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High glucose recovery from direct enzymatic hydrolysis of bisulfite-pretreatment on non-detoxified furfural residues

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HIGHLIGHTS

- Non-detoxified FRs were directly pretreated with bisulfite for ethanol production.
- Direct bisulfite pretreatment eliminated sulfuric acid injection and washing step.
- Direct bisulfite pretreatment and enzymatic hydrolysis improved glucose recovery.
- The surface tension of supernatants was decreased by lignin sulfonation.
- High-solids loadings of 16.5% showed excellent performance with unwashed FRs.

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ABSTRACT

This study reports four schemes to pretreat wet furfural residues (FRs) with sodium bisulfite for production of fermentable sugar. The results showed that non-detoxified FRs (pH 2–3) had great potential to lower the cost of bioconversion. The optimal process was that unwashed FRs were first pretreated with bisulfite, and the whole slurry was then directly used for enzymatic hydrolysis. A maximum glucose yield of 99.4% was achieved from substrates pretreated with 0.1 g $NaHSO_3$/g dry substrate (DS), at a relatively low temperature of 100 °C for 3 h. Compared with raw material, enzymatic hydrolysis at a high-solid of 16.5% (w/w) specifically showed more excellent performance with bisulfite treated FRs. Direct bisulfite pretreatment improved the accessibility of substrates and the total glucose recovery. Lignosulfonate in the non-detoxified slurry decreased the non-productive adsorption of cellulase on the substrate, thus improving enzymatic hydrolysis.

1. Introduction

Some huge obstacles existed in pretreatment process make industrial waste residues difficult and expensive to produce bioethanol. FRs, are one of the abundant lignocellulosic residues from commercial furfural production (Feng et al., 2013). The non-detoxified FRs have an initial pH of 2–3 and have a moisture content of 50–60%. To remove toxic products and eliminate the acidity of FRs, the washing process requires a large amount of water and sodium hydroxide (Bu et al., 2011). Based on the features of FRs (acid, carbohydrate-rich, and inexpensive), to explore a simple pretreatment method could be beneficial to the improvement of economic efficiency.

Because of high lignin content in materials, various pretreatment methods have been explored to increase the substrate accessibility to cellulose (Galbe and Zacchi, 2007; Leu and Zhu, 2013). The inhibitory effect of lignin (non-productive adsorption to cellulase) and sugar loss are key issues during ethanol production. In the furfural industry, corncobs are heated at 175–185 °C under acidic conditions (5–8% dilute sulfuric acid) to hydrolyze all the arabinoxylans (hemicelluloses) into xylose and partial cellulose to glucose (Yu et al., 2013). There exists not only xylose but also glucose in the wet FRs resulting from degradation of hemicellulose and cellulose. The traditional washing process removed the sugar in the FRs, which could be converted to ethanol or other chemicals. From an economic standpoint, it is of great necessity to reduce the sugar loss during the process. Although most pretreatments for converting FRs into biofuels have been resulted in high cellulose conversion (~70%), they are based on expensive chemical pretreatment processes that consume water and generate many effluents. Pretreatment that requires fewer chemicals and little water is promising for biofuel production. Alkaline pretreatments due to

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the mild conditions and low inhibitions have attracted extensive attention for removal of lignin in the recent years (Yan et al., 2015). However, detoxification of pretreatment residues is heavy and expensive due to the large amount of water consumption. It seems that modification of lignin under an acidic circumstance is an approach that can be explored. Acidic bisulfite pretreatment produces fewer inhibitors and is a more suitable pretreatment method for bioethanol production (Bu et al., 2012; Zhu et al., 2009).

In acidic bisulfite processes, nucleophilic substitution at the α-carbon and the sulfonation of lignin are the two main lignin reactions (McDonough, 1993). Acidic bisulfite pretreatment facilitates delignification through lignin sulfonation resulting in a highly digestible substrate. Moreover, lignosulfonate produced during lignin sulfonation increases the hydrophilicity of lignin, thus improving the enzymatic hydrolysis (Bu et al., 2012). Furthermore, lignosulfonate has a low affinity to cellulase and acts as a surfactant to enhance enzymatic saccharification (Wang et al., 2013a; Zhou et al., 2013). Therefore, the washing of FRs can be eliminated, while reducing water consumption and facilitating direct enzymatic saccharification of the pretreated whole slurry. Recently, "pH profiling" concept and low temperature (165 °C) was used in bisulfite pretreatment (Zhang et al., 2014; Cheng et al., 2015). The delayed application of acid and the lower temperature can reduce sugar degradation and furan formation. The hemicelluloses in FRs are mostly removed during furfural production, so there is hardly any furan formation and sugar degradation. A low temperature of 100 °C was applied to balance saccharification during bisulfite pretreatment without the addition of acid. Previous studies showed that acid addition was not necessary in applying sulfite pretreatment because of the formation of acetic acid, through deacetylation of the acetyl groups during pretreatment (Wang et al., 2009). Corncobs contain acetyl groups that can be converted to acetic acid during furfural production. Acetic acid in the wet FRs was detected, so effective bisulfite pretreatment may be achieved to produce lignosulfonate and used in delignification even without the addition of acid.

Despite many efforts to develop FRs for biofuel production, few studies have investigated the potential of non-detoxified furfural residues (FRs) for bioethanol production. Bisulfite pretreatment was used to increase glucose recovery and reduce water consumption. Enzymatic hydrolysis of pretreated whole slurry of FRs, without washing detoxification, was also evaluated. The aim of this study was to compare the sugar recoveries of FRs, and determine the effect of direct bisulfite pretreatment on the chemical composition and enzymatic hydrolysis of substrates. Simultaneously, the surface tension in various supernatants after hydrolysis was compared.

2. 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 freezer at −4 °C until used.

2.2. Bisulfite pretreatment

As received FRs material without energy consumption for the size reduction of 10 g was directly used for pretreatment. Bisulfite pretreatment of FRs with or without washing were conducted at 100 °C for 3 h at a liquor to solid ratio of 15:1 using 200 mL jar on a roller bottle at 35 rpm. Two levels of sodium bisulfite loadings of 0.1–0.6 g $NaHSO_3$/g dry substrate (DS) were used. No additional sulfuric acid was applied in pretreatment. 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 natural to remove the lignosulfonate and residual acid, and then enzymatically saccharified for 72 h. Based on the different processes, four schemes of pretreatment combined with enzymatic hydrolysis were obtained, called scheme WBWC, scheme WBC, scheme BWC, and scheme BC. The details for the four schemes are showed in Fig. 1. Moreover, after pretreatment, some of the washed samples were dried in an oven at 105 °C for 6 h to evaluate the yield. 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 2.5% (w/w) at pH 4.8, using a shaking incubator at 150 rpm. The filter paper activity of cellulose (Celluclast 1.5 L, Sigma Co., St. Louis, MO, USA) was 95 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 FRs without pretreatment was performed as a control. At various intervals, hydrolysates (about 2 mL) were taken and centrifuged at 10,000*g* for 5 min. The supernatants were filtered through 0.2 μm filters and were 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 a 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. However, the total glucose recovery of the substrate was calculated by adding the original glucose in raw material.

2.4. Effect of solids loadings on scheme SC

Processes under scheme BC were accomplished at high-solids-loadings using 200-mL jar on a roller bottle at 35 rpm. A certain quality of substrates was added to the 200-mL jar and diluted with distilled water to obtain a final substrate concentration of 7.5%, 12.5%, 16.5%, and 20% (w/w). Other conditions were the same as mentioned previously (Sections 2.2 and 2.3).

2.5. 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 and A., 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