JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

Cite This: J. Agric. Food Chem. 2018, 66, 10225−10232

pubs.acs.org/JAFC

An Approach of Utilizing Water-Soluble Carbