



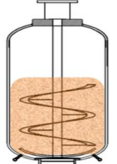
General Method to Correct the Fluctuation of Acid Based Pretreatment Efficiency of Lignocellulose for Highly Efficient Bioconversion

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S Supporting Information

Base pH Approaching Method
Simply and Quickly Correcting the Fluctuating Pretreatment Efficiency

Ash sharply changes pretreatment efficiency	Approaching the base pH value	Adjusted acid usage for each batch of feedstock	Stable and high bioconversion for various biorefining cases
			

ABSTRACT: Acid based pretreatment efficiency frequently changes with the varying properties of raw lignocellulose feedstock and creates disturbances in industrial biorefining applications. This study revealed that high alkaline ash content in feedstocks neutralized partial acid catalyst and consequently reduced the pretreatment efficiency. The ash content varies in each batch of feedstocks by differences in growing regions, seasons, irrigation, soil, fertilizer use, harvest operation, transportation, storage, and prehandling operations. These differences lead to frequent and significant fluctuations of acid based pretreatment efficiency. The practical solution to the fluctuation should be simple and fast, preferably without performing a time-consuming pretreatment operation assay. Here we proposed a Base pH Approaching method to correct the fluctuating pretreatment efficiency by a simple titration procedure. The method contained only two simple steps in flasks. The first step is the measurement of the inherent base pH value of the thoroughly washed feedstock slurry, and the second step is the adjustment of sulfuric acid usage by titration of the feedstock slurry to the base pH value. The method is universally effective to different feedstocks (corn stover, wheat straw, or rice straw) using different strains (yeasts or bacteria) for different products (ethanol and lactic acid) and different fermentation types (SSF or SSCF).

KEYWORDS: Lignocellulose, Ash content, Pretreatment efficiency, Repeatability, pH value

INTRODUCTION

Poor repeatability of pretreatment efficiency is commonly encountered in biorefinery processing of lignocellulose biomass. The unstable pretreatment efficiency creates great difficulties in both bench scale research and industrial applications by its randomly fluctuating hydrolysis yield and consequent fermentation performance. Since the pretreatment was conducted under the same reactors and operation parameters, the fluctuation of pretreatment efficiency should be highly related to the feedstock properties. Among many possible reasons, ash content changes frequently and significantly from 3% to 20% of the overall biomass weight, depending on crop species, growing regions, seasons, irrigation, soil, fertilizer use, harvest operation, transportation, storage, and the prehandling operation.¹ Ash in lignocellulose biomass is composed of various inorganic substances either the particles sedimented on the surface of biomass (exogenous ash), or the intracellular compounds required for plant growth (physiological ash).^{2–5} At the start of

biorefinery operations, the prehandling treatment only removes partial exogenous ash and the differences in ash content still maintain among different batches of feedstocks.^{1,6} Ash in biomass feedstock generally behaves in a strong acid buffering capacity by neutralizing the acid catalyst, and the pretreatment efficiency of acid based pretreatment is negatively affected correspondingly.^{7–10}

In the conventional dilute acid pretreatment, this phenomenon had been frequently and reasonably ignored because the acid catalyst was in large supply and the acid loss by ash neutralization was negligible compared to the need for pretreatment.^{9–11} As pretreatment technologies have advanced in industrial applications, acid catalyst usage has been sharply reduced with significant increases of feedstock solid loading for

Received: December 5, 2017

Revised: January 18, 2018

Published: January 25, 2018

the purpose of wastewater generation reduction.^{12,13} In such a low acid usage scenario, the loss of acid by ash neutralization is becoming a key factor on catalysis effectiveness. In other words, the pretreatment efficiency is more sensitive to ash content in advanced pretreatment technologies with very low acid catalyst usage. The difference in acid loss by ash in each batch of feedstocks significantly affects the stability of pretreatment efficiency. Therefore, the pretreatment parameters should be readjusted for each batch of feedstocks to compensate for the difference of acid loss.

Adjustment of pretreatment parameters requires a series of pretreatment operations, assessments, and calculations, thus it is not applicable to the changing feedstocks in industrial operations as a quick response. A simple, fast, and universally applicable method is required to calibrate the fluctuation of pretreatment efficiency. Here we show such an acid usage adjustment method by a simple and quick pH titration of feedstock suspension slurry in flasks before pretreatment. The method is universally effective for the high ash containing raw feedstocks to different feedstock types (corn stover, wheat straw, or rice straw) using different strains (yeasts or bacteria) for different products (ethanol and lactic acid) and different fermentation types (SSF or SSCF). This work provided an important and practical approach to maintain the stable pretreatment efficiency in industrial applications by its simple and fast sulfuric acid usage adjustment procedure before pretreatment.

MATERIALS AND METHODS

Raw Materials. Corn stover was harvested from Bayan Nur, Inner Mongolia, China, in the Fall of 2015 containing 31.5% cellulose, 21.3% hemicellulose, 15.7% lignin, and 6.7% ash by weight percentage. Wheat straw was harvested from Jinan, Shandong, China, in the Summer of 2015 containing 34.0% cellulose, 25.7% hemicellulose, 19.0% lignin, and 10.0% ash. Rice straw was harvested in Yichun, Jiangxi, China, in the Summer of 2015 containing 34.0% cellulose, 21.8% hemicellulose, 18.2% lignin, and 11.7% ash. The composition of the feedstock was measured according to NREL protocol LAP-002.^{14,15} The feedstock was ground using a hammer crusher to pass it through the 10 mm (diameter) apertures, then it was sealed in plastic bags and stored at room temperature until use.

Strains and Media. The biodetoxification strain *Amorphotheca resiniae* ZN1 was isolated in our previous study and stored in the China General Microorganisms Collection Center (CGMCC), Beijing, China, as CGMCC 7452.¹⁶ *A. resiniae* ZN1 was maintained on potato–dextrose–agar (PDA) slant prepared by boiling 200 g of peeled and sliced potatoes in 1 l of deionized water for 30 min with the addition of 20 g of glucose and 15 g of agar.

The ethanol fermenting strain *Saccharomyces cerevisiae* DQ1 (without xylose utilization) was cultured in the synthetic medium containing 20 g/L of glucose, 1 g/L of yeast extract, 2 g/L of KH_2PO_4 , 1 g/L of $(\text{NH}_4)_2\text{SO}_4$, 1 g/L of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$.^{7,17} *S. cerevisiae* XH7 (with xylose utilization) was cultured in YPD medium used for seed culture contained 20 g/L of glucose, 20 g/L of peptone, and 10 g/L of yeast extract.^{18,19}

The lactic acid bacterium (LAB) *Pediococcus acidilactici* TY112 was stored in CGMCC with the number 8664 and used for L-lactic acid fermentation.²⁰ The simplified MRS medium for seed culture contained 20 g/L of glucose, 10 g/L of yeast extract, 10 g/L of peptone, 2 g/L of diammonium hydrogen citrate, 5 g/L of sodium acetate, 0.3 g/L of MgSO_4 , 2 g/L of K_2HPO_4 , and 0.23 g/L of MnSO_4 .

Enzyme and Reagents. Commercial cellulase Cellic CTec 2.0 was purchased from Novozymes (China), Beijing, China. The filter paper activity was 203.2 FPU/mL determined using the NREL protocol LAP-006,²¹ the cellobiase activity was 4900 CBU/mL using the

method of Ghose,²² and the protein concentration was 87.3 mg/mL determined by the Bradford method.²³

Glucose, KH_2PO_4 , $(\text{NH}_4)_2\text{SO}_4$, $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{K}_2\text{HPO}_4 \cdot 3\text{H}_2\text{O}$, $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, NaOH, $\text{Ca}(\text{OH})_2$, H_2SO_4 , diammonium hydrogen citrate, sodium acetate were analytical pure and obtained from Lingfeng Chemical Reagent Co., Shanghai, China. Yeast extract and peptone were purchased from Oxoid, Basingstoke, Hampshire, UK.

Dry Sulfuric Acid Pretreatment and Biodetoxification. Corn stover, wheat straw, or rice straw was pretreated using the dry acid pretreatment method.^{13,24,25} Briefly, the feedstock and the dilute sulfuric acid solution were cocurrently fed into the 20 L pretreatment reactor at the solid/liquid ratio of 2:1 (w/w) and 175 °C for 5 min. The pretreatment efficiency was assayed by measuring the hydrolysis yield of cellulose according to the NREL LAP-009 protocol.²⁶ Briefly, 2.5% (w/w) of the pretreated feedstock solids was hydrolyzed using 26 mg cellulase protein/g cellulose (20 FPU/g DM) at pH 4.8, 50 °C for 72 h. The experiments were carried out in duplicate.

The ash content effect was evaluated by thoroughly washing (200 times of fresh water to the feedstock) to remove the exogenous ash and water-soluble ash before pretreatment. The sulfuric acid usage (mg H_2SO_4 per gram of dry feedstock) of the thoroughly washed feedstock determined by the consequent hydrolysis, ethanol fermentation was defined as the “base acid usage” for the specific feedstock (corn stover, wheat straw, or rice straw).

The pretreated feedstocks were biodetoxified to remove the inhibitor compounds.^{16,27} Briefly, the feedstocks were neutralized using 20% (w/w) $\text{Ca}(\text{OH})_2$ suspension to pH 5–6 and then disk milled to remove the extra-long fibers. *A. resiniae* ZN1 was inoculated at 10% (w/w), 28 °C for 36–48 h in a 15 L bioreactor with 0.8 vvm of aeration (the ratio of the air volumetric flow rate in liters per minute to the feedstock volume in liters).

Fermentation Operations. Simultaneous saccharification and ethanol fermentation (SSF for ethanol, without xylose utilization) was carried out using the pretreated and biodetoxified feedstocks at pH 5.5 in the 5 L helical agitated bioreactor.¹⁷ After 12 h prehydrolysis at 50 °C, *S. cerevisiae* DQ1 was inoculated into the slurry at 10% (v/v) after the adaptation step and SSF was maintained at 37 °C for 60 h.²⁴

Simultaneous saccharification and ethanol cofermentation (SSCF for ethanol, with xylose utilization) was carried out under similar conditions. After 12 h prehydrolysis at 50 °C, the xylose-utilizing *S. cerevisiae* XH7 was inoculated at 20% (v/v) ratio after short adaption into the slurry, and the SSCF was performed at 30 °C for 96 h. A short adaption before inoculation was carried out using the pretreated corn stover as the carbon source.²⁸

Simultaneous saccharification and L-lactic acid fermentation (SSF for L-lactic acid, without xylose utilization) was carried out using the pretreated and biodetoxified corn stover at pH 5.5 by 5 M NaOH solution in the 5 L bioreactor.²⁹ After 6 h prehydrolysis at 50 °C, *P. acidilactici* TY112 was inoculated at 10% (v/v) inoculum and SSF was lasted for 72 h at 42 °C. When the original feedstock (without washing) was used, 10 g/L of yeast extract, 10 g/L of peptone, 2 g/L of diammonium hydrogen citrate, and 0.23 g/L of MnSO_4 were added. When the thoroughly washed corn stover was used, 10 g/L of yeast extract, 10 g/L of peptone, 2 g/L of diammonium hydrogen citrate, 0.28 g/L of MgSO_4 , 2 g/L of K_2HPO_4 , and 0.23 g/L of MnSO_4 were added. One vial of *P. acidilactici* TY112 stock was inoculated into the 20 mL seeding medium and cultured at 42 °C for 12 h, then 10% (v/v) of the culture was added into the fresh seed medium at 42 °C for 5 h before the final inoculation into the corn stover hydrolysate.

Base pH Approaching Adjustment of Lignocellulose Feedstock. A suspension slurry was prepared by mixing 2 g of dry feedstock with 100 mL of deionized water in a 250 mL flask and, then, shaken vigorously at 30 °C for 30 min, and the pH value of the slurry was measured by pH sensor. When the suspension slurry was prepared from the thoroughly washed feedstock, sulfuric acid was added at the base acid usage and shaken vigorously at 30 °C for 30 min; the pH value of the slurry was measured as the “base pH value” for this feedstock.

