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Effect of bisulfite treatment on composition, structure, enzymatic hydrolysis and cellulase adsorption profiles of sugarcane bagasse

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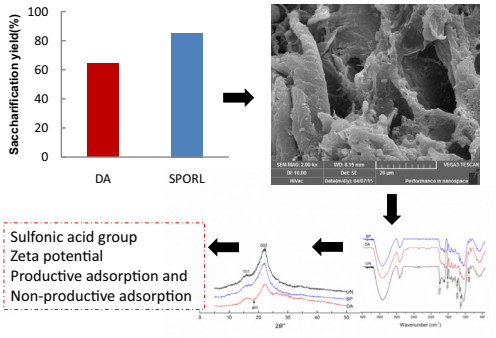
HIGHLIGHTS

- SPORL treated SCB had a higher saccharification yield than DA treated SCB.
- Compositions and SEM pictures of DA and SPORL SCBs were not significantly different.
- FTIR and XRD determinations of these two treated SCBs showed some differences.
- The sulfonic acid group content and Zeta potential of SPORL treated SCB were higher.
- The non-productive adsorption of cellulase to SPORL treated SCB was less.

GRAPHICAL ABSTRACT



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ABSTRACT

The effect of sulfite pretreatment to overcome recalcitrance of lignocellulose (SPORL) on composition, structure, enzymatic hydrolysis and cellulase adsorption profiles of sugarcane bagasse (SCB) was investigated. SPORL gave a higher SCB hydrolysis yield (85.33%) compared to dilute acid pretreatment (DA) (64.39%). The SEM pictures showed that SPORL SCB structure became more disordered and looser, suggesting SPORL SCB was more accessible to cellulase. The zeta potential of SPORL SCB suspension (−21.89 mV) was significantly different from that of DA SCB (−12.87 mV), which demonstrated the lignin in SPORL SCB was more hydrophilic. With regard to cellulase adsorption profiles, SPORL SCB had a lower non-productive adsorption (14.87 mg/g lignin) and a higher productive adsorption (37.67 mg/g carbohydrate) compared with DA SCB (17.05 mg/g lignin; 25.79 mg/g carbohydrate). These results indicated that SPORL SCB had better accessibility to cellulase and the higher productive cellulase adsorption of SPORL SCB had improved hydrolysis.

1. Introduction

Sugar and starch-based ethanol biofuel has been widely studied and used in many countries including US, Brazil, Europe, Japan and China (Harris et al., 2014; Ajanovic and Haas, 2014; Robl et al., 2015; Chen and Qiu, 2010). Due to the scarcity and unavailability of certain food materials required in the production of food-based fuel, lignocellulose-based bioethanol gained a substantial interest among many researchers (Gnansounou and Dauriat, 2010). However, the obstacle of improving the low saccharification efficiency of substrate still existed in bioconversion of lignocellulose because of the recalcitrance of native lignocellulose biomass. Therefore, pretreatment methods are essential to open the native

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structures of lignocellulose and make cellulases accessible to substrate (Kumar and Wyman, 2009; Luo et al., 2014; Pu et al., 2013).

So far, many researchers have focused on the pretreatment methods of lignocellulose biomass in lignocellulosic ethanol biorefinery, including dilute acid pretreatment, alkali pretreatment, ionic liquid pretreatment, steam explosion pretreatment, etc. (Zhu and Pan, 2010). To efficiently remove the recalcitrance of lignocellulose substrate in the saccharification process, a pretreatment method named Sulfite Pretreatment to Overcome Recalcitrance of Lignocellulose (SPORL) was developed (Zhu et al., 2009a). SPORL method has some significant advantages, such as producing less water pollution, consuming less energy and being able to obtain higher enzymatic saccharification efficiency of lignocellulose (Zhu et al., 2010; Zhu and Pan, 2010). This pretreatment method has been successfully used in many kinds of woody lignocellulose substrates, like Aspen, Lodgepole pine, Red pine and Spruce (Lan et al., 2013; Zhu et al., 2009a), however, was hardly used in non-wood lignocellulosic biomass.

Sugarcane bagasse (SCB) is a non-wood lignocellulosic biomass, a solid residue from the sugar milling of a shrubby tropical plant sugarcane. SCB is also an abundant renewable resource and its main constituents are cellulose and hemicellulose, thus making it viable as raw material in lignocellulose bioethanol industry. Maitan-Alfenas et al. have conducted extensive researches on SCB-based bioethanol, including pretreatment methods, enzymatic hydrolysis, and fermentation (Maitan-Alfenas et al., 2015; Cao and Aita, 2013; Rabelo et al., 2014). However, in these studies, the enzymatic saccharification yield was still low and the pretreatment methods often consumed more energy and caused more pollution. Therefore, a less energy-consuming and pollution-producing SCB pretreatment method should be explored to obtain a higher saccharification yield in a bioethanol refinery.

The aim of this study was, thus, to investigate the saccharification potential of SCB treated by SPORL. In this study, DA and SPORL pretreatment methods were initially conducted. Subsequent enzymatic saccharification experiments of the two SCBs were done at pH 5.3. To further explain the reason for the higher saccharification yield of SPORL SCB, the morphology and cellulose crystallinity of the two SCBs were studied by SEM, XRD and FTIR analysis, and then the Zeta potential of substrate suspension and the adsorption profiles of cellulase onto SCB substrates were also measured.

