

Fractionation of the main components of corn stover by formic acid and enzymatic saccharification of solid residue

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ABSTRACT

In this work, three main components (cellulose, hemicellulose, and lignin) of corn stover were fractionated effectively by formic acid. The effects of reaction temperature and time on the fractionation were investigated. It was found that the suitable conditions for the fractionation of corn stover were: the formic acid concentration was 88 wt.%, the ratio of solid to liquid was 1:13, and the treatment temperature was 80 °C with the duration of 3 h. Under such conditions, the retention yield of cellulose, extraction yields of hemicellulose and lignin were 89.5%, 79.8% and 65.6%, respectively. Subsequently, the enzymatic hydrolysis of the solid residue was conducted by using the enzyme mixture of cellulase (20 FPU/g dry substrate) and β-glucosidase (10 IU/g dry substrate). The enzymatic hydrolysis rate of glucan and xylan could reach 62.8% and 79.4%, respectively. In addition, to study the property changes of corn stover before and after treatment, SEM, XRD and TGA analyses were performed, and the lignin extracted by formic acid was also characterized by TGA and GPC.

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1. Introduction

Due to the rapid depletion of fossil fuels and the increased energy requirement, energy crises are getting more and more serious around the world. Thus, the abundant, renewable and biodegradable lignocellulosic biomass has been received much attentions for the development of biofuels and biochemical (Sanderson, 2011). To achieve a complete and profitable utilization of biomass, many efforts have been made to develop new processes (Zhang and Shahbazi, 2011), including physical (chipping, milling), chemical (acid, alkaline, ammonia) (Kim et al., 2003; Lenihan et al., 2010; Li et al., 2012) and physico-chemical (steam explosion, AFEX, SPORL) methods (Alizadeh et al., 2005; Chen and Liu, 2007; Shuai et al., 2010). Each treatment method has both advantages and disadvantages. For instance, dilute acid treatment can dissolve most of hemicellulose, but cannot remove lignin efficiently, and usually high temperature (over 140 °C) is needed with the erosion for equipment; alkaline pretreatment can remove lignin efficiently, but it is difficult to fractionate the whole components and may cause environmental pollution; steam explosion has high request of equipment and it is not easy to realize continuous operation at large scale.

Organosolv extraction is considered as an effective method for fractionation of lignocellulosic biomass. Especially, formic acid (FA)

shows potential for its ability to reach an extensive delignification with simultaneous extraction of hemicellulose and good retention of cellulose (Kupiainen et al., 2012; Zhang et al., 2010), and FA treatment is environmental friendly because the FA can be readily and almost completely recycled by distillation for reuse. Also, the fractionated cellulose can be used for bioethanol, papermaking or other cellulosic materials (Shen et al., 2010; Van De Vyver et al., 2011), hemicellulose can be prepared for xylitol or furfural (Moreau et al., 1998), and the lignin extracted can be produced to cycloalkanes as aviation fuel by catalytic hydrogenation or other modified chemical products (e.g. concrete additives) (Yan et al., 2010; Yu et al., 2013).

Zhang et al. (2010) studied the fractionation of corn cob by the use of 88 wt.% FA with 0.2 wt.% HCl as a catalyst, but they did not provide the digestibility tests of the FA-treated biomass. Corn stover is also an abundant and renewable agricultural waste in China, and it has been considered as a promising biomass for the sustainable production of biofuels or biomaterials (Liu et al., 2013). In the present work, corn stover was treated by 88 wt.% of FA without any catalyst. The three main components of corn stover were fractionated effectively with relatively mild reaction conditions. The overall process of the fractionation of corn stover by FA is given in Fig. 1. It is seen that, corn stover was subjected to FA treatment to dissolve hemicellulose (degraded to mono- and oligosaccharides) and lignin. Subsequently, the treated stock went to filtration to separate solid residual (mainly cellulose) and liquid (mixture of hemicellulose and lignin). The liquid fraction could be distilled to recover FA and thus hemicellulose plus lignin were precipitated simultaneously. After that, the precipitated solid could be washed and then filtrated to get hemicellulose (liquid fraction)

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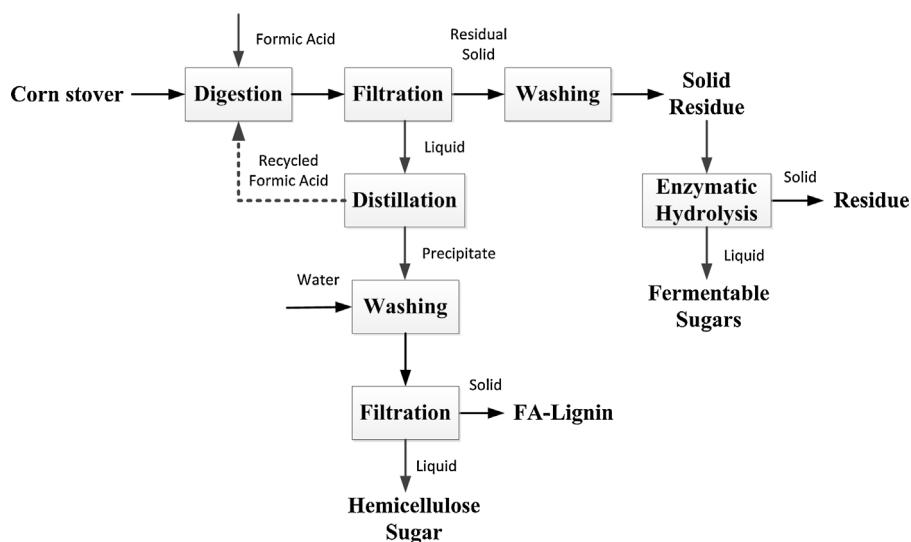


Fig. 1. Flowchart of the fractionation of corn stover by 88% formic acid.

and FA-lignin (solid fraction). Furthermore, the digestibility of the FA-treated corn stover was evaluated via enzymatic hydrolysis, the characteristic changes of corn stover before and after FA treatment were investigated by SEM, XRD and TGA, and the molecular weight and thermal stability of FA-lignin were also studied for its potential utilizations.

