Comparison of different alkali-based pretreatments of corn stover for improving enzymatic saccharification

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HIGHLIGHTS

▶ ASSP of corn stover leads to good enzymatic digestigility.
▶ Temperatures over 140 °C are not needed for ASSP.
▶ High carbohydrate recovery can be obtained by ASSP.
▶ A total sugars yield of 78.2% can be obtained via ASSP and enzymatic hydrolysis.

ARTICLE INFO

ABSTRACT

Corn stover was treated with NaOH, NaOH + anthraquinone (AQ), NaOH + Na_2SO_3 (alkaline), NaOH + Na_2SO_3 (neutral), and NaOH + Na_2S, respectively. The treated corn stover was subjected to hydrolysis with cellulase (20 FPU/g dry biomass) and β-glucosidase (10 IU/g dry biomass). Compared with other pretreatment methods, alkaline sodium sulfite pretreatment (ASSP) at a relatively low temperature of 140 °C provided for the best lignin removal of about 92%. After ASSP with 10 wt.% of the total alkali charge (Na_2SO_3:NaOH = 1:1) at 140 °C for 30 min and subsequent enzymatic hydrolysis, a total sugar yield of 78.2% was obtained on the basis of the amount of glucose and xylose released from raw corn stover. This yield was 24.0% higher than that achieved with NaOH only under the same conditions. Therefore, the supplement of Na_2SO_3 in alkali pretreatment can facilitate delignification and significantly improve the enzymatic saccharification.

1. Introduction

The enzymatic conversion of cellulose and hemicellulose in lignocellulosic biomass to fermentable sugars is difficult as these sugar-based polymers are compactly associated with lignin (Agbor et al., 2011; Liu et al., 2011). As the level of crystallinity of cellulose, degree of polymerization, available surface area, as well as the bonding of hemicellulose to lignin and cellulose, also impacts enzymatic hydrolysis (Hendriks and Zeeman, 2009; Mosier et al., 2005; Ohgren et al., 2007), lignocellulosic biomass requires pretreatment to break the lignin seal and disrupt the crystalline structure of cellulose.

Despite a great deal of work on various pretreatment methods (Alvira et al., 2010; Mosier et al., 2005; Wang et al., 2012), commercially viable bioconversion of lignocellulosic materials has not yet been achieved mainly due to high energy consumption and low conversion efficiency (McIntosh and Vancov, 2011; Tao et al., 2011). Alkali pretreatment, which can utilize pulping equipment, showed a satisfactory performance (Banerjee et al., 2011; Macdonald et al., 1983; Pang et al., 2008; Varga et al., 2002; Zhang et al., 2011), as alkali pretreatment efficiently breaks the ester bonds crosslinking lignin and xylan via solvation and saponification (Sun and Cheng, 2002), leading to a more porous structure for enzyme access.

In the pulping industry, chemicals like anthraquinone, Na_2SO_3, and Na_2S are typical additives to enhance carbohydrate recovery and lignin removal; however, the use of NaOH combined with anthraquinone, Na_2SO_3, or Na_2S has not been reported in biomass pretreatment, and how to reduce hemicellulose degradation and improve enzymatic digestibility during alkali-based pretreatment still needs to be further explored. In the current study, five alkali-based pretreatments of corn stover using different combinations of chemicals (i.e. NaOH, NaOH + AQ, NaOH + Na_2SO_3 (alkaline), NaOH + Na_2SO_3 (neutral), and NaOH + Na_2S, respectively) were carried out, and their impact on enzymatic hydrolysis was studied.

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Table 1
Alkali-based pretreatment method with different combinations of chemicals.

Trial ID	Pretreatment methods[a] and chemicals	NaOH[b] (%)	AQ[b] (%)	Na$_2$SO$_3$[b] (%)	Na$_2$S[b] (%)
1	SHP (NaOH)	7.0/10.0	–	–	–
2	SH-AQP (NaOH + AQ)	7.0/10.0	0.05/0.05	–	–
3	ASSP (NaOH + Na$_2$SO$_3$)	5.6/8.0	–	2.2/3.2	–
4	NSSP (NaOH + Na$_2$SO$_3$)	2.1/3.0	–	7.7/11.0	–
5	KPP[c] (NaOH + Na$_2$S)	5.9/8.5	–	–	1.1/1.5

[a] SHP: NaOH pretreatment; SH-AQP: NaOH + AQ pretreatment (AQ: anthraquinone); ASSP: alkaline sodium sulfite pretreatment; NSSP: neutral sodium sulfite pretreatment; KPP: traditional Kraft pulping pretreatment.
[b] Data before and after backslash were the 7% and 10% (w/v) total alkali charge (based on the dry weight of biomass), respectively, which were calculated as NaOH, i.e. 1 g Na$_2$SO$_3$ = (80/126) g NaOH; 1 g Na$_2$S = (80/78) g NaOH. The mass ratio of NaOH to Na$_2$SO$_3$ (calculated as NaOH) was 4:1 and 3:7 for ASSP and NSSP, respectively.
[c] The sulfidity of KPP liquid was 15%.