2. Materials and methods

2.1. Materials

SCB samples were collected from Guitang Sugar Refinery (Guangxi, China). The size of SCB was about from $0.1 \times 0.1 \times 0.1$ to $0.1 \times 1.0 \times 3.0$ cm^3. The cellulase CTec2 was provided by Novozymes (Tianjin, China), and the enzymatic activity was 147 FPU/mL that was assayed by the description from IUPAC (Ghose, 1987). The chemical reagents for HPLC analysis were of HPLC grade. All the other chemicals were of analytical grade.

2.2. Pretreatment

The first step of the pretreatment process was to place the complex (SCB, chemicals and distilled H_2O) into the stainless steel, steam-jacketed rotating pressure vessel (ZQS1-15 Model, Machinery Works in Shanxi University of Science and Technology, Shanxi, China). For the SPORL pretreatment, the complex was 50 g dried SCB, 3 g $NaHSO_3$, 0.55 g H_2SO_4 and 400 mL distilled H_2O; for the DA pretreatment, the complex was 50 g dried SCB, 0.55 g H_2SO_4 and 400 mL distilled H_2O. The contents were heated at 160 °C for 30 min and at a speed of 1 rpm to mix. After cooling, the complex was separated by filtration and squeezing into the solid part and the liquid part (pretreatment liquor). The solid part was still wet and included a lot of pretreatment liquor. The solid part was subsequently milled for 2 min by a Joyoung Mill (JYL-350 Model, Joyoung Co., Ltd, Hangzhou, China) for size reduction without washing, and then, sealed in a plastic bag and stored at 4 °C for the next analysis.

2.3. Determination of SCB composition

The chemical composition (cellulose, hemicellulose, lignin and ash) of pretreated and untreated SCB was determined according to the method recommended by National Renewable Energy Laboratory (Sluiter et al., 2012). The formulas in the literature (Qiu et al., 2012) were referred to for the calculation of cellulose and hemicellulose contents in the treated and untreated SCB. All experiments and assays were performed in triplicate.

2.4. Enzymatic hydrolysis

After pretreatment, the milled solid part including about 75% water was used to conduct the enzymatic hydrolysis. Enzymatic hydrolysis of SCB was conducted at 2% (w/v) dry substrate solids in a 100 mL shake flask. The total volume of citrate buffer (50 mM, pH 5.3), CTec2 cellulase (7.5 FPU/g dried substrate) and the water in the solid part was 50 mL. The dry weight of the solid part was 1 g. The mixture was incubated at 50 °C with agitation at 140 rpm for 72 h. The reducing sugar (RS) in hydrolysate after 72 h was determined by the dinitrosalicylic acid (DNS) method (Ghose, 1987). All experiments and assays were performed in triplicate. The saccharification yield was calculated based on the amount of the reducing sugars obtained in the hydrolysate divided by the total carbohydrate present in SCB and times 0.9 (to correct the increased weight from hydrolysis), as shown in the following equation:

$$\text{Saccharification yield}\,(\%) = 0.9 \times \text{RS(g)} \times 100/\text{total carbohydrate(g)}$$

2.5. High performance liquid chromatography (HPLC)

The glucose, xylose, cellobiose, mannose, arabinose and galactose in hydrolysates were measured with a HPLC system (Agilent, 1260) equipped with a refractive index detector (Agilent, G1362AX, 30 °C). The column was a Carbomix Pb-NP10:5% (7.8 × 300 mm, 10 μm, Sepax), and the column temperature was 75 °C. The mobile phase was deionized water at a flow rate of 0.45 mL/min. All experiments and assays were performed in triplicate.

2.6. Scanning electron microscopy (SEM) analysis

The samples of pretreated and untreated SCB were first oven-dried at 70 °C to constant weight. The completely dried samples were coated with gold to make the fibers conductive, avoiding degradation and buildup of charge on the specimen before scanning electron micrographs were taken using a scanning electron microscope (TESCAN, VEGA 3 SBH). The scanning electron microscope was operated at 20 kV to image the samples.

2.7. X-ray diffraction (XRD) analysis

The treated and untreated SCB samples were determined by XRD using a diffractometer (D/Max, 2200) and Cu-K_α radiation (λ = 1.54 Å) generated at 36 kV and 30 mA. The samples were

scanned from 5° to 50° with a step size 0.02°. Data reduction was accomplished with JADE 5.0. The crystallinity index (CrI) was calculated from the following equation below:

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

where I_{002} is the scattered intensity of diffraction (002) plane and I_{am} is the scattered intensity due to the amorphous portion evaluated as the minimum intensity between the main and secondary peaks (Qiu et al., 2012).

2.8. Fourier transform infrared spectroscopy (FTIR) analysis

Fourier transform infrared spectroscopy (FTIR) was performed using FTIR Spectrometer (Bruker, TENSOR27). SCB samples were analyzed by grinding with KBr (1:20, w/w) and pressing into slices. Scans were conducted at 400–4000 cm^{-1} with a resolution of 4 cm^{-1} and at 10 scans per sample.

2.9. Sulfur-content analysis

The sulfur content of the pretreated SCB samples was determined using inductively coupled plasma mass spectrometry (ICP-MS) (Horiba Jobin-Yvon, Ultima model). The samples were digested at 145 °C for 15 min in a microwave oven using 5 mL 70% HNO_3 and 3 mL 30% H_2O_2 before analysis. The sulfur contents were then calculated to get sulfonic acid group contents. All experiments and assays were performed in triplicate.