2. Materials and methods

2.1. Materials

Air-dried corn stover 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 20 and 40 mesh. The main components (dry weight basis) of the ground corn stover were determined according to the National Renewable Energy Laboratory (NREL) analytical procedure for the determination of structural carbohydrates and lignin in biomass (Sluiter et al., 2008b), and the results are shown in Table 1. Formic acid with concentration of 88 wt.% was purchased from Fuyu Chemical Reagent Co. Ltd. China. Commercial enzymes, cellulase (Celluclast 1.5L) and β -glucosidase (Novozyme 188) were obtained from Sigma-Aldrich China Inc. All chemicals and enzymes were used as received.

2.2. FA treatment

Fig. 1 shows the overall processes of fractionation of corn stover by using formic acid with FA recycling, and the operations for each step are illustrated as below. The ground corn stover was placed in a sealed glass flask, and mixed with 88 wt.% FA at the solid to liquid ratio of 1:13, which can achieve a smoothly magnetic agitation. The actual FA dosage in treatment was 87.42%, as the moisture content of raw corn stover is 7.93% (Table 1). The flask was heated in an oil bath with stirring for the desired time. After reaction the mixture was filtered. The solid residue was firstly washed with 88 wt.% FA, and then washed to neutral pH by deionized water to get the pulp mainly consisted of cellulose. FA was recycled through rotary vacuum evaporator at 70 °C and the recycled FA was cooled with liquid nitrogen in cold trap; simultaneously the mixture of lignin and hemicellulose sugar was deposited at the bottom of flask so that the deposited solid could be separated with FA liquor by filtration. At last, the deposited solid was washed with deionized water.

Thus, the hemicellulose sugar was dissolved and the solid lignin (FA-lignin) was precipitated, and then the solid/liquid mixture was filtrated to separate the hemicellulose sugar liquor and FA-lignin. The hemicellulose sugar liquor was further hydrolyzed to monosaccharide by 4 wt.% sulfuric acid at 121 °C for 1 h in an autoclave to determine the extraction yield of hemicellulose. Blank tests (using standard xylose solution) were also conducted for calibration. The lignin was washed with water and then vacuum dried at 70 °C for later characterization. After separation, the enzymatic hydrolysis of obtained cellulose pulp was carried out to get fermentable sugars.

2.3. Enzymatic hydrolysis of residual corn stover

The activities of cellulase and β -glucosidase were 192 FPU (filter paper unit)/mL and 741 IU (International Unit)/mL, respectively, as measured according to standard methods (Ghose, 1987). The corn stover residue after treatment at the optimized conditions was used as substrates for enzymatic hydrolysis. The enzymatic hydrolysis was conducted with solid consistency of 2% (w/v). A mixture of cellulase (with desired enzyme loading, i.e. 5, 10, 20, 30 FPU/g dry substrate, respectively) and β -glucosidase (10 IU/g dry substrate) was added together with 20 mL of 0.05 M sodium citrate buffer (pH 4.8) and 0.02% sodium azide, and the hydrolysis took place at 50 °C for 72 h in serum bottles (25 mL) placed in an air bath incubator shaker at 90 rpm. During hydrolysis, hydrolysate was sampled at desired intervals for sugar analysis.

2.4. Analytical methods

The composition of treated and untreated corn stover was measured according to the NREL procedures. Acid and enzymatic hydrolyzates (0.22 μ m filtered) were analyzed by a high performance liquid chromatography (HPLC) system (Model 1200, Agilent, USA) equipped with a Bio-Rad Aminex HPX-87H column (300 mm \times 7.8 mm) and refractive index detector. The column was operated at 55 °C with 0.005 M/L H₂SO₄ solution as the mobile phase at a flow rate of 0.5 mL/min. The quantitative analysis was performed using a calibration with external standards of known concentration. All samples were analyzed in duplicate and the average was reported.

The content of furfural and 5-hydroxymethyl furfural (HMF) was measured by HPLC (Waters 2489, USA) with ultraviolet detector (284 nm) and SunFire C18 (4.6 mm \times 250 mm) chromatographic

Table 1

Components content of raw corn stover.

Glucan (%)	Xylan (%)	Araban (%)	Lignin (%)	Extractives ^a (%)	Ash (%)	Moisture (%)
35.93	20.64	4.01	22.37	18.4	2.25	7.93

^a Total water soluble and ethanol soluble substances.

column. Ethanol/water (volume ratio of 1:4) was used as mobile phase with flow rate of 1 mL/min at 35 °C.

