Efficient Fractionation of Corn Stover by Bisulfite Pretreatment for the Production of Bioethanol and High Value Products

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Fractionation of corn stover (CS) was carried out by bisulfite pretreatment in order to improve the production of bioethanol and high-value chemicals. Firstly, the optimum bisulfite pretreatment conditions of CS (170 °C, 30 min, 7% $NaHSO_3$, 1% H_2SO_4) were identified. Next, a biorefinery process of bisulfite pretreatment for CS was proposed. CS was separated into solid and liquor components using such pretreatment. The solid components were employed for bioethanol production by quasi-simultaneous saccharification and fermentation (Q-SSF). The bisulfite liquor was fractionated into hemicellulosic sugars and lignin by different types of resins. It was shown that CS components could be effectively fractionated through bisulfite pretreatment in combination with resin separation to produce bioethanol, hemicellulosic sugars, and lignosulfonate.

Keywords: Corn stover; Bioethanol; Bisulfite pretreatment; Lignosulfonate; Biorefinery

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INTRODUCTION

Lignocellulosic biofuels are recognized as a potential alternative to fossil fuels (Ho *et al.* 2019; Milano *et al.* 2016). Many studies have been conducted to develop efficient technologies to convert lignocellulosic polysaccharides to bioethanol (Alvira *et al*. 2010). Corn stover (CS) is one of the most promising lignocellulosic biomasses for bioethanol production (Zabed *et al*. 2016). CS (the leaves, stalks, and husk left over after corn is harvested) is composed of high contents of cellulose and hemicellulose, and much attention has been paid to CS due to its low-cost in production of bioethanol and other by-products (Kadam *et al.* 2008; Buruiana *et al.* 2014; Uppugundla *et al.* 2014).

Unlike starch-based ethanol, the major problem in producing lignocellulosic bioethanol is that CS has a low conversion due to the recalcitrance of the material (Zhao *et al.* 2012; Meng *et al.* 2016). Biomass recalcitrance is thought to largely arise due to the spatial network of cellulose, hemicellulose, and lignin as a protective bulwark that restricts enzyme accessibility (Zhang *et al.* 2011). Several pretreatment processes have been developed to overcome biomass recalcitrance, such as dilute acid pretreatment (Liu *et al.* 2016), steam explosion pretreatment (Liu *et al.* 2013), organic acid pretreatment (Huang *et al.* 2018), hot water pretreatment (Li *et al.* 2014; Li *et al.* 2015), ammonia fiber expansion pretreatment (AFEX) (Kumar *et al.* 2009; Sundaram *et al.* 2015), and ionic liquid (IL) pretreatment (Papa *et al.* 2017). However, different pretreatments present various challenges due to the costs generated by using expensive chemicals, water, chemical recovery, energy, and the needed equipment (Brodeur *et al.* 2011).

Previous work has shown that bisulfite pretreatment is a promising process that can be used in the effective conversion of different lignocellulose polysaccharides into bioethanol as a liquid fuel source (Zhu *et al.* 2009; Tan *et al.* 2013; Li *et al.* 2014; Liu *et al.* 2017). Wang *et al.* (2011) and Yu *et al.* (2015) showed that the enzymatic hydrolysis of CS for ethanol production can be significantly improved using bisulfite pretreatment. However, to date, there has been a lack of studies exploring the biorefinery of CS using bisulfite pretreatment. In biorefinery, all the major components of CS (including cellulose, hemicellulose, and lignin) should be fractionated to liquid fuels and other high-value products. Significant hemicellulose degradation and sulfonation of lignin occur during bisulfite pretreatment (Tan *et al.* 2016), which improves the enzymatic digestibility of pretreated lignocellulosic solids. However, only part of the xylan and sulfonated lignin are spontaneously dissolved into the spent liquor, leading to the waste of these components. Therefore, the spent liquor is considered as a feedstock to produce high-value chemicals, such as xylose, xylan, and lignosulfonate, by which the biorefinery of CS can be realized. Furthermore, industrial-scale technology is in the early stage of development (Aditiya *et al.* 2016; Chen and Fu 2016), and pretreatment process and overall technological facilities are still under investigation in producing bioethanol from CS. Bisulfite pretreatment is derived from the traditional sulfite pulping process (Zhu *et al.* 2009), which has been performed in industry practice for more than a century. As a mature technology, the use of bisulfite pretreatment has a great advantage because the equipment and infrastructure for bisulfite pretreatment process have been widely used in the pulp and paper industries, which may greatly accelerate the process of industrial studies and the technologies for bioethanol production from CS and other lignocellulosic biomasses.

The present study aimed to separate cellulose, hemicellulose, and lignin from CS using the bisulfite pretreatment. Firstly, bisulfite pretreatment was applied on CS based on the chemical components of CS. Secondly, cellulose could be transformed into bioethanol through the quasi-simultaneous saccharification and fermentation (Q-SSF) process. Finally, different types of resins were employed to separate hemicellulose and lignin in the spent liquor in order to produce potential products, such as hemicellulosic sugars and lignosulfonate.

EXPERIMENTAL

Materials

CS was purchased from Weifang, Shandong Province, P. R. China. The processed CS was air-dried until constant moisture content was achieved (about 10%). Dried CS was cut to small pieces of 2-3 cm in length, sealed in bags, and then stored at room temperature.