2.10. Zeta potential measurement

The zeta potentials of the SCB samples treated by DA and SPORL methods were measured in the citrate buffer (50 mM, pH 5.3) by using a shaker/incubator at 50 °C and 180 rpm for 2 h. Next, the mixture of the treated SCB sample and the buffer solution was allowed to stand at 4 °C overnight, and then was centrifuged at 10,000 rpm for 10 min. The SCB in the buffer solution was 2% (w/v). The supernatant was tested by using a Zeta Potential Analyzer (Brookhaven, ZetaPALS). The measurement procedure from the study conducted by Lan et al. was referred to (Lan et al., 2013). All zeta potential measurements were performed in triplicate with seven readings in each experiment.

2.11. Cellulase adsorption

Cellulase adsorption experiments were conducted in citrate buffer solutions of pH 5.3 with the substrate at a solids consistency of 1% (w/v). Lignin residues and pretreated SCB samples were respectively used to determine the non-productive and total adsorption of cellulase. Lignin residues were made according to the previous method (Lou et al., 2013). The initial concentration of CTec2 was 80 mg protein/g substrate. After incubation at 50 °C for 2 h, the solution was centrifuged at 12,000 rpm for 10 min. The protein concentration of the final supernatant was measured by using Bradford method. The amount of cellulase adsorption onto the substrate was calculated by subtracting the amount of free protein in the supernatant from the total amount of initial protein. All experiments and assays were performed in triplicate.

2.12. Statistical analysis

The means and standard deviations were calculated. All data were analyzed by one-way analysis of variance (one-way ANOVA). The statistical software Origin 8.5 (OriginLab, Northampton, MA, USA) was used for data analysis.

3. Results and discussion

3.1. Composition of SCB

Substrate pretreatment is an important process in order to obtain effective saccharification yield of lignocellulosic biomass in a lignocellulosic ethanol industry (Zhu and Pan, 2010). In this study, the effects of DA pretreatment and SPORL pretreatment on the composition of SCB were initially compared, and the results are shown in Table 1.

The extractives in the untreated and treated SCB samples were respectively 1.33 ± 0.27% (UN), 4.67 ± 0.09% (DA) and 4.51 ± 0.11% (SPORL). The contents of extractive in SCB samples are often different according to the types and sources of sugarcane. The extractive content of 1.33 ± 0.27% in untreated SCB was reasonable. The contents of extractive in DA and SPORL treated SCB samples were significantly ($p < 0.05$) higher than that in untreated SCB, which might be due to the looser structures of treated SCBs that caused more extractives to be extracted. The total composition of untreated and treated SCBs was respectively 99.65%, 104.30% (DA) and 102.21% (SPORL). These data on total composition were not 100%, but this was acceptable (Tsuchida et al., 2014; Rezende et al., 2011).

The pretreated SCB samples had significantly ($p < 0.05$) less hemicellulose and ash, and significantly ($p < 0.05$) more cellulose and lignin as compared with the untreated SCB. DA pretreatment removed a lot of hemicellulose and hemicellulose content of DA treated SCB decreased to 10.5% from 24.2% of untreated SCB, which was consistent with the results obtained by Zhu et al., in which DA treatment mainly removed hemicellulose by breaking down the structure of hemicellulose (Zhu et al., 2009b). For the SCB treated by SPORL, the hemicellulose content was 11.2% which was similar with that of DA treated SCB 10.5% (Table 1). In the process of pretreatment, both solutions prepared for DA and SPORL pretreatments contained dilute sulfuric acid. The sulfuric acid present in SPORL method gave similar hemicellulose removal (no significant difference, $p > 0.05$) compared to the dilute acid method. Lan et al. studied the chemical composition of lodgepole pine and aspen treated by SPORL and found that SPORL pretreatment could removed most of the xylan and mannan (Lan et al., 2013). For example, xylan and mannan contents of untreated and treated lodgepole pine by SPORL were 5.5% and 11.7% vs. 1.0% and 0.9%, respectively. Xylan and mannan contents of untreated and treated aspen by SPORL were 16.4% and 1.4% vs. 1.9% and 0.3%, respectively.

In this study, the ash contents of untreated and treated SCB samples were determined and the results are shown in Table 1. The ash contents in DA and SPORL SCB samples were respectively decreased to 2.0% and 3.6% from 9.5% of untreated SCB. It has been known that non-woody biomass has higher ash content than woody biomass and the ash content of crop straw is about 8–15% (Yu and Chen, 2010). Raveendran and Ganesh (1998) found the ash content in sugarcane bagasse was 2.9% and Cao and Aita (2013) found that of SCB was 6.5%, indicating that the ash contents are significantly different according to the type and original place etc. of SCB. The ash cations, K^+, Mg^{2+}, Fe^{3+}, Ca^{2+}, Cu^{2+}, Zn^{2+} etc. existing in biomass can affect cellulase activities because its alkalinity may affectively neutralize some acids, reducing effectiveness (Demeyer et al., 2001). Therefore, removing the ashes in treated substrate can help to stabilize the pH during next experiments and washing treated substrate with water is often used to remove ashes to improve hydrolysis (Yu and Chen, 2010). In this study, water washing was not conducted in order to reduce liquid wastes and keep the other components in the pretreatment liquor that could improve enzymatic hydrolysis.