The recycled FA was diluted 100 times and the concentration was determined by titration with NaOH standard solution (0.1 mol/L), and it was calculated according to the following formula:

$$C_{\text{formic acid}} = \frac{C_{\text{NaOH}} \times V_{\text{NaOH}}}{V_{\text{formic acid}}}$$

where C_{NaOH} is the concentration of NaOH standard solution (mol/L); V_{NaOH} is the consumed volume of NaOH standard solution (mL); $V_{\text{formic acid}}$ is the volume of titrated formic acid solution (mL).

The yield of solid residue, retention yield of cellulose, and extraction yield of hemicellulose or lignin were calculated by the equations below, respectively:

$$Y_{\text{residual solid}} (\%) = (W_{\text{residual solid}} / W_{\text{raw corn stover}}) \times 100$$

$$Y_{\text{cellulose}} (\%) = (W_{\text{glucan in the solid residue}} / W_{\text{glucan in the raw corn stover}}) \times 100$$

$$Y_{\text{hemicellulose}} (\%) = (W_{\text{(xylan+araban) in the solution}} / W_{\text{(xylan+araban) in the raw corn stover}}) \times 100$$

$$Y_{\text{lignin}} (\%) = (W_{\text{lignin solid extracted}} / W_{\text{lignin in the raw corn stover}}) \times 100$$

wherein W is the mass of corresponding components (g).

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

$$E_{\text{glucan}} (\%) = (W_{\text{glucose in hydrolysate}} \times 0.9 / W_{\text{glucan in substrate}}) \times 100$$

$$E_{\text{xylan}} (\%) = (W_{\text{xylose in hydrolysate}} \times 0.88 / W_{\text{xylan in substrate}}) \times 100$$

2.5. Scanning electron microscopy

Scanning electron microscopy (SEM) of corn stover before and after treatment was carried out with a Hitachi S-4800 (Hitachi Co., Japan) at 3.0 kV. Dried samples were sputter coated with a thin gold layer prior to analysis.

2.6. Crystallinity index

Crystallinity of intact and treated corn stover was analyzed by a Bruker D8 ADVANCE X-ray diffractometer (XRD, Bruker Co., Germany) equipped with Ni-filtered Cu K α radiation generated at 40 kV and 40 mA. The scattering angle range was 5–60° with a scan rate of 4° min⁻¹. The crystallinity index (CrI) was calculated according to the empirical method developed by Segal et al. (1959) using the following equation:

$$\text{CrI} = \frac{I_{002} - I_{\text{amorph}}}{I_{002}}$$

where I_{002} is the maximum intensity of the (0 0 2) lattice diffraction and I_{amorph} is the intensity diffraction at 18 degrees.

2.7. Thermal properties

Pyrolysis of intact corn stover, residual solid and FA-lignin were performed by a thermo-gravimetric analyzer (TGA, Rubotherm-DYNTHERM-HP, Rubotherm Co., Germany) with temperature range from room temperature to 600 °C at a heating rate of 10 °C min⁻¹.

under nitrogen (25 mL/min). The samples were dried in a vacuum oven at 70 °C overnight before TGA analysis.

2.8. Molecular weight and distribution

The molecular weight and distribution of FA-lignin were characterized by Gel Permeation Chromatography (GPC) (HELEOS System, Wyatt, USA). Samples were dissolved to a concentration of 0.5% in 8% lithium bromide/*N,N*-dimethylformamide (LiBr/DMF) and stirred with magneton for 5 days. Then the solutions were diluted to concentration of 0.05% with 0.1% LiBr/DMF. The separations were performed on a system consisting of laser detector (DAWN, HELEOS-II, Wyatt) and UV/Visible detector (Waters-2489) equipped with MZ-Gel SD plus 10E 3 Å 10 μm guard column. The mobile phase was 0.1% LiBr/DMF at a flow rate of 1.00 mL/min. The injection volume was 200 μL and samples were filtered by 0.22 μm PTFE filter before injected. PS (polystyrene) standards were used to calibrate the columns.

3. Results and discussion

3.1. Effect of FA treatment on the composition change of corn stover

The composition of corn stover residue after treatment is listed in Table 2. As can be seen, after treatment under all conditions used, the main components of the solid residue were glucan (about 50–67%) and lignin (about 10–17%), while the content of xylan and araban reduced more than half compared to the original contents (20.64% of xylan and 4.01% of araban, as shown in Table 1). Particularly, a large number of hemicellulose (xylan + araban) and partial lignin were dissolved out by high concentration of FA under relatively low temperature. For example, after FA treatment at 80 °C for 3 h, the content of xylan, araban, and lignin was 8.42%, 1.37%, and 12.48%, respectively, which were much lower compared to the original corn stover (Table 1). On the contrary, the extraction effect on cellulose by FA was not obvious (Table 2). This is because cellulose is more stable compared to heterogeneous hemicellulose and lignin due to its uniform and crystalline structure (Jahan et al., 2006; Mamman et al., 2008). In addition, with the

Table 2
Composition of residual corn stover treated by 88% formic acid.

