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De-ashing treatment of corn stover improves the efficiencies of enzymatic hydrolysis and consequent ethanol fermentation



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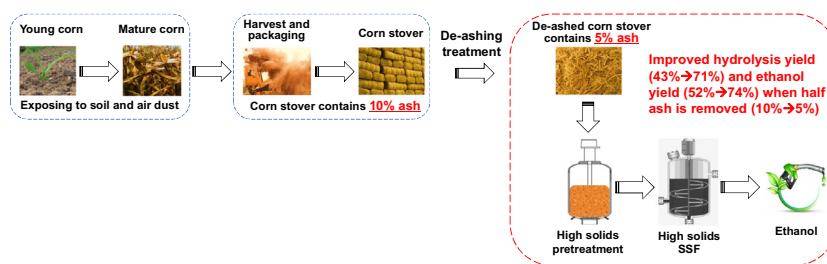
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HIGHLIGHTS

- De-ashing improves the yields of hydrolysis and ethanol fermentation of corn stover.
- De-ashing affects the pretreatment efficiency significantly in high solids content pretreatment.
- Alkaline compounds in ash neutralize sulfuric acid used as catalyst in pretreatment.

GRAPHICAL ABSTRACT



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ABSTRACT

In this study, corn stover with different ash content was pretreated using dry dilute acid pretreatment method at high solids loading of 67% (w/w). The results indicate that the hydrolysis yield of corn stover is increased from 43.30% to 70.99%, and ethanol yield is increased from 51.74% to 73.52% when ash is removed from 9.60% to 4.98%. The pH measurement of corn stover slurry indicates that the decrease of pretreatment efficiency is due to the neutralization of sulfuric acid by alkaline compounds in the ash. The elemental analysis reveals that the ash has the similar composition with the farmland soil. This study demonstrates the importance of ash removal from lignocellulose feedstock under high solids content pretreatment.

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1. Introduction

Agricultural residues such as corn stover, wheat straw, and rice straw are grown in farmland exposing to the dusts from soil and air. The dusts attached on their leaves and husks are defined as the extractable ash, because it can be removed by extraction or washing, while inorganic substances existing inside its plant organism are defined as the structural ash (Sluiter et al., 2005). The total content of the dusts and inorganic substances, called

“ash”, is estimated to be around 10% in lignocellulose biomass (Sluiter and Sluiter, 2011). In pretreatment operations, the fine ash particles absorb more steam, water, dilute acid solution, or solvent than the relatively larger lignocellulose fibers. As the result, the pretreatment efficiency is reduced because the liquid is moved onto the inert ash particles instead of lignocellulose fibers. Therefore, water washing or mechanic vibration is usually applied to remove the soil or air dust portions of ash (extractable ash) for maintaining the pretreatment efficiency (Zhu et al., 2004, 2005; Yu and Chen, 2010; Qiu and Chen, 2012).

In many publications of pretreatment study, de-ashing operation seems to be an optional step, and even no descriptions are given to this step. Two reasons might be responsible for the

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phenomenon: (1) the total ash content detected in the specific biomass is relatively low, so that no significant impact on pretreatment efficiency is found, for instance, in the case of wood biomass utilization; (2) the ash is completely saturated by sufficient liquid solution used in the impregnation step, so that the impact is hindered, for instance, in the case of most dilute acid pretreatment, alkaline solution treatment, and steam explosion (steam absorption).

One of the recent trends in pretreatment technology is the increase of lignocellulose solids content as high as possible, in order to reduce fresh water usage and waste water generation (Linde et al., 2008; Sassner et al., 2008; Zhang et al., 2011; Modenbach and Nokes, 2012). The ash content in lignocellulose feedstock is also increased to a high level with the increase of lignocellulose solids in pretreatment operations. Chen et al. (2012) noticed that the solid loading at 30% (w/w) already resulted in a decrease to the efficiency of dilute acid pretreatment. In the recently proposed “dry” dilute acid pretreatment, the solids content is increased to 70% (w/w) (Zhang et al., 2011; He et al., 2014). Both the lignocellulose feedstock and the pretreated lignocellulose product are “dry” solid with no any waste water release. As the outcome, the ash content is also increased to a considerably high content. In this circumstance, it is worth a detailed investigation of the impact of ash content and de-ashing on the pretreatment efficiency and the consequent ethanol fermentability.

In this study, the dilute acid pretreatment of corn stover with different ash content was conducted at high solids content close to 70% (w/w). The impact of ash removal on the hydrolysis yield of the pretreated corn stover and the ethanol yield in the consequent fermentation was investigated and analyzed. This study provides an important proof for the ash removal in the pre-handling step of dilute acid pretreatment processes.

2. Methods

2.1. Raw materials and ash removal operation

The virgin corn stover was grown in Dancheng, Henan, China and harvested in fall 2011, which contained 35.85% cellulose content, 5.82% xylan content and 9.60% ash content. Corn stover was de-ashed by washing with tap water at 10, 30, 50, and 100 folds weight of the dry corn stover. Corn stover was soaked in the water and stirred manually for 1 h in a box. The corn stover floating on the surface of water was harvested to remove the stone and metals and pressed in a cylinder pressing machine to remove the successive water, then dried at 105 °C till constant weight. The unwashed corn stover was used as the control feedstock. The dry corn stover was milled coarsely using a beater pulverizer and screened through a mesh sieve with the circles of 10 mm in diameter and stored in sealed plastic bags until use. The soil sample was taken from the same region of the corn stover in Dancheng, Henan, China.

2.2. Microorganisms and enzymes

The biodetoxification strain, *Amorphotheca resinae* ZN1, is stored at Chinese General Microorganisms Collection Center, Beijing, China with the registration number of CGMCC 7452. The biodetoxification for the removal of inhibitors from the pretreated corn stover is described in He et al. (2014).

The ethanol fermentation strain, *Saccharomyces cerevisiae* DQ1, is stored at Chinese General Microorganisms Collection Center, Beijing, China with the registration number of CGMCC 2528 (Zhang et al., 2010a; Chu et al., 2012). *S. cerevisiae* DQ1 was first adapted in the pretreated corn stover hydrolysate for 3 times and then inoculated into the bioreactors to start the fermentation according to the procedure in He et al. (2014).

Cellulase enzyme Youtell #6 was kindly provided by Hunan Youtell Biochemical Co. (Yueyang, Hunan, China). The filter paper activity was 135 FPU/g determined by NREL LAP-006 (Adney and Baker, 1996), and the cellobiase activity was 344 CBU per gram determined by the method described by Ghose (1987). The protein concentration of the cellulase used was 90 mg/g cellulase power determined by the Bradford assay using BSA as protein standard.

2.3. Pretreatment reactor and operation

1400 g of corn stover (dry base) was impregnated with 700 g of 5% (w/w) dilute sulfuric acid solution for 12 h at ambient temperature of 18–25 °C. The impregnated corn stover was pretreated in the helically agitated reactor as described in He et al. (2014). The pretreatment condition was fixed at 185 °C with the sulfuric acid usage of 2.5% (2.5 g sulfuric acid per 100 g of dry corn stover) for 3 min residual time in the reactor.

The pretreatment efficiency was assayed using the NREL LAP-009 protocol (Brown and Torget, 1996). 1.0 g corn stover (dry base) was added into the 100 mL flask followed by the addition of deionized water with the adjusting of pH value to 4.8 by 5 M NaOH solution. Then the 0.1 M citrate buffer (pH 4.8) and 0.08 ml tetracycline were added into the flask to prepare 5% (w/w) solids slurry, in which the total liquid was 19 ml containing water in the corn stover, water added into the flask, citrate buffer, NaOH solution and tetracycline. The enzyme dosage was 15 FPU/g DM (10 mg protein per/g DM) and the enzymatic hydrolysis was conducted at 50 °C, pH 4.8 and 150 rpm shaking. Materials without cellulase addition as well as cellulase without materials addition were carried out at 5% solids loading, 50 °C, pH 4.8 as the controls of saccharification. The materials used for enzymatic hydrolysis was directly added into flask without any treatments such as washing or other detoxification process. After 72 h hydrolysis, the conversion of cellulose and xylan to the glucose and xylose was calculated as below:

$$\text{Cellulose conversion}(\%) = \frac{(C_{glu} - C_0) \times V}{m \times f_{cellulose} \times 1.111 + \frac{m \times C_{oligtglu}}{1000}} \times 100\% \quad (1)$$

where C_{glu} was the glucose concentration after 72 h hydrolysis (g/L), C_0 was glucose concentration in the substrate and enzyme blank (g/L), V was the volume of the total liquid in the hydrolysis process (L), m was the weight of dry corn stover added in the initial of hydrolysis (g), $f_{cellulose}$ was the cellulose content of dry corn stover (%), $C_{oligtglu}$ was the glucan oligomers content in the dry corn stover (mg/g DM).

