furfural market. With approximately 85% of global furfural production capacity and 75% of world consumption, China will continue to drive the overall market. Furfural is mainly produced from corncob in China. Production of one ton of furfural can produce 13 tons of FRs (Dai et al., 2010). About 2.3 million tons of FRs are annually generated in China (Feng et al., 2013). Utilization of these residues

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is highly desirable as it may not only reduce environmental issue but also increases the economic viability of the furfural industry (Zhao et al., 2015; Zhu et al., 2016). As a cellulose-rich waste, FRs can be used to produce ethanol by fermentation (Xing et al., 2016). Removal of acidic/toxic compounds seems necessary for efficient enzymatic hydrolysis and fermentation (Bu et al., 2012). It is estimated that the acid content in FRs was 14–15% (Jönsson and Martín, 2016). Water-washing detoxification is the most common method, but it is difficult to implement at the manufacturing scale.

To minimize water usage, a bisulfite pretreatment technology was developed (Xing et al., 2015; Zheng et al., 2015). Unwashed FRs was first pretreated with bisulfite, and the whole slurry was then directly used for enzymatic hydrolysis. Without detoxification of FRs, water can be saved and acid in the wet FRs facilitates lignin sulfonation (Xing et al., 2015). Due to these advantages, bisulfite pretreatment can be effectively applied to non-detoxified FRs. The Lignosulfonate in the non-detoxified slurry decreased the non-productive adsorption of cellulose on the substrate and enhanced enzymatic hydrolysis. Apart from high glucose yield, high glucose concentration is also necessary for realizing commercial application. When enzymatic hydrolysis is performed at high-solids substrates, the reaction mixture becomes more viscous. High amounts of free water are required for high-solids enzymatic hydrolysis. A recent study found that pretreated materials by sulfonation of lignin have increased water retention values (WRV) (Chandra et al., 2015) due to the hydrophilic nature of lignosulfonates. Low amount of free water can render a negative effect on cellulose conversion. To investigate the potential of FRs for bioethanol production, high-solids enzymatic hydrolysis of FRs was discussed at high-solids loadings.

The objective of this work is to improve the glucose concentration by using bisulfite pretreatment on non-detoxified FRs. The zeta potentials in the hydrolysis systems were compared to verify the effect of washing on the high-solids enzymatic hydrolysis. The enzymatic hydrolysis residues (residues obtained from enzymatic hydrolysis) were further analyzed by TG, XPS, and SEM to determine the reasons for the hydrolysis results and potential application of enzymatic hydrolysis residues. The lignocellulose biorefinery based on the sugar and lignin platform was also discussed.

2. Materials and methods

2.1. Materials

FRs were kindly provided by Chunlei Furfural Corporation (Hebei, China). FRs had a pH of 2–3 and high moisture content (54.2%) initially. The particle size of the FRs was uneven and ranged from 60-mesh to 20-mesh. The residues were stored in a freezer at -4°C until used.

Celluclast 1.5 L, a cellulase preparation from *Trichoderma reesei*, and Novozyme 188, a β -glucosidase preparation from *Aspergillus niger*, were purchased from Novozymes Investment Co, Ltd. (Shanghai, China). The activity of Celluclast 1.5 L was detected to be 95 FPU/mL, and that of Novozyme 188 was 175 BGU/mL.

2.2. Bisulfite pretreatment

No size reduction was needed before pretreatment in our experiments. All bisulfite pretreatments of FRs with or without washing were conducted at 100°C for 3 h at a liquor-to-solid ratio of 7:1 using a 200 mL jar on a roller bottle at 35 rpm. The sodium bisulfite loading was 0.1 g NaHSO_3/g dry substrate (DS). No additional sulfuric acid was applied in all the pretreatment. The pretreatment

condition was based on the previous studies (Xing et al., 2015; Zheng et al., 2015). After pretreatment, the whole slurry was directly used for enzymatic hydrolysis. As a control, some of the solids after pretreatment were washed with distilled water until neutral to remove the lignosulfonate and residual acid, and then enzymatically saccharified for 72 h. Four schemes of pretreatment combined with enzymatic hydrolysis were obtained based on the different processes: scheme WBWE, scheme WBE, scheme BWE, and scheme BE. The details for the four schemes are shown in Fig. 1. After pretreatment, some of the washed samples were dried in an oven at 105°C for 6 h to evaluate the moisture content and the solid yield. The mass of total solid residues would be recorded. The solid yield was calculated using the following equation:

$$\text{Solid yield} = \frac{\text{mass of pretreated dry solid(g)}}{\text{mass of untreated dry solid(g)}} \times 100$$

The composition percentage of bisulfite pretreated FRs based on the initial FRs can be obtained by multiplying the yield by the percentage of each composition of bisulfite pretreated FRs.

2.3. Enzymatic hydrolysis

Enzymatic hydrolysis was performed at 50°C for 72 h. The reaction was carried out with a substrate consistency of 12.5% (w/w) at pH 4.8, using a shaking incubator at 150 rpm. The enzyme loading for the substrate was 18 FPU/g-cellulose for cellulase and 27 CBU/g-cellulose for β -glucosidase. The hydrolysis of FRs without pretreatment was performed as a control. At various intervals, hydrolysates (about 2 mL) were taken and centrifuged at $10,000g$ for 5 min. The supernatants were filtered through $0.2\ \mu\text{m}$ filters and diluted properly for further sugar analysis. All experiments were performed at least in duplicate, and the standard errors or deviations of the analyses were observed to be lower than 5%. The glucose yield of the substrate was calculated according to the glucose content as a percentage of the theoretical glucose available in the substrates. A cellulose:glucose ratio of 1:1.11 (g/g) was considered. To ensure the applicability and accuracy of the experimental data, glucose in the initial FRs was eliminated from the calculation of the substrate saccharification yield.

2.4. Analysis of substrate composition and sugar concentration

The Klason lignin and carbohydrate contents of raw and pretreated materials were carried out according to the National Renewable Energy Laboratory (NREL) lignin analysis method for biomass (Sluiter et al., 2008). The Klason lignin content was taken as the ash free residue after acid hydrolysis. The filtrate was collected to detect the carbohydrate and inhibition concentration by HPLC (Waters2695e, USA) equipped with a refractive index detector (RID) (Waters 2414, USA). Glucose and xylose were separated (AminexHPX-87P; Bio-Rad Laboratories) at 85°C . Acetic acid and formic acid were separated (AminexHPX-87H; Bio-Rad Laboratories) at 65°C . The flow rate was 0.6 mL/min using a MilliQ-water as eluent at. The injection volume of the sample was $10\ \mu\text{L}$ and the temperature set for RID was 35°C . Each sample is analyzed in duplicates.

2.5. Characterization of hydrolysis substrates

2.5.1. Surface tensions

The surface tensions of the supernatant samples were determined according to Jian's method on the automatic tension meter (model JK99B, Zhongchen digital technology equipment Co, Ltd, Shanghai, China) at 20°C in an aqueous medium (Jian et al., 2011).

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