Cellic® CTec2 was obtained from the commercially available source (Novozymes). The filter paper activity was determined to be 100 FPU/mL. Sodium bisulfite, sulfuric acid, and ethanol were of analytical grade. Other reagents, such as D-(+)glucose, D-(+)xylose, furfural, and 5-hydroxy-2-furaldehyde (HMF), were of chromatographic grade. Macroporous adsorption resins AB-8, DM130, CAD-40, and DM301 were provided by Hecheng New Material Co., Ltd., Zhengzhou, China.

Bisulfite Pretreatment of CS

Bisulfite pretreatment was carried out in a rotary electrothermal pressure digester (ZQS-3, Qinggong Jixie Factory of Shanaxi University of Science and Technology, Xianyang, Shaanxi Province, P. R. China) as follows. The reaction was performed at 170 °C for 30 min in a solution of sodium bisulfite consisting of 3%, 4%, 7%, or 8% $NaHSO_3$ and 1% H_2SO_4 (based on weight of dry CS, w/w). The reaction pressure was about 0.7 to 0.8 MPa. The ratio of CS (dry weight, g) to liquor (volume, mL) was 1:4. Subsequently, the insoluble solids were filtered using bag-type strainer and reserved for subsequent analyses.

Enzymatic Hydrolysis

The CS and the pretreated samples were hydrolyzed by Cellic® CTec2 in 100-mL flasks to evaluate their enzymatic digestibility under following conditions: 2% solid concentration (dry matter basis), pH of 4.8 (0.05 M sodium acetate buffer), 48 °C, 150 rpm in a shaker, and cellulase dosage of 10 FPU/g dry sample. The supernatant was used to determine the contents of glucose and xylose by the high-performance liquid chromatography (HPLC, Shimadzu, Japan) equipped with a refractive index detector (Shimadzu) on an Aminex HPX-87H column (Bio-Rad, USA). Briefly, 5 mM H_2SO_4 was employed as eluent at a flow rate of 0.6 mL/min, and the column temperature was maintained at 65 °C. The conversions of cellulose and xylan in enzymatic hydrolysis process were calculated using the equations as follows:

$$\text{Conversion of cellulose}(\%) = \frac{\text{Gluose released from enzymatic hydrolysis(mg)} \times 0.9}{\text{Sample weight(mg)} \times \text{Glucan content }(\%)} \times 100\% \tag{1}$$

$$\text{Conversion of xylan}(\%) = \frac{\text{Xylose released from enzymatic hydrolysis(mg)} \times 0.88}{\text{Sample weight(mg)} \times \text{Xylan content }(\%)} \times 100\% \tag{2}$$

Q-SSF

The Q-SSF experiment was performed in 100-mL anaerobic bottles using a commercial yeast strain of *Saccharomyces cerevisiae* (Angel Yeast Corporation Ltd., Hubei, China) according to the authors' previous work (Tan *et al.* 2013). Briefly, approximately 0.22 g of yeast was first activated. Subsequently, fermentation was carried out with 0.45 g glucose and 10 mL sterile water at 30 °C and 200 rpm for 2 h. The solid concentration of Q-SSF was 15%. The Q-SSF process consisted of pre-hydrolysis and subsequent fermentation process. Cellic® CTec2 (cellulase loading of 10 FPU/g dry solid sample) was added in the pre-hydrolysis phase, followed by incubation for 6 h. Next, the activated yeast (5 mg yeast/g dry sample) was supplemented into the fermentation for Q-SSF. The ethanol content was determined using the HPLC equipped with a refractive index detector (Shimadzu) on an Aminex HPX-87H column (Bio-Rad, USA). Briefly, 5 mM H_2SO_4 was employed as eluent at a flow rate of 0.6 mL/min, and the column temperature was maintained at 65 °C. The cellulose conversion in Q-SSF process was calculated as follows (Dowe and McMillan 2008),

$$\text{Cellulose conversion}(\%) = \frac{[\text{EtOH}]}{f \times biomass \times 1.11 \times 0.51} \times 100\% \tag{3}$$

where [EtOH] is the ethanol concentration at the end of the fermentation (g/L), *biomass* is the dry biomass concentration at the beginning of the fermentation (g/L), *f* is the cellulose fraction in dry biomass (g/g), 0.51 is the conversion factor for glucose to ethanol based on stoichiometric biochemistry of yeast, and 1.11 is the conversion factor for cellulose to the equivalent glucose

Resin Separation for Fractionation of Spent Bisulfite Pretreatment Liquid

In the present study, the hemicellulose and lignin constituents in the spent liquid were separated using various macroporous adsorption resins, such as AB-8, DM130, CAD-40, and DM301. Table 1 lists the physical properties of the four resins. Briefly, the impurities and monomers inside the pores of resins were removed by pretreating the resins with NaOH and HCl solutions. Subsequently, all resins were washed with deionized water. A chromatographic column (50 cm in length and 1.5 cm in diameter) was filled with different types of resin. The spent bisulfite pretreatment liquor was loaded onto the column at a constant speed, and then the column was washed with five volumes of deionized water. The filtered liquid (mainly consisting of sugars, named as carbohydrate constituent, CCS) was collected, and its chemical compositions were determined. The chromatographic column was then eluted with five volumes of 95% methanol, and all the eluent (mainly consisting of the lignosulfonate, named as lignosulfonate constituent, LCS) was pooled and analyzed. The recovery efficiencies of reducing sugar and lignosulfonate in CCS and LCS from different resins were determined, which were expressed as a proportion of the substance content in CCS or LCS to that in spent pretreatment liquor.