Table 2
Composition of corn stover after alkali-based pretreatments.

Trial ID	Pretreatment methods[a] and chemicals	Pretreatment conditions		Solid yield[b] (%)	Principle components of pretreated biomass[b] (%)		
		Temperature (°C)	Total alkali charge (%)		Glucan	Xylan	Lignin
–	–	–	–	100[c]	35.0[c]	19.9[c]	22.5[c]
1	SHP (NaOH)	140	7	63.1	51.3	24.0	13.3
			10	53.5	59.9	25.6	6.4
		160	7	56.6	54.1	22.4	13.1
			10	50.7	61.9	24.9	6.6
2	SH-AQP (NaOH + AQ)	140	7	65.4	52.8	24.0	13.7
			10	54.4	60.6	25.5	6.7
		160	7	57.8	55.3	22.4	11.6
			10	50.8	61.0	24.7	7.3
3	ASSP (NaOH + Na$_2$SO$_3$)	140	7	61.2	53.8	24.4	9.4
			10	54.0	61.3	25.9	4.1
		160	7	54.4	59.2	23.7	7.1
			10	47.0	62.4	25.1	4.9
4	NSSP (NaOH + Na$_2$SO$_3$)	140	7	72.7	46.9	24.7	12.0
			10	64.0	54.8	25.6	8.1
		160	7	57.3	57.1	25.4	7.4
			10	52.1	59.9	25.2	3.5
5	KPP (NaOH + Na$_2$S)	140	7	64.1	50.8	23.6	14.4
			10	55.6	60.5	25.2	8.2
		160	7	59.0	53.1	22.4	15.3
			10	52.5	61.2	24.3	7.6

[a] SHP: NaOH pretreatment; SH-AQP: NaOH + AQ pretreatment (AQ: anthraquinone); ASSP: alkaline sodium sulfite pretreatment; NSSP: neutral sodium sulfite pretreatment; KPP: traditional Kraft pulping pretreatment.
[b] Values represented are the averages of the results from duplicated experiments.
[c] Data presented were the main composition of the original corn stover.

2. Methods

2.1. Materials

Air-dried corn stover, harvested in October, 2010, was obtained from Qingdao, Shandong Province, China. The corn stover was milled using a plant grinder (Model FZ102, Beijing Zhongxingweiye Instrument Co. Ltd., China) and screened to obtain particle sizes between 2 and 0.425 mm. The screened corn stover was stored in sealed plastic bags at room temperature. The main components (dry weight basis) of the ground corn stover included 35.0% glucan, 19.9% xylan, 22.5% lignin, and 18.4% total extractives (water soluble and ethanol soluble substances).

Sodium hydroxide, anthraquinone (AQ), sodium sulfite, and sodium sulfide were purchased from Sinopharm Chemical Reagent Co. Ltd. Commercial enzymes, Celluclast 1.5L (cellulase) and Novozyme 188 (β-glucosidase), were obtained from Sigma–Aldrich China Inc. All chemicals and enzymes were used as received.

2.2. Pretreatment of corn stover

The pretreatments of the milled corn stover (15 g oven dried) were conducted in Parr Hastelloy reactors (Series 4560, Moline, Illinois, USA) at a stirring speed of 200 rpm with a solid concentration of 10 wt.%. The dosages for NaOH pretreatment (SHP), NaOH + AQ pretreatment (SH-AQP), alkaline sodium sulfite pretreatment (ASSP), neutral sodium sulfite pretreatment (NSSP), and the traditional Kraft pulping pretreatment (KPP) are listed in Table 1. The total alkali charges of the pretreatments were 7% and 10% (w/w), calculated on the basis of the amount of NaOH. In KPP, the sulfidity, which can affect delignification, was defined as the amount of sodium sulfide in the KPP liquid divided by the amount of active alkali (NaOH + Na$_2$S). Chemicals used for each pretreatment were added simultaneously.

