Effect of Physicochemical Pretreatments and Enzymatic Hydrolysis on Corn Straw Degradation and Reducing Sugar Yield

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Straw lignocelluloses were converted to reducing sugar for possible use for bioenergy production *via* physicochemical pretreatments and enzymatic hydrolysis. The experiment was divided into 2 steps. The first step focused on breaking the crystal structure and removing lignin in corn straw. The lignin, hemicellulose, and cellulose degradation rates observed were 92.2%, 73.7%, and 4.6%, respectively, after corn straw was treated with sodium hydroxide (3% w/w) plus high-pressure steam (autoclave), 74.8%, 72.5%, and 4.3% after corn straw was treated with sodium hydroxide (8%, w/w) plus wet steam explosion, compared with native corn straw ($P < 0.05$). The second step was enzymatic hydrolysis for the pretreated straw. The enzymatic hydrolysis could yield 576 mg/g reducing sugar and significantly degrade cellulose and hemicellulose contents by 93.3% and 94.4% for the corn straw pretreated with sodium hydroxide plus high-pressure steam. For the corn straw pretreated with sodium hydroxide plus wet steam explosion, the enzymatic hydrolysis could yield 508 mg/g reducing sugar, and degrade cellulose and hemicellulose contents by 83.5% and 84.2%, respectively, compared with the untreated corn straw ($P<0.05$). Scanning electron microscopy showed that the physicochemical pretreatments plus enzymatic hydrolysis degraded corn straw to many small molecules. Thus, physicochemical pretreatments plus enzymatic hydrolysis converted lignocellulose to reducing sugar effectively.

Keywords: Corn straw; Physicochemical pretreatment; Enzymatic hydrolysis; Reducing sugar yield; Ultramicroscopic structure

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INTRODUCTION

Corn straw is one of the most abundant agricultural residues in the world, and it represents an ideally cheap, renewable, and widely available feedstock for bioconversion to fuels and chemicals (Sánchez and Cardona 2008). However, most corn straw is burned or buried in the fields as waste due to the shortage of effective treatment methods (Jiang *et al.* 2012). Crop straw is mainly composed of cellulose, hemicellulose, and lignin, which can be converted to reducing sugar and other low-molecular weight carbohydrates for biofuel and other high-value biomaterial products (Lambert *et al.* 1990; Himmel *et al.* 2007; Kumar *et al.* 2009). Nevertheless, the crystal structure of straw inhibits enzyme accessibility and hydrolysis for reducing sugar. Lignin is a macromolecule and a highly branched polymer, which forms the lignin sheath and surrounds hemicellulose and cellulose to protect them from degradation by cellulase and hemicellulase (Bellido *et al.* 2014; Wang *et al.* 2015a; Zhang *et al.* 2015). Therefore, lignin removal from straw is one of the most important steps for thorough degradation. Of course, the synchronous degradation of lignin, cellulose, and hemicellulose increases the effectiveness of enzymatic hydrolysis for reducing sugar. To solve this problem, many pretreatment methods such as physical, chemical, and physicochemical treatments have been applied (Chang *et al.* 2012; Kim and Han 2012; Joe *et al*. 2015; Qin *et al*. 2015).

It is extremely difficult to achieve good results by adopting a single method. In recent studies, two or three methods have been combined together for processing different crop straws to achieve the desired effects (Sun and Cheng 2002; Singh *et al.* 2014; Singh *et al.* 2015; Wang *et al.* 2015b). Steam explosion and high-pressure steam (autoclave) are the more effective pretreatment methods for crop straw degradation because they have the more potential for energy efficiency, lower environmental impact, and more soluble carbohydrate production than other pretreatment technologies (Liu *et al.* 1999; Viola *et al.* 2008; Alvira *et al.* 2010). Even so, steam explosion and high-pressure steam are not able to increase lignin degradation to a high level (Liu *et al.* 1999; Chang *et al.* 2012). Hence, sodium hydroxide (NaOH) is used together with steam explosion or high-pressure steam in this study to degrade crop straw completely. Biological treatments of crop straw including microbial fermentation and enzymatic hydrolysis are safe, environmentally friendly, and less energy intensive than other methods (Dinis *et al.* 2009). However, they are restricted by the pretreatment methods, enzyme activity, enzyme price, microbial species, hydrolysis reaction effectiveness, microbial fermenting period, *etc*.; great improvement is needed for commercial applications (Sun and Cheng 2002).