The eluent was dried to lignosulfonate solids (LSS) by vacuum distillation, followed by structural and property analyses. The spent bisulfite pretreatment liquid was vacuum freeze-dried to obtain control sample, which was designated as water-soluble substance (WSS) and directly compared with LSS. Above-mentioned experiment was carried out at least in duplicate, and the results were expressed as the means of repeated experiments.

Table 1. The Physical Properties of Four Macroporous Resins

Macroporous Resin	AB-8	DM301	DM130	CAD-40
Structure	Polystyrene	Polystyrene	Polystyrene	Polystyrene
Micropore area (m^2/g)	480-520	330-380	500-550	450-500
Micropore volume (ml/g)	0.73-0.77	0.7-0.9	0.81-0.88	0.73-0.77
Pore diameter (nm)	13-14	14-17	9-10	5-6
Polarity	Weak polar	Moderate polar	Weak polar	Weak polar

WSS and LSS Analyses

The contents of C, H, N, and S were determined by elemental analyses of LSS and WSS (Vario EL Ⅲ, Germany). Fourier transform infrared spectroscopy (FT-IR) (ALPHA, Germany) was employed to analyze the FT-IR spectra of the LSS and WSS samples using KBr discs.

Analytical Methods

The contents of cellulose (glucan), xylan, and lignin in untreated and pretreated CS samples were assessed according to NREL methods (Sluiter *et al.* 2008). The acid-soluble lignin was determined by UV-vis spectroscopy at a wavelength of 320 nm with an absorptivity of 30 (L/g cm). An Aminex HPX-87H column (Bio-Rad, USA) was used to separate furfural and 5-HMF (Loow *et al.* 2018). Briefly, 5 mM H_2SO_4 was employed as eluent at a flow rate of 0.5 mL/min, and the column temperature was maintained at 40 °C. A UV-vis spectrophotometer (UV-5500PC, METASH, China) with a typical absorption peak at 280 nm was used to determine the lignosulfonate content in the spent liquor (Li *et al.* 2012). The content of reducing sugar in bisulfite pretreatment liquid was examined by the 3,5-dinitrosalicylic acid (DNS) colorimetric method (Breuil and Saddler 1985).

RESULTS AND DISCUSSION

Bisulfite Pretreatment of CS

Previous studies have shown that bisulfite pretreatment is an efficient pretreatment, exhibiting great enzymatic cellulose conversion and ethanol production (Tan *et al.* 2013; Tian *et al.* 2011; Wang *et al.* 2011). In the present study, such a process was selected for the CS pretreatment, after which CS was separated into the cellulosic solids and spent liquor.

It has been found that $NaHSO_3$ dosage and H_2SO_4 dosage are the important factors affecting the bisulfite pretreatment (Tan *et al.* 2016). Previous studies have shown that 1% acid almost completely removes the xylan in the raw material (Tan *et al.* 2013; Wang *et al.* 2011). Therefore, the effect of $NaHSO_3$ dosage was investigated relative to bisulfite pretreatment of CS. The major composition changes of CS upon bisulfite pretreatment were analyzed with different $NaHSO_3$ dosages (3%, 4%, 7%, or 8%) and 1% acid at 170 °C for 30 min, and Table 2 lists the results.

The contents of cellulose, hemicellulose, and lignin in untreated CS were about 27%, 13%, and 20%, respectively. As shown in Table 2, the contents of extractives in the bisulfate-pretreated CS (about 9 to 10%) were significantly increased compared with the untreated samples (2.29%). Acidification appeared to be the main reason for the precipitation of extractives on the substance surfaces (Koljonen *et al.* 2004). Some lignin-derivatives might be precipitated from the pretreatment liquid and adsorbed on the substance surfaces (Dai *et al.* 2006), leading to the increased extractives in the bisulfite-pretreated samples. Moreover, the hemicellulose content of the solid substrates was increased with the decrease of $NaHSO_3$ dosage. More xylan was dissolved at higher $NaHSO_3$ dosage.

When $NaHSO_3$ dosage was increased from 3% to 8%, the solubility of xylan was increased from 66.19% to 75.8%, indicating that a large portion of hemicelluloses was removed during bisulfite pretreatment. Previous study has demonstrated that hemicelluloses can be removed through the cleavage of glycosidic ether linkages between sugar units during bisulfite pretreatment (Tan *et al.* 2015). The degree of delignification was increased from 17.9% to 39.5% when the $NaHSO_3$ dosage was increased from 3% to 8%. Sulfonation of lignin occurred during bisulfite pretreatment, which could be attributed to the sulfonation of Cα and/or Cγ in the phenyl-propane unit of lignin (Zhan 2009). The sulfonation of lignin could enhance the hydrophilicity of lignin, resulting in the dissolution of lignin during bisulfite pretreatment. Furthermore, it was found that the glucan loss was less than 6% when the $NaHSO_3$ dosage was within the range of 3 to 8%, indicating that most of cellulose in CS remained as solid during bisulfite pretreatment.