Table 1
The compositions of SCBs untreated and pretreated. The values following ± were standard deviations. All experiments and assays were performed in triplicate.

SCBs	Composition (%)					
	Extractive	Cellulose	Hemicellulose	Lignin	Ash	Total
Untreated	1.33 ± 0.27[a]	41.54 ± 0.45[c]	24.24 ± 1.92[e]	23.06 ± 0.28[g]	9.50 ± 0.50[j]	99.65 ± 2.25
DA	4.67 ± 0.09[b]	55.05 ± 2.81[d]	10.51 ± 1.69[f]	32.06 ± 0.75[h]	2.01 ± 0.11[k]	104.30 ± 4.12
SPORL	4.51 ± 0.11[b]	54.36 ± 1.08[d]	11.17 ± 0.82[f]	28.60 ± 0.93[i]	3.57 ± 0.15[l]	102.21 ± 2.02

Contrasting letters at superscript position within a column denote a statistically significant difference ($p < 0.05$).

After pretreatment, the cellulose contents of DA and SPORL SCBs increased to respectively 55.1% and 54.4% from 41.5% of untreated SCB (Table 1), demonstrating that most of cellulose in SCB samples remained after pretreatment. The lignin contents of DA and SPORL SCBs were respectively 32.1% and 28.6% that were significantly ($p < 0.05$) higher than 23.1% of untreated SCB (Table 1), which might be attributed to that dilute acid can efficiently remove hemicellulose (Singh et al., 2015). Alkali facilitates removing lignin and therefore lignin removed by DA and SPORL pretreatment methods was little, and the increase of lignin content was due to the decrease of total solid content after pretreatment.

In general, SPORL and DA pretreatment methods had the similar cellulose and hemicellulose contents (54.4% and 11.2% vs. 55.1% and 10.5%). SPORL pretreatment had a lower lignin content of 28.6% and a higher ash content of 3.6% as compared with 32.1% and 2.0% of DA.

3.2. Enzymatic hydrolysis of SCB

Table 2 shows the sugar contents in the hydrolysates and the saccharification yields of SCBs treated by DA and SPORL methods. As can be seen in Table 2, the SPORL treated SCB had a significantly ($p < 0.05$) higher saccharification yield of 85.3% than the DA pretreated SCB (64.4%). The total reducing sugar contents of the two hydrolysates were respectively 12.4 g/L for SPORL pretreatment and 9.4 g/L for DA pretreatment. The contents of total reducing sugar from dry SCB samples were respectively 0.47 (DA) and 0.62 (SPORL) g/g dry SCB. These results were potential because 1 g of DA or SPORL treated SCB included about 0.35 g of materials that could not be converted into sugars. An analysis by HPLC was conducted to identify the sugars present in the two hydrolysates, including glucose, xylose, cellobiose, mannose, arabinose and galactose. However, in the two hydrolysates of SCBs treated by SPORL and DA, no mannose, arabinose and galactose were detected. The major sugars in the hydrolysates of SCBs treated by SPORL and DA were glucose (8.1 g/L vs. 7.0 g/L), xylose (2.7 g/L vs. 2.0 g/L) and cellobiose (0.3 g/L vs. 0.3 g/L). The existing of cellobiose in the hydrolysates showed that the cellobiase in CTec2 was insufficient for the SCB samples used in this study. For the SPORL treated SCB, the total content of glucose, xylose and cellobiose was 11.0 g/L that was a little lower than the reducing sugar content of 12.4 g/L, which suggested that there were some other reducing sugars not detected by HPLC method. For the DA treated SCB, the similar result was observed. HPLC data showed that more glucose and xylose were released from the SPORL treated SCB than DA treated SCB. The reducing sugar content in the hydrolysate of SPORL treated SCB obtained using DNS method was higher than that of DA treated SCB. These results suggested that the higher saccharification yield of SPORL SCB was attributed to the more glucose and xylose in the reducing sugars from the hydrolysate.

The results of saccharification yields of SCB samples from reported literatures and this study were compared in Table 3. As can be seen in Table 3, Gao et al. (2013) obtained a high glucan digestibility of 97.5% by using a high energy-consuming pretreatment (1% NaOH (80 °C, 180 min) – washing – liquid hot water (180 °C, 20 min) – washing) and a very high cellulase loading of 50 FPU/g substrate. When the pretreatment condition was milder (liquid hot water (180 °C, 20 min) – washing), the glucan digestibility was just about 70%. Aita et al. (2011) used 28% ammonia to treat SCB at 160 °C for 60 min and got a cellulose digestibility of 87% when cellulase loading was 60 FPU/g SCB. However, the cellulose digestibility was just 77% when cellulase loading was 30 FPU/g SCB. It is well known that low energy-consuming pretreatment and low enzyme loading are often desirable in efficient pretreatment and hydrolysis of lignocellulosic biomass (Sun et al., 2016; Xing et al., 2016). In this study, the saccharification yield of 85.3% for SPORL substrate was higher compared with the results in other studies (Table 3). Furthermore, the enzyme loading of 7.5 FPU/g dried SCB was lower and the pretreatment condition (160 °C, 30 min) was mild.

The results showed SPORL pretreatment was a potential method to get a desirable SCB's saccharification yield. On the basis of the results in this study, some further research can be performed, e.g. optimization on pretreatment and enzymatic hydrolysis. Generally more enhanced saccharification yield of SPORL SCB can be expected by further investigation of SPORL pretreatment.