Physical treatment is more effective than typical chemical approaches in breaking up the straw crystal structure (Chang *et al.* 2012). Chemical treatment can degrade lignin effectively (Kim and Han 2012), while microbial fermentation and enzymatic hydrolysis are more effective to convert straw to reducing sugar and low-molecular weight carbohydrates effectively based on the physicochemical pretreatments (Chang *et al.* 2012; Wang 2015c). In order to increase crop straw application, physicochemical pretreatments and enzymatic hydrolysis were combined together in this study to provide new alternative methods for converting crop straw to reducing sugar effectively.

EXPERIMENTAL

Materials Preparation

The air-dried corn straw was ground in a FW 100 hammer crusher (Beijing Junhao Technological Development Co., Ltd, Beijing, China), passed through a 40-mesh screen, and stored at room temperature before use. The corn straw was pretreated with QBS-80B steam explosion machine (Hebi Zhengdao Biological Energy Co., Ltd, Hebi, China) and LDZX-30KBS high-pressure steam sterilization pot(Shanghai Shenan Medical Apparatus Factory, Shanghai, China), respectively. The combination of cellulase and hemicellulase was purchased from Shandong Zesheng Biological Technology Co. Ltd., Taian, China. The activities of cellulase and hemicellulase were determined as 323 FPU/g (142 mg protein/g) and 3069 U/g (4 mg protein/g) according to the NREL Laboratory Analytical Procedure (IUPAC 1987). The liquid cellulase and hemicellulase solution was prepared as follows: A certain quantity of enzyme powder was weighed, placed in distilled saline at ratio of 1:10 (enzyme power: saline), shaken at 200 RPM for 3 h, filtrated with filter paper, and passed through a 0.25 μm filter membrane. Cellulase and hemicellulase activities were determined as 32 FPU/mL and 307 U/mL under the conditions of pH 4.8 and 40 °C.

Straw Pretreatments

According to a previous study (Wang 2015c), a three-factor and three-level orthogonal experiment (NaOH concentrations (w/w): 2.0%, 2.5%, 3.0%; solid-liquid ratios: 1:6.0, 1:7.5, 1:9.0 (w:v); autoclave time: 15 min, 30 min, 45 min) was conducted to determine the optimal conditions for removing lignin in corn straw. The amount of NaOH was weighed according to corn straw weight (w/w) and dissolved in water at the corresponding solid-liquid ratios (corn straw:water, w/v). Corn straw was soaked in NaOH solution in a flask and autoclaved at 121 °C for the corresponding time. After cooling to room temperature, the pH was adjusted to 7.0 with 9 M hydrochloric acid, and the material was dried at 65 °C. There were three replicates for each treatment condition in the experiment.

For the pretreatment of NaOH-wet steam explosion, 0%, 2%, 4%, 6%, and 8% NaOH was weighed according to the weight of corn straw and dissolved in water at the ratio of 1:2 (*i.e.*, corn straw:water = 1:2, w/v). The NaOH solution was sprayed evenly on corn straw, which was placed in a steam chamber. The steam pressure was adjusted to 2.5 MPa, kept for 200 s, and then suddenly released at the end of the treatment to give the explosion effect. The exploded samples were collected and dried at 65 °C. The pretreatment of NaOH-dry steam explosion was prepared according to the above protocol modified by evenly spraying the different proportions of solid NaOH without dissolution in water on corn straw respectively.

The Optimal Conditions of Enzymatic Hydrolysis

The corn straw pretreated with NaOH plus autoclave was used as the substrate (50 g/L) under the condition of 200 RPM. A four-factor and four-level orthogonal experiment (enzyme activity (cellulase activity: 6.45, 12.90, 19.35, 25.80 FPU/g biomass plus hemicellulase activity: 61.88, 123.76, 185.64, 247.52 U/g biomass); pH: 4.2, 4.8, 5.4, 6.0; reaction time: 24 h, 48 h, 72 h, 96 h; reaction temperature: 30, 40, 50, 60 °C) was used to optimize the enzymatic hydrolysis conditions. The 0.1 M citric acid-sodium citrate buffers of pH 4.2, 4.8, 5.4, and 6.0 were used to make the different enzyme solutions with pH values of 4.2, 4.8, 5.4, and 6.0. The reaction pH values were adjusted to 4.2, 4.8, 5.4, and 6.0 with 9 M hydrochloric acid. The ratio of liquid and corn straw in the reaction volume was adjusted to 20:1 (v/w) with the different pH buffers of citric acid-sodium citrate. The enzymatic reaction was terminated by placing the flask in boiling water for 15 min with stirring. The mixture was then cooled to room temperature with cold water.

