

Effects of biomass particle size on steam explosion pretreatment performance for improving the enzyme digestibility of corn stover

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ABSTRACT

Size reduction is an essential process for corn stover biomass utilization. Effects of biomass particle size on the efficiency of pretreatment and enzymatic hydrolysis are systematically investigated in the present study. Sugar recoveries and conversions of the biomass with particle sizes at 2.5, 2.0, 1.5, 1.0 and 0.5 cm were compared. The highest sugar recovery reached 99.6% for glucan and 67.0% for xylan at the particle size of 1.0 and 0.5 cm, respectively, but the highest sugar conversion (100% for glucan and 83% for xylan) was observed at the particle size of 2.5 cm. The enzymatic hydrolysis rate and conversion of pretreated biomass obviously increased with increasing biomass particle size. With increase of biomass particle size, the specific surface area of pretreated biomass significantly increased and the crystallinity index of pretreated biomass apparently decreased. In studied extent of particle sizes, the utilization of larger biomass particles would be desirable to achieve the high pretreatment efficiency and hence improve subsequent enzymatic hydrolysis performance compared with the smaller ones.

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1. Introduction

Energy security and environmental concerns have increased the interests in alternative, nonpetroleum-based sources of energy. Lignocellulose-based renewable energy, such as cellulosic ethanol, will potentially play an important role in mitigating the dependence on fossil oil and reducing CO₂ emission (Alvira et al., 2010; Zhong et al., 2010). However, the inefficient feedstock conversion process represents hurdles for large-scale deployment of biomass-to-biofuel technologies. Corn stover (CS) is considered as potential feedstock for bioethanol production due to its high glucan and xylan contents and abundant amount in China (about 300 million tons per year) (Zhong et al., 2010). Owing to structural recalcitrance of lignocellulosic biomass, pretreatment is a crucial step for breaking down the lignin-carbohydrate-complex (LCC) structure and disrupting the crystalline structure of cellulose, which enhance cellulose accessibility to enzymes in saccharification (Alvira et al., 2010; Mosier et al., 2005; Wyman et al., 2005). Steam explosion is one of the most widely employed physicochemical pretreatments.

After auto-hydrolysis and explosive depressurization stages, particle size distribution, chemical composition and micro-structure of the biomass feedstock are altered (Mosier et al., 2005). In addition, steam explosion pretreatment not only provides high sugar yield and little amount of byproducts, but also offers low capital investment with nearly no hazardous process chemicals and conditions applied (Avellar and Glasser, 1998).

Size reduction of biomass before pretreatment is an energy-intensive and expensive process, but it is necessary for cellulose bioconversion (Zhu et al., 2009). Biomass particle size obviously impacts the design of handling, transportation and conversion facilities (Obernberger and Thek, 2004). Suitable biomass particle size will significantly improve the efficiency of pretreatment due to the high efficient mass and heat transfer. During steam explosion pretreatment, heat transfer issue may result in overcooking the surface part of the larger biomass particles and incomplete pretreatment of the interior part (Ladisich, 1989; Brownell et al., 1986). For smaller biomass particles, hemicellulose may be prone to degrade into byproducts due to the intense degree of heat. Therefore, optimizing biomass particle size is crucial in terms of achieving high sugar conversion and low production cost (Zhu et al., 2009; Ballesteros et al., 2000). However, to the best of our knowledge, there is no systematic research on evaluating the effects of corn

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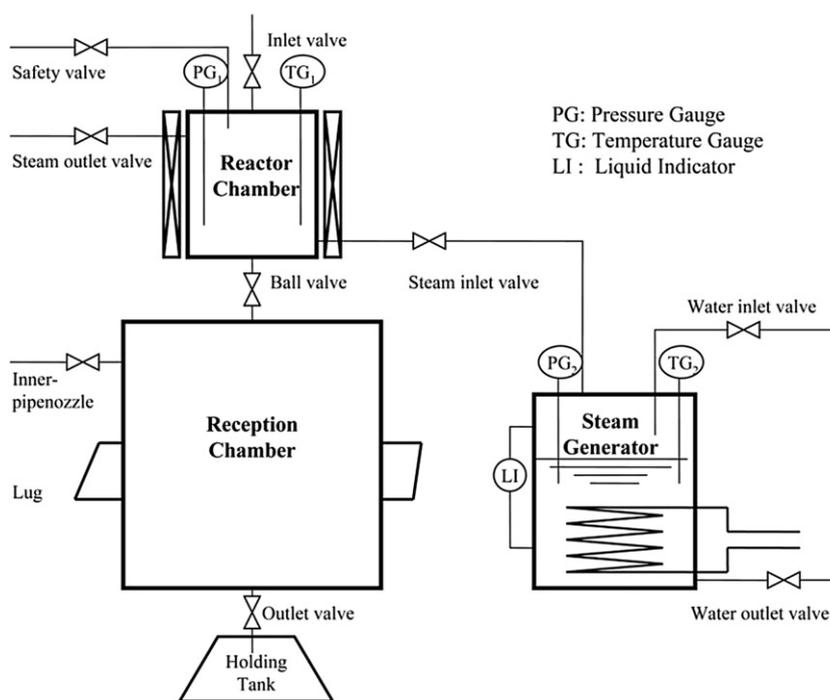


Fig. 1. Schematic of the steam explosion pretreatment reactor system.

stover biomass particle size on pretreatment performance. In addition, in-depth understanding the effects of particle size on biomass micro-structure changes after pretreatment and their correlations with enzymatic hydrolysis performance is necessary for understanding pretreatment mechanisms.

The present study aims to identify the effect of biomass particle sizes on pretreatment and its correlation with subsequent enzymatic hydrolysis performance. The effects of biomass particle size on the sugar recoveries during pretreatment and the sugar conversions in enzymatic hydrolysis were investigated. The mass balance around the whole saccharification process was also performed. Meanwhile, the features (including crystallinity, porosity and micro-structure) of corn stover biomass with varied particle sizes were characterized. Biomass physicochemical features combined with sugar conversions and yields showed insights into the mechanisms of the effects of biomass particle size on lignocellulose bioconversion.