Table 2. Chemical Components of Pretreated Corn Stover from Bisulfite Pretreatment

$NaHSO_3$ dosage (%) *	pH	Yield (%)	Extractives (%)	Glucan (%) (% loss**)	Xylan (%) (% loss**)	Total lignin (%) (% loss**)
Untreated EFB	-	-	2.29±0.20	27.0±1.11	13.02±0.62	19.85±0.91
3	1.85	62.71	8.97±0.40	40.59±1.81 (5.78)	7.02±0.50 (66.19)	25.98±1.21 (17.93)
4	1.93	59.30	10.12±0.11	44.32±1.50 (2.70)	6.38±0.41 (70.93)	27.12±1.40 (18.97)
7	2.14	53.87	10.80±0.32	48.72±1.31 (2.85)	6.25±0.80 (74.12)	24.21±1.81 (34.31)
8	2.18	53.67	10.19±0.53	48.67±1.22 (3.30)	5.87±0.52 (75.80)	22.37±1.01 (39.51)

* $NaHSO_3$ dosage was based on untreated corn stover;
**glucan, xylan and lignin losses were based on the glucan, xylan and lignin contents in the untreated corn stover;
*** pretreatment conditions: 1% H_2SO_4, 170 °C, 30 min.

Fermentability of Bisulfate-pretreated and Enzymatically Hydrolyzed CS

Figure 1(a) shows the enzymatic digestibility of untreated and bisulfate-pretreated samples. The untreated CS exhibited a poor cellulose digestibility, which could only reach about 30% after 48 h of enzymatic hydrolysis. The enzymatic cellulose conversion of the pretreated cellulosic solids was increased with the increase of $NaHSO_3$ dosage. After pretreatment with 3% $NaHSO_3$ and 1% H_2SO_4, the enzymatic cellulose conversion was 65.6% with a cellulase dosage of 10 FPU/g dry sample after 48 h enzymatic hydrolysis (Fig. 1a). In the bisulfite pretreatment with 7% or 8% $NaHSO_3$, the conversion of cellulose could reach 85.9% and 86.0%, respectively. In this experiment, a $NaHSO_3$ dosage of 7% might be suitable for the CS bisulfite pretreatment due to the low chemical dosage and high cellulose conversion. In addition, the xylan conversion was higher than 85% when the cellulosic solid was hydrolyzed for 48 h.

Under the bisulfite pretreatment conditions of 7% $NaHSO_3$ and 1% H_2SO_4, the yield of the cellulosic solids was 53.9% based on the oven dry weight of untreated CS. The cellulosic solids of pretreated CS were used to produce bioethanol by Q-SSF at a solid concentration of 15%. Figure 1(b) shows that the ethanol production was enhanced to 29 g/L after 24 h of fermentation. The cellulose conversion was about 70%, and the ethanol productivity was about 1.229 g/L/h. In the system, the ethanol production was elevated to 36 g/L after 48 h of fermentation. The cellulose conversion was about 86%, and the ethanol productivity was about 0.752 g/L/h. After 72 h fermentation, the ethanol production reached about 37 g/L. However, the ethanol productivity from the Q-SSF of pretreated EFB became 0.514 g/L/h at 72 h, which was less than that at 24 h and 48 h. Saha *et al.* (2013) have reached an ethanol concentration of about 21 g/L and an ethanol productivity of about 0.29 g/L/h by using hydrothermally pretreated CS. Cai *et al.* (2016) have used a combinative technology of alkali and N-methylmorpholine-N-oxide (NMMO) to pretreat CS in order to improve the ethanol fermentation. The fermentation of the pretreated CS resulted in an ethanol yield of 64.6% through the separate hydrolysis and fermentation process, while it was only 18.8% for untreated samples with the cellulase loading of 15 FPU/g substrate. The results indicated that bisulfite pretreatment was an effective method for improving enzymatic hydrolysis of CS even at a low cellulase loading, by which a high conversion of cellulose to ethanol could be achieved.