Enzymatic Hydrolysis of the Different Pretreated Corn Straw

The condition and process of enzymatic hydrolysis were conducted according to results obtained from the above experiment, *i.e.*, pH 4.8, cellulase activity 25.8 FPU/mL, hemicellulase activity 247.5 U/mL, 40 °C, and 96 h reaction in shaking incubator at 200 RPM. Total reducing sugar in the reaction liquid was estimated by the dinitrosalicylic acid (DNS) method (Miller 1959), and then the pH was adjusted to 7.0 with 5% potassium hydroxide (w/v). The biomass after enzymatic hydrolysis was dried at 65 °C to

90% dry matter, which was ground for further analysis. The cellulose, hemicellulose, and lignin fractions in the samples were determined according to the method of Van Soest *et al.* (1991). The experimental design is listed in Table 1.

Table 1. The Different Physicochemical Pretreatments of Corn Straw for Enzymatic Hydrolysis

Groups	Different Physicochemical Treatments
1	Control group (untreated corn straw)
2	NaOH (3%, w / w) + autoclave (straw:NaOH solution = 1: 9, w / v)
3	Dry steam explosion without water addition
4	NaOH (4%, w / w) + dry steam explosion
5	NaOH (8%, w / w) + dry steam explosion
6	Wet steam explosion (straw:water = 1:2, w / v)
7	NaOH (4%, w / w) + wet steam explosion (straw:NaOH solution = 1:2, w / v)
8	NaOH (8%, w / w) + wet steam explosion (straw:NaOH solution = 1:2, w / v)

Surface Morphology of the Treated Corn Straw

Physical changes in the native and treated corn straw were analyzed by scanning electron microscopy (SEM). The SEM images were taken at 1000× magnification using an S-3400NIISEM instrument (Hitachi Co. Ltd., Tokyo, Japan) at acceleration voltages of 10 kV. All samples were mounted on conductive adhesive tape and coated with gold-palladium (SC7640, Quorum Technology, Newhaven, UK).

Statistical Analysis

The data were expressed as mean ± standard errors (SE) for calculating chemical compositions and reducing sugar yield of the pretreated corn straw with different physicochemical methods plus enzymatic hydrolysis. The data were determined for each of the three replicates per treatment, which was averaged to give a single value of each sample for subsequent statistical analysis. The data were analyzed using the ANOVA in SPSS 20.0 software (IBM, Armonk, NY, USA). Tukey's multiple range test was employed to evaluate the differences. Differences were considered statistically significant at $P < 0.05$.

RESULTS AND DISCUSSION

Optimal Conditions of NaOH–Autoclave Pretreatment for Lignin Removal in Corn Straw

The orthogonal experiment in Table 2 indicated that the optimal conditions of NaOH-autoclave pretreatment were as follows: 3% (w/v) NaOH, solid-liquid ratio at 1:9 (w:v), autoclaved at 121 °C for 15 min. The impact order was: NaOH concentration > solid-liquid ratio > autoclaving time. The minimal lignin content was 0.61% in group 9 ($P < 0.05$). The lignin content in NaOH-autoclave treated corn straw was further reduced to 0.50% (lignin degradation rate was 92.21%) under the above optimal conditions, in which hemicellulose and cellulose degradation rates were 73.21% and 4.6%, respectively, compared with the native corn straw ($P < 0.05$) (Table 3). There are few reports about NaOH-autoclave pretreatment of crop straw. Previous research showed that acid-sprayed or acid-soaked barley straw with 1.0% H_2SO_4 (w/w) was kept at 220 °C for 5 min to result in a high glucose yield with enzymatic hydrolysis (Linde *et al.* 2006). Whether the alkali or acid pretreatment is conducted, the main function of alkali or acid plus high-pressure steam was to destroy straw crystal structure for the further enzymatic hydrolysis.