Condition	Temp. (°C)	Time (h)	Component content ^a (%)			
			Glucan	Xylan	Araban	Lignin
	60	3	50.52	9.43	1.89	17.29
	60	5	53.50	9.16	1.64	16.95
	60	10	57.10	9.11	1.42	16.25
	70	3	60.72	9.16	1.88	16.46
	70	5	62.06	8.76	1.58	15.98
	70	10	63.52	8.17	1.69	15.24
	80	2	62.04	8.45	1.60	16.23
	80	3	66.73	8.42	1.37	12.48
	80	5	66.47	7.59	1.22	12.14
	80	10	67.29	7.07	0.96	11.01
	90	3	66.76	7.32	0.72	10.08
	100	3	67.00	6.63	0.67	9.86
	110	3	66.77	6.35	0.63	9.87

^a Based on dry weight of residue.

intensification of treatment conditions, the glucan content of solid residue keeps increasing, while hemicellulose and lignin contents keep decreasing. For instance, when the reaction time increased from 2 to 3 h at 80 °C, the glucan content increased about 8%, though the just slight increase could be obtained as the reaction time was longer than 3 h. Also, at the same reaction time of 3 h, the glucan content increased about 32% when the temperature raised from 60 to 80 °C, while the corresponding contents of hemicellulose and lignin reduced about 14% and 28%, respectively. However, over high temperature (above 80 °C) may result in more glucan loss, despite more hemicellulose and lignin can be dissolved as well.

3.2. Effect of FA treatment on fractionation effectiveness

The effect of reaction time of FA treatment on fractionation effectiveness is presented in Fig. 2, which presents that solid yields of treated corn stover decreased when extending reaction time at the same temperature. This is due to the dissolution of hemicellulose and lignin during FA treatment, and it is in good agreement with the results of component analysis listed in Table 2. Thus, increasing reaction time at constant temperature benefits the extraction of hemicellulose and lignin, while it also leads to slight degradation of cellulose. However, there was slight decrease of the extraction yield of hemicellulose as the reaction time was over 5 h. For instance, the extraction yield of hemicellulose (78.6%) after FA treatment at 80 °C for 10 h was lower than that (80.5%) after treatment for 5 h at the same temperature. The slight reduction of extraction yield of hemicellulose is probably due to the conversion of hemicellulose to furfural or HMF (Lloyd and Wyman, 2005). As measured, about 0.2% and 0.15% of hemicellulose (based on the weight of hemicellulose in raw corn stover) in the sugar fraction was converted to furfural and HMF respectively, for the treatment by FA at 80 °C for 3 h, while about 1.5% and 0.6% of hemicellulose was converted to furfural and HMF respectively when the FA treatment was prolonged to 5 h. Also, similar trends for solid yields, retention yield of cellulose, extraction yields of hemicellulose and lignin can also be obtained at different temperature (60, 70, and 80 °C), as exhibited in Fig. 2(a)–(c). In addition, Fig. 2(c) displays that there was no clear increase of extraction yields of hemicellulose and lignin when reaction time prolonged from 3 to 5 h, while obvious cellulose loss can be obtained. Therefore, over long reaction time (longer than 3 h) is not needed.

Fig. 3 shows the effect of reaction temperature of FA treatment on fractionation effectiveness. It is seen that, the solid yield reduced with the increase of temperature. This was caused by the dissolution of hemicellulose and lignin as well as the degradation of cellulose, which is in good agreement with the trends of retention yield of cellulose, extraction yield of hemicellulose and lignin (Fig. 3). However, when the temperature was over 80 °C, the increasing of the extraction yield of lignin was getting slower, and the extraction yield of hemicellulose descended slightly, which is because the over high temperature could also lead to the dehydration of monosaccharides to generate furfural or HMF under acid catalysis condition (Mansilla et al., 1998). For example, about 0.6% and 0.18% of hemicellulose (based on the weight of hemicellulose in raw corn stover) in the sugar fraction was converted to furfural and HMF respectively after FA treatment at 90 °C for 3 h, and the conversion was higher compared to the one treated at 80 °C for 3 h (0.2% and 0.15% for furfural and HMF, respectively). Therefore, 80 °C was suitable reaction temperature.

After FA treatment at 80 °C for 3 h, the solid yield, retention yield of cellulose, extraction yield of hemicellulose and lignin was 48.19%, 89.5%, 79.8% and 65.6%, respectively. Comparable retention