$$\text{Xylan conversion}(\%) = \frac{(C_{xyl} - C_0) \times V}{m \times f_{xylan} \times 1.136 + \frac{m \times C_{olixyl}}{1000}} \times 100\% \quad (2)$$

where C_{xyl} was the xylose concentration after 72 h hydrolysis (g/L), C_0 was xylose concentration in the substrate and enzyme blank (g/L), V was the volume of the total liquid in the hydrolysis process (L), m was the weight of dry corn stover added in the initial of hydrolysis (g), f_{xylan} was the cellulose content of dry corn stover (%), C_{olixyl} was the glucan oligomers content in the dry corn stover (mg/g DM). The experiments were carried out in duplication and the data used here was taken from average of two parallel experiments.

2.4. Biological detoxification of pretreated corn stover

The pretreated corn stover was biologically detoxified by *A. resinae* ZN1 to remove the main inhibitors in the pretreated corn stover such as furan derivatives and organic acids (Zhang et al., 2010b; He et al., 2014) prior to the SSF process. Seed culture of *A. resinae* ZN1 was carried out on the corn stover pretreated at 185 °C, 2.0% acid usage and 3 min residue time by inoculation of the spore suspension washed from the PDA slant at 10%

inoculation (v/w). The pretreated corn stover was adjusted to pH 4.5 by adding 20% (w/w) Ca(OH)₂ solution. Then the seed culture was cultured for 7 days till furfural, 5-hydroxymethyl furfural (5-HMF), and acetic acid were completely removed, then the seed was transferred into the materials used for SSF feedstock and the biodetoxification started. The biodetoxification was maintained statically at 28 °C, pH 5.0 and lasted for 7 days for inhibitors removal and sugars retention.

2.5. Simultaneous saccharification and ethanol fermentation (SSF)

Simultaneous saccharification and ethanol fermentation was carried out using the pretreated corn stover with different ash content at 25% solids loading, 15 FPU/g DM, pH 5.5 and 150 rpm in the 5 L helical agitated bioreactors described by Zhang et al. (2010a). Temperature was controlled at 50 °C during 12 h prehydrolysis and 37 °C during SSF. Samples were taken periodically and prepared for ethanol detection on HPLC. The ethanol yield after 96 h was calculated by the method proper for high solids loading SSF process described by Zhang and Bao (2012). The experiments were carried out in duplication and the data used here was taken from average of two parallel experiments.

2.6. Measurements of cellulose and xylan in corn stover

Cellulose and xylan contents of corn stover were measured by two-step acid hydrolysis according to NREL LAP protocols (Sluiter et al., 2008a,b). 100 mg of the thoroughly washed and dried corn stover was added to 1 ml 72% (w/w) H₂SO₄ for 60 min reaction at 30 °C. Then the acid was diluted to 4% (w/w) by the addition of 28 ml deionized water. The mixture of corn stover and dilute acid was hydrolyzed at 121 °C in the autoclave for 60 min. The hydrolysate was neutralized by CaCO₃ powder to pH 5.0 and then centrifuged at 14500g for 5 min to remove the solids. The supernatant was taken for sugars determination to calculate the cellulose and xylan content. The experiments were carried out in duplication and the data used here was taken from average of two parallel experiments.

Oligomers of cellulose and xylan were measured using the method modified from NREL LAP (Sluiter et al., 2008b). 5.0 g of the pretreated corn stover were mixed with 50 ml deionized water and shaken at 180 rpm for 2 h in a 250 ml flask. 5 ml supernatant was taken out and reacted in 4% sulfuric acid concentration in a cap tube at 121 °C for 1 h. Then the hydrolysate neutralized by CaCO₃ was taken to measure the sugar concentration. The difference of the sugar concentration before and after acid hydrolysis was defined as the oligosaccharides content. The experiments were carried out in duplication and the data used here was taken from average of two parallel experiments.

2.7. Measurements of ash content in corn stover

Ash content was determined using the method described by NREL protocol (Sluiter et al., 2005). About 1 g of dried corn stover was completely burned in the muffle furnace by the temperature gradients from room temperature to 105 °C, then to 250 °C at the rate of 10 °C per minute and hold at 250 °C for 30 min; finally to 575 °C at the rate of 20 °C per minute and kept for 3 h. The temperature was cooled naturally to 105 °C and the burned corn stover ashes were removed from the furnace to desiccator for weighing. The weight percentage of the combustion residues to the original dry corn stover sample was defined as the ash content. The experiments were carried out in duplication and the data used here was taken from average of two parallel experiments.

2.8. Acid absorption of corn stover

Corn stover with different ash content was titrated by 5% (w/w) dilute sulfuric acid solution to pH 2.4. The acid consumption in the titration was used to evaluate the capacity of ash absorption and de-ashing degree. 1 g dry corn stover was added into 20 ml deionized water, then 5% (w/w) sulfuric acid solution was used to titrate the corn stover slurry until pH value was 2.4. The amount of sulfuric acid used (gram per 100 g CS) was recorded. Same experiment was carried out using the washed corn stover sieved into the size 10, 20, 40, and 60 meshes per square inch. The experiments were carried out in triplication and the data used here was taken from average of three parallel experiments.

2.9. Elemental analysis in the corn stover

The fines after the combustion of dry corn stover in the muffle furnace was collected and used for the determination of the elements by Scanning Electron Microscopy (JSM-6360LV, Joel Co., Japan)/Energy Disperse Spectrometer (Falcon, Edax Co., USA). The results were expressed as the elements content in the dry corn stover.

2.10. Analysis of sugars and ethanol

Sugars and ethanol concentration was detected using the HPLC (LC-20AD, RID-10A refractive index detector, Shimadzu, Kyoto, Japan) equipped with HPX-87H column (Bio-Rad, Hercules, CA, USA). The flow phase used here was 5 mM H₂SO₄ and the flow rate was set to 0.6 ml/min. The samples were filtered through 0.22 μm membrane before analysis on HPLC.

3. Results and discussion

3.1. Impact of ash content in corn stover feedstock on pretreatment efficiency

The pretreatment of corn stover at different ash content was conducted at 185 °C, 2.5% sulfuric acid usage for 3 min. The corn stover solids content was 67% (w/w, dry base), which was equivalent to the solid/liquid ratio of 2:1. The extractable ash was removed from the virgin corn stover by washing using different amount of tap water. The ash content in corn stover decreased with increasing water usage from the original 9.60% ± 0.40% (un-washed corn stover) to 7.75% ± 0.49%, 6.79% ± 1.97%, 5.30% ± 0.38%, and 4.98% ± 0.28% with the water usage of 10, 30, 50, and 100 folds to the corn stover weight, respectively. Fig. 1 shows the conversion yield of cellulose and xylan of the pretreated corn stover in the enzymatic hydrolysis assay. Cellulose conversion increased steadily from 43.30% to 45.56%, 53.39%, 65.26%, and 70.99% when the ash content was reduced from 9.60% (un-washed corn stover) to 7.75%, 6.79%, 5.30%, and 4.98%, respectively. Similar tendency was observed in the increase of xylan conversion (from 56.83% to 57.09%, 66.36%, 81.69%, and 90.07%). The result suggests that the ash content in the corn stover feedstock significantly affects its enzymatic hydrolysis yield and the de-ashing operation is an inevitable step of pretreatment process.

The insoluble and soluble contents in the pretreated corn stover under different ash content of corn stover are shown in Table 1. Insoluble cellulose content increased from 35.38% (un-washed) to 36.99%, 37.15%, 37.13%, and 37.61% when the ash content was reduced from 9.60% (un-washed) to 7.75%, 6.79%, 5.30%, and 4.98%, respectively. In the contrast, the xylan content decreased slightly from 5.82% (un-washed) to 4.32%, 4.41%, 3.69% and 3.08%, respectively. The lower xylan content and higher cellulose

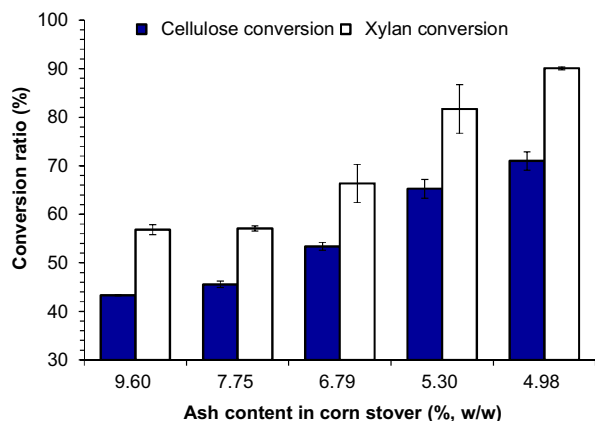


Fig. 1. Cellulose conversion and xylan conversion after 72 h enzymatic hydrolysis using the pretreated corn stover with different ash content. Conditions of enzymatic hydrolysis: 5% solids loading, 15 FPU/g DM (equivalent to 10 mg protein/g DM), pH 4.8, 50 °C, 150 rpm. The experiments were carried out in duplication and the data used here was taken from average of two parallel experiments.

content after pretreatment indicate the higher pretreatment efficiency with the reducing ash content in the corn stover feedstock.