Table 2. The Orthogonal Design of NaOH-Autoclave Pretreatments for Lignin Removal in Corn Straw

Groups	NaOH Concentrations (%) Factor A	Solid–liquid Ratio (g/mL) Factor B	Autoclaving Time (min) Factor C	Lignin Contents (%)
1	2.0	1:6.0	15	3.87±0.008^{A}
2	2.0	1:7.5	30	3.73±0.050^{A}
3	2.0	1:9.0	45	2.41±0.076^{B}
4	2.5	1:6.0	30	2.37±0.140^{B}
5	2.5	1:7.5	45	1.75±0.120^{C}
6	2.5	1:9.0	15	0.68±0.019^{F}
7	3.0	1:6.0	45	1.38±0.013^{D}
8	3.0	1:7.5	15	0.90±0.067^{E}
9	3.0	1:9.0	30	0.61±0.029^{F}
K1	10.01	7.62	5.45	
K2	4.80	6.38	6.71	
K3	2.89	3.70	5.54	
k1	3.34	2.54	1.82	
k2	1.60	2.13	2.24	
k3	0.96	1.23	1.85	
R	2.38	1.31	0.42	
Important Order	$R_A>R_B>R_C$			
Optimal Levels	3.0	1:9.0	15	

Note: The data followed by the different capital letters in the same column are significantly different from each other (*P*< 0.05), while the data followed by the same capital letters in the same column are insignificantly different from each other (*P*> 0.05).

Effect of Different Physicochemical Treatments on Lignocellulose Degradation of Corn Straw

Table 3indicated that steam explosion could significantly increase cellulose, hemicellulose, and lignin degradation rates of corn straw ($P < 0.05$). NaOH addition during the dry steam explosion increased cellulose and hemicellulose degradation ($P < 0.05$), but did not have any effect on lignin degradation ($P > 0.05$). However, NaOH addition during the wet steam explosion significantly increased the degradation of lignin ($P < 0.05$) but not cellulose or hemicellulose ($P > 0.05$). The maximal lignin degradation rate was 74.8% in the treated corn straw with 8% NaOH-wet steam explosion, in which hemicellulose and cellulose degradation rates were 72.5% and 4.3%, respectively, compared with the native corn straw ($P < 0.05$). The function of NaOH is to permeate into the interface between lignin and hemicellulose under the help of high pressure and water to cause lignin degradation (Li *et al.* 2007); therefore, alkali solution treatment has more effect on lignin degradation than cellulose and hemicellulose degradation.

According to this result, NaOH concentrations of 4% and 8% (w / w) were selected for further studies.

Physicochemical pretreatments are effective in degrading the crystal structure of straw prior to microbial fermentation and enzymatic hydrolysis (Viola *et a*l. 2008; Alvira *et al.* 2010; Chang *et al.* 2012; Toquero and Bolado 2014), but it has some limits for high lignin degradation. It was reported that lignin degradation rate of rice straw are 52.6% under the conditions of 2.96% NaOH, 81.8 °C, and 56.7 min reaction, with a glucose yield of 254 g/kg after enzymatic hydrolysis (Kim and Han 2012). Another study showed that the lignin degradation rate of corn straw was 77.9% in an alkaline reaction (1.5% NaOH, 100 °C, 1.5 h) followed by steam explosion (Guo *et al.* 2013).

The previous results indicate that alkaline hydrolysis requires high temperature and a long reaction time, which can degrade lignin to some extent. This study combines the physical and chemical reactions together to save time and increase lignin degradation effectively. The combined treatments of NaOH (3%) plus autoclave or NaOH (8%) plus wet steam explosion significantly decreased lignin content by 92.2% (6.42% *vs.* 0.5%) or 74.8% (6.42% *vs.* 1.62%), respectively, indicating superiority of the combined treatments together. It is emphasized that NaOH-wet steam explosion is very useful and economical for straw pretreatment due to saving time(200 s)and making later dry processing easy(*i.e.*, corn straw:water = 1:2, w/v).Even so, the combined treatments have no significant effect on cellulose degradation, which will require the further enzymatic hydrolysis.