After the measurement of the base pH value of the specific feedstock, the suspension slurry of the same feedstock containing

Table 1. Composition of Dry Acid Pretreated Corn Stover Containing Different Ash^a

ash (%)	cellulose (%)	xylan (%)	glucose (mg/g DM)	xylose (mg/g DM)	o-glu ^b (mg/g DM)	o-xyl ^c (mg/g DM)	acetate (mg/g DM)	HMF (mg/g DM)	furfural (mg/g DM)
(A) Pretreated Corn Stover under the Constant Sulfuric Acid Usage (20 mg Sulfuric Acid per g Dry Corn Stover)									
3.26	38.85 ± 1.49	3.62 ± 0.05	19.54 ± 0.24	137.11 ± 0.54	11.18 ± 1.19	62.69 ± 3.59	17.88 ± 1.33	1.85 ± 0.01	1.89 ± 0.15
4.33	37.54 ± 0.48	4.79 ± 0.23	16.30 ± 0.37	96.44 ± 1.79	20.70 ± 3.35	88.84 ± 6.24	13.98 ± 0.82	1.97 ± 0.09	1.56 ± 0.02
4.63	37.19 ± 0.50	5.76 ± 0.01	15.87 ± 0.72	94.62 ± 2.03	20.82 ± 1.53	90.02 ± 4.52	13.45 ± 0.02	1.92 ± 0.07	1.65 ± 0.03
5.30	36.64 ± 0.56	6.23 ± 0.12	16.45 ± 0.25	91.59 ± 1.08	20.75 ± 1.77	92.85 ± 4.87	12.06 ± 1.94	2.42 ± 0.03	1.47 ± 0.09
6.65	33.08 ± 0.35	9.01 ± 0.30	19.83 ± 0.16	46.96 ± 1.17	27.08 ± 0.87	95.05 ± 2.01	10.56 ± 0.24	3.54 ± 0.11	0.82 ± 0.02
(B) Pretreated Corn Stover under the Adjusted Sulfuric Acid Usage									
3.26	38.85 ± 1.49	3.62 ± 0.05	19.54 ± 0.24	137.11 ± 0.54	11.18 ± 1.19	62.69 ± 3.59	17.88 ± 1.33	1.85 ± 0.01	1.89 ± 0.15
4.33	38.05 ± 0.95	3.29 ± 0.09	26.37 ± 0.24	124.58 ± 1.47	14.72 ± 0.13	56.93 ± 3.67	16.85 ± 0.78	3.59 ± 0.01	2.92 ± 0.01
4.63	37.03 ± 0.47	3.05 ± 0.15	28.63 ± 0.03	124.87 ± 2.23	13.70 ± 0.44	52.66 ± 0.31	19.43 ± 0.08	4.18 ± 0.12	3.16 ± 0.13
5.30	36.44 ± 0.22	3.18 ± 0.03	32.45 ± 0.20	124.31 ± 1.96	16.53 ± 2.93	50.26 ± 3.96	19.90 ± 0.53	5.76 ± 0.14	3.42 ± 0.03
6.65	33.17 ± 0.25	3.09 ± 0.22	35.70 ± 0.47	116.66 ± 1.67	16.37 ± 2.82	50.02 ± 2.55	18.25 ± 0.20	7.28 ± 0.03	3.09 ± 0.02
(C) Pretreated Corn Stover under the Constant Sulfuric Acid Usage (20 mg Sulfuric Acid per g Dry Corn Stover) after Soluble Salts Addition									
3.26 ^d	37.80 ± 0.79	3.44 ± 0.09	18.06 ± 0.35	131.07 ± 1.10	14.64 ± 2.99	58.53 ± 2.43	18.35 ± 0.72	1.91 ± 0.04	2.06 ± 0.08

^aCorn stover was washed by 0, 10, 30, 50, 200 times (w/w) water to the samples with different ash content, then pressed and dried until the constant weight. The experiments were carried out in duplicate, and the data used here was taken from average of two parallel experiments. ^bOligomer of glucan. ^cOligomer of xylan. ^d34.31 mg/g DS KCl, 1.85 mg/g DS Na₂SO₄, and 9.02 mg/g DS MgSO₄ were added into the thoroughly washed corn stover, and pretreatment was conducted at 175 °C with 20.0 mg/g DM sulfuric acid usage for 5 min.

different ash was titrated by sulfuric acid to the base pH value. More sulfuric acid was used than the base acid usage due to the neutralization of ash. This adjusted sulfuric acid usage (mg H₂SO₄/g dry feedstock) was applied to the practical dry acid pretreatment. The block diagram of the Base pH Approaching method was shown in Figure 9.

Analysis of Sugars, Ethanol, L-Lactic Acid, and Inhibitors. Cellulose and hemicellulose contents were determined using a two-step sulfuric acid hydrolysis method.¹⁵ Oligomers of glucan and xylan were measured according to the one-step sulfuric acid hydrolysis method.³⁰ Glucose, xylose, ethanol, lactic acid, acetic acid, furfural, and HMF were measured using HPLC (LC-20AD pump, RID-10A detector, Shimadzu, Kyoto, Japan) with a Bio-Rad Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 65 °C and 0.6 mL/min of 5 mM H₂SO₄ solution.

Analysis of Element Composition. Metal ions contents were measured by inductively coupled plasma atomic emission spectrometry (725 ICP-OES, Agilent, CA, USA) using the simultaneous CCD detector at 1.2 kW, 15 L/min of plasma gas flow, 1.5 L/min of auxiliary gas flow, 0.75 L/min of nebulizer flow, 15 rpm of pump speed, 35 s of sample delay time, and 10 s of stabilization time. The samples were prepared by mixing 0.1 g of the ions containing solids or hydrolysates with 3 mL of nitric acid and 1 mL of perchloric acid, then boiled on an electric furnace for 4 h. All the liquid was then transferred into a flask and diluted with deionized water to 25 mL before used for detection.