3.3. SEM, XRD and FTIR

The results in this study showed that the saccharification yield of SCB treated by SPORL method was significantly higher than that of DA and the differences of substrate composition between SPORL and DA were not significant. Therefore, the composition might not be the main reason for the improvement seen in the saccharification yield of SPORL treated SCB and the improvement may be attributed to other factors.

Table 2
Sugar contents of hydrolysates, sugar contents from dry SCB samples and saccharification yields of SCB samples treated by DA and SPORL. The values following ± were standard deviations. All experiments and assays were performed in triplicate.

	Sugar content of hydrolysate (g/L)		Sugar content from dry SCB (g/g)	
	DA	SPORL	DA	SPORL
Glucose	7.04 ± 0.11[a]	8.07 ± 0.27[b]	0.35 ± 0.01	0.40 ± 0.01
Xylose	2.01 ± 0.02[c]	2.72 ± 0.15[d]	0.10 ± 0.00	0.14 ± 0.01
Cellobiose	0.28 ± 0.03[e]	0.25 ± 0.02[e]	0.01 ± 0.00	0.01 ± 0.00
Total reducing sugar	9.38 ± 0.28[f]	12.42 ± 0.13[g]	0.47 ± 0.01	0.62 ± 0.02
Saccharification yield (%)	64.39 ± 0.95[h]	85.33 ± 2.41[i]	64.39 ± 0.95	85.33 ± 2.41

Contrasting letters at superscript position within a row denote a statistically significant difference ($p < 0.05$).

Table 3
Comparison of saccharification yields of SCB samples from reported literatures and this study.

Treatment	Solid consistency (%)	Enzyme loading (FPU/g substrate)	Saccharification (%)	References
Milling-NaOH (120 °C, 60 min)-washing	8	10	23.26	Maitan-Alfenas et al. (2015)
Milling-H_2SO_4 (120 °C, 60 min)-washing	8	10	10.58	Maitan-Alfenas et al. (2015)
Ball milling (120 min)	5	15	82.0	Silva et al. (2010)
LHW[a] (180 °C, 20 min)-wash	5	50[b]	70[c]	Gao et al. (2013)
NaOH (80 °C, 3 h)-wash-LHW[a] (180 °C, 20 min)-wash	5	50[b]	97.5[c]	Gao et al. (2013)
Dilute H_3PO_4 (4 h)-steam explosion (180 °C, 10 min)	2	110	71.76[c]	Zeng et al. (2014)
28% ammonia (160 °C, 60 min)	4	30	77[d]	Aita et al. (2011)
28% ammonia (160 °C, 60 min)	4	60	87[d]	Aita et al. (2011)
NMMO[e] (100 °C, 420 min)	1	5	74.0	Kuo and Lee (2009)
SPORL (0.75% $NaHSO_3$, 0.14%H_2SO_4, 160 °C, 30 min)	2	7.5	85.3	This study
DA (0.14% H_2SO_4, 160 °C, 30 min)	2	7.5	64.4	This study

[a] LHW-liquid hot water pretreatment.
[b] Glucan loading.
[c] Glucan digestibility.
[d] Cellulose digestibility.
[e] NMMO: N-methylmorpholine-N-oxide.

Scanning electron microscopy (SEM) was used to monitor the differences in morphology of SCB samples untreated and treated by SPORL and DA. Fig. S1 depicts the general, cross-sectional and vessel's morphologies of these three SCB samples. As can be seen in Fig. S1A, the general morphologies of two treated SCB samples were obviously rougher than that of untreated SCB, which indicated that these two pretreatment methods could effectively break the structure of SCB. In the SEM pictures of two treated SCB samples (Fig. S1B), it was found that the gaps and pores of vessels were filled with many SCB fragments. The vessel surfaces of two treated SCB samples were rougher and the surface of vessel of untreated SCB was very smooth. In Fig. S1C, the cross-sectional morphology of untreated SCB was more rigid and ordered than those of two treated SCB samples. The DA and SPORL treated SCB samples showed looser and softer structures which could increase the accessibility of cellulases to cellulose. However, the SEM images of DA and SPORL treated SCB samples were not significantly different. Therefore, compared with DA pretreatment, the higher saccharification yield of SPORL treated SCB was not mainly due to the changes of morphology of SCB samples.

In order to investigate the structures of untreated, DA and SPORL treated SCB samples, FTIR analysis was conducted. Fig. S2 shows the FTIR spectra of these three SCB samples. As can be seen in Fig. S2, the absorption at 3400 cm^{-1} related to the stretching of intermolecular O—H bonds (Xiao et al., 2012) was weaker for the SPORL treated SCB than the DA treated SCB, which indicated that more intermolecular O—H bonds were broken among cellulose molecules for the SPORL treated SCB. The peak of C—H stretching near 2900 cm^{-1} are the distinguished features of cellulose (Sindhu et al., 2010). For the SPORL treated SCB, the absorption near 2900 cm^{-1} was lower compared with DA treated SCB, which suggested SPORL treated SCB had more disordered and incompact cellulose structure. The bands at 1737 cm^{-1} and 1636 cm^{-1} are attributed to the stretching of C=O in hemicelluloses and lignin, respectively (Zhang et al., 2011). The absorptions of the SPORL treated SCB at these two peaks were both weaker than that of the DA treated SCB. These indicated that more C=O bonds in the hemicelluloses and lignin of the SCB treated by SPORL method were broken. The band at 1516 cm^{-1} represents the stretching of the phenyl ring in lignin. Therefore, for the SPORL treated SCB, the less absorption was indicative of the greater damage of lignin structure which made cellulase more accessible to cellulose. The characteristic peak at near 901 cm^{-1} represents the stretching vibration at the β-(1,4)-glycosidic linkages (Zhang et al., 2011). Compared with DA treated SCB, the peak at near 901 cm^{-1} on the FTIR spectra of SPORL treated SCB was weaker. This result might mean that cellulose structure of SPORL treated SCB was looser.