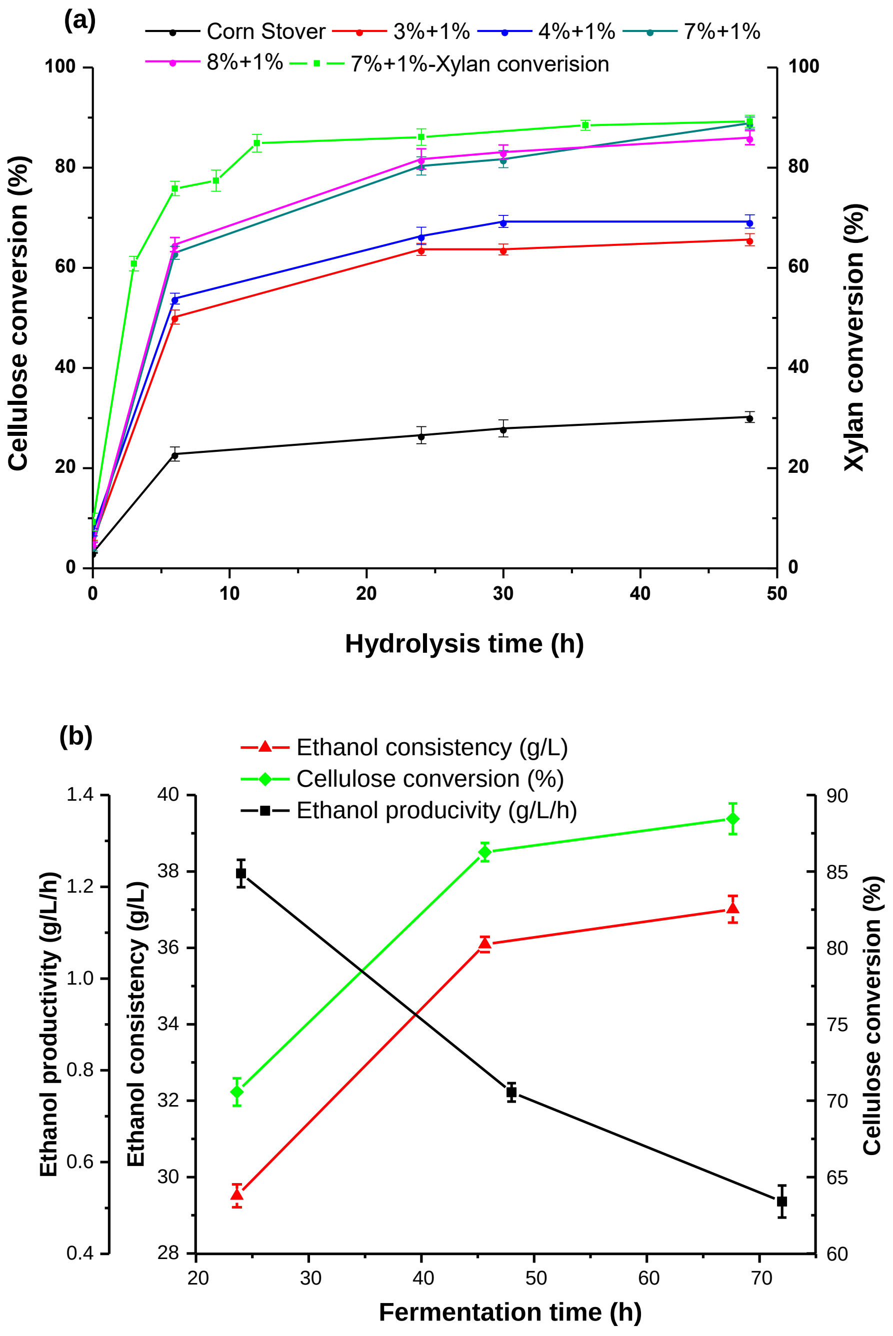

Fig. 1. Enzymatic digestibility (a) and ethanol productivity during Q-SSF (b) of bisulfite pretreated corn stover. (a) 2% solid concentration (dry matter basis), pH of 4.8 (0.05 M sodium acetate buffer), 48 °C, 150 rpm in a shaker, and cellulase dosage of 10 FPU/g dry sample. (b) The solid concentration of Q-SSF was 15%. Q-SSF process consisted of pre-hydrolysis and subsequent fermentation process. Cellic® CTec2 (cellulase loading of 10 FPU/g dry solid sample) was added in the pre-hydrolysis phase, followed by incubation for 6 h.

Resin Separation for Fractionation of Spent Bisulfite Pretreatment Liquid and Preliminary Mass Balance of the Biorefinery Process

Chemical components of spent bisulfite pretreatment liquid

In order to progressively improve the effective utilization of spent bisulfite pretreatment liquid, the major chemical components of the spent liquor were analyzed (including glucose, xylose, and lignin) (Table 3). It was found that the increase in $NaHSO_3$ (from 3% to 8%, w/w) could remove more xylose and lignin from bisulfite pretreatment at an H_2SO_4 dosage of 1% (w/w). The concentrations of xylose and lignin in the spent bisulfite pretreatment liquid were about 6.75 g/L and 9.52 g/L, respectively, when pretreatment was conducted with 3% bisulfite and 1% sulfuric acid. However, the concentrations of xylose and lignin were about 10.10 g/L and 14.50 g/L, respectively, when the pretreatment was conducted with 7% bisulfite and 1% sulfuric acid. The concentrations of major chemical components existing in the bisulfite pretreatment liquid were obtained, which were consistent with the chemical components of the cellulosic solids.

Furthermore, the contents of furfural and 5-HMF were determined, since these are known to be the major fermentation inhibitors in the spent bisulfite pretreatment liquid (Table 3). 5-HMF is derived from the dehydration of glucose, and furfural is formed from the dehydration of xylose during sugar degradation by thermal acid (Larsson *et al.* 1999). These compounds, which inhibit the ethanol production by the yeast, are designated as fermentation inhibitors. The formation of furfural and 5-HMF was dependent on the combined effect of $NaHSO_3$ dosage and acid dosage (Tan *et al*. 2016). Table 3 reveals that the increase of $NaHSO_3$ dosage resulted in the increased furfural and 5-HMF concentrations in the spent liquor. These data suggested that the concentrations of furfural and 5-HMF in the spent liquor were 10.22 g/L and 3.11 g/L after bisulfite pretreatment, respectively, when using 7% bisulfite and 1% sulfuric acid.