Soluble glucose, xylose, oligosaccharides, and inhibitors in the pretreated corn stover also indicate the efficiency of pretreatment (Sassner et al., 2008; Zhang et al., 2011; He et al., 2014). Table 1 shows that the soluble sugars' content in the pretreated corn stover increased but the oligosaccharides gradually decreased with enhanced de-ashing. The content of xylose from hemicellulose degradation increased from 15.38 mg/g DM to 28.45, 28.88, 31.75 and 38.89 mg/g DM when the ash content decreased from 9.60% to 7.75%, 6.79%, 5.30%, and 4.98%, respectively. The content of the three major inhibitors, furfural, 5-hydroxymethylfurfural (HMF), and acetic acid, increased with the reducing ash content, also indicated that the intensity of pretreatment was enhanced by de-ashing operation.

3.2. pH change and elemental composition analysis of the ash in corn stover

Table 2 shows that the pH value of corn stover at different ash content when the corn stover was completely mixed with sufficient amount of water to form liquid slurry. The pH value of the un-washed corn stover decreased from the original pH of 9.10 to 8.61, 8.60, 8.51, and 8.28 gradually but slightly when the

ash was removed from corn stover at different water usage. When corn stover was impregnated with dilute sulfuric acid in the weight solids/liquid ratio of 2:1 for 12 h, the pH value decreased significantly from 4.15 to 3.61, 3.45, 3.37, and 2.78 with the reduction of ash content from 9.60% (unwashed) to 7.75%, 6.79%, 5.30% and 4.98%, respectively. The results indicate that the existence of alkaline compounds in the ash caused the higher pH of corn stover and the impregnated sulfuric acid was used to neutralize the alkaline compounds. This result may suggest the reason of pretreatment efficiency decrease: the sulfuric acid used as catalyst is neutralized into inert neutral salts and lose the capacity to catalyze the structural change reactions of lignocellulose in the pretreatment, thus the pretreatment efficiency is reduced.

Two more experiments were carried out to give further evidences of pretreatment efficiency reduction: (1) sulfuric acid usage when the corn stover slurry was titrated to pH 2.4 (pH value of the thoroughly de-ashed corn stover after impregnation in the previous study of dry dilute acid pretreatment); (2) elemental composition measurement of the ash. The acid usage changed from 3.70 g ± 0.07 g to 3.75 g ± 0.07 g, 3.28 g ± 0.38 g, 2.55 g ± 0.07 g, and 2.05 g ± 0.07 g for titrating 100 g of the dry corn stover when the ash content was reduced from 9.60% (un-washed) to 7.75%, 6.79%, 5.30%, and 4.98%, respectively. In the dry dilute acid pretreatment (Zhang et al., 2011; He et al., 2014), only 2.5 g sulfuric acid per 100 g dry corn stover is used. The result indicates that significant amount of sulfuric acid is consumed to neutralize the alkaline ash, instead of to catalyze the lignocellulose deconstruction reactions, if the ash is not sufficiently removed from corn stover.

To find the size distribution of the ash, the milled corn stover was sieved into five fractions: >10, 10–20, 20–40, 40–60, and <60 mesh per square inch using norm sample sieves. Then the thoroughly de-ashed and virgin corn stover was titrated by dilute sulfuric acid solution and the sulfuric acid usage was recorded in Fig. 2. For the un-washed virgin corn stover, the sulfuric acid usage decreased significantly from 5.93 to 3.73, 3.20, 2.80, and 2.63 g per 100 g dry corn stover with the reduced size from <60, 40–60, 20–40, 10–20, >10 meshes, respectively; for the thoroughly washed corn stover, the sulfuric acid also decreased but not significantly from 3.08 g to 2.43 g, 2.33 g, 2.10 g, and 1.78 g per 100 g dry corn stover with the same size increase. The result indicates that the ash existed more in small size in both un-washed and washed corn stover materials. Even when corn stover was thoroughly washed out and more than half of ash was removed, the increasing tendency of sulfuric acid usage still continued with the decreasing particle size. Clearly, over-milled corn stover is mixed with ash then negatively affects the pretreatment efficiency.