The pretreatment included 20 min of heating to reach the desired temperature, and 30 min of maintaining the temperature at 140 or 160 °C. After pretreatment, the samples were removed from the reactors and the solids were washed on a Buchner funnel with deionized water to minimize the re-deposition of lignin (Pedersen et al., 2010). In order to reduce the losses of fines during washing, the filtrate was collected and reused to wash the sample pad. The washed substrates were completely transferred to a pre-weighed plastic bag, weighed, sealed, and stored at 4 °C. In addition, for the optimization of chemical ratios for ASSP at 140 °C for 30 min with 10 wt.% total alkali charge, the ratio of NaOH to Na$_2$SO$_3$ was adjusted from 10:1 to 0.5:1 (w/w), i.e. Na$_2$SO$_3$ concentration was varied from 0.1% to 1.0% (w/v) accordingly.
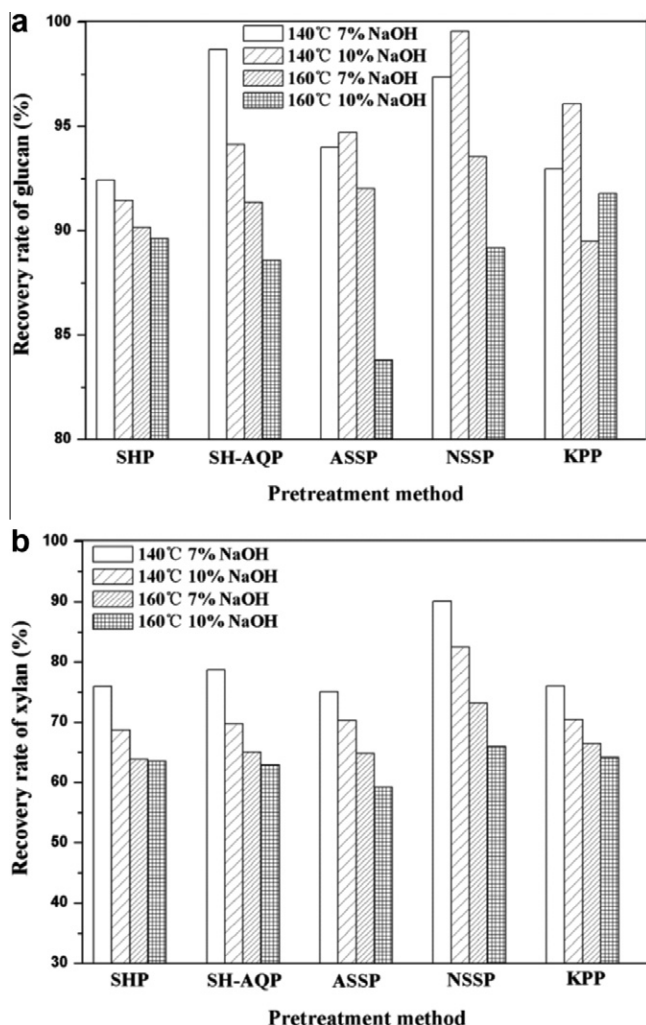
Fig. 1. Recovery rates of glucan (a) and xylan (b) after alkali-based pretreatments. Pretreatment conditions: solid concentration of 10 wt.%; 140 °C or 170 °C for 30 min; alkali charge of 7% or 10% for different pretreatment methods. SHP: NaOH pretreatment; SH-AQP: NaOH + AQ pretreatment (AQ: anthraquinone); ASSP: alkaline sodium sulfite pretreatment; NSSP: neutral sodium sulfite pretreatment; KPP: traditional Kraft pulping pretreatment. Values represented are the averages of the results from duplicated experiments.

2.3. Enzymatic hydrolysis

The activities of cellulases (Celluclast 1.5L) and β-glucosidase (Novozyme 188) were 117 FPU (filter paper unit)/mL and 426 IU (International Unit)/mL, respectively, as measured according to standard methods (Ghose, 1987).

Enzymatic hydrolysis of different substrates was carried out at a substrate consistency of 2% (w/v). A mixture of cellulase (20 FPU/g dry biomass) and β-glucosidase (10 IU/g dry biomass) was added together with 20 mL of 0.05 M sodium citrate buffer (pH 4.8) and 0.02% sodium azide and hydrolysis took place at 50 °C for 48 h in serum bottles (25 mL) placed in an incubator shaker at 160 rpm. The hydrolyzate was centrifuged at 4983g for 10 min, and the supernatant was collected and frozen at −17 °C. As a control, enzymatic hydrolysis of untreated corn stover was also conducted. An average of duplicates was reported as the results of all the pretreatments, enzymatic hydrolysis, and sugar analyses.

2.4. Analysis methods

Total solids, ash and extractives of raw corn stover were measured according to the National Renewable Energy Laboratory
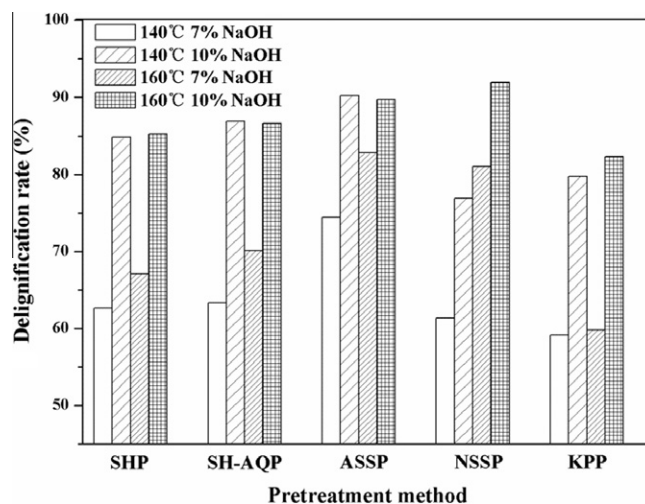