2. Materials and methods

2.1. Feedstock preparation and analysis

Corn stover used in the present study was harvested from the suburb of Tianjin, China. The feedstock was air-dried to the moisture content of 5–10%. For composition analysis, the feedstock was milled by knife mill (YS-08, BYZME, China), passed through a screen of 2 mm and then stored in sealed bags at 4 °C. The composition analysis was carried out according to the laboratory analysis protocol (LAP) of National Renewable Energy Laboratory (NREL), Colorado, USA (NREL, 2004). Moisture content was analyzed using a Sartorius MA 35 moisture analyzer (Sartorius, Germany). Prior to pretreatment, the feedstock was cut manually into an average size of 2.5 cm (2.5 cm L × 1.0 cm W × 1.0 cm H), 2.0 cm (2.0 cm L × 1.0 cm W × 1.0 cm H), 1.5 cm (1.5 cm L × 0.5 cm W × 0.5 cm H) and milled into an average size of 1.0 cm (1.0 cm L × 0.2 cm W × 0.2 cm H), 0.5 cm (0.5 cm L × 0.1 cm W × 0.1 cm H), respectively. Samples were sprayed with deionized water to the moisture content of 30% and

then stored at room temperature until steam explosion pretreatment.

2.2. Steam explosion pretreatment (SEP)

Steam explosion pretreatment was adopted to break down the structure of the lignocellulosic matrix to facilitate the bioconversion. The pretreated reactor system consists of a reactor chamber (15 L working volume), a reception chamber (150 L working volume) and a steam generator (Fig. 1). During pretreatment, 150 g corn stover (dry basis) was top-loaded into the reactor chamber. High-pressure steam supplied by the steam generator was then filled into the reactor until the temperature reached 200 °C (1.6 MPa). After 5 min of exposure to the saturated steam, corn stover was exploded into the reception chamber by the ball-valve. The pretreatment condition (200 °C, 5 min) was optimized in our previous studies (Pang et al., 2013). After pretreatment, the pretreated biomass was separated from the liquid fraction by vacuum filtration using a Buchner funnel and then washed with 3.0 L water. Monomeric, oligomeric sugars and byproducts were determined for the liquid fractions to calculate the overall sugar recoveries.

2.3. Enzymatic hydrolysis

The NREL standard protocol (LAP-009) was followed for enzymatic hydrolysis. Commercial cellulase (Accellerase 1500, Genencor) was a generous gift from Genecor (Suzhou, China). Novozyme 188 was purchased from Sigma-Aldrich (St. Louis, MO). The cellulase activity of Accellerase 1500 is 77 FPU/mL, and the β -glucosidase activity of Novozymes188 is 250 pNPGU/mL. The pretreated biomass was hydrolyzed at a glucan loading of 1% or 6% (w/v) in a 0.05 M citrate buffer solution (pH 4.8) with an Accellerase 1500 loading of 15 or 60 FPU/g glucan and a β -glucosidase loading of 64 pNPGU/g glucan. Samples were hydrolyzed at 50 °C and 200 rpm for 168 h and then the slurry were centrifuged at 12,000 rpm for 5 min to separate solids from liquids. The solid residues were washed with a volume of water equal to 50 times the dry weight of the pretreated biomass. The liquid fractions were

collected for sugar analysis. Enzymatic kinetics experiments at 1% glucan loading were conducted. Glucan and xylan conversions were calculated based on glucan and xylan in the initial pretreated biomass of enzymatic hydrolysis. All these experiments were conducted with two replicates.

2.4. Analytical methods

The byproducts, monosaccharides and oligosaccharides included glucose oligomers (GO), xylose oligomers (XO), arabinose oligomers (AO) in liquid fraction were measured according to the NREL standard protocol (“Determination of Sugars, Byproducts, and Degradation Products in Liquid Fraction Process Samples”).

The samples were analyzed by HPLC consisted of a Waters (Milford, MA) 1515 pump, a Waters 2414 refractive index detector and an Aminex HPX-87H carbohydrate analysis column (Bio-rad, Hercules, CA) equipped with a Cation H cartridge (Bio-rad) at 65 °C with 5 mM H₂SO₄ as the mobile phase at a flow rate of 0.6 mL/min.

2.5. Characteristics analysis of corn stover biomass

The specific surface area (SSA) was determined by N₂ adsorption/desorption isotherms applying the Brunauer–Emmett–Teller (BET) method at 77 K on an Automatic Intelligent 3H-2000A Volumetric System (Beishide Instrument-S&T Co., Ltd., China). The biomass crystallinity of the samples was measured using a D8 Fucus X-ray Diffractometer (Bruker AXS Co., Germany). The crystallinity index (CrI) was calculated according to the method of Segal et al. (1959). The FT-IR spectra were obtained using a Nicolet IZ10 Fourier transform infrared spectroscopy and KBr disc containing about 1% finely ground samples. Micrographs were carried out on a field emission SEM (S4800, Hitachi, Co., Japan) after the samples were sputtered with a thick layer of gold. Bulk density measurement was performed according to a slightly modified method from Mani et al. (2006). Mass per unit volume gave the bulk density of the biomass in g/L.

2.6. Mass balance calculations

Mass balances were determined around pretreatment and enzymatic hydrolysis. Glucan and xylan recovery in pretreatment and glucose and xylose yield of the whole process (pretreatment + enzymatic hydrolysis) were calculated as follows:

Glucan recovery(%)

$$= \frac{\text{Glucan}_{\text{Pretreated solid}} \times (180/162) + (\text{Glucose} + \text{GO})_{\text{Liquid fraction}}}{\text{Glucan}_{\text{Feedstock}} \times (180/162) + \text{Glucose}_{\text{Feedstock}}} \quad (1)$$

Xylan recovery(%)

$$= \frac{\text{Xylan}_{\text{Pretreated solid}} \times (150/132) + (\text{Xylose} + \text{XO})_{\text{Liquid fraction}}}{\text{Xylan}_{\text{Feedstock}} \times (150/132) + \text{Xylose}_{\text{Feedstock}}} \quad (2)$$

$$\text{Glucose yield}(\%) = \frac{(\text{Glucose} + \text{GO})_{\text{Liquid fraction}} + \text{Glucose}_{\text{Hydrolysate}}}{\text{Glucan}_{\text{Feedstock}} \times (180/162) + \text{Glucose}_{\text{Feedstock}}} \quad (3)$$

$$\text{Xylan yield}(\%) = \frac{(\text{Xylose} + \text{XO})_{\text{Liquid fraction}} + \text{Xylose}_{\text{Hydrolysate}}}{\text{Xylan}_{\text{Feedstock}} \times (150/132) + \text{Xylose}_{\text{Feedstock}}} \quad (4)$$

In which GO stands for glucose oligomers, XO stands for xylose oligomers. (180/162) is the correction coefficient between molecular weights of glucan and glucose, and (150/132) is the correction coefficient between molecular weights of xylan and xylose.