The X-ray diffraction profiles of untreated and treated SCB samples are shown in Fig. S3. In lignocellulosic biomass, the CrI represents the relative amount of crystalline cellulose in the total solid and the lower CrI is, the less the relative amount of crystalline cellulose is (Cao and Aita, 2013). In this study, the CrIs of untreated SCB, DA treated SCB and SPORL treated SCB were 51.7%, 55.20% and 57.69%, respectively. The CrI of untreated SCB was slightly (no significant difference, $p > 0.05$) less than the treated SCB samples, which suggested that treated SCB samples had higher relative amount of crystalline cellulose because of the removal of lignin, hemicellulose and amorphous cellulose from the native SCB. The earlier reports by Binod et al. showed a similar result that the CrI of native lignocellulosic biomass was less compared to the pretreated samples (Binod et al., 2012; Sindhu et al., 2010; Cao and Aita, 2013). In this study, the CrI of SPORL treated SCB was the highest and the saccharification yield of SPORL treated SCB was also the highest, which was in accordance with the results in other researches (Cao and Aita, 2013; Binod et al., 2012; Sindhu et al., 2010). In the study conducted by Cao and Aita, the sugarcane bagasse treated by Tween 80 had the highest CrI and the highest cellulose digestibility. Binod et al. found that the Microwave-alkali-acid treated sugarcane bagasse with the highest CrI gave the highest reducing sugar yield in hydrolysates. In Sindhu et al.'s research, the CrI of sugarcane bagasse treated with formic acid-H_2SO_4 was the highest and the reducing sugar yield of this sample was also the highest.

3.4. Sulfonic group, Zeta potential and cellulase adsorption

In order to investigate the reason for the higher saccharification yield of SPORL treated SCB compared to DA treated SCB, the sulfonic acid group contents of these two treated substrates were measured (Table 4). The results showed that the SPORL treated SCB had a significantly ($p < 0.05$) higher sulfonic acid group content of 10.92 mg/g lignin compared with DA treated SCB (7.66 mg/g lignin). In this study, SPORL treated SCB had also a significantly ($p < 0.05$) higher saccharification yield of 85.33% than that of DA treated SCB (64.39%). This was in accordance with the other reports that sulfonic acid groups in lignin could function as a surfactant to reduce the nonproductive adsorption of cellulase to lignin due to its strong hydrophilicity and be beneficial to the enzymatic hydrolysis of lignocellulose biomass (Lan et al., 2013; Lou et al., 2013).

The previous studies found that there is a positive relationship between the value of zeta potential and the hydrophilicity of sample. The high zeta potential (absolute value) of sample solution presents high surface charge of sample, and the sample with high surface charge is more hydrophilic (Lan et al., 2013; Lou et al.,

Table 4
Sulfonic group contents, Zeta potential values and cellulase adsorption content of DA and SPORL treated SCB samples. The values following ± were standard deviations. All experiments and assays were performed in triplicate.

	DA	SPORL
Sulfonic acid group (mg/g lignin)	7.66 ± 0.22[a]	10.92 ± 0.97[b]
Zeta potential (mV)	−15.18 ± 1.72[c]	−24.38 ± 0.79[d]
Total adsorption (mg pro/g dry SCB)	22.37 ± 0.60[e]	28.94 ± 0.09[f]
Non-productive adsorption (mg pro/g lignin)	17.05 ± 0.37[g]	14.87 ± 0.91[h]
Productive adsorption (mg pro/g carbohydrate)	25.79 ± 1.16[i]	37.67 ± 0.69[j]

Contrasting letters at superscript position within a row denote a statistically significant difference ($p < 0.05$).

2013). In order to investigate the hydrophilicity of the lignins in DA and SPORL treated SCB samples, the zeta potential values of these two samples in buffer solution were measured and the data were listed in Table 4. The zeta potential of suspension of DA treated SCB was 15.18 mV (absolute value) significantly ($p < 0.05$) lower than that of SPORL treated SCB 24.38 mV (absolute value). Therefore, the lignin in SPORL treated SCB with a higher zeta potential (absolute value) had a higher surface charge and was more hydrophilic or less hydrophobic than that of DA treated SCB. Haynes et al. found that hydrophobic interactions are an important driving force in protein adsorption process (Haynes and Norde, 1994). Therefore, it could be hypothesized that the lignin in SPORL treated sample with a higher hydrophilicity or lower hydrophobicity had a poorer cellulase adsorption property, and the SPORL treated SCB had thus a lower non-productive adsorption compared with the DA treated SCB. Our previous research also showed that the SPORL treated lodgepole pine had the highest saccharification yield at pH 6.2 where the suspension of this substrate had the highest zeta potential (absolute value) (Lan et al., 2013).