Fig. 2. Lignin removal after alkali-based pretreatments. Pretreatment conditions: solid concentration of 10%; 140 °C or 170 °C for 30 min; alkali charge of 7% or 10% for different pretreatment methods. SHP: NaOH pretreatment; SH-AQP: NaOH + AQ pretreatment (AQ: anthraquinone); ASSP: alkaline sodium sulfite pretreatment; NSSP: neutral sodium sulfite pretreatment; KPP: traditional Kraft pulping pretreatment. Values represented are the averages of the results from duplicated experiments.

(NREL) analytical procedure (Sluiter et al., 2005a,b, 2008a). Glucan, xylan, and lignin content of untreated and pretreated corn stover was determined by the NREL procedure for the determination of structural carbohydrates and lignin in biomass (Sluiter et al., 2008b). Acid and enzymatic hydrolyzates (0.45 μm filtered) were analyzed by a high performance liquid chromatography (HPLC) system (Model 1200, Agilent, USA) equipped with a Sugar Pak 1 column (300 mm × 6.5 mm) and refractive index detector. The column was operated at 80 °C with 0.05 g/L EDTA-Ca-2Na solution as the mobile phase at a flow rate of 0.5 mL/min, and the quantitative analysis was performed using a calibration with external standards of known concentration.

2.5. Calculations

The solid yield of pretreated biomass was calculated by the following equation:

$$Y_{solid}(\%) = (\text{Pretreated biomass (g)}/\text{Original biomass (g)}) \times 100$$

The recovery rates of glucan, or xylan, as well as the delignification rate after pretreatment were calculated as follows:

$$R_{glucan/xylan}\ (\%) = (Y_{solid} \times C_{glucan/xylan\ of\ pretreated\ biomass})$$
$$/C_{glucan/xylan\ of\ original\ biomass}$$

$$R_{delignification}(\%) = 1 - (Y_{solid}$$
$$\times C_{lignin\ of\ pretreated\ biomass})/C_{lignin\ of\ original\ biomass}$$

where, C is the content of the corresponding component (wt.%).

The enzymatic hydrolysis rate of glucan or xylan was calculated by the following equations:

$$E_{glucan}(\%) = (M_{glucose\ in\ hydrolyzate} \times 0.9/M_{glucan\ in\ pretreated\ biomass}) \times 100$$

$$E_{xylan}(\%) = (M_{xylose\ in\ hydrolyzate} \times 0.88/M_{xylan\ in\ pretreated\ biomass}) \times 100$$

wherein, M is the mass of sugars (g).

The total sugar yields (g/g original biomass) were expressed as the total mass (g) of glucose and xylose divided by the original

biomass (g) for the samples after pretreatment and enzymatic hydrolysis.

3. Results and discussion

3.1. Comparison of different pretreatments for corn stover

3.1.1. Solid yield and chemical composition of biomass

The composition of corn stover after different alkali-based pre-treatments is shown in Table 2. The solid yield of pretreated bio-mass decreased significantly as temperature and alkali dosage increased, and the highest solid yield of 72.7% was obtained after NSSP at 140 °C with 7 wt.% alkali charge. The weight loss was mainly due to the removal of lignin and extractives during pre-treatment. The utilization of AQ together with NaOH improved so-lid recovery only slightly, likely due to the inhibition of carbohydrate degradation by AQ, and simultaneous promotion of lignin removal (Fatehi et al., 2009).

Compared to untreated corn stover, the glucan and xylan con-tent of the pretreated corn stover increased notably. For instance, the glucan and xylan contents of alkaline sulfite-pretreated bio-mass were 61.3% (75.1% higher than that of the original corn sto-ver) and 25.9% (30.2% increment compared to the original corn stover), respectively, after ASSP at 140 °C with 10 wt.% alkali charge. This outcome was not only the result of delignification, but also a consequence of the dissolution of a large amount of extractives. In addition, with increasing total alkali charge at the same temperature, the lignin content decreased significantly, which could benefit the enzymatic conversion of carbohydrate. However, increasing temperature with the same alkali charge only had a minor impact on lignin removal (Table 2).