Table 1
Corn stover composition.

Component	Dry matter (%)	SD (%)
Glucan	31.7	1.1
Xylan	17.1	0.7
Arabinan	2.60	0.2
Acetyl	2.90	0.1
Acid-insoluble lignin (ALL)	12.6	0.6
Acid-soluble lignin (ASL)	0.80	0.0
Water extractives	19.3	1.0
Free glucose	5.80	0.1
Free xylose	1.70	0.1
Ethanol extractives	4.20	0.2
Ash	4.30	0.0
Total	95.5	–

All data in the table are mean values of duplicate experiments; SD means standard deviations.

Solid recovery was calculated on dry solid basis as the percentage of recovered pretreated biomass of the feedstock weight. Lignin composition was difficult to determine for the liquid fraction, therefore lignin recovery was calculated based on the lignin in the solid fraction.

3. Results and discussion

3.1. Composition analysis of the feedstock

The results of composition analysis (Table 1) showed that glucan, xylan and lignin content of corn stover used in this study were 31.7%, 17.1%, 12.6%, respectively, which were obviously lower than the literature reports (33.6% glucan, 25.3% xylan and 16.7% lignin) (Jin et al., 2012a,b; Kaparaju and Felby, 2010). This result may reflect a difference in corn stover itself. However, it is noticeable that the content of free glucose and xylose which was apt to degrade to byproduct during pretreatment was 5.80% and 1.70% on dry solid basis, respectively. The value of total extractives which were easy to dissolve into the liquid fraction during pretreatment was approximate 24%. The acetyl content (2.9%) was higher compared with the literature reports (2.2%), while ash content (4.3%) was lower than that of the previous analysis (7.8%) (Tamaki and Mazza, 2010; Yang and Wyman, 2004). As previous study, the efficacy of auto-hydrolysis is positive correlated with the amount of acids and salts generated from the plant cell (Öhgren et al., 2007). Higher acetyl content will result in a higher acetic acid concentration during pretreatment, which helps auto-hydrolysis.

3.2. Compositions of the pretreated biomass and liquid fraction

The compositions of the pretreated biomass and liquid fraction with different biomass particle sizes are listed in Tables 2A and 2B. The mass recovery of pretreated biomass and their compositions were obviously different depending on biomass particle size. The mass recovery of glucan increased from 29.05 to 34.45 g/100 g feedstock and xylan from 3.28 to 4.76 g/100 g feedstock, respectively, with biomass particle size decreased from 2.5 to 1.0 cm, but then slightly decreased for both glucan and xylan at particle size of 0.5 cm. Most of the arabinan was solubilized in liquid under all tested conditions. The lignin composition, another important component in biorefinery process, increased with increasing biomass particle size.

In liquid fractions (Table 2B), the concentration of soluble glucose (monomer plus oligomers) was found increasing with reduced biomass particle size except for the particle size of 2.5 cm which gave the highest value of this concentration. Both xylose and furfural amounts decreased with decreasing biomass particle size, but

Table 2A
Compositions of pretreated biomass and physical properties of feedstock and pretreated biomass.

Particle size (cm)	Composition of pretreated biomass (g/100 g feedstock)				Physical properties of feedstock and pretreated biomass					
	Glucan (g)	Xylan (g)	Arabinan (g)	Lignin (AIL) (g)	SSA (m ² /g)		CrI (%)		Bulk density (g/L)	
					FS	PB	FS	PB	FS	PB
2.5	29.05(0.46)	3.28(0.37)	0.32(0.02)	14.52(0.26)	2.35(0.06)		45.9(1.8)		39.1(2.5)	46.8(1.2)
2.0	31.78(0.85)	4.77(0.17)	0.39(0.02)	13.39(0.27)	2.31(0.09)		46.2(1.1)		46.9(2.9)	52.0(1.2)
1.5	34.41(0.90)	4.63(0.15)	0.53(0.03)	13.41(0.52)	1.00(0.05)	2.14(0.07)	51.2(0.7)		62.4(1.2)	68.2(1.5)
1.0	34.45(0.47)	4.76(0.08)	0.42(0.02)	13.23(0.18)	2.11(0.03)		47.5(1.9)		102.2(1.6)	62.3(1.7)
0.5	31.50(0.23)	4.59(0.16)	0.43(0.02)	12.89(0.29)	2.07(0.01)		48.8(1.2)		126.0(1.8)	82.5(1.3)

Pretreatment conditions: 200 °C, 5 min; standard deviations are shown in parentheses. SSA stands for specific surface area; CrI stands for crystallinity index; FS stands for feedstock; PB stands for pretreated biomass.

Table 2B
Compositions of liquid fraction during steam explosion pretreatment.

Particle size (cm)	Compositions of liquid fractions (g/100 g feedstock)									
	Glucose (g)	GO (g)	Xylose (g)	XO (g)	Arabinose (g)	AO (g)	Formic acid (g)	Acetic acid (g)	HMF (g)	Furfural (g)
2.5	2.09(0.03)	0.81(0.03)	5.01(0.02)	1.95(0.04)	1.37(0.01)	n.d.	2.97(0.17)	3.00(0.15)	0.78(0.02)	1.12(0.13)
2.0	1.32(0.03)	0.92(0.03)	4.13(0.01)	3.86(0.02)	1.20(0.03)	0.32(0.05)	2.82(0.05)	2.92(0.10)	0.33(0.02)	1.06(0.07)
1.5	1.28(0.05)	1.00(0.01)	4.15(0.09)	3.67(0.09)	1.17(0.16)	n.d.	2.87(0.02)	3.01(0.02)	0.31(0.02)	1.05(0.15)
1.0	1.55(0.02)	0.93(0.20)	4.42(0.02)	3.33(0.04)	1.30(0.02)	n.d.	2.86(0.09)	2.97(0.20)	0.41(0.02)	0.99(0.26)
0.5	1.89(0.01)	0.92(0.03)	3.57(0.02)	4.76(0.03)	0.69(0.02)	0.48(0.03)	2.93(0.05)	2.84(0.04)	0.46(0.01)	0.82(0.04)

GO stands for glucose oligomers; XO stands for xylose oligomers; AO stands for arabinose oligomers. Pretreatment conditions: 200 °C, 5 min; standard deviations are shown in parentheses; n.d. means not determined.

xylose oligomer amount increased with decreasing biomass particle size. It was interesting to note that the conditions generating high amount of glucose and xylose in the liquid fraction typically also generated high amount of HMF and furfural, respectively. No significant differences of formic acid and acetic acid amount were observed among the feedstocks with different biomass particle sizes. As a result, the feedstock with particle size of 2.5 cm lead to higher cellulose and hemicellulose solubilization and degradation compared with the smaller particles at the same pretreatment condition.

Table 3A shows the component recoveries during pretreatment as a function of the biomass particle size. The recoveries of glucan and solid biomass increased with particle size reduced from 2.5 to 1.0 cm, but slightly decreased at 0.5 cm. The xylan and arabinan recoveries only varied from 51.1% to 67.0% and 65.0% to 60.4% with decreasing biomass particle size, respectively. These relatively low recoveries of xylan and arabinan might be explained by the hemicellulose solubilization and degradation to byproducts (e.g. formic acid and furfural) during pretreatment (Varge et al., 2004). The literature results showed the opposite trend of above component recoveries working with softwood and herbaceous agricultural wastes (Ballesteros et al., 2002, 2000). This difference might be due to not only the feedstock type but also heat transfer patterns in the biomass during pretreatment. In addition, the sieved operation during the preparing material process in previous research resulted in the different components of the feedstock at different biomass particle sizes, which may significantly influence the pretreatment process. Solubilization or degradation

of lignin during pretreatment was not found. On the contrary, lignin recovery increased from 102.2% to 115.1% with increasing biomass particle size. This phenomenon had been attributed to the formation of lignin-like compounds (“pseudo-lignin”) from recondensation reactions especially between carbohydrates or carbohydrates degradation products and other components from the water extractives, which also explained the low xylan recovery (Sannigrahi et al., 2011; Li et al., 2007). These above results infer that the corn stover with larger biomass particle size is subjected to more sufficient pretreatment reaction than smaller biomass particle size in the scope from 2.5 to 0.5 cm, due to higher pretreatment severity leading to degradation products of smaller molecules in the glucan and xylan degradation pathway (Qin et al., 2012).

3.3. Enzymatic hydrolysis

The corn stover with different biomass particle sizes was steam explosion pretreated and then hydrolyzed using commercial enzymes at two glucan loadings (1 and 6%) and two enzyme loadings (15 and 60 FPU/g glucan) (Fig. 2) (in this study, the biomass particle size refers to the ones before pretreatment). The conversions of glucan and xylan, which reflect the digestibility of the pretreated biomass, obviously increased with increasing biomass particle size at almost all tested conditions (different glucan loadings and different enzyme loadings). Enzymatic hydrolysis at 1% glucan loading with the enzyme loading of 60 FPU/g glucan achieved a glucan conversion and a xylan conversion as high

Table 3A
Component recoveries during steam explosion pretreatment at different biomass particle sizes.

Particle size (cm)	Component recoveries during pretreatment (%) ^a				
	Glucan recovery	Xylan recovery	Arabinan recovery	Lignin recovery	Solid recovery
2.5	86.0 (1.3)	51.7 (0.9)	65.0 (2.1)	115.1 (2.2)	49.0 (2.5)
2.0	91.8 (1.6)	65.8 (1.3)	69.0 (1.8)	106.2 (2.1)	54.8 (2.1)
1.5	99.1 (1.5)	64.1 (1.2)	65.7 (2.0)	106.4 (1.6)	54.2 (1.9)
1.0	99.6 (1.2)	64.3 (1.6)	66.0 (1.3)	104.9 (1.9)	55.3 (2.3)
0.5	92.4 (1.8)	67.0 (1.2)	60.4 (2.3)	102.2 (1.5)	53.7 (2.8)

^a Pretreatment conditions: 200 °C, 5 min; standard deviations are shown in parentheses.

as 99.0% and 82.8% at the particle size of 2.5 cm. However, these two values for the particle size of 0.5 cm were as low as 73.5% and 37.1%. Considering the economics, high sugar concentration is required for industrial process (Jin et al., 2012a,b). The maximum glucose and xylose concentrations reached to 59.7 and 6.2 g/L, respectively, at particle size of 2.5 cm by enzymatic hydrolysis at 6% glucan loading with an enzyme loading of 60 FPU/g glucan. Thus, corn stover with larger biomass particle size achieved the better enzymatic hydrolysis performance, which reflected the higher pretreatment efficiency. From the biorefinery and economy point of view, the larger biomass particles producing high sugar conversions and reducing the electric power consumption during biomass size reduction process can be considered as the best ones for biomass conversion process.

The efficiency of enzyme–substrate interactions was strongly influenced by specific surface area (SSA). The data in Table 2A showed that SSA of pretreated biomass was substantially increased compared to the untreated one. Meanwhile, the SSA of pretreated biomass increased with increasing biomass particle size, which matches the trend of glucan and xylan conversions during enzymatic hydrolysis. The previous studies also reported that the rice straw after dilute-acid and steam explosion pretreatment achieved the high SSA of biomass, which resulted in the high glucan and xylan conversion (Chen et al., 2011). In addition, biomass crystallinity was also believed as an important factor affecting enzymatic saccharification of glucan and xylan (Zhang and Lynd, 2004). Steam explosion pretreatment apparently decreased the biomass crystallinity index and hence improved biomass digestibility (Table 2A).