Table 1

Components in the corn stover under different washing conditions after pretreatment.

Ash in corn stover (%)	Cellulose content (%)	Xylan content (%)	Glucose (mg/g DM)	Xylose (mg/g DM)	O-Glu ^a (mg/g DM)	O-Xyl ^b (mg/g DM)	Acetate (mg/g DM)	HMF (mg/g DM)	Furfural (mg/g DM)
<i>Before pretreatment</i>									
9.60	31.54 ± 0.24	18.10 ± 0.31	/	/	/	/	/	/	/
7.75	33.67 ± 0.33	18.81 ± 0.62	/	/	/	/	/	/	/
6.79	34.04 ± 0.88	19.89 ± 0.80	/	/	/	/	/	/	/
5.30	35.05 ± 3.44	21.26 ± 1.13	/	/	/	/	/	/	/
4.98	36.18 ± 0.05	19.83 ± 0.35	/	/	/	/	/	/	/
<i>After pretreatment</i>									
9.60	35.38 ± 0.74	5.82 ± 0.17	3.24 ± 0.21	15.38 ± 0.44	17.38 ± 0.78	87.90 ± 3.44	6.91 ± 0.06	0.91 ± 0.01	0.53 ± 0.12
7.75	36.99 ± 0.28	4.32 ± 0.07	4.56 ± 0.12	28.45 ± 0.37	15.90 ± 0.76	77.00 ± 0.72	6.94 ± 0.05	1.52 ± 0.20	1.27 ± 0.04
6.79	37.15 ± 0.61	4.41 ± 0.28	3.60 ± 0.26	28.88 ± 0.11	14.36 ± 2.56	81.95 ± 3.40	8.73 ± 0.08	1.37 ± 0.02	1.45 ± 0.39
5.30	37.13 ± 0.34	3.69 ± 0.73	4.00 ± 0.37	31.75 ± 2.09	12.05 ± 0.77	74.57 ± 1.33	8.34 ± 0.33	1.42 ± 0.12	1.67 ± 0.05
4.98	37.61 ± 0.34	3.08 ± 0.85	4.52 ± 0.22	38.89 ± 0.21	10.36 ± 0.04	70.19 ± 1.30	8.80 ± 0.88	1.66 ± 0.32	1.54 ± 0.15

Corn stover was pretreated for 3 min at 185 °C with 2.5% sulfuric acid usage at 50 rpm agitation rate. Unit here was defined as micrograms components in the per gram dry corn stover. The experiments were carried out in duplication and the data used here was taken from average of two parallel experiments.

^a O-Glu represents the oligomer of glucan.

^b O-Xyl represents the oligomer of xylan.

Table 2
pH measurement of corn stover slurry with different ash content.

Corn stover with different ash content (%)	pH of un-washed corn stover (–)	pH of corn stover impregnated by acid (–)
9.60	9.10 ± 0.04	4.15 ± 0.11
7.75	8.61 ± 0.01	3.61 ± 0.06
6.79	8.60 ± 0.06	3.46 ± 0.06
5.30	8.51 ± 0.03	3.37 ± 0.09
4.98	8.28 ± 0.05	2.78 ± 0.01

Impregnation of corn stover by 5% (w/w) sulfuric acid was carried out at a solid to liquid ratio of 2:1 to reach the acid usage 2.5%. 1 g dry material (dry base) was added into 20 ml deionized water and the pH of slurry was determined by pH sensor. The experiments were carried out in duplication and the data used here was taken from average of two parallel experiments.

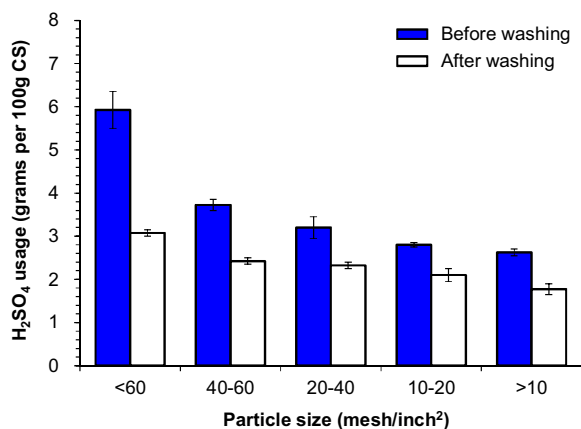


Fig. 2. Titration using corn stover with different size. The corn stover without de-ashing and containing 4.98% ash content were used for this experiment. Both corn stover were sieved through the mesh sieve to different size. 1 g dry corn stover was added into 20 ml deionized water, then 5% (w/w) sulfuric acid solution was used to titrate the corn stover slurry until pH value was 2.4. The amount of sulfuric acid used (gram per 100 g CS) was recorded. The experiments were carried out in triplication and the data used here was taken from average of three parallel experiments.