3.1.2. Carbohydrate recovery

The recovery rate of carbohydrate (expressed as glucan and xy-lan) after different pretreatments is presented in Fig. 1. Carbohy-drate retention after SHP and SH-AQP decreased as temperature and chemical dosage increased, and the application of AQ together with NaOH facilitated carbohydrate recovery under the same con-ditions, particularly at the lower temperature of 140 °C. For in-stance, as shown in Fig. 1, the recovery rates of glucan and xylan after SH-AQP with 7 wt.% alkali charge and 0.05 wt.% AQ at 140 °C were improved by 6.2% and 2.7%, respectively, compared to SHP. For ASSP and NSSP, a higher glucan recovery could be ob-tained when the alkali dosage was increased (at a fixed ratio of NaOH to Na_2SO_3) at the same temperature of 140 °C. As shown in Fig. 1a, the recovery rate of glucan after NSSP at 140 °C with 10 wt.% alkali charge was as high as 99.5%, which was 8.9% higher than that after SHP under the same conditions. However, the glu-can recovery decreased when the alkali charge was increased after ASSP or NSSP at 160 °C, probably due to easier degradation of glu-can by NaOH at the elevated temperature. Fig. 1a also shows that, in the case of KPP, glucan recovery can be improved by increasing the alkali charge at both 140 and 160 °C, while the xylan recovery had the opposite trend (Fig. 1b). Xylan recovery was reduced with the increase in temperature or alkali dosage in all cases of pretreat-ments (Fig. 1b), indicating that hemicellulose was still easier to de-grade than cellulose, even with added protection (AQ, Na_2SO_3, or Na_2S).

3.1.3. Lignin removal

The delignification rates after different alkali-based pretreat-ments are given in Fig. 2. Delignification was improved by increas-ing temperature or alkali dosage, but the increase in alkali had a stronger effect than temperature. Also, at the same temperature with the same alkali dosage, a higher delignification could be ob-
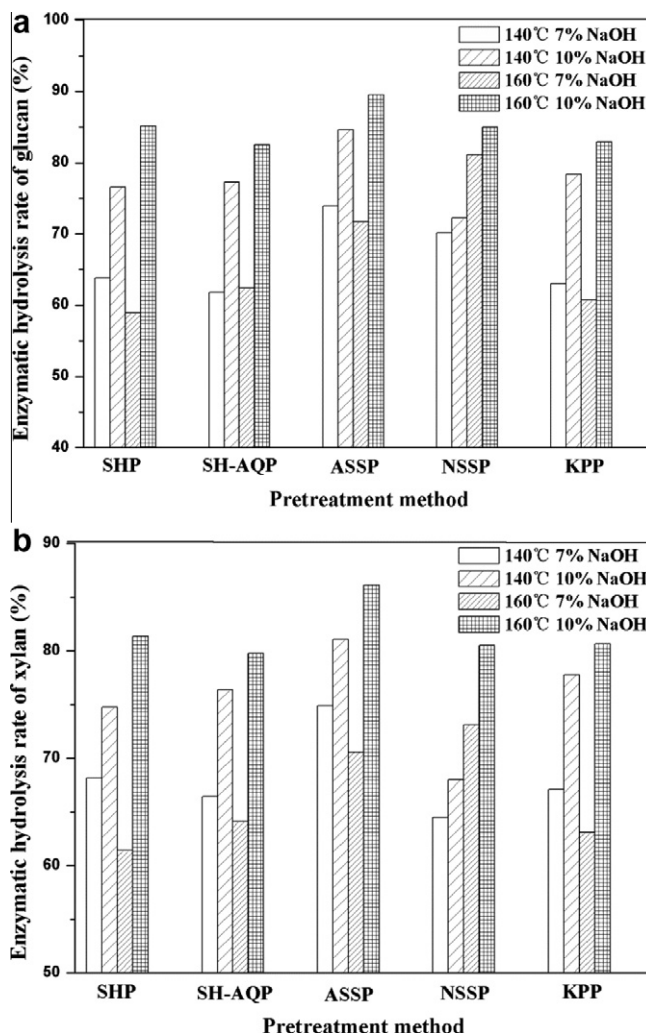

Fig. 3. Enzymatic hydrolysis rates of glucan (a) and xylan (b) for pretreated corn stover. Enzymatic hydrolysis conditions: 50 °C for 48 h with 20 FPU of cellulase dosage and 10 IU of β-glucosidase dosage (on per gram of dry biomass). SHP: NaOH pretreatment; SH-AQP: NaOH + AQ pretreatment (AQ: anthraquinone); ASSP: alkaline sodium sulfite pretreatment; NSSP: neutral sodium sulfite pretreatment; KPP: traditional Kraft pulping pretreatment. Values represented are the averages of the results from duplicated experiments.

tained by ASSP as the dissolubility of lignin is significantly en-hanced through the sulphonation. However, to reach a higher delignification, a higher temperature was needed for NSSP with the same total alkali dose (Fig. 2), as a higher temperature en-hances the solubility of lignin (Bobleter, 1994).