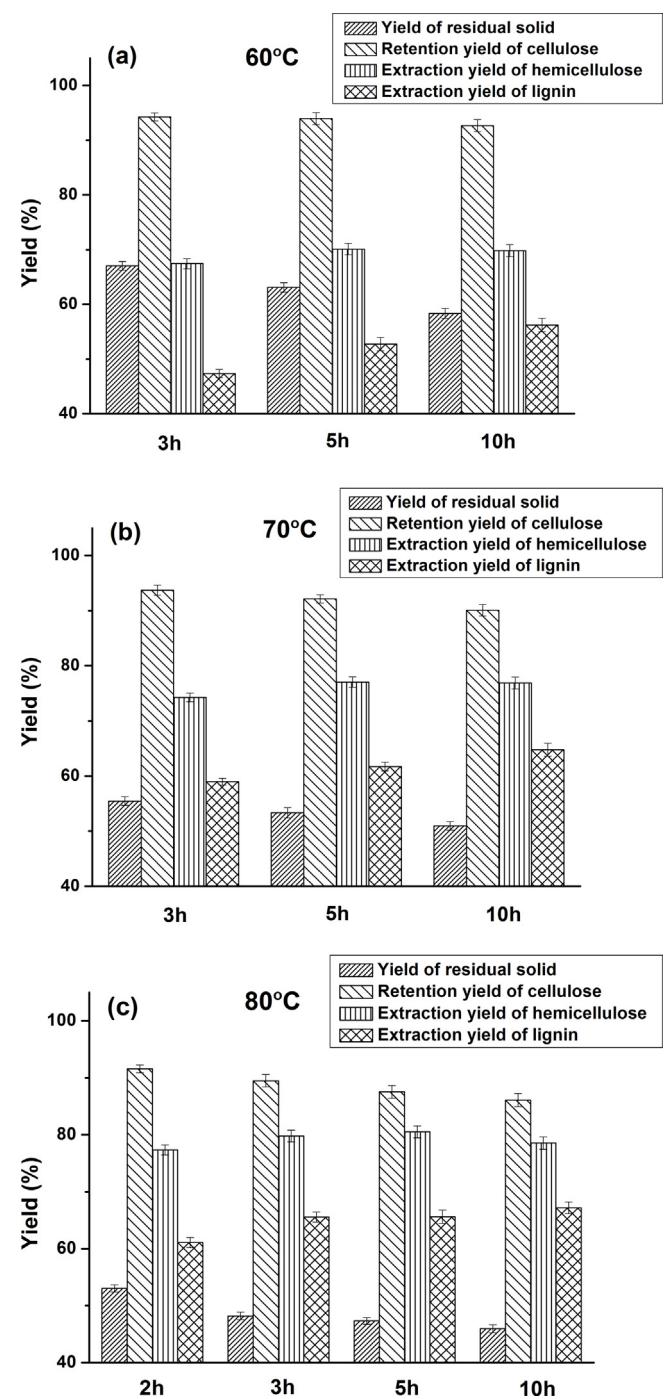


Fig. 2. Effect of reaction time on the FA fractionation of corn stover at the same reaction temperature (All reactions were conducted using 88% formic acid with solid to liquid ratio of 1:13; a: 60 °C; b: 70 °C; c: 80 °C.).

yield of cellulose was also reported by using low concentration of formic acid (about 0.24 wt.%) to treat corn stover at high temperature (195 °C) for 15 min (Xu et al., 2009), but the corresponding extraction yield of hemicellulose (45%) was much lower than our result (79.8%). In addition, we also tried lower concentration of FA (such as 20 wt.%), but the effect of fractionation was not remarkable (the total extraction yield of hemicellulose plus lignin was only about 20% under the same conditions). Hence, high concentration (88 wt.%) of FA was selected for effective fractionation of corn stover.

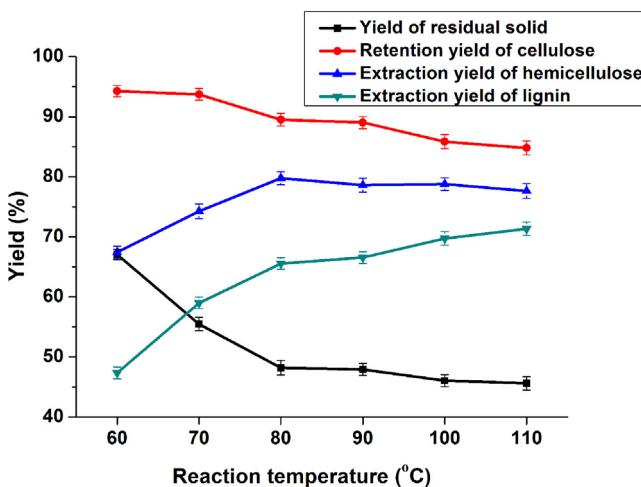


Fig. 3. Effect of reaction temperature on the FA fractionation of corn stover under the same reaction time (All reactions were conducted for 3 h using 88% formic acid with solid to liquid ratio of 1:13).

3.3. Effect of digestion conditions on enzymatic hydrolysis

The enzymatic hydrolysis of residual corn stover after FA treatment was carried out, and the effect of dosage of cellulase and hydrolysis time on the enzymatic hydrolysis rate of glucan and xylan was investigated. It can be seen from Fig. 4, in general, the more dosage of enzymes added, the higher of the enzymatic hydrolysis rate could be obtained. Fig. 4(a) and (b) shows the efficiency of enzymatic hydrolysis for both glucan and xylan before 24 h was much higher than that with longer hydrolysis duration (over 24 h), and the enzymatic hydrolysis rate of glucan and xylan basically kept steady after 48 h hydrolysis. This is mostly because of the feedback inhibition of high concentration of monosaccharides in saccharification (Jing et al., 2009). In addition, Fig. 4 presents that the increase of enzymatic hydrolysis rate of glucan was limited (just 5.14% improvement with 48 h hydrolysis) by increasing cellulase dosage from 20 to 30 FPU/g substrate, and the benefits can be flattened out by the cost of enzyme. Although obvious increase of enzymatic hydrolysis rate of xylan could be achieved by increasing cellulase dosage, the content of xylan in solid residue after FA treatment is very low (below 9%, Table 2). Thus, the suitable cellulase dosage is 20 FPU/g substrate and the appropriate hydrolysis time is 48 h. Under this condition, the enzymatic hydrolysis rates of glucan and xylan were 62.8% and 79.4%, respectively. The relatively low enzymatic hydrolysis rate of glucan is possibly due to the intact structure of cellulose (Agbor et al., 2011).