Table 3. Effect of Different Pretreatments on the Main Compositions of Corn Straw (%)

NaOH levels	Hemicellulose	Cellulose	Lignin
Control	30.85±1.12^{A}	37.38±0.93^{A}	6.42±0.16^{A}
NaOH + Dry Steam Explosion			
0	8.33±0.76^{B}	35.85±0.30^{B}	5.51±0.12^{B}
2	5.39±0.52^{D}	35.97±0.45^{B}	5.69±0.16^{B}
4	5.24±0.61^{D}	34.26±0.26^{C}	5.12±0.26^{B}
6	6.82±0.22^{C}	31.66±0.22^{D}	5.44±0.26^{B}
8	5.90±0.49^{CD}	29.50±1.31^{E}	5.35±0.22^{B}
NaOH + Wet Steam Explosion			
0	7.18±0.16^{C}	36.80±0.16^{AB}	5.39±0.38^{B}
2	8.44±0.43^{B}	36.87±1.18^{AB}	5.49±0.07^{B}
4	8.37±0.15^{B}	36.89±0.40^{AB}	4.11±0.20^{C}
6	8.45±0.28^{B}	35.67±1.47^{B}	2.92±0.09^{D}
8	8.49±0.10^{B}	35.78±1.66^{B}	1.62±0.19^{E}
NaOH + Autoclave	8.11±0.49^{B}	35.66±0.19^{B}	0.50±0.03^{F}

Note: Data followed by different capital letters in the same columns are significantly different from each other (P< 0.05), while the data followed by the same capital letters in the same columns are insignificantly different from each other (P> 0.05).

Optimal Conditions of Enzymatic Hydrolysis for the Corn Straw Pretreated with NaOH plus Autoclave

The orthogonal experiment in Table 4 showed that the optimal conditions of enzymatic hydrolysis were as follows: cellulase activity, 25.80 FPU/g; hemicellulase activity, 248 U/g; pH 4.8; substrate concentration, 50 g/L; reaction time, 96 h; and temperature, 40 °C. The impact order was: pH > temperature > enzyme activity >

enzymatic reaction time. Under the above optimal conditions of enzymatic hydrolysis, the maximal reducing sugar yield was 575.5 mg/g biomass ($P < 0.05$). According to the principle of enzymatic reaction kinetics, when the substrate concentration is fixed, the catalytic reaction rate increases with increasing enzyme dosage (Liao *et al.* 2008), which increases the cost of bioconversion. Therefore, selecting the appropriate enzyme dosage for converting cellulose materials economically and effectively is very important. This study provides a better alternative method than the previous studies for straw degradation and reducing sugar yield (Yu *et al.* 2009; Kim and Han 2012; Wang *et al*. 2015b).

Table 4. Optimal Conditions of Enzymatic Hydrolysis for the Corn Straw Pretreated with NaOH plus Autoclave

Groups	Enzyme Dosage* (FPU/g + U/g)	pH	Reaction Time (h)	Temperature (°C)	Reducing Sugar Yield (mg/g)
	Factor A	Factor B	Factor C	Factor D	
1	6.45+61.8	4.2	24	30	208.18±0.70^{H}
2	6.45+61.8	4.8	48	40	385.91±6.04^{E}
3	6.45+61.8	5.4	72	50	164.34±2.25^{J}
4	6.45+61.8	6.0	96	60	75.57±2.37^{L}
5	12.90+123.76	4.2	48	50	483.78±2.25^{B}
6	12.90+123.76	4.8	24	60	209.34±1.86^{H}
7	12.90+123.76	5.4	96	30	135.82±6.27^{K}
8	12.90+123.76	6.0	72	40	181.06±6.27^{I}
9	19.35+185.64	4.2	72	60	289.27±6.43^{F}
10	19.35+185.64	4.8	96	50	571.57±1.86^{A}
11	19.35+185.64	5.4	24	40	406.81±0.85^{D}
12	19.35+185.64	6.0	48	30	154.75±5.54^{J}
13	25.80+247.52	4.2	96	40	567.39±14.61^{A}
14	25.80+247.52	4.8	72	30	448.86±21.03^{C}
15	25.80+247.52	5.4	48	60	183.77±9.98^{I}
16	25.80+247.52	6.0	24	50	250.05±4.06^{G}
K1	834.00	1548.62	1074.38	947.61	
K2	1010.00	1615.68	1208.21	1541.17	
K3	1422.40	890.74	1083.53	1469.74	
K4	1450.07	661.43	1350.35	757.95	
k1	208.50	387.16	268.60	236.90	
k2	252.50	403.92	302.05	385.29	
k3	355.60	222.68	270.88	367.44	
k4	362.52	165.36	337.59	189.49	
R	154.02	238.56	68.99	195.80	
Important Order	$R_B > R_D > R_A > R_C$				
Optimal Levels	25.80	4.8	96	40	

*Enzyme dosage includes cellulase (CFU / g) and hemicellulase (U / g).The data followed by the different capital letters in the same column are significantly different from each other ($P < 0.05$), while the data followed by the same capital letters in the same column are insignificantly different from each other ($P > 0.05$).