3.2. Enzymatic hydrolysis

3.2.1. Enzymatic hydrolysis rate of pretreated corn stover

Enzymatic hydrolysis rates of glucan and xylan for pretreated corn stover are shown in Fig. 3. Increasing alkali dosage signifi-cantly improved enzymatic hydrolysis rates of polysaccharides at the same temperature in all cases. The maximum enzymatic hydrolysis rates of glucan and xylan were 89.5% and 86.1%, respec-tively, for ASSP treated samples (160 °C with 10 wt.% alkali charge), while only 85% and 81.6% of enzymatic hydrolysis rates could be achieved for glucan and xylan, respectively, for SHP treated sam-ples under the same conditions, as the addition of Na_2SO_3 facili-tated lignin removal (90%) (Fig. 2), leading to higher accessibility to enzymes. However, carbohydrate degraded extensively after the ASSP at 160 °C, and the glucan and xylan recovery were only
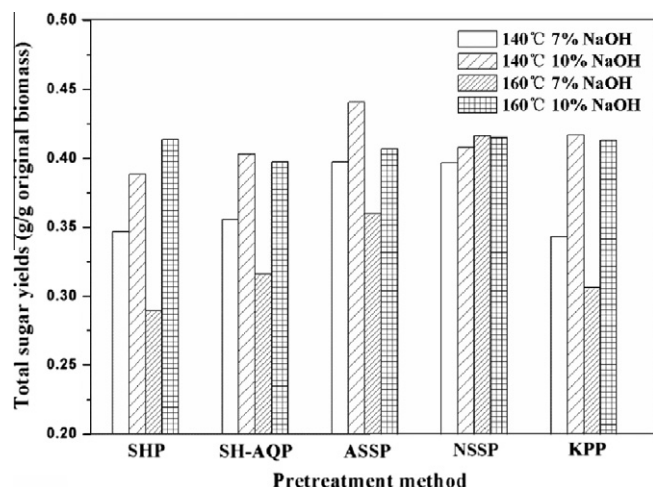
Fig. 4. Effects of alkali-based pretreatments on total sugar yields (glucose + xylose). Enzymatic hydrolysis conditions: 50 °C for 48 h with 20 FPU of cellulase dosage and 10 IU of β-glucosidase dosage (on per gram of dry biomass). SHP: NaOH pretreatment; SH-AQP: NaOH + AQ pretreatment (AQ: anthraquinone); ASSP: alkaline sodium sulfite pretreatment; NSSP: neutral sodium sulfite pretreatment; KPP: traditional Kraft pulping pretreatment. Values represented are the averages of the results from duplicated experiments.

about 83% and 59%, respectively (Fig. 1b). Therefore, a temperature over 140 °C is not needed for ASSP. In addition, a high enzymatic hydrolysis rate of xylan (60–85%) could be achieved, possibly because the cellulase might have contained some hemicellulases, which promoted the hydrolysis of hemicelluloses.

3.2.2. Total sugar yields

The total sugar yields after pretreatments with additives (AQ, Na_2SO_3, or Na_2S) at 140 °C was higher than that after SHP under the same conditions (Fig. 4), indicating that the addition of additives during alkaline pretreatment can improve enzymatic digestibility at a relatively low temperature. The highest total sugar yields of 0.44 g/g original biomass could be achieved for ASSP-treated samples (140 °C with 10 wt.% alkali charge), and the corresponding enzymatic hydrolysis rates of glucan and xylan were as high as 84.7% and 81.1% (Fig. 3), respectively.

3.3. Optimization of chemical ratios for alkaline sodium sulfite pretreatment

Based on the above results, ASSP demonstrated several advantages: (1) relatively low temperature requirement; (2) efficient lignin removal; and (3) high enzymatic hydrolysis efficiency. Therefore, it was necessary to optimize the proportion (w/w) between NaOH and Na_2SO_3 for achieving a better pretreatment performance.
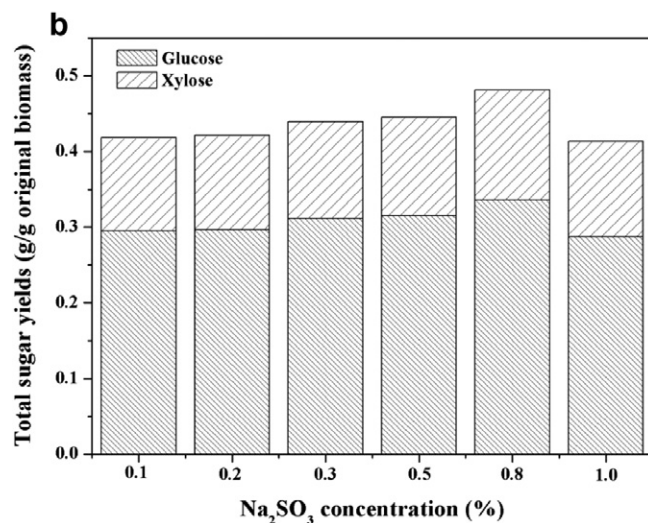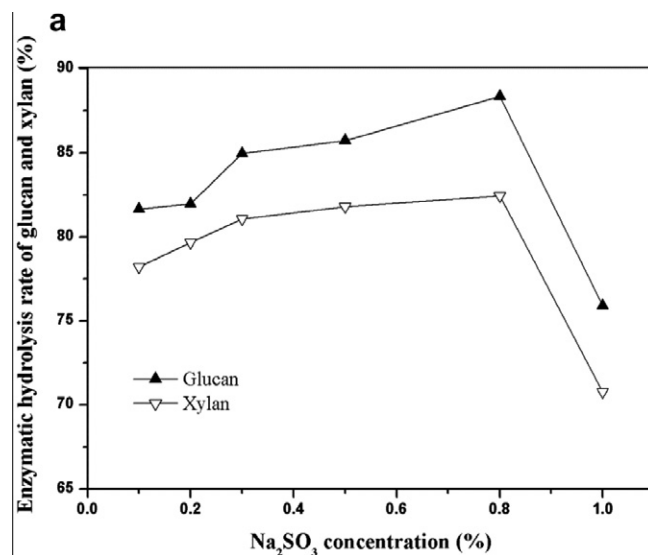