In order to verify this hypothesis, the adsorption profiles of these two SCB samples were determined and the data on total adsorption, non-productive adsorption and productive adsorption of cellulase onto SCB samples were shown in Table 4. As can be seen in Table 4, the total adsorption content of cellulase onto DA treated SCB (22.37 mg protein/g dry SCB) was significantly ($p < 0.05$) lower than that of SPORL treated SCB (28.94 mg protein/g dry SCB). The non-productive adsorption contents of cellulase onto the lignin of DA and SPORL treated SCB samples were respectively 17.05 and 14.87 mg protein/g lignin. The productive adsorption content of cellulase onto SPORL treated SCB (37.67 mg protein/g carbohydrate) was significantly ($p < 0.05$) higher than that of DA treated SCB (25.79 mg protein/g carbohydrate). These results were consistent with the hypothesis mentioned above. Lou et al. reported a similar result that the lignin from the SPORL treated lodgepole pine had a higher zeta potential (absolute value) and a lower non-productive cellulose adsorption compared to that of DA treated lodgepole pine (Lou et al., 2013).

4. Conclusions

This study expanded the application of SPORL treatment, because we focused on the SCB, a non-wood biomass, and determined the structural properties, solving the problems that the previous SPORL studies mainly focused on woody substrates and the structure profiles of SPORL treated biomass were hardly studied. The results in this study showed SPORL treated SCB had a significantly higher saccharification yield compared with DA, and SPORL had not a significant effect on SCB's structure. Enhanced saccharification yield by SPORL might be attributed to that the more sulfonic acid groups and stronger hydrophilicity caused less nonproductive adsorption of cellulase to lignin.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2016.10.029.

References

Aita, G.A., Salvi, D.A., Walker, M.S., 2011. Enzyme hydrolysis and ethanol fermentation of dilute ammonia pretreated energy cane. Bioresour. Technol. 102, 4444–4448.
Ajanovic, A., Haas, R., 2014. On the future prospects and limits of biofuels in Brazil, the US and EU. Appl. Energy 135, 730–737.
Binod, P., Satyanagalakshmi, K., Sindhu, R., Janu, K.U., Sukumaran, R.K., Pandey, A., 2012. Short duration microwave assisted pretreatment enhances the enzymatic saccharification and fermentable sugar yield from sugarcane bagasse. Renewable Energy 37, 109–116.
Cao, S., Aita, G.M., 2013. Enzymatic hydrolysis and ethanol yields of combined surfactant and dilute ammonia treated sugarcane bagasse. Bioresour. Technol. 131, 357–364.
Chen, H., Qiu, W., 2010. Key technologies for bioethanol production from lignocellulose. Bioresour. Technol. 28 (5), 556–562.
Demeyer, A., Voundi Nkana, J.C., Verloo, M.G., 2001. Characteristics of wood ash and influence on soil properties and nutrient uptake: an overview. Bioresour. Technol. 77, 287–295.
Gao, Y., Xu, J., Zhang, Y., Yu, Q., Yuan, Z., Liu, Y., 2013. Effects of different pretreatment methods on chemical composition of sugarcane bagasse and enzymatic hydrolysis. Bioresour. Technol. 144, 396–400.
Ghose, T.K., 1987. Measurement of cellulase activities. Pure Appl. Chem. 59, 257–268.
Gnansounou, E., Dauriat, A., 2010. Techno-economic analysis of lignocellulosic ethanol: a review. Bioresour. Technol. 101, 4980–4991.
Harris, P.V., Xu, F., Kreel, N.E., Kang, C., Fukuyama, S., 2014. New enzyme insights drive advances in commercial ethanol production. Curr. Opin. Chem. Biol. 19, 162–170.
Haynes, C.A., Norde, W., 1994. Globular proteins at solid/liquid interfaces. Colloids Surf., B 2, 517–566.
Kumar, R., Wyman, C.E., 2009. Does change in accessibility with conversion depend on both the substrate and pretreatment technology? Bioresour. Technol. 100, 4193–4202.
Kuo, C., Lee, C., 2009. Enhanced enzymatic hydrolysis of sugarcane bagasse by N-methylmorpholine-N-oxide pretreatment. Bioresour. Technol. 100, 866–871.
Lan, T.Q., Lou, H., Zhu, J.Y., 2013. Enzymatic saccharification of lignocelluloses should be conducted at elevated pH 5.2–6.2. Bioenergy Res. 6, 476–485.
Lou, H., Zhu, J.Y., Lan, T.Q., Lai, H., Qiu, X., 2013. pH-induced lignin surface modification to reduce nonspecific cellulase binding and enhance enzymatic saccharification of lignocelluloses. ChemSusChem 6, 919–927.
Luo, J., Fang, Z., Smith Jr., R.L., 2014. Ultrasound-enhanced conversion of biomass to biofuels. Prog. Energy Combust. Sci. 41, 56–93.
Maitan-Alfenas, G.P., Visser, E.M., et al., 2015. The influence of pretreatment methods on saccharification of sugarcane bagasse by an enzyme extract from *Chrysoporthe cubensis* and commercial cocktails: a comparative study. Bioresour. Technol. 192, 670–676.
Pu, Y., Hu, F., Huang, F., Davison, B.H., Ragauskas, A.J., 2013. Assessing the molecular structure basis for biomass recalcitrance during dilute acid and hydrothermal pretreatments. Biotechnol. Biofuels 6, 15.
Qiu, Z., Aita, G.M., Walker, M.S., 2012. Effect of ionic liquid pretreatment on the chemical composition, structure and enzymatic hydrolysis of energy cane bagasse. Bioresour. Technol. 117, 251–256.
Rabelo, S.C., Andrade, R.R., Filho, R.M., Costa, A.C., 2014. Alkaline hydrogen peroxide pretreatment, enzymatic hydrolysis and fermentation of sugarcane bagasse to ethanol. Fuel 136, 349–357.
Raveendran, K., Ganesh, A., 1998. Adsorption characteristics and pore-development of biomass-pyrolysis char. Fuel 77 (7), 769–781.
Rezende, C.A., Lima, M.A., Maziero, P., Azevedo, E.R., Garcia, W., Polikarpov, I., 2011. Chemical and morphological characterization of sugarcane bagasse submitted to a delignification process for enhanced enzymatic digestibility. Biotechnol. Biofuels 4, 54.
Robl, D., Costa, P.D., Buchli, F., Lima, D.J.D., Delabona, P.D., Squina, F.M., Pimentel, I. C., Padilla, G., Pradella, J.G.D., 2015. Enhancing of sugar cane bagasse hydrolysis by *Annulohypoxylon stygium* glycohydrolases. Bioresour. Technol. 177, 247–254.
Silva, A.S., Inoue, H., Endo, T., Yano, S., Bon, E.P.S., 2010. Milling pretreatment of sugarcane bagasse and straw for enzymatic hydrolysis and ethanol fermentation. Bioresour. Technol. 101, 7402–7409.