Table 3. Concentrations of Major Chemical Components and Fermentation Inhibitors in Bisulfite Pretreatment Liquid

$NaHSO_3$ dosage (%)	H_2SO_4 dosage (%)	Xylose (g/L)	Lignin (g/L)	Furfural (g/L)	5-HMF (g/L)
3	1	6.75±0.21	9.52±0.30	9.21±0.20	1.61±0.19
4	1	9.31±0.30	10.32±0.11	9.79±0.44	2.48±0.11
7	1	10.10±0.51	14.50±0.21	10.22±0.51	3.11±0.20
8	1	9.49±0.40	14.73±0.10	10.53±0.20	2.67±0.32

5-HMF: 5-hydroxymethyl-2-furaldehyde.
Experiments were performed in duplicate.

Resin separation

The carbohydrate and lignin components in spent bisulfite pretreatment liquor were separated using four types of macroporous adsorption resin and 95% methanol as eluent. Figure 2A shows that the color of CCS was much lighter than that of pretreatment liquor, especially for the CCS under AB-8 separation. The recovery efficiencies of the carbohydrate and lignin components using different resins are listed in Fig. 2B-2C. Figure 2B reveals that the recovery efficiencies of the reducing sugar in CCS from the chromatographic column with macroporous adsorption resins AB-8 and CAD-40 were higher (over 80%) compared with DM130 and DM301.

Moreover, the recoveries of reducing sugar using resin AB-8 were above 90%. The lignosulfonate content in CCS was approximately 10%, 19%, 27%, and 41% when using resins AB-8, DM 130, DM 301, and CAD-40, respectively. In addition, resin AB-8 had higher efficiency in removing lignin components in CCS compared with other resins because of the higher carbohydrate content (above 90%) and the lower lignosulfonate content (only about 10% of recovery of lignosulfonate). Besides, the eluent, named as LCS, was also analyzed (Fig. 2C). Recovery efficiencies of lignosulfonate in LCS were approximately 86%, 81%, 63%, and 56% for resins AB-8, DM 301, DM 130, and CAD-40, respectively, whereas the recovery efficiencies of reducing sugar in LCS were all below 10%. AB-8 resin was able to effectively separate the sugar and lignosulfonate components in the bisulfite pretreatment liquor based on these results. Therefore, the resin AB-8 might be selected for separating the carbohydrate and lignosulfonate constituents in the spent bisulfite pretreatment liquor. The authors' previous work has shown that the effective separation of spent bisulfite pretreatment liquid of empty fruit bunch from oil palm can be achieved by using the resin DM130, while this work revealed that the resin AB-8 was the best choice. This discrepancy might be caused by the different chemical compositions of the cell wall polymers (cellulose, hemicelluloses, and lignin) in different plant cell walls (Demartini *et al.* 2013).

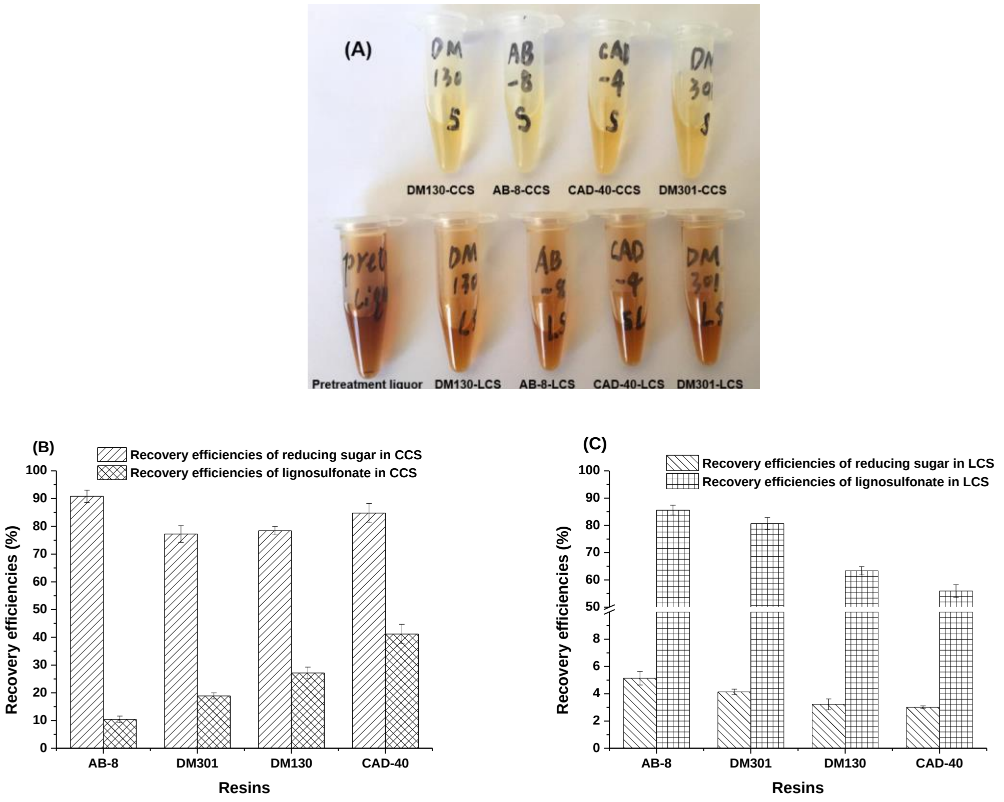

Fig. 2. (A) Photograph of carbohydrate constituent (CCS) and lignosulfonate constituent (LCS) by resin separation of the spent pretreatment liquid; (B) Recovery efficiencies of different constituents in CCS; (C) Recovery efficiencies of different constituents in LCS using different resins