The Effect of Different Pretreatments plus Enzymatic Hydrolysis on Chemical Compositions of Corn Straw and Reducing Sugar Yield

The contents of cellulose, hemicellulose, and lignin in the corn straw pretreated with physicochemical methods plus enzymatic hydrolysis are shown in Table 5, and the corresponding reducing sugar yields are shown in Table 6. Enzymatic hydrolysis significantly decreased cellulose and hemicellulose contents in corn straw ($P < 0.05$), without any effect on lignin degradation ($P > 0.05$). One explanation is that enzyme powder contains only cellulase and hemicellulase but not ligninase. After enzymatic hydrolysis, the degradation rates of cellulose and hemicellulose were increased by 93.3% and 94.4%, respectively, and reducing sugar yield was 575.5mg/g biomass in the corn straw pretreated with NaOH (3%) plus autoclave ($P < 0.05$); 83.5%, 84.2%, and 508.2mg/g in the corn straw pretreated with NaOH (8%) plus steam explosion ($P < 0.05$); 21.9%, 11.5%, and 249.3 mg/g in the native corn straw ($P < 0.05$). This result indicated that physicochemical pretreatment significantly increased reducing sugar yield, as noted previously (Ko *et al.* 2009; Carrasco *et al*. 2011; Kim and Han 2012). The reasons are that the pretreatments provide more chances or accessibility for enzymatic hydrolyzation (Kim and Holtzapple 2005; Mosier *et al.* 2005; Hendriks and Zeeman 2009; Holopainen-Mantila *et al.* 2013), as well as increase hydrophilic straw surface to promote the interaction between enzyme and straw (Yun 2014). The higher yield of reducing sugar from the pretreated corn straw indicates the effectiveness of these kinds of pretreatments in this study.

Surface Morphology and SEM

The surface morphology and SEM images indicated that the surface of the untreated corn straw was unbroken (Fig. 1); however, the treated corn straw showed that all the methods could break up the smooth surface of native straw (Figs. 2 to Fig. 5).

NaOH-autoclave and NaOH-wet explosion were effective in breaking up corn straw, in which the straw surface was rougher and covered with a destroyed wax layer, and the size of corn straw tended to be smaller. After enzymatic hydrolysis, the pretreated straw turned into powder or many smaller molecules, indicating that the optimal physicochemical pretreatments followed by enzymatic hydrolysis are more effective in altering the structure of corn straw than the single pretreatments.

The physicochemical pretreatments formed many craters by silica dissolution, exposed the composition of fiber bundle, which made the enzymatic hydrolysis more efficient (Li *et al*. 2007), in agreement with this study. Generally, lignin and hemicellulose encase cellulose, which prevents cellulase from reaching cellulose fibrils. Previous studies showed that lignin appeared on the outer surface of straw after explosion to expose more internal cellulose surfaces for enzyme accessibility (Selig *et al.* 2007; Kristensen *et al.* 2008).

Other reports indicated that the lignin layer of the exploded corn straw was easily removed because lignin was less strongly bound to carbohydrate polymers (Liu *et al.* 1999; Chang *et al.* 2012), especially when NaOH was combined with physical pretreatment together to increase the lignin removal and enhance cellulose digestibility. Cellulose and hemicellulose accessibility was improved by creating pores and breaking the lignin-carbohydrate complex (Mooney *et al.* 1998). Therefore, it is convenient for enzymes to attack the cellulose and hemicellulose fractions effectively, which was demonstrated by higher reducing sugar yield under the condition that lignin in corn straw is removed by NaOH-autoclave or NaOH-wet explosion pretreatments in this study.