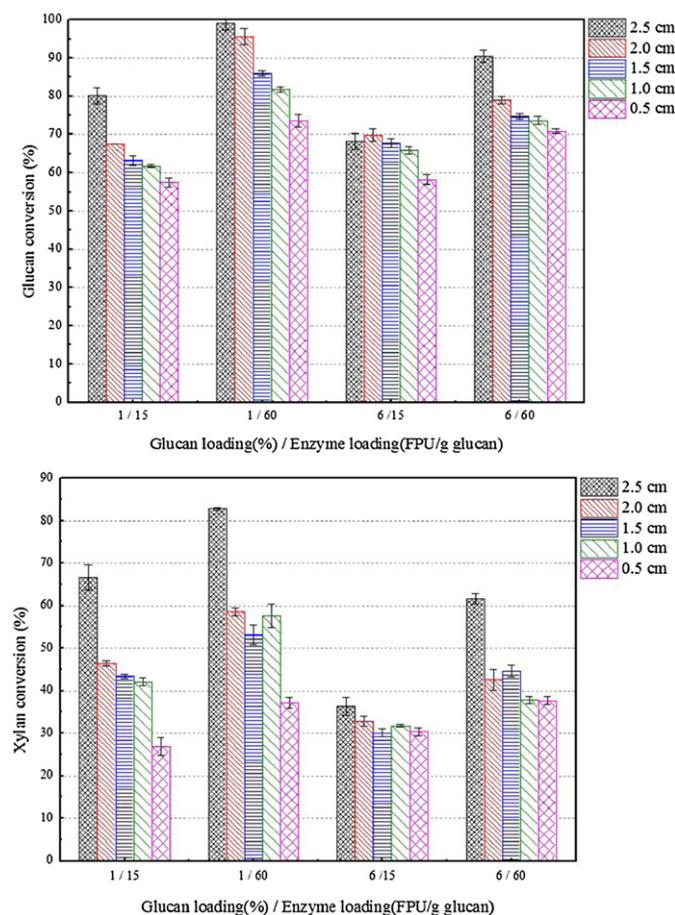


Fig. 2. Glucan and xylan conversions of enzymatic hydrolysis on the pretreated biomass at different particle sizes. Enzymatic hydrolysis conditions: glucan loading: 1% or 6%; enzyme loading: 15 or 60 FPU/g glucan, 50 °C and 200 rpm.

The crystallinity index of the pretreated biomass decreased with increasing biomass particle size, which also explains why larger biomass particle size had a higher sugar conversion. Lignin and lignin-like compounds had been considered as another major impeding factor in enzymatic hydrolysis due to mostly non-productive binding to cellulases (Rollin et al., 2011; Chang and Holtzapfle, 2000). However, despite a larger lignin fraction in the pretreated biomass at larger particle sizes, it did not affect the biomass digestibility much. Therefore, larger biomass particles after pretreatment showed the higher SSA and the lower biomass crystallinity, resulting in the high digestibility efficiency of pretreated biomass compared with the smaller ones.

Fig. 3 shows the time course of glucan conversion for the pretreated solid biomass as a function of particle size. Glucan conversion followed the typical batch hydrolysis pattern, with a rapid rate at the beginning of enzymatic hydrolysis, and then the rate slowed down. It was hypothesized that the initial enzymatic hydrolysis rate was a function of enzyme accessible surface area, while the slowdown of hydrolysis in the later stages was due to the difficulty of hydrolyzing highly crystalline part of cellulose (Fan et al., 1980). The initial enzymatic hydrolysis rate increased with increasing biomass particle size at both enzyme loadings. The previous studies showed the opposite trend, which may be due to the different pretreatment, feedstock preparation and style (Zeng et al., 2007; Ballesteros et al., 2002). The maximum initial rates found at particle size of 2.5 cm were 0.89 g and 1.94 g glucose/(Lh) at the

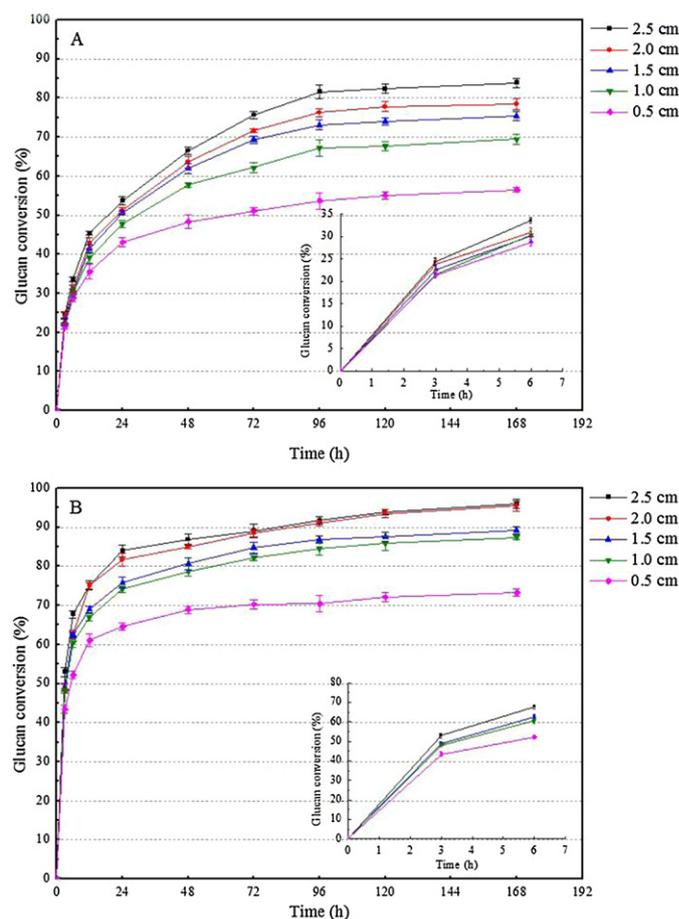


Fig. 3. Kinetic profiles of enzymatic hydrolysis on the pretreated biomass at different particle sizes. The initial enzymatic hydrolysis rate is calculated based on the hydrolysis that occurs in the first 3 h. Enzymatic hydrolysis conditions: 1% glucan loading with enzyme loading of 15 FPU/g (A) and 60 FPU/g (B) glucan at 50 °C and 200 rpm.

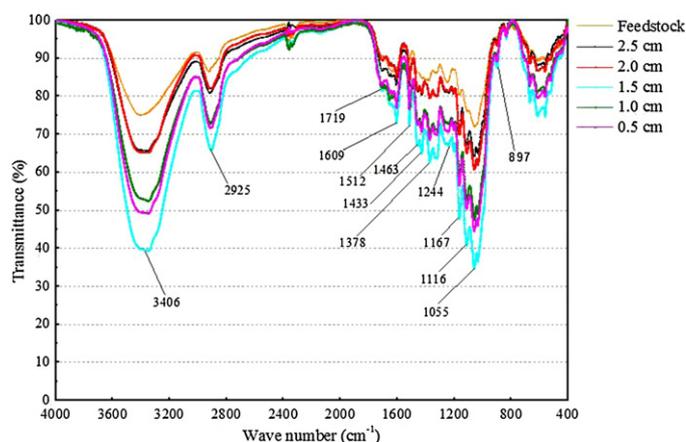


Fig. 4. FT-IR spectrums of the untreated and pretreated biomass at different particle sizes.

enzyme loading of 15 FPU and 60 FPU/g glucan, respectively. The difference of hydrolysis rate between enzyme loadings of 60 FPU and 15 FPU/g glucan for all biomass particle sizes was large at the initial stage of the hydrolysis (0–3 h) (the rate for 60 FPU/g glucan is two times higher) and such difference decreased as the hydrolysis proceeded. Glucan conversions for particle sizes >2 cm reached >85% and >63% at the enzyme loading of 60 FPU/g and 15 FPU/g glucan at 48 h, respectively. It implied that the high enzyme loading resulted in the high initial enzymatic hydrolysis rate and high glucan conversion. The enzymatic kinetics also showed that the utilization of the larger biomass particles during pretreatment would be suitable for improving the enzymatic hydrolysis performance due to the time saved by high enzymatic hydrolysis rate.