Preliminary mass balance of the biorefinery process

Figure 3 shows the preliminary mass balance of the overall process under bisulfite pretreatment when the Q-SSF process was conducted with 15% solids and a cellulase loading of 10 FPU/g substance. As a result, the ethanol yield of 162 L/ton CS was achieved through Q-SSF of the solid substrate from bisulfite pretreatment. Moreover, the yields of the lignin and xylan in the spent liquor were 58 and 76 kg, respectively, for 1,000 kg of CS. For biorefinery of CS, the lignin and xylose/xylan fractions in the spent liquor were separated to produce lignosulfonate and xylose/xylan products by resin separation (resin AB-8 and 95% methanol system). Lignosulfonates and xylose/xylan products could be sold as a commodity for their characteristics.

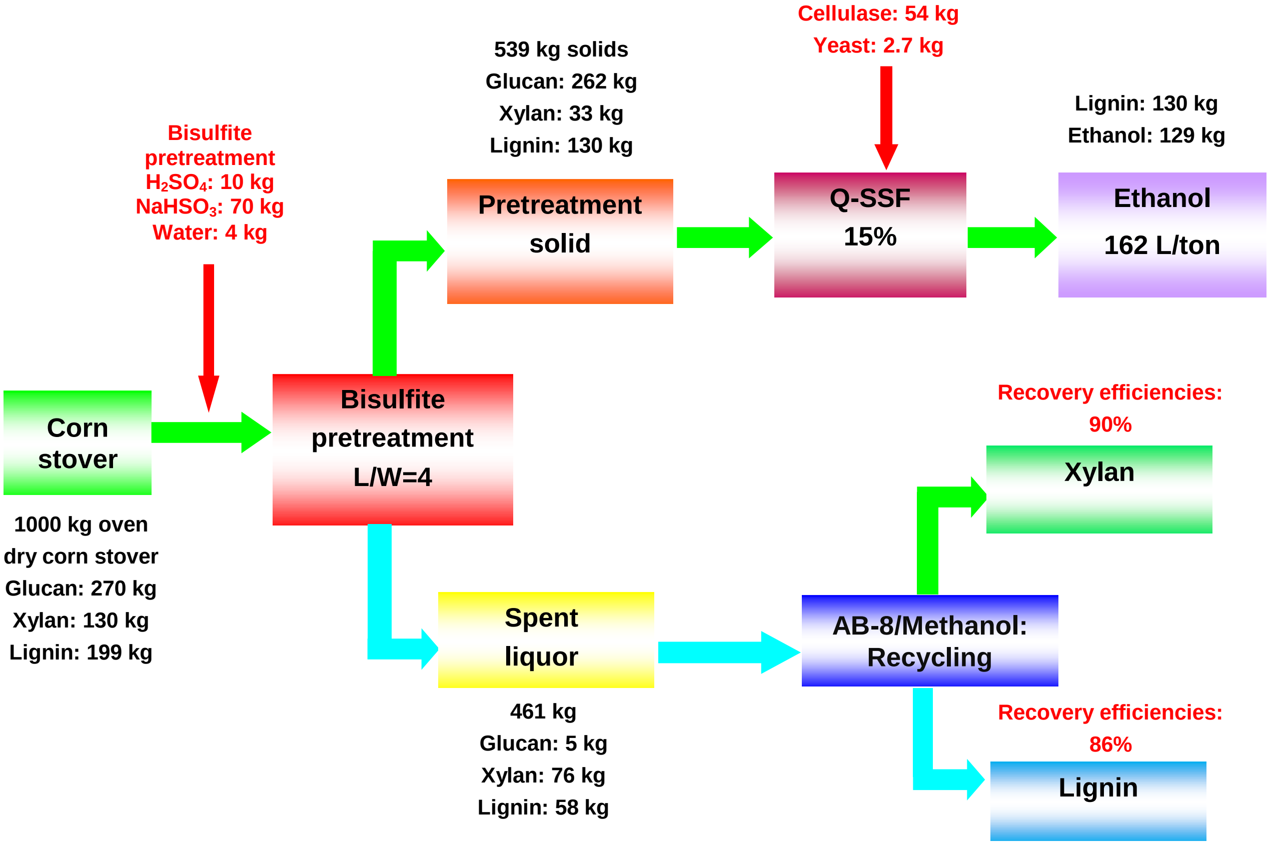

Fig. 3. Preliminary mass balance of biorefinery of corn stover with bisulfite pretreatment
* pretreatment was conducted at 170 °C for 30 min with sodium bisulfite and sulfuric acid dosages of 7% and 1%, respectively, on oven dry corn stover. Q-SSF was conducted at solid consistency of 15% and cellulase dosage of 10 FPU/g substrates