3.4. Mass balance of the fractionation process by FA treatment

An ideal fractionation is able to maximally and efficiently recover all available main components in feedstock with relatively low cost for different utilization purposes. To investigate the fractionation effectiveness of FA treatment, a mass balance analysis of the fractionation process was performed (Fig. 5). As shown, 35.73 g glucose in solid residue, 18.6 g xylose in liquor fraction, and 14.67 g FA-lignin can be obtained after FA fractionation under the suitable conditions (treated at 80 °C for 3 h, enzymatic hydrolysis with 20 FPU/g substrate for 48 h). By the use of formic acid fractionation, the extraction yields of hemicellulose (79.8%) was equivalent to that by using steam explosion/ethanol extraction method (80%), while the corresponding retention yield of cellulose (89.5%) and extraction yield of lignin (65.6%) were lower than that by using steam explosion/ethanol extraction method (94% and 75%, respectively) (Chen and Liu, 2007). Nevertheless, the extraction yields

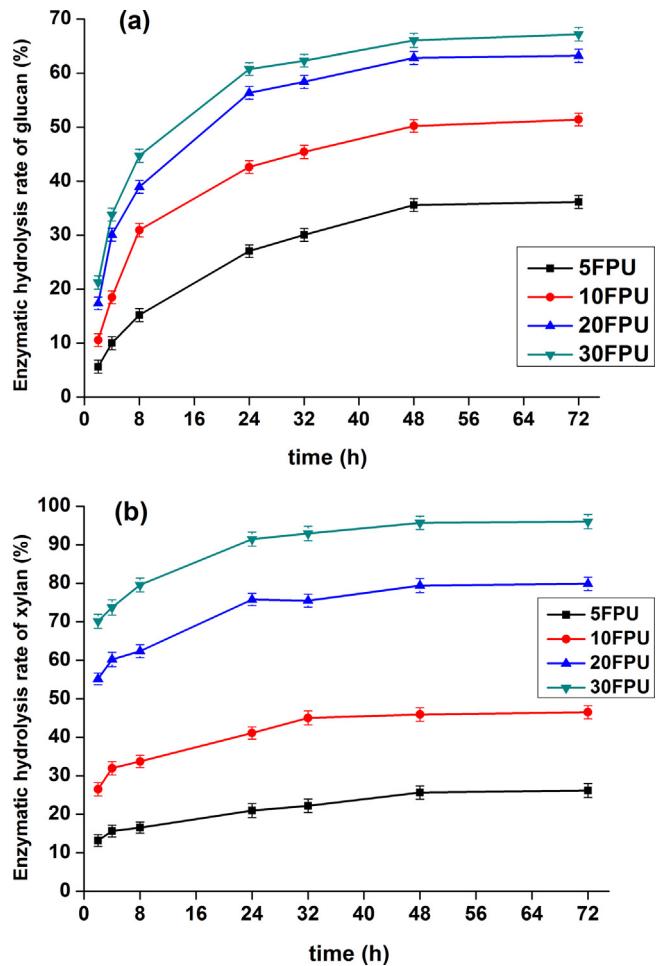


Fig. 4. Effect of digest time and cellulase dosage on enzymatic hydrolysis rate of glucan (a) and xylan (b) (The hydrolysis was conducted with solid consistency of 2% (w/v), β -glucosidase (10 IU/g dry substrate) at 50 °C for 72 h in an air bath incubator shaker at 90 rpm.).

of hemicellulose and lignin were higher than that by using phosphoric acid/acetone method (70.8% and 50% for extraction yields of hemicellulose and lignin, respectively) (Zhang et al., 2007), and the extraction yield of hemicellulose was also much higher than that (about 45%) by using low concentration of formic acid (Xu et al., 2009). The fractionated solid residue can be subjected to enzymatic hydrolysis to produce fermentable sugar for the production of bioethanol or other biochemical, or it can be used to produce paper products (Shen et al., 2010). Fig. 5 also shows that the enzymatic hydrolysis rate of glucose is 62.8%. The color of hemicellulose sugar liquor was yellow due to some acid-dissolved lignin. After purification, the xylose can be used to produce xylitol (Moreau et al., 1998). FA-lignin can be utilized to manufacture value-added products or subjected to the generation of power (Yan et al., 2010; Yu et al., 2013). In addition, in this process $96.2 \pm 0.1\%$ of formic acid could be readily recovered and the concentration of the recovered FA is 84.6 ± 0.1 wt.%. Hence, the whole process has great feasibility for large application of the fully utilization of main components of lignocellulosic biomass.

3.5. Characterization of corn stover before and after FA treatment

The changes in the microstructure of corn stover before and after treatment by formic acid are displayed in Fig. 6. As can be observed, the treated corn stover was destructed, and the porosity increased after FA treatment. This is due to the efficient removal