The Cl⁻ and PO₄³⁻ contents were measured by ion chromatography (Dionex ICS-1100, Thermo Fisher Scientific, MA) consisting of a conductivity detector (AERS 500 (4 mm), recycle mode), a IonPac AS11 analytical column (4 × 250 mm), and a IonPac AG11 Guard (4 × 50 mm) (Thermo Fisher Scientific) at 30 °C and the eluent flow rate of 1.0 mL/min at the following gradient: 10 mM KOH solution for 12 min, then switched to 25 mM KOH solution in 6 s and held for 12 min, and then again switched to 10 mM KOH solution in 0.1 min and held for 6 min.

RESULTS AND DISCUSSION

Fluctuating Pretreatment Efficiency Caused by the Varied Ash Content in Corn Stover. To quantitatively assess the impact of ash content in corn stover on pretreatment efficiency, the ash was removed from the raw corn stover feedstock using different amounts of fresh water (0, 10, 30, 50, 200 times, w/w, based on the raw corn stover) to generate an ash content gradient from 3.26%, 4.33%, 4.63%, 5.30%, to

6.65% (w/w). The higher ash content led to the significant increase of the residual xylan and xylo-oligomer content (Table 1A), and the decrease of cellulose conversion yield (Figure 1A). The ethanol fermentation assay was conducted by the method of simultaneous saccharification and fermentation (SSF) by *S. cerevisiae* DQ1 (without xylose utilization) at the same cellulose content (11.7%, w/w). As the ash content increased from 3.26% to 6.65%, the initial glucose generation in the prehydrolysis decreased from 112.2 to 81.6 g/L and the ethanol generation in the SSF decreased from 67.1 to 50.7 g/L (Figure 1B). The results clearly revealed that the high ash content in the feedstock resulted in the sharp decrease of pretreatment efficiency indicated by the hydrolysis yield and ethanol fermentation.

The reasons for the reduced pretreatment efficiency were investigated. First, the impact of the soluble cations in the ash on pretreatment efficiency was assayed (Table 2). The soluble salts of KCl, Na₂SO₄, MgSO₄ were added into the thoroughly washed corn stover (31.56 mg of KCl, 1.71 mg of Na₂SO₄, and 13.88 mg of MgSO₄ per gram of dry feedstock) to reach the equivalent cation content in the raw corn stover feedstock. The soluble salts addition did not change the pH value of the feedstock because of the neutral property of the salts added. The thoroughly washed corn stover after the addition of soluble salts was dry acid pretreated under the same operation conditions (20 mg/g of sulfuric acid, 175 °C for 5 min) and the pretreatment efficiency was found to be essentially the same with that of the thoroughly washed feedstock without salts addition (92.82% ± 1.87% with the salts addition vs 93.29% ± 1.17% without salts addition). The composition and inhibitor generation were also essentially the same between the two pretreated feedstocks (Table 1). The results indicate that the existence of the soluble ions (K⁺, Na⁺, Mg²⁺) in the feedstock was not the direct reason for the changing pretreatment efficiency, when the pH value of the feedstocks remained the same.

The second examined factor is the existence of anions such as carbonate, oxide and hydroxide in the feedstocks because of their strong pH buffer capacity.^{7,31} The pH values of the slurries prepared from corn stover feedstocks containing

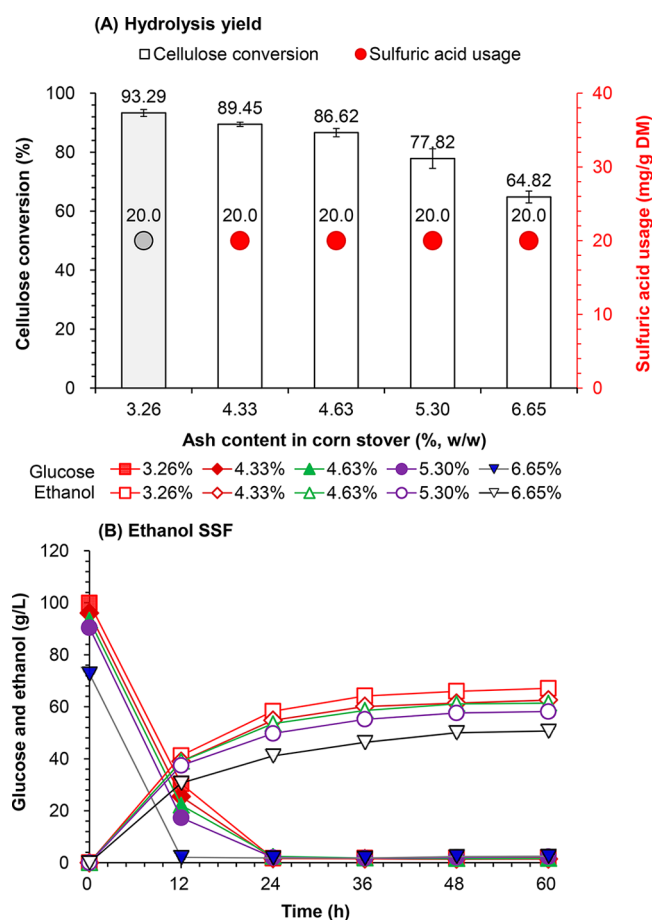


Figure 1. Pretreatment efficiency assay of corn stover feedstocks containing different ash under constant sulfuric acid usage (20 mg/g DM). (A) Hydrolysis yield of the pretreated corn stover; (B) SSF of the pretreated and biodegraded corn stover. The SSF was conducted at 11.7% (w/w) of cellulose content (w/w), 14 mg cellulase protein/g cellulose, 50 °C, pH 5.5 for 12 h in the prehydrolysis stage, and 37 °C, pH 5.5 for 60 h by *S. cerevisiae* DQ1 (without xylose utilization) in the SSF stage. The experiments of enzymatic hydrolysis were carried out in duplicate and the data used here was taken from the average of two parallel experiments.

different ash were almost the same (6.65–6.83), but the addition of sulfuric acid at the base acid usage (20 mg/g DM) led to the different final pH values from 2.30 to 3.21 due to the different neutralization capacity of such anions compounds in ash (Figure 2). The higher ash content led to the higher sulfuric acid loss and the higher pH value of the feedstock slurry, and finally led to the decreased pretreatment efficiency (Figure 3 and Table S1).