Structure and Properties of LSS and WSS

Figure 4 illustrates the FT-IR spectra of the LSS and WSS. LSS and WSS all contained functional groups, such as aromatic ring, alcoholic hydroxyl, methoxy, methyl, G–lignin, S–lignin, and other groups. The wide absorption around 3,404 to 3,410 cm^{-1} could be attributed to the O–H stretching vibration in –OH groups, while the signals at 2,937 cm^{-1} could be assigned to the C–H stretching vibrations in methyl groups. The bands at 1,517 cm^{-1} and 1,427 cm^{-1} corresponded to the aromatic ring vibrations. A small peak at 1,460 cm^{-1} corresponded to asymmetric C–H deformations in CH_3 and CH_2. The vibrations caused by aliphatic CH stretching in CH_3 at 1,384 cm^{-1} could be observed in the two spectra. The absorption peaks at 1,195 cm^{-1} were assigned to C-H vibrations in methoxy groups. Absorptions of syringyl (S) units and guaiacyl (G) units were obviously observed at 1,123 cm^{-1} and 1,044 cm^{-1}, respectively (Li *et al.* 2012). Certain changes were observed in LSS after separation when using resin AB-8 compared with WSS. The conjugated carboxyl absorption at 1,637 cm^{-1} was missing in the WSS spectra. In contrast, the band at 1,664 cm^{-1} due to C=O stretch of conjugated *p*-substituted aryl ketones was observed in the LSS spectra. The signal at 1,246 cm^{-1} which was assigned to C–O stretching vibrations in syringyl groups was much more prominent in LSS compared with WSS.

The sulfur content of WSS and LSS was determined using elemental analysis method. The contents of C, H, S, and N in WSS were 33.91%, 4.89%, 5.34%, and 1.49%, respectively, while those values became 49.08%, 6.01%, 2.88% and 2.41% in LSS, respectively. The results indicated that both LSS and WSS contained relatively high contents of sulfur. The decrease of sulfur content in LSS might be due to the removal of inorganic sulfur in WSS during resin separation, suggesting that lignin was better purified after resin separation (Tan *et al.* 2016). These results showed that LSS obtained from the spent bisulfite pretreatment liquor of CS was pure, and it consisted of relatively high content of sulfur, implying that LSS could be used as surfactants, chemical products, or in other industrial applications.

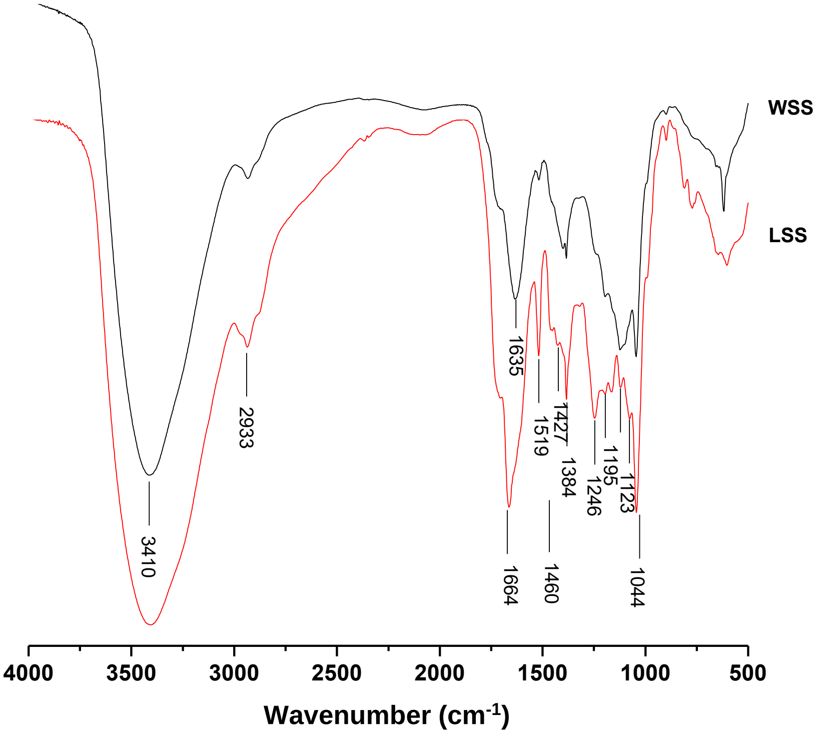

Fig. 4. FT-IR spectra of water-soluble substances in bisulfite pretreatment liquid (WSS) and lignosulfonate solids after resin separation (LSS)

CONCLUSIONS

1. Corn stover (CS) was fractionated into cellulosic solids, hemicellulosic sugars, and lignosulfonates following application of bisulfite pretreatment.

2. The cellulosic solids were transformed into bioethanol by a quasi-simultaneous saccharification and fermentation (Q-SSF) process. The bisulfite pretreatment liquid was effectively separated into hemicellulosic sugars and lignosulfonates with the macroporous adsorption resin AB-8 and a 95% methanol system.
3. The resulting lignosulfonate was pure, and it contained relatively high content of sulfur, suggesting that the lignosulfonate could be used as a surfactant or in other industrial applications.

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