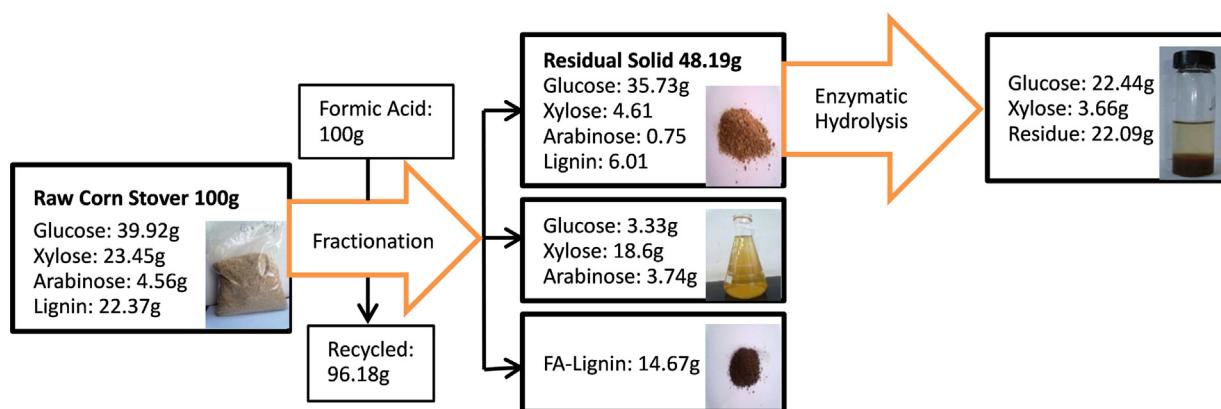


Fig. 5. Mass flow through fractionation and enzymatic hydrolysis based on dried corn stover (Fractionation condition: 88% formic acid, solid to liquid ratio of 1:13, under 80 °C for 3 h; enzymatic hydrolysis condition: cellulase 20 FPU/g substrate, β-glucosidase 10 IU/g dry substrate, 50 °C for 48 h).

of lignin and hemicellulose, and is also in agreement with composition analysis (Table 2). Furthermore, Fig. 6 displays that the long bunch cellulose was able to be seen clearly before and after FA treatment. However, some globular and gel-like material can be observed (Fig. 6(c) and (d)) after treatment, and they are most likely the re-precipitated lignin, or released polysaccharides (Mou et al., 2013). The minor degradation of cellulose and efficient dissolution of lignin and hemicellulose suggest that high concentration of formic acid could play a significant role in removing the hemicellulose and lignin, but just have little impact on the degradation of cellulose under relatively mild conditions.

The crystallinity changes of corn stover after FA treatment were characterized by XRD analysis which allows rapid comparison of cellulose samples (Park et al., 2010), and the XRD profiles of the untreated and treated corn stover are shown in Fig. 7. As can be seen that, after FA treatment, the peak (I_{101} and I_{002}) of crystalline cellulose became more intense and the CrI increased with the elevating

of treatment temperature. This is due to the removal of amorphous hemicellulose and lignin. Similar results are also reported in previous research (Yang et al., 2013; Zhang et al., 2010).

The thermal stability of untreated and treated corn stover as well as FA-lignin is shown in Fig. 8. It is seen from Fig. 8 that, the decomposition temperature (about 160 °C) of untreated corn stover was lower than that (about 220 °C) of treated corn stover. The maximum rates of weight loss for untreated and treated corn stover are about 260–300 °C and 330–350 °C, respectively. This is because there are a large amount of lignin (22.37%) and hemicellulose (24.65%) in untreated corn stover, and the higher thermal stability of treated corn stover is due to the removal of lignin and hemicellulose (Zhang et al., 2010). It is known that pyrolysis of lignin and hemicellulose begins at 160 and 220 °C, respectively; while cellulose is more stable and its decomposition temperature is at about 315 °C (Wanga et al., 2008; Yang et al., 2007). The decomposition temperature of FA-lignin is at about 160 °C as well (Fig. 8).

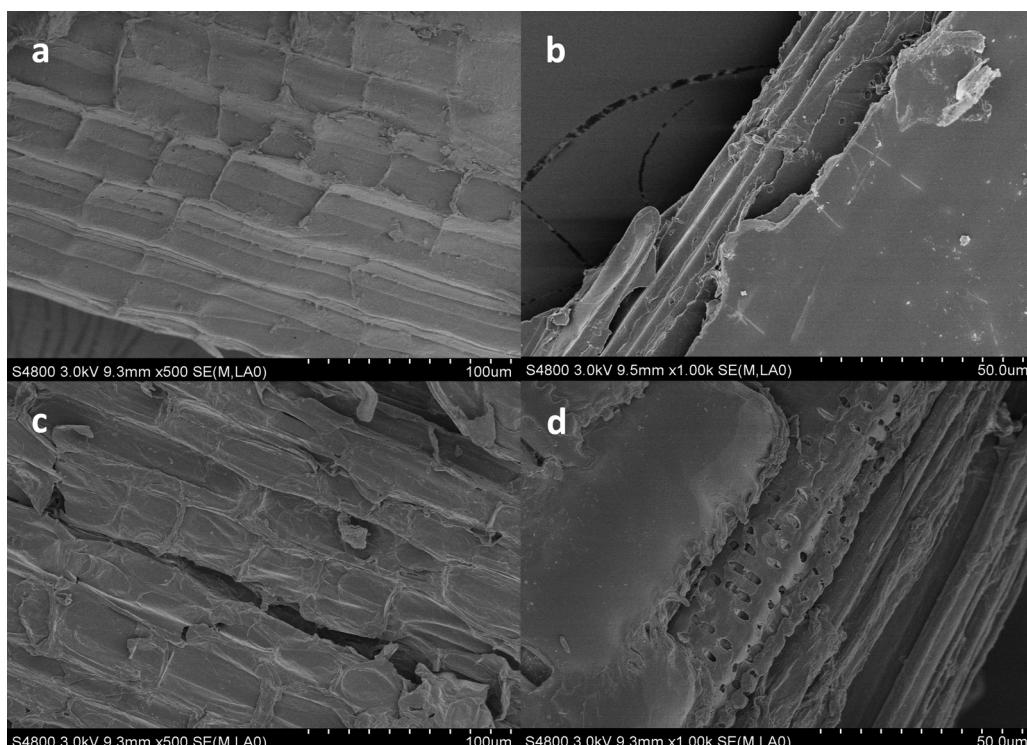


Fig. 6. SEM photomicrograph of untreated corn stover (a and b) and solid residues after treatment by 88% formic acid with 80 °C for 3 h (c and d).