Fig. 5. Effect of Na_2SO_3 concentration on enzymatic hydrolysis rate (a) and total sugar yields (b). Enzymatic hydrolysis condition: 50 °C for 48 h with 20 FPU of cellulase dosage and 10 IU of β-glucosidase dosage (on per gram of dry biomass). Values represented are the averages of the results from duplicated experiments.

3.3.1. Changes in solid recovery and biomass components after ASSP

The main composition of corn stover after ASSP with different Na_2SO_3 concentration is presented in Table 3. The solid yield increased as the Na_2SO_3 concentration increased from 0.1% to 1.0% (w/v) at 140 °C for 30 min and 10 wt.% total alkali charge, and

Table 3
Composition of corn stover after ASSP with varied Na_2SO_3 concentration.[a]

Na_2SO_3 concentration[b] (w/v%)	NaOH/Na_2SO_3 (w/w)	Solid yield[c] (%)	Composition of solid fraction[c] (%)			
			Lignin	Glucan	Xylan	Ash
Original biomass	–	100.0	22.5	35.0	19.9	1.0
0.1	10:1	53.0	5.8 (13.7)	61.4 (93.0)	26.0 (69.4)	0.7
0.2	6:1	53.2	2.6 (6.2)	61.2 (93.0)	25.9 (69.3)	0.7
0.3	4:1	53.8	4.2 (10.0)	61.3 (94.3)	25.7 (69.6)	0.8
0.5	2:1	55.2	4.6 (11.2)	59.6 (94.1)	26.0 (72.1)	0.7
0.8	1:1	59.3	5.3 (13.9)	57.7 (97.9)	26.1 (77.7)	0.8
1.0	0.5:1	62.7	6.6 (18.4)	54.4 (97.4)	24.9 (78.6)	1.0

[a] Pretreatment conditions: 140 °C for 30 min; solid to liquid ratio (w/w) of 1:10; total alkali charge of 10 wt.%. 1 g Na_2SO_3 (calculated as NaOH) = (80/126) g NaOH. Data in the bracket next to the composition number were the recovery rates for each component after pretreatments.

[b] Na_2SO_3 concentration was the weight of used Na_2SO_3 divided by the volume of total liquid.

[c] Values represented are the averages of the results from duplicated experiments.
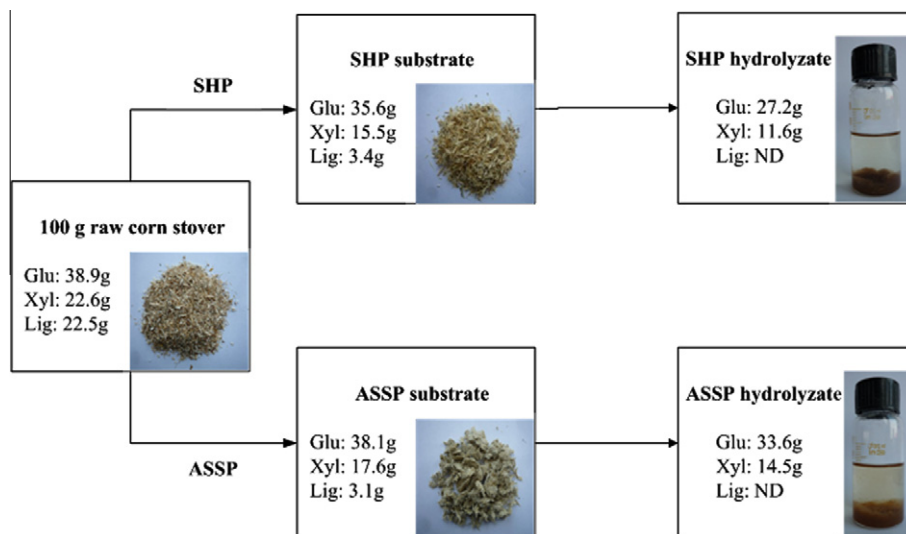
Fig. 6. Comparative effects of SHP and ASSP on enzymatic saccharification. The pretreatment conditions: the ratio of solid to liquid was 1:10; 10 wt.% total alkali charge (NaOH: Na_2SO_3 = 1:1 for ASSP); soaking for 30 min at 140 °C. Enzymatic hydrolysis condition: 50 °C for 48 h with 20 FPU of cellulase dosage and 10 IU of β-glucosidase dosage (on per gram of dry biomass). Mass of glucose (Glu), xylose (Xyl) and lignin (Lig) recovered was expressed by g per 100 g oven dry raw corn stover. ND: not detected. SHP: NaOH pretreatment; ASSP: alkaline sodium sulfite pretreatment. Values represented are the averages of the results from duplicated experiments.