Equal acid catalyst content after neutralization by ash is crucially important for maintaining the constant pretreatment efficiency. Therefore, the acid catalyst usage for the feedstocks containing different ash must be readjusted before pretreatment to compensate for the acid loss in neutralization. However, the adjustment of pretreatment parameters by practical pretreatment experimentation is complicated and time-consuming, thus it is not applicable to respond quickly to the change of ash content in the feedstocks. A simple, fast, and universally applicable method is required to calibrate the fluctuation of pretreatment efficiency.

Acquisition of Pretreatment Efficiency Repeatability by Acid Usage Adjustment. A quick and simple acid usage

Table 2. Soluble Cation and Anion Contents in the Dry Acid Pretreated Corn Stover Containing Different Ash

cation contents		ash in hydrolysate (mg/L)		ash equivalent in corn stover (mg/g DM)	
		raw feedstock	thoroughly washed feedstock	raw feedstock	thoroughly washed feedstock
K ⁺ Mg ²⁺ Na ⁺ Al ³⁺ Cu ²⁺ Fe ³⁺ Mn ²⁺ Zn ²⁺	K ⁺	7600	500	17.73	1.17
	Mg ²⁺	1800	610	4.2	1.42
	Na ⁺	270	32	0.63	0.075
	Al ³⁺	10	6	0.023	0.014
	Cu ²⁺	<5	<5	<0.012	<0.012
	Fe ³⁺	40	36	0.093	0.084
	Mn ²⁺	15	12	0.035	0.028
	Zn ²⁺	3.5	3.5	0.008	0.008
Anion contents	Cl ⁻	5051.7	176	11.79	0.41
	PO ₄ ³⁻	217.9	191	0.51	0.45

^aDry acid pretreated and biodegraded corn stover was hydrolyzed at 30% (w/w) solids loading, 14 mg cellulase protein/g cellulose for 48 h at 50 °C. The water-insoluble solids were removed by centrifugation at 10 000 rpm for 10 min, and the supernatant was obtained to detect the ions content.

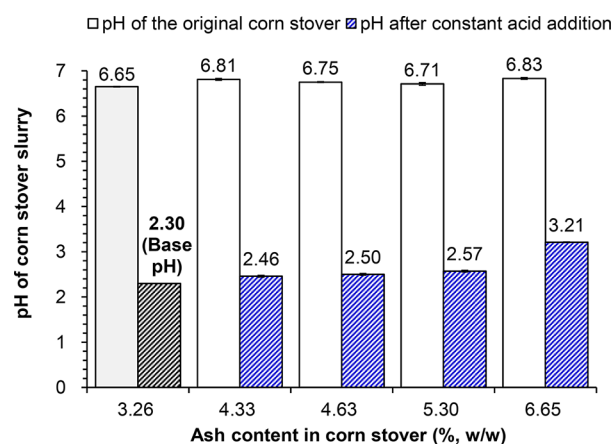


Figure 2. pH change of corn stover suspension slurry before and after sulfuric acid addition. The corn stover suspension slurry was prepared by mixing 2 g of dry feedstock with 100 mL of deionized water in 250 mL flask, shaking vigorously at 30 °C for 30 min, and measuring the pH value of the slurry by pH sensor. Sulfuric acid was then added into the slurries at the dosage of base acid usage (20 mg H₂SO₄/g DM) and shaken vigorously again at 30 °C for 30 min. The pH value of the slurry was measured by pH sensor and the pH value for the thoroughly washed feedstock (3.26% of ash, w/w) was defined as the base pH value. The experiments were carried out in triplicate, and the data used here was taken from the average of three parallel experiments.

adjustment method without operating the practical pretreatment experiment is an ideal solution. Here we proposed such a sulfuric acid usage adjustment method on the raw feedstock to compensate for the acid loss by ash neutralization before pretreatment operation.

The maximum pretreatment efficiency for the specific feedstock should be defined in the early stage of biorefining experiments. For the corn stover feedstock used in this work, the ash was thoroughly washed to the minimum (3.26%, w/w) and the sulfuric acid usage in dry acid pretreatment was at the minimum level (20 mg/g DM) considering both the hydrolysis yield and inhibitor generation (Figure 3 and Table S1). This

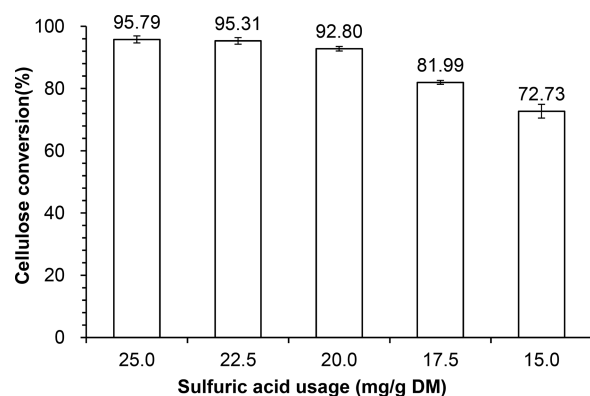


Figure 3. Impact of sulfuric acid usage on pretreatment efficiency in dry acid pretreatment. Thoroughly washed corn stover was pretreated at 175 °C for 5 min under different sulfuric acid usages. The experiments were carried out in duplicate, and the data used here was taken from the average of two parallel experiments.