Table 5. Main Compositions of the Different Chemically Treated Corn Straw With or Without Enzymatic Hydrolysis (%, dry matter)

Group		Cellulose			Hemi-Cellulose		Lignin	
	Y1	Y2	Y3	Y1	Y2	Y3	Y1	Y2
1	37.60 ± 1.01^{Aa}	29.36 ± 0.62^{Ab}	21.91	30.13 ± 1.43^{Aa}	26.67 ± 0.41^{Ab}	11.48	5.96 ± 0.70^{Aa}	6.26 ± 0.54^{Aa}
2	33.26 ± 1.50^{Ca}	2.22 ± 0.22^{Gb}	93.33	7.65 ± 0.59^{Ba}	0.43 ± 0.03^{Eb}	94.38	0.59 ± 0.08^{Da}	0.58 ± 0.06^{Da}
3	34.45 ± 0.74^{BCa}	12.87 ± 0.35^{Cb}	62.64	7.74 ± 0.59^{Ba}	3.80 ± 0.43^{BCb}	50.90	5.42 ± 0.07^{ABa}	5.44 ± 0.36^{ABa}
4	33.19 ± 0.41^{Ca}	10.28 ± 0.73^{Db}	69.03	5.84 ± 0.26^{Ca}	4.09 ± 0.26^{Bb}	29.97	5.58 ± 0.48^{ABa}	5.21 ± 0.13^{ABa}
5	29.04 ± 1.62^{Da}	7.39 ± 0.71^{Eb}	74.55	5.68 ± 0.15^{Ca}	3.01 ± 0.36^{Cb}	47.01	5.50 ± 0.11^{ABa}	5.11 ± 0.05^{ABa}
6	35.63 ± 0.43^{Ba}	15.35 ± 1.11^{Bb}	56.92	7.45 ± 0.54^{Ba}	3.58 ± 0.34^{BCb}	51.95	5.59 ± 0.28^{ABa}	5.38 ± 0.17^{ABa}
7	35.79 ± 0.57^{Ba}	11.59 ± 1.09^{CDb}	67.62	8.23 ± 0.16^{Ba}	3.09 ± 0.27^{Cb}	62.45	4.49 ± 0.16^{Ba}	4.57 ± 0.87^{Ba}
8	33.67 ± 1.14^{Ca}	5.56 ± 0.50^{Fb}	83.49	8.19 ± 0.45^{Ba}	1.29 ± 0.22^{Db}	84.25	1.60 ± 0.22^{Ca}	1.50 ± 0.43^{Ca}

Note: The corn straw pretreatment design in group 1-8 is listed in Table 1.Y1, lignocellulose contents after physicochemical treatments; Y2, lignocellulose contents after physicochemical treatments plus enzymatic hydrolysis; Y3, degradation rates of lignocellulose after enzymatic hydrolysis. The data followed by the different capital letters in the same columns are significantly different from each other ($P< 0.05$), while the data followed by the same capital letters in the same columns are insignificantly different from each other ($P> 0.05$). The data followed by the different lowercase letters between the two rows of pre–enzymatic hydrolysis and post–enzymatic hydrolysis are significantly different from each other ($P< 0.05$), while the data followed by the same lowercase letters between the two rows of pre–enzymatic hydrolysis and post–enzymatic hydrolysis are insignificantly different from each other ($P> 0.05$).

Table 6. Effect of Enzymatic Hydrolysis on Reducing Sugar Yield (mg / g biomass)

Groups	Before Enzymatic Hydrolysis	After Enzymatic Hydrolysis
1	92.63 ± 0.18^{Ab}	249.33 ± 16.99^{Ea}
2	11.99 ± 0.53^{Eb}	575.51 ± 7.04^{Aa}
3	47.30 ± 0.94^{Bb}	360.39 ± 7.80^{Da}
4	27.15 ± 2.02^{Db}	374.22 ± 22.11^{Da}
5	13.54 ± 0.35^{Eb}	356.95 ± 1.62^{Da}
6	91.69 ± 5.82^{Ab}	389.23 ± 4.36^{Da}
7	38.38 ± 3.04^{Cb}	451.75 ± 20.46^{Ca}
8	11.41 ± 1.11^{Eb}	508.17 ± 9.54^{Ba}

Note: The corn straw pretreatment design in group 1-8 is listed in Table 1.The data followed by the different capital letters in the same columns are significantly different from each other ($P< 0.05$), while the data followed by the same capital letters in the same columns are insignificantly different from each other ($P> 0.05$). The data followed by the different lower–case letters in the same rows are significantly different from each other ($P< 0.05$), while the data followed by the same lower–case letters in the same rows are insignificantly different from each other ($P> 0.05$).

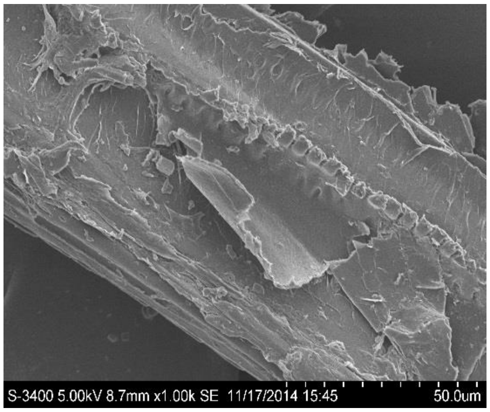

Fig. 1. Native corn straw

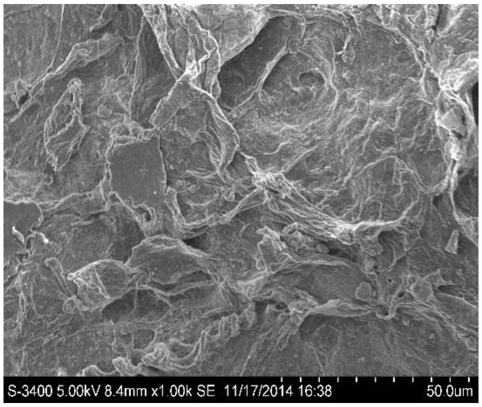

Fig. 2. Corn straw treated with sodium hydroxide (3%) + autoclave

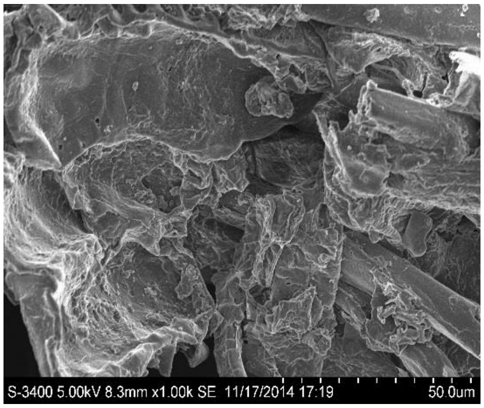

Fig. 3. Corn straw treated with sodium hydroxide (8%) + wet steam explosion

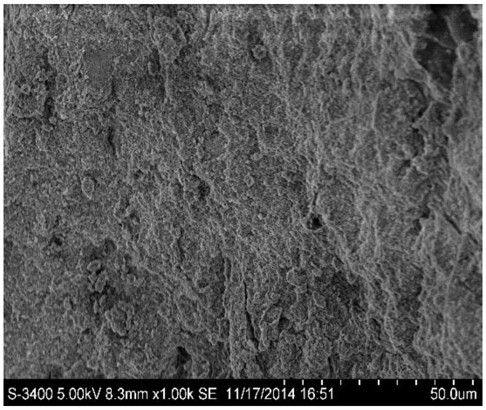

Fig. 4. Corn straw treated with sodium hydroxide (3%) + autoclave plus enzymatic hydrolysis

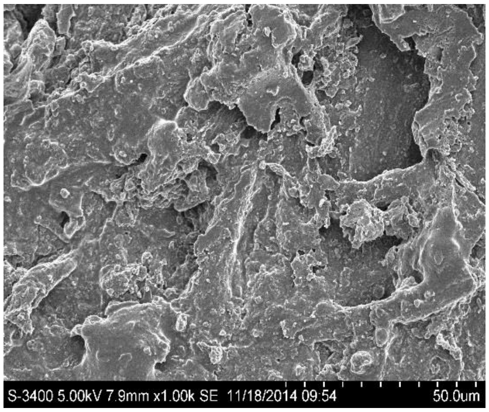

Fig. 5. Corn straw treated with sodium explosion (8%) + wet steam explosion plus enzymatic hydrolysis

CONCLUSIONS

1. The physicochemical pretreatments evaluated in this work were able to degrade hemicellulose and lignin in corn straw and provide more chances for accessibility and hydrolysis of enzymes.
2. These experiments showed that cellulase and hemicellulase could significantly convert cellulose and hemicellulose to reducing sugar for bioenergy and other biomaterial production based on the effective pretreatments.

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