the solid yield (%) as a function of Na_2SO_3 concentration (% w/v) can be expressed by the following equation:

$$y = 10.096x^2 - 0.2065x + 52.905, (R^2 = 0.9994).$$

where, y is the solid yield (%) and x is the Na2SO3 concentration (% w/v).

Table 3 also shows that, as the Na_2SO_3 concentration increased, carbohydrate recovery increased to some extent, and the highest recovery rates of glucan and xylan were 97.9% and 78.6%, respectively. The highest lignin removal (lignin content of 6.2%) was achieved with 0.2% (w/v) Na_2SO_3.

3.3.2. Effect of Na_2SO_3 concentration on enzymatic efficiency

The enzymatic hydrolysis rates of glucan and xylan were enhanced when the Na_2SO_3 concentration was increased from 0.1% to 0.8% (w/v) (Fig. 5a). In particular, though lignin removal (Table 3) was not high with 0.8% (w/v) Na_2SO_3, the highest enzymatic hydrolysis rates of glucan (88.4%) and xylan (82.4%) were obtained. Since the highest lignin removal with 0.2% (w/v) Na_2SO_3 did not lead to the highest enzymatic hydrolysis rate, it is possible that lignin removal during ASSP was not the only variable influencing enzymatic hydrolysis. Although the sulphonation increases the dissolution of lignin, the crystallinity of cellulose is another important barrier which cannot be neglected during enzymatic saccharification (Laureano-Perez et al., 2005; Mirahmadi et al., 2010).

As shown in Fig. 5b, the total sugar yields increased as the Na_2SO_3 concentration increased from 0.1% to 0.8% (w/v). With an Na_2SO_3 concentration of 0.8% (w/v), the total sugar yields (glucose and xylose) were as high as 0.48 g/g original biomass, which corresponded to the highest enzymatic hydrolysis rates of glucan and xylan (Fig. 5a). The 78.2% total sugar conversion based on the amount of glucose and xylose in raw biomass was 24.0% higher than that for the SHP-treated samples under the same conditions (Fig. 6).

3.4. Comparison of saccharification between SHP and ASSP

The carbohydrate recovery (55.7%) for ASSP-treated samples was higher than that for SHP-treated samples (51.1%) under the

same conditions (Fig. 6). Also, the corresponding sugar yields of fermentable glucose and xylose were 0.34 g/g original biomass and 0.15 g/g original biomass, respectively, after the ASSP with optimized chemical ratio and subsequent enzymatic hydrolysis. However, in the case of SHP, only 0.27 g/g original biomass of glucose yield and 0.12 g/g original biomass of xylose yield could be achieved after enzymatic hydrolysis. In addition, the recovery of the effluent derived from the process of sulfite pretreatment should be taken into account. It has been reported that the unconsumed alkali can be reused in the process, and there was no significant impact on the effectiveness of the treatment observed, so that the chemical consumption can be reduced and the environmental impact can be minimized (Mirahmadi et al., 2010; Sills and Gossett, 2011). Sodium lignosulphonate can be readily recovered by spray drying and modified to value-added products, such as concrete additives and phenol–formaldehyde resins (Doherty et al., 2011).

4. Conclusion

The comparative performance of five alkali-based pretreatments of corn stover with different combinations of chemicals (i.e. NaOH, NaOH + AQ, NaOH + Na_2SO_3 (alkaline), NaOH + Na_2SO_3 (neutral), and NaOH + Na_2S, respectively) was studied. Alkaline sodium sulfite pretreatment at 140 °C led to a high lignin removal and carbohydrate recovery with better enzymatic digestibility, compared to the other pretreatments. Particularly, a high total sugar yields (glucose and xylose) of 0.48 g/g original biomass could be achieved after the optimized alkali pretreatment with the supplement of Na_2SO_3 and the consistent enzymatic hydrolysis.

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