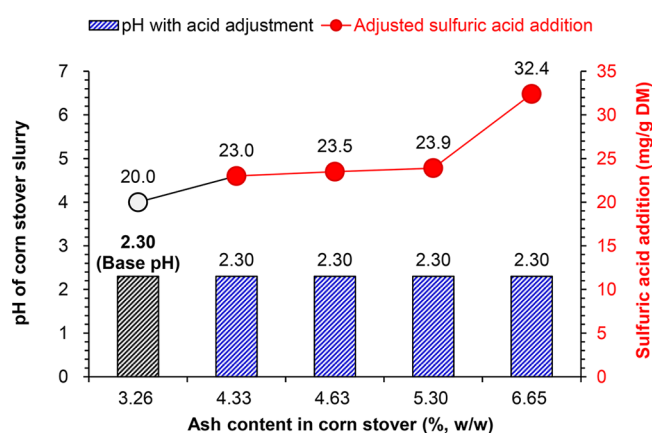


Figure 4. pH approach to the base pH value of corn stover by adjusting sulfuric acid usage. The corn stover suspension slurry was prepared by mixing 2 g of dry feedstock with 100 mL of deionized water in 250 mL flask and, then, shaking vigorously at 30 °C for 30 min. Sulfuric acid was then added into the slurries at the dosage of base acid usage (20 mg H₂SO₄/g DM) and shaken vigorously again at 30 °C for 30 min. The pH value for the thoroughly washed feedstock (3.26% of ash, w/w) was defined as the base pH value. The suspension slurry of the raw feedstock containing different ash was titrated by sulfuric acid until the final pH value of the suspension slurry was equal to the base pH value. The adjusted sulfuric acid usage (mg H₂SO₄ per gram of dry feedstock) was recorded for each feedstock. The experiments were carried out in triplicate and the data used here was taken from the average of three parallel experiments.

minimum sulfuric acid usage was defined as the base acid usage for the corn stover feedstock used and the pretreatment efficiency of the thoroughly washed corn stover at the base acid usage was used as the control.

On the step for correcting the fluctuation of pretreatment efficiency, the suspension slurries were prepared from the thoroughly washed corn stover with addition of sulfuric acid at the base acid usage (20 mg/g DM). The corresponding pH value of the slurry was measured as 2.30 (the base pH value for the corn stover feedstock used) (Figure 2). We predicted that the gap of pretreatment efficiency could be filled by adding more sulfuric acid to neutralize the ash components until the pH value of the corn stover slurries containing different ash was equal to the base pH value (base pH approaching). With the corresponding adjusted sulfuric acid usage, the effective sulfuric

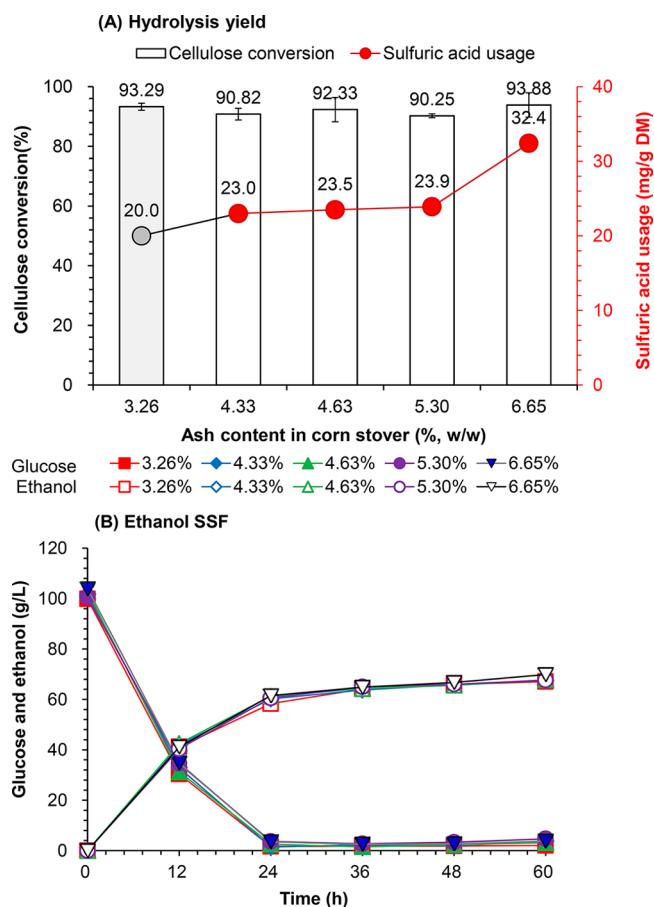


Figure 5. Pretreatment efficiency assay of corn stover feedstocks containing different ash under adjusted sulfuric acid usage. (A) Hydrolysis yield of the pretreated corn stover. (B) SSF evaluation of the pretreated and biodegraded corn stover. The SSF was conducted at 11.7% (w/w) of cellulose content (w/w), 14 mg cellulase protein/g cellulose, 50 °C, and pH 5.5 for 12 h in the prehydrolysis stage and 37 °C and pH 5.5 for 60 h by *S. cerevisiae* DQ1 (without xylose utilization) in the SSF stage. The experiments of enzymatic hydrolysis were carried out in duplicate, and the data used here was taken from the average of two parallel experiments.

acid usage for the feedstocks containing different ash was equivalent to the base acid usage for the thoroughly washed feedstock. The sulfates formed in the neutralization were neutral and would have negligible effect on pretreatment efficiency (Table 1).

In this study, the adjusted sulfuric acid usage for the corn stover feedstock containing 4.33, 4.63, 5.30, and 6.65% (w/w) of ash was 23.0, 23.5, 23.9, and 32.4 mg sulfuric acid per gram of dry feedstock, respectively, to render the pH value of the feedstock slurry to the base pH value (2.30) (Figure 4). Exactly as we predicted, the pretreated corn stover containing different ash after acid usage adjustment was essentially the same, either in the hydrolysis yield (Figure 5A) or in the ethanol fermentation yield (Figure 5B). The residual xylan content, xylose/xylo-oligomer ratio, acetic acid, and furfural content in the pretreated corn stover containing different ash amounts were also very close, except that HMF and furfural increased with the increasing ash content (Table 1B) due to the direct conversion from the water-soluble sugars in the raw corn stover feedstock.^{32,33} However, HMF was completely biodegraded