The elemental composition of corn stover was measured after completely combustion of the carbohydrates, proteins, and lipid in it. Table 3 shows that the elemental species of the ash were similar to that of soils, especially the silica and oxygen elements accounting for 68% in soil, and 46–68% in the ash at different washing intensity, corresponding to the major soil component of silica dioxide. The elements which are easy to form soluble compounds, such as K and Mg, quickly decreased with the enhanced washing; on the other hand, the elements easy to form insoluble compounds, such as Ca and Al, maintained relatively stable concentrations or even increased due to the weight loss of soluble elements in the ash. The result suggest that most of the water soluble salts in the ash were removed in water washing step, and the water insoluble salts were left behind. The wash-out portion could not be simply

Table 3
Elemental compositions in the ash of corn stover after complete combustion.

	Si (%)	Ca (%)	Mg (%)	K (%)	Al (%)	O (%)	S (%)	P (%)
<i>Elements in the soil</i>	33.23	5.45	2.18	3.17	11.57	34.56	/	/
<i>Elements in the ash</i>								
Corn stover with 9.60% ash	19.00	13.81	11.94	16.02	1.87	26.84	1.81	2.88
Corn stover with 7.75% ash	25.79	15.31	8.92	11.54	1.60	29.49	2.35	2.67
Corn stover with 6.79% ash	30.48	13.60	8.97	6.72	1.93	32.35	2.07	2.58
Corn stover with 5.30% ash	34.77	11.61	7.55	7.61	0.96	32.64	1.91	1.72
Corn stover with 4.98% ash	32.81	16.11	7.36	2.58	1.31	34.30	1.75	2.63

attributed to extractable ash because the insoluble portion is also part of extractable ash. Also, this could be changed when different de-ashing method such as vibration or cyclone machine is used.

An interesting phenomenon can be found that the residue ash after thoroughly washing (4.98% ash content) has the most similar composition to the soil. This may imply that the contribution of agricultural residues to the surface farmland soil composition is significant besides the absorption of inorganic salts by crops from soil during the growth. The massive removal of agricultural residues from farmland might produce a potential impact on farmland soil composition. The ecological effect should be considered when biorefinery industry is practiced in large scale.

3.3. Simultaneous saccharification and ethanol fermentation (SSF)

To further evaluate the efficiency of pretreatment, simultaneous saccharification and ethanol fermentation was carried out using the pretreated corn stover with different ash content at the solids content of 25% (dry materials), 15 FPU/g DM cellulase dosage. Prior to the ethanol fermentation, the pretreated corn stover was detoxified biologically to remove the main inhibitors by the fungus *A. resiniae* ZN1 in the solid state culture (Zhang et al., 2010b; He et al., 2014). Fig. 3 shows that the ethanol fermentability was improved on both glucose release and ethanol yield when the ash was removed from corn stover. The initial glucose concentration after 12 h prehydrolysis increased from 44.97 g/L to 57.06 g/L, 58.57 g/L, 57.38 g/L, and 66.06 g/L when the ash was removed 9.60–7.75%, 6.79%, 5.30%, and 4.98%, respectively. At the same time, the ethanol titer increased to 28.52 g/L, 33.64 g/L, 38.23 g/L, 38.80 g/L, and 43.03 g/L, and the ethanol yield reached 51.74%, 58.40%, 66.11%, 67.13%, and 73.52%, correspondingly.