Sindhu, R., Binod, P., Satyanagalakshmi, K., Janu, K.U., Sajna, K.V., Kurien, N., Sukumaran, R.K., Pandey, A., 2010. Formic acid as a potential pretreatment agent for the conversion of sugarcane bagasse to bioethanol. Appl. Biochem. Biotechnol. 162 (8), 2313–2323.

Singh, J., Suhag, M., Dhaka, A., 2015. Augmented digestion of lignocellulose by steam explosion, acid and alkaline pretreatment methods: a review. Carbohyd. Polym. 117, 624–631.

Sluiter, A., Hames, B., Ruiz, R., Scarlata, C., Sluiter, J., Templeton, D., Crocker, D., 2012. Determination of Structural Carbohydrates and Lignin in Biomass: NREL Laboratory Analytical Procedure (Version 08-03-2012).

Sun, S.N., Sun, S.L., Cao, X., Sun, R., 2016. The role of pretreatment in improving the enzymatic hydrolysis of lignocellulosic materials. Bioresour. Technol. 199, 49–58.

Tsuchida, J.E., Rezende, C.A., Oliveira-Silva, R., Lima, M.A., Eurydice, M.N., Polikarpov, I., Bonagamba, T.J., 2014. Nuclear magnetic resonance investigation of water accessibility in cellulose of pretreated sugarcane bagasse. Biotechnol. Biofuels 7, 127.

Xiao, W., Yin, W., Xia, S., Ma, P., 2012. The study of factors affecting the enzymatic hydrolysis of cellulose after ionic liquid pretreatment. Carbohyd. Polym. 87, 2019–2023.

Xing, Y., Bu, L., Sun, D., Liu, Z., Liu, S., Jiang, J., 2016. Enhancement of high-solids enzymatic hydrolysis and fermentation of furfural residues by addition of *Gleditsia saponin*. Fuel 177, 142–147.

Yu, B., Chen, H., 2010. Effect of the ash on enzymatic hydrolysis of steam-exploded rice straw. Bioresour. Technol. 101, 9114–9119.

Zeng, J., Tong, Z., Wang, L., Zhu, J.Y., Ingram, L., 2014. Isolation and structural characterization of sugarcane bagasse lignin after dilute phosphoric acid plus steam explosion pretreatment and its effect on cellulose hydrolysis. Bioresour. Technol. 154, 274–281.

Zhang, J., Ma, X., Yu, J., et al., 2011. The effects of four different pretreatments on enzymatic hydrolysis of sweet sorghum bagasse. Bioresour. Technol. 102 (6), 4585–4589.

Zhu, J.Y., Pan, X.J., 2010. Woody biomass pretreatment for cellulosic ethanol production: technology and energy consumption evaluation. Bioresour. Technol. 101, 4992–5002.

Zhu, J.Y., Pan, X.J., Wang, G.S., Gleisner, R., 2009a. Sulfite pretreatment (SPORL) for robust enzymatic saccharification of spruce and red pine. Bioresour. Technol. 100, 2411–2418.

Zhu, Z., Sathitsuksanoh, N., Vinzant, T., Schell, D.J., McMillan, J.D., Zhang, Y.-H.P., 2009b. Comparative study of corn stover pretreated by dilute acid and cellulose solvent-based lignocellulose fractionation: enzymatic hydrolysis, supramolecular structure, and substrate accessibility. Biotechnol. Bioeng. 103 (4), 715–724.

Zhu, J.Y., Pan, X., Zalesny Jr., R.S., 2010. Pretreatment of woody biomass for biofuel production: energy efficiency, technologies, and recalcitrance. Appl. Microbiol. Biotechnol. 87, 847–857.