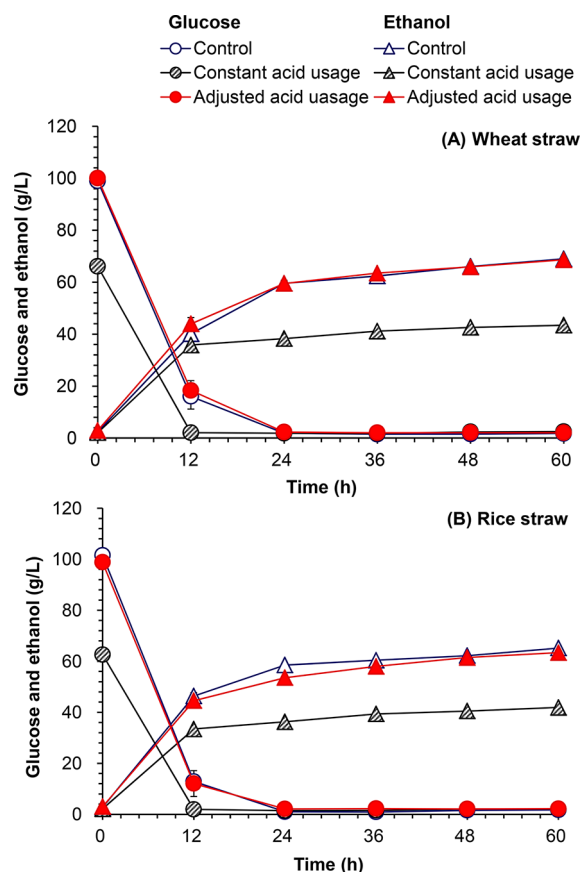


Figure 6. Application of the base pH approaching method on extension of lignocellulose feedstocks (wheat straw and rice straw). (A) Wheat straw. (B) Rice straw. (control) Thoroughly washed wheat straw and rice straw were pretreated at 175 °C for 5 min with the base acid usage (20 mg H₂SO₄/g DM). (constant acid usage) Raw feedstocks with 20 mg H₂SO₄/g DM. (adjusted acid usage) Raw feedstocks with adjusted acid usage. The SSF was conducted at the cellulose content of 11.4% (w/w) for wheat straw and 10.9% (w/w) for rice straw at the 14 mg cellulase protein/g cellulose, 50 °C and pH 5.5 for 12 h in the prehydrolysis stage and 37 °C and pH 5.5 for 60 h by *S. cerevisiae* DQ1 (without xylose utilization) in the SSF stage. The experiments were carried out in duplicate, and the data used here was taken from the average of two parallel experiments.

and no difference in HMF content was found in the consequent bioconversions.

The results suggest that the adjustment of sulfuric acid usage by the base pH approaching method successfully compensated for the sulfuric acid loss as well as declined efficiencies of pretreatment and bioconversion.

Expanding the Base pH Approaching Method to Different Feedstocks and Fermentations. The base pH approaching method was found to be highly effective for correcting the fluctuation of pretreatment efficiency of corn stover feedstock and elevating the consequent ethanol fermentation performance. Here we further evaluated the effectiveness of the method in several typical biorefining cases.

The first case is the extension of feedstock options from corn stover to different feedstock types (wheat straw and rice straw). The ash in raw feedstocks was washed thoroughly from 10.04% to 5.87% for wheat straw, and from 11.69% to 8.06% for rice straw, respectively. The base acid usage for the thoroughly washed wheat straw and rice straw were 20 mg sulfuric acid per gram of dry feedstock according to our previous study,¹⁹ and

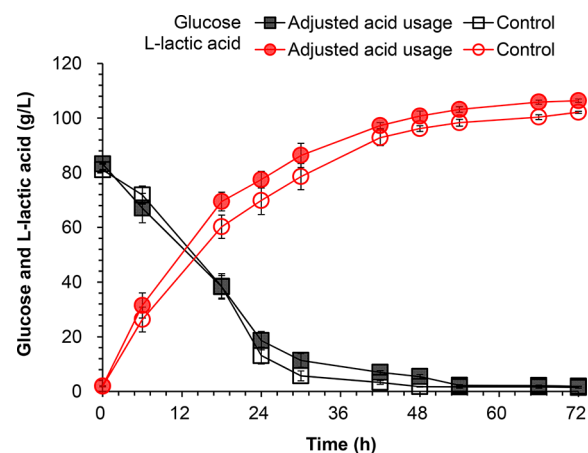


Figure 7. Application of the base pH approaching method on extension of fermenting strain and product (the engineered lactic acid bacterium and L-lactic acid). (adjusted acid usage) Raw corn stover with high ash content of 6.65% (w/w) (without washing and other deashing operation) was pretreated with the adjusted sulfuric acid usage of 32.4 mg/g DM. (control) Thoroughly washed corn stover with ash content of 3.26% (w/w) was pretreated with the base sulfuric acid usage of 20.0 mg/g DM. The SSF was conducted using the pretreated and biodetoxified corn stover at 11.7% (w/w) of cellulose content, 14 mg cellulase protein/g cellulose, 50 °C, and pH 5.5 for 6 h in the prehydrolysis stage and 42 °C and pH 5.5 for 72 h by the engineered LAB strain *P. acidilactici* TY112 in the SSF stage. The experiments were carried out in duplicate, and the data used here was taken from the average of two parallel experiments.

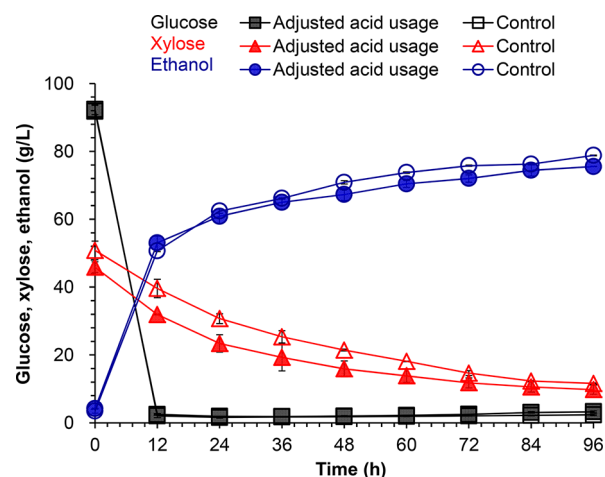


Figure 8. Application of the base pH approaching method on extension of fermentation operation type (SSCF with xylose utilization for ethanol). (adjusted acid usage) Raw corn stover with high ash content of 6.65% (w/w) (without washing and other deashing operation) was pretreated with the adjusted sulfuric acid usage of 32.4 mg/g DM. (control) Thoroughly washed corn stover with ash content of 3.26% (w/w) was pretreated with the base sulfuric acid usage of 20.0 mg/g DM. The SSCF was conducted using the pretreated and biodetoxified corn stover at 11.7% (w/w) of cellulose content (w/w), 14 mg cellulase protein/g cellulose, 50 °C, and pH 5.5 for 12 h in the prehydrolysis stage and 30 °C and pH 5.5 for 96 h by *S. cerevisiae* XH7 (with xylose utilization) in the SSCF stage. The experiments were carried out in duplicate, and the data used here was taken from the average of two parallel experiments.