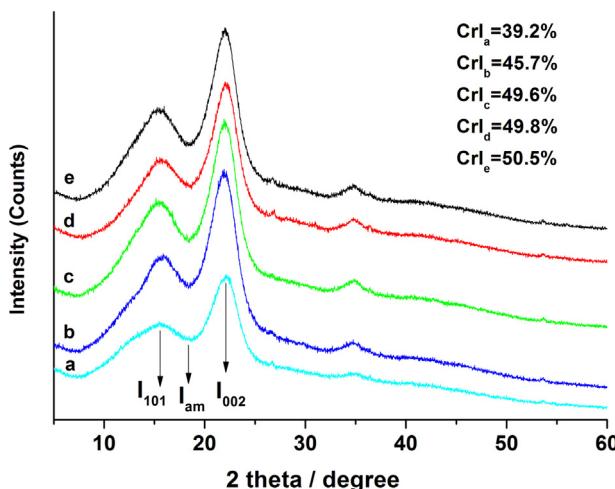


Fig. 7. X-ray diffraction images of untreated corn stover (a) and solid residues after treatment by 88% formic acid for 3 h with 60 °C (b), 70 °C (c), 80 °C (d), 90 °C (e).

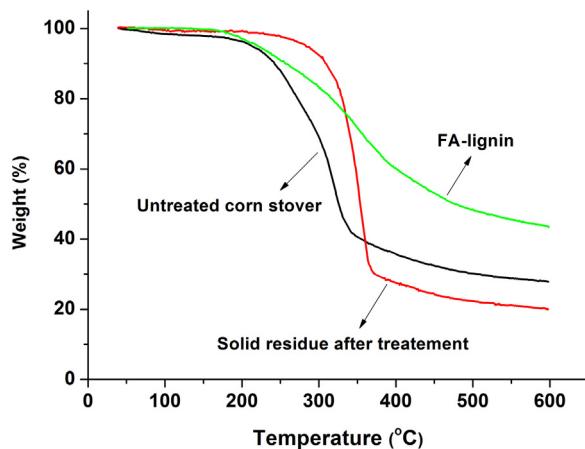


Fig. 8. TG curves of untreated corn stover, solid residue after treatment and FA-lignin (treated by 88% formic acid at 80 °C for 3 h).

Fig. 8 also presents that about 28% (untreated corn stover) and 19% (treated corn stover) of solid residues were remained at 600 °C. This is because the lignin content in treated corn stover was much lower compared to untreated corn stover. In addition, as shown in **Fig. 8**, about 43.5% of mass residue for FA-lignin was remained at 600 °C, which is probably due to the relatively high stability of highly condensed aromatic structures in lignin (Khan and Ashraf, 2007).

The relative molecular weight and distribution of FA-lignin extracted under different conditions were measured as well, and the results are shown in **Table 3**. As can be seen, the M_w (weight-average molecular mass) and M_n (number-average molecular mass) of the FA-lignin decreased obviously with the increase of temperature. It was probably due to the fact that more intensive formation of ester groups under lower temperature when lignin reacted with formic acid made lignin crowded (Zhang et al., 2010). On the other hand, higher temperature would also result in the

Table 3
Molecular weight and distribution of FA-lignin extracted with 88% formic acid.

Temp. (°C)	Time (h)	M_w	M_n	PDI
80	3	14250	9829	1.45
90	3	8128	6352	1.28
100	3	5390	4568	1.18

degradation of lignin (Yu et al., 2013). The polydispersity index (PDI) of FA-lignin reduced as the temperature increased, indicating the molecular weight distribution became more homogeneous. Compared with other lignin products (like lignosulfonate with molecular weight of about 2000–5000), the molecular weight of FA-lignin is much larger which may be more appropriate in some utilization areas (e.g. synthesis of phenol-formaldehyde resin and concrete water-reducer) (Tejado et al., 2007; Yu et al., 2013).

4. Conclusions

The three main components of corn stover were effectively separated by FA method. After treatment with 88 wt.% of FA at 80 °C for 3 h, the retention yield of cellulose, extraction yields of hemicellulose and lignin were 89.5%, 79.8% and 65.6%, respectively. The significant increase of crystalline index and thermal stability of corn stover after FA treatment is attributed to the efficient removal of lignin and hemicellulose. Also, the solid residue after FA treatment can be subjected to enzymatic hydrolysis, the enzymatic hydrolysis rates of glucan and xylan can reach 62.8% and 79.4%, respectively, with the addition of cellulase (20 FPU/g dry substrate) and β-glucosidase (10 IU/g dry substrate). In addition, about 96.2% of FA used can be recovered in the process developed. Therefore, the fractionation of corn stover by high concentration of FA can be considered as a promising and green process.

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