Generally, the ash content below 10% of the dry weight of biomass is acceptable when the initial solid content in dilute acid pretreatment is low (Dien et al., 2006; Jensen et al., 2010; Humbird et al., 2011; Sluiter and Sluiter, 2011). However, in this study, the ash content was less than 10% but the pretreatment efficiency demonstrated a great change with the decrease of ash content. The main reason was due to the extremely high solids loading (close 70%) in the pretreatment operation (“dry” dilute acid pretreatment). Then the minimum amount of sulfuric acid was absorbed by the fine ash particles, rather than penetrated into corn stover materials. After the extensive de-ashing treatment, the ash content in the corn stover feedstock was reduced to 4.98% in weight percentage, and the ethanol yield in SSF at 25% solids loading reached a relatively high level of 73.52%. To combine the results of SSF with that of enzymatic hydrolysis, it could be observed that the glucose releasing by cellulase in the SSF was impacted by ash content more than the fermentation process. In the previous study by Qiu and Chen (2012), the inorganic ions in the ash significantly affect the activity of cellulase and then the hydrolysis was inhibited. In this study, the cellulase is also likely to be inhibited by inorganic salts, but the data from the results of

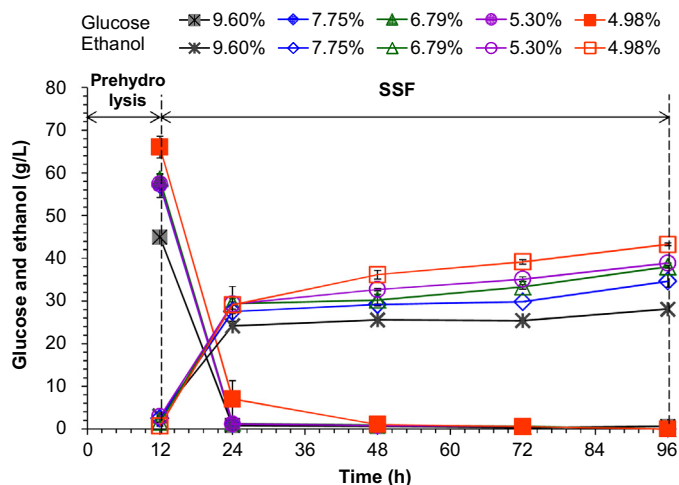


Fig. 3. Simultaneous saccharification and ethanol fermentation using the pretreated corn stover with different ash content. SSF conditions: 25% solids loading (dry materials), 15 FPU/g DM (equivalent to 10 mg protein/g DM), 150 rpm, 50 °C, pH 4.8 during the prehydrolysis and 37 °C, pH 5.5 during SSF. The experiments were carried out in duplication and the data used here was taken from average of two parallel experiments.

the corn stover after pretreatment show that the ash not only impacted the hydrolysis but also the pretreatment efficiency. Therefore, this study demonstrated the importance of de-ashing treatment in the dilute acid pretreatment at very high solids content, and perhaps the conclusion applies to other pretreatment operations with the direct contact of chemicals in liquid phase with lignocellulose biomass, such as ammonia fiber explosion, steam explosion, alkaline treatment.

For the ash removal method, water washing is used in this study. Obviously, this method does not work in large scale operations because of the huge water usage. Alternatively, mechanic vibration shaking or cyclone separator might be suitable choices, because most of the ash components exist in very fine particles as shown in this study (Fig. 2). De-ashing of agriculture residues by mechanic vibration and cyclone separator (generally combined together) is a commonly practiced operation in animal feed industry. However, in lab scale it is rarely used because of the noise and space occupation. NREL has taken into account the vibration machine and cyclone separator into its process design (Humbird et al., 2011). The Canadian company, SunOpta Inc., designed the vibration and cyclone separator process in the feedstock handling in their steam explosion pretreatment technology (<http://www.sunopta.com/>). In these practices, the de-ashing operation is realized without consuming massive amount of water. Considering the efficiency of different de-ashing methods, water-washing is effective to remove the water soluble components, including soluble compounds in ash, and perhaps soluble sugars also. On the other hand, vibration/cyclone method removes both heavy particles (such as sands, stones, and metal pieces) and fine dusts effectively. However, the ash components attached relatively firmly on leaves and stalks might not be easily removed. The loss of fine feedstock particles might be another drawback of vibration/cyclone method depending on the machine used.

4. Conclusion

Ash in the corn stover significantly affected the pretreatment efficiency and ethanol yield in SSF at very high solid loading. The hydrolysis yield of corn stover is increased from 43.30% to 70.99%, and ethanol yield is increased from 51.74% to 73.52%

when ash is removed from 9.60% to 4.98%. Cellulose and xylan conversion after pretreatment and hydrolysis increased with the decreasing ash content in corn stover. The inorganic ions existed in the ash were contributed for the low pretreatment efficiency. This study provided an important proof for ash removal prior to the pretreatment.

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