the base pH values were measured as 2.33 for wheat straw and 2.29 for rice straw. The sulfuric acid usage was 33.3 mg/g DM for wheat straw and 31.3 mg/g DM for rice straw to approach

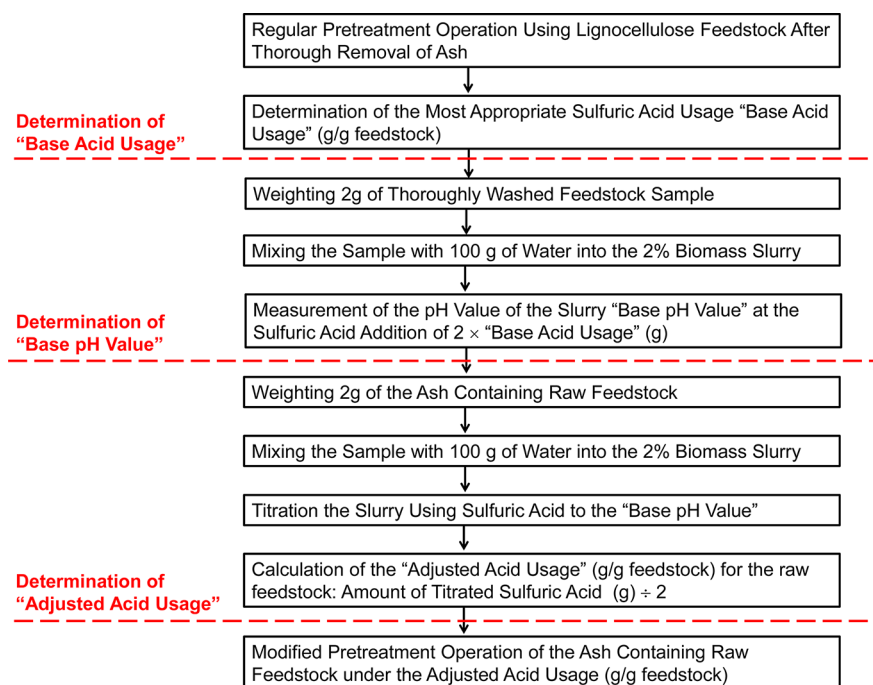


Figure 9. Diagram of the base pH approaching method on correcting fluctuation of pretreatment efficiency.

their corresponding base pH value and the adjusted acid usages were used in dry acid pretreatment for these two raw feedstocks. The residual xylan and xylo-oligomer contents, as well as the acetic acid and furfural formation in the pretreated wheat straw and rice straw containing different ash were very close to the control (thoroughly washed feedstocks with base acid usage) (Table S2). The ethanol fermentation evaluation shows the very close final ethanol titers for the acid usage adjusted feedstock and the control: 68.6 vs 69.0 g/L for wheat straw (Figure 6A) and 63.4 vs 65.2 g/L for rice straw (Figure 6B). The ethanol titers of the feedstocks after acid usage adjustment were all significantly greater than those without acid usage adjustment (Figure 6).

The second case is the extension of ethanol fermentation to L-lactic acid fermentation from the ash containing corn stover. Similar to ethanol fermentation, 32.4 mg/g DM of sulfuric acid was used for raw corn stover in dry acid pretreatment. The pretreated and biodetoxified corn stover feedstock was simultaneously saccharified and fermented to L-lactic acid by *P. acidilactici* TY112 at the same cellulose content (11.66%, w/w). L-Lactic acid fermentation using the high ash containing corn stover after acid usage adjustment (106.4 g/L) was close to that using the thoroughly washed corn stover (102.1 g/L) (Figure 7).

The third case is the extension to cofermentation of xylose to ethanol from the ash containing corn stover. Again, 32.4 mg/g DM of sulfuric acid was used for raw corn stover in dry acid pretreatment. The pretreated and biodetoxified corn stover was sent for simultaneous saccharification and cofermentation (SSCF) by a xylose utilizing strain *S. cerevisiae* XH7 at the same cellulose content (11.66%, w/w). The initial glucose (91.8 g/L) and xylose (45.9 g/L) in prehydrolysis, and the final ethanol generation (76.9 g/L) in SSCF were essentially the same to those using the thoroughly washed corn stover (92.4, 50.8, and 80.1 g/L), respectively (Figure 8). The conversion trends on glucose and xylose to ethanol were very close during the SSCF.

These application cases suggest that the base pH approaching method is generally effective for high ash containing raw feedstocks, including for different feedstock types (corn stover, wheat straw, or rice straw), different fermenting strains (yeasts or bacteria), different fermentation products (ethanol and lactic acid), and different fermentation types (SSF or SSCF).

CONCLUSION

Poor repeatability of acid based pretreatment efficiency was found to be determined by the changing ash content in the feedstock. A base pH approaching method was proposed to correct the varied pretreatment efficiency by performing a simple titration of ash containing feedstock suspension slurry to an inherent base pH value (Figure 9). The method could be generally applicable to different feedstocks (corn stover, wheat straw, or rice straw) for production of different products (ethanol and lactic acid) at different fermentation types (SSF or SSCF). This study provided an important and practical approach to maintain stable pretreatment efficiency in industrial applications.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.7b04601.

Impact of the effective acid usage on pretreatment efficiency and composition of dry acid pretreated wheat straw and rice straw (PDF)

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was partially supported by the National Basic Research Program of China (2011CB707406).

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