3.4. FT-IR analysis of the untreated and pretreated biomass

Fig. 4 shows the FT-IR spectrums of the untreated and pretreated biomass. In general, the smaller biomass particles (0.5, 1.0 and 1.5 cm) after pretreatment obviously changed the intensities of peaks at 3406 and 2925 cm^{-1} which attributed to O–H and C–H stretching, corresponding to the aliphatic moieties in lignin and polysaccharides, respectively. These modifications may indicate that hydrogen bonds, methyl and methylene portions of cellulose were altered and might help the change of biomass crystallinity (He et al., 2008). The larger biomass particles (2.5 and 2.0 cm) after pretreatment had higher impact on the intensity changes of 1719 cm^{-1} peak attributed to hemicellulose acetyl and uronic ester groups or linkages in lignin or ester hemicellulose ferulic and *p*-coumaric acid carboxylic groups, which means the important ester linkages were dissociated (Sun et al., 2005). Interestingly, the smaller biomass particles (0.5, 1.0 and 1.5 cm) after pretreatment intensified the peak at 1609 cm^{-1} represented aromatic skeletal vibration and the peaks at 1512 and 1433 cm^{-1} attributed to the aromatic C=C stretch from aromatic ring of lignin. These indicate that large amounts of residual lignin still bond to carbohydrates, which corresponds with the residual lignin content of pretreated biomass. Pretreated biomass at particle size of 2.5 and 2.0 cm reduced the intensity of the 1244 cm^{-1} band attributed to the C–O stretching band (guaiacyl units) (Windeisen et al., 2007). The information indicates that the relative content of guaiacyl lignin units in larger biomass particles have significant changes compared with smaller ones. In addition, pretreated biomass obviously changed the intensities of peaks at 1167 and 1116 cm^{-1} arise from C–O anti-symmetric bridge stretching and C–OH skeletal vibration and at 1055 cm^{-1} attributed to the C–O–C stretching typical of glucan and xylan, respectively. These modifications may correspond to the

degradation of hemicelluloses and help the increase of SSA. As a result, the high pretreatment efficiency at large biomass particle size was achieved due to the serious disrupted important ester linkages between carbohydrates and lignin and the increased cellulose content and decreased hemicellulose content compared with untreated biomass and smaller ones.

3.5. Scanning electron microscopy (SEM)

Micro-structure of untreated biomass, pretreated biomass and solid residues after enzymatic hydrolysis at different biomass particle sizes is shown in Fig. 5. The untreated biomass exhibited highly rigid and ordered fibers and the surfaces of untreated biomass were more-or-less smooth (Fig. 5a1–a3). A scanning electron micrograph of stem cross-sections of untreated biomass showed relatively high thickness of the plant cell wall (Fig. 5a4). After pretreatment, the orderly fibers of corn stover at particle size of 1.5, 1.0 and 0.5 cm were slightly disrupted (Fig. 5e1 and f1), but the fibers of pretreated biomass at particle size of 2.5 and 2.0 cm obviously separated from the initially connected structure and inner parts of the cell were fully exposed, which means the external surface area and the porosity of biomass were increased (Fig. 5b1, c1, and d1). At a higher magnification, length-wise grooves and small holes could be seen clearly in photos, especially for larger biomass particles after pretreatment (Fig. 5b3 and c3). It indicated that steam explosion pretreatment apparently disrupt the micro-structure and increased the surface area of pretreated biomass. In addition, many droplets can be found on nearly any surface of larger biomass particles after pretreatment compared with the untreated biomass and smaller ones (Fig. 5a2–f2 and a3–f3). The previous study demonstrated that the droplets composed of lignin and lignin–carbohydrate complex migrated from the cell walls and re-deposited on the surface of pretreated solid biomass (Donohoe et al., 2008). These above results were in accordance with SSA and CrI analysis of pretreated biomass. After 168 h hydrolysis with >95% glucan conversions at particle size of 2.5 and 2.0 cm (Fig. 5b4 and c4), the plant cell walls were found collapsed and obviously thinner compared with untreated biomass and other particle sizes (Fig. 5a4, e4, and f4). Consequently, steam explosion pretreatment obvious caused profound lignin re-localization and serious degraded the fibrillar structure of larger biomass particles compared with untreated biomass and smaller ones, which were associated with the decreased CrI and the increased SSA.

3.6. Glucose and xylose yields and overall mass balance

Many techno-economic analyses of lignocellulose pretreatment had emphasized the importance of glucose and xylose yields on the optimization of the process and ensuring maximum biomass utilization. Glucose and xylose yields from pretreatment and enzymatic hydrolysis are given in Table 3B. The glucose yields at 1% glucan loading with an enzyme loading of 15 FPU/g glucan and at 6% glucan loading with an enzyme loading of 60 FPU/g glucan increased with increasing biomass particle size. However, the maximum glucose yield at 1% glucan loading with an enzyme loading 60 FPU/g glucan and at 6% glucan loading with an enzyme loading 15 FPU/g glucan was obtained at particle sizes of 2.0 and 1.5 cm, respectively. As for xylose yields, the maximum value at 1% glucan loading were obtained at particle size of 2.0 cm. Thus, the relatively high glucose and xylose yields was obtained at the particle size of 2.5 and 2.0 cm, which also implied that the utilization of larger biomass particles resulted in the higher pretreatment and enzymatic hydrolysis efficiency.

Mass balance is essential for evaluating the effect of biomass particle sizes on cellulosic ethanol production (Jin et al., 2010; Li et al., 2010). Overall mass balance at each particle size was

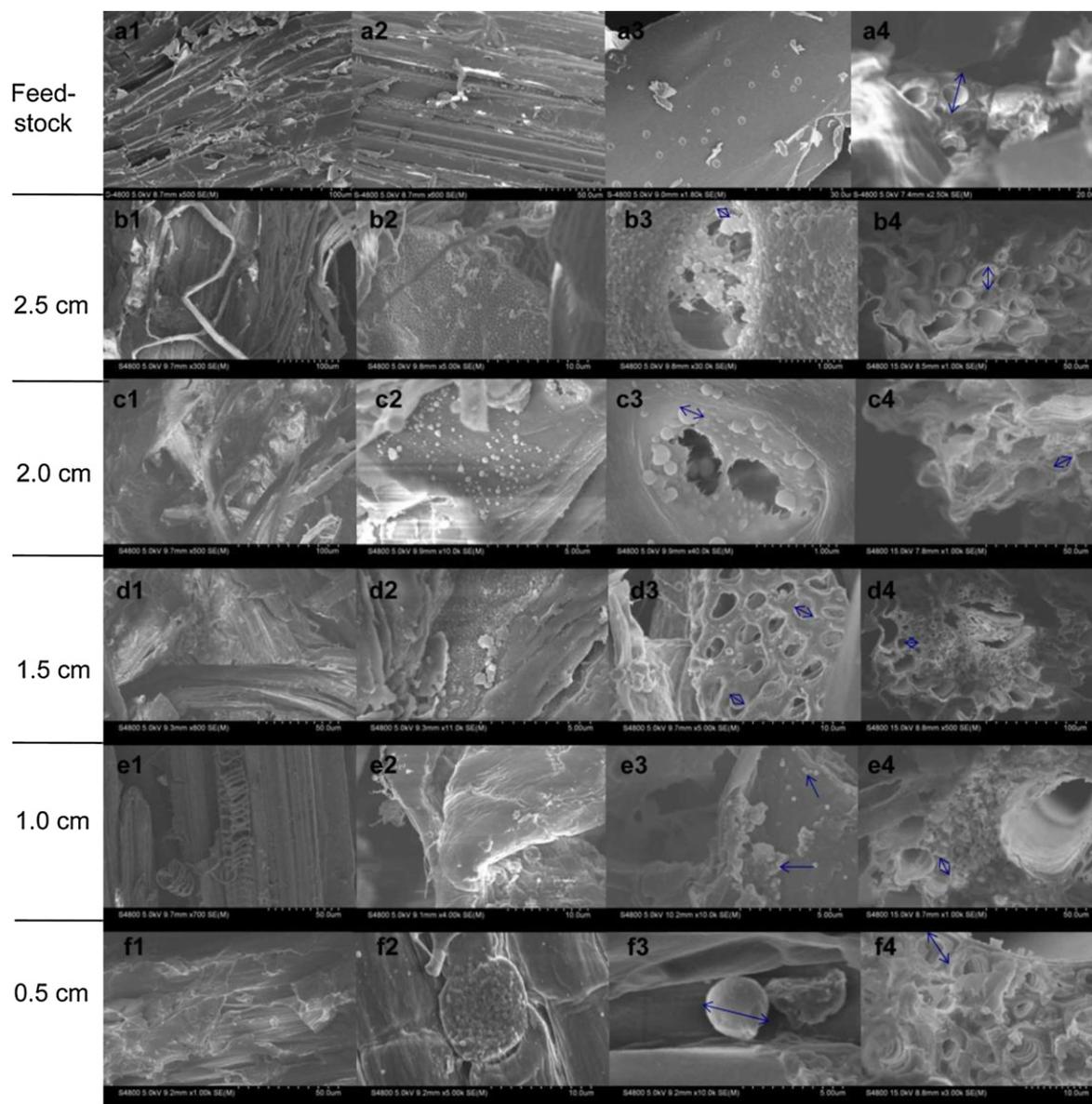


Fig. 5. SEM images of the untreated biomass (a1–a4), pretreated biomass (b1–b3; c1–c3; d1–d3; e1–e3; f1–f3) and solid residues (b4, c4, d4, e4, and f4) after enzymatic hydrolysis (1% glucan loading and 60 FPU/g glucan) at different particle sizes.

performed by using matter recovery and composition analysis after each step. Details of mass balance at particle size of 2.0 cm are given in Fig. 6. There is no evident mass loss for glucan, but most of xylan and arabinan was solubilized and degraded during pretreatment. Approximately 88% and 55% of glucan and xylan was hydrolyzed to

glucose and xylose, respectively, through pretreatment and enzymatic hydrolysis at particle size of 2.0 cm by 1% glucan loading with an enzyme loading of 60 FPU/g glucan. Mass balance around the enzymatic hydrolysis step achieved 100.48% and 107.52% closure for glucose and xylose, respectively. However, it should be noted

Table 3B

Glucose and xylose yields of the whole process (pretreatment + enzymatic hydrolysis) at different biomass particle sizes.

Particle size (cm)	Component yields of the whole process (%) ^a							
	Glucose yield ^b	Xylose yield ^b	Glucose yield ^c	Xylose yield ^c	Glucose yield ^d	Xylose yield ^d	Glucose yield ^e	Xylose yield ^e
2.5	70.3 (2.4)	45.9 (2.9)	85.2 (2.1)	48.7 (1.4)	61.0 (2.6)	40.5 (2.6)	78.4 (2.1)	45.0 (1.4)
2.0	63.8 (1.8)	52.1 (1.5)	87.9 (2.3)	55.2 (1.8)	65.9 (2.1)	48.6 (1.7)	73.7 (1.9)	51.1 (2.7)
1.5	64.8 (1.9)	50.0 (1.8)	85.9 (1.7)	52.4 (2.6)	68.9 (1.6)	46.7 (1.3)	75.4 (1.8)	50.3 (1.5)
1.0	63.9 (1.8)	49.7 (1.5)	82.6 (1.8)	53.4 (2.9)	67.8 (1.8)	46.8 (1.7)	75.0 (1.6)	48.4 (1.8)
0.5	56.1 (2.0)	49.0 (2.4)	69.8 (2.1)	51.5 (1.8)	56.7 (2.0)	49.9 (1.3)	67.6 (1.9)	51.7 (1.3)

^a Pretreatment conditions: 200 °C, 5 min

^b Enzymatic hydrolysis conditions: 1% glucan loading (w/v), 15 FPU/g glucan.

^c Enzymatic hydrolysis conditions: 1% glucan loading (w/v), 60 FPU/g glucan.

^d Enzymatic hydrolysis conditions: 6% glucan loading (w/v), 15 FPU/g glucan.

^e Enzymatic hydrolysis conditions: 6% glucan loading (w/v), 60 FPU/g glucan. Standard deviations are shown in parentheses.

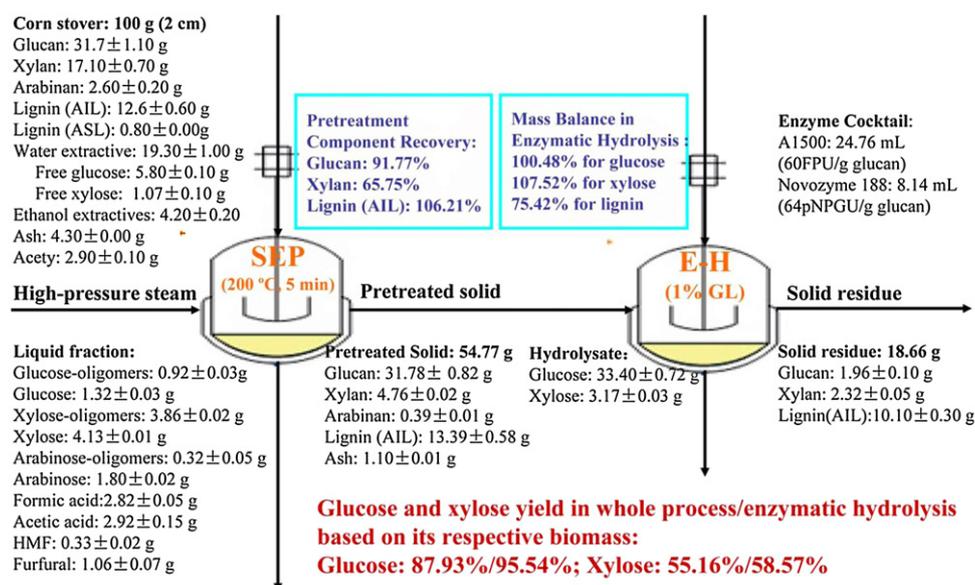


Fig. 6. Overall mass balance around steam explosion pretreatment and enzymatic hydrolysis of corn stover at particle size of 2.0 cm. SEP stands for steam explosion pretreatment; EH stands for enzymatic hydrolysis; GL stands for glucan loading.

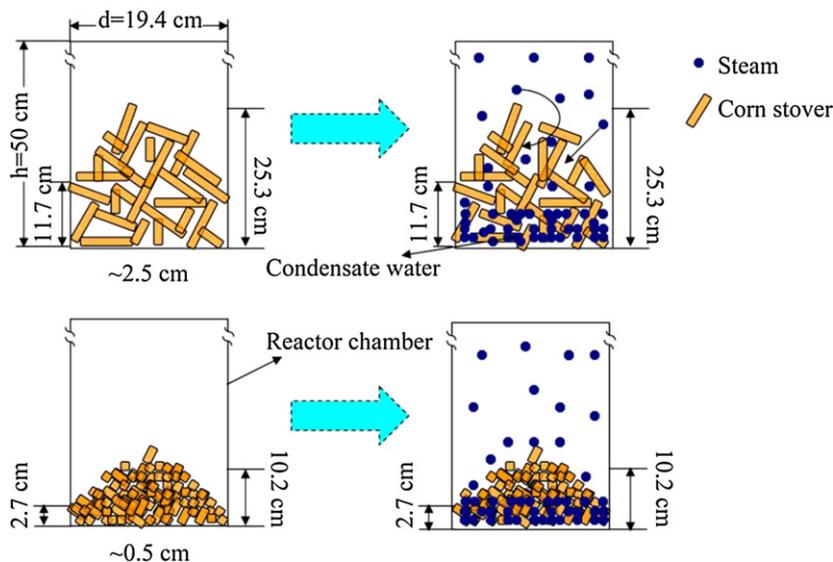


Fig. 7. Proposed mechanistic model for steam explosion pretreatment of corn stover at different biomass particle sizes.

that lignin recovery around the enzymatic hydrolysis step was about 75%. The reason could be that lignin or lignin-like compounds dissolved into hydrolysates due to the physical shear force during enzymatic hydrolysis.

3.7. Proposed mechanistic model for pretreatment of different biomass particles

In the SEP, there are two most important steps (auto-hydrolysis and explosion) after penetration of high-pressure steam into the plant cell wall. At auto-hydrolysis step, hemicellulose was dissolved and degraded, and lignin was solubilized and recondensed. At instantaneous de-compression step, the particles were exploded into small pieces, and the crystalline structure of cellulose was disrupted. Meanwhile, the porosity of corn stover biomass greatly increased. However, the porosity of the pile of corn stover biomass at larger particle size was higher than that of smaller particle size, due to the lower bulk density of corn stover biomass at larger

particle size (Table 2A). High-pressure steam penetrated easier the interior of the pile of corn stover at larger particle size due to the high porosity, resulting in the efficient auto-hydrolysis and explosion (Fig. 7). On the other hand, there was about 2.0 L condensate water at the bottom of the reactor chamber before depressurization step, and the volume of pile submerged in condensate water was larger for the smaller particles than that for the larger ones. The plant cells of corn stover biomass submerged in condensate water were full of water, resulting in the poor efficiency of explosive depressurization on the biomass solids. Consequently, after the pretreatment, corn stover biomass at larger particle size obtained higher SSA and lower crystallinity, which resulted in higher sugar conversions and yields compared with the smaller particles.

4. Conclusions

Our results clearly showed the importance of the choice of particle size for corn stover biomass utilization process. The amount

of byproduct was higher and the sugar recoveries were lower for the larger biomass particles during pretreatment, but the sugar conversions and yields were higher during enzymatic hydrolysis. With increasing particle size, the SSA of larger biomass particles obviously increased and the CrI of larger biomass particles apparently decreased. The results indicated the larger biomass particles were better mixed and reacted with high-pressure steam. Therefore, the utilization of larger corn stover biomass particles in steam explosion pretreatment would be more suitable to reduce process cost compared with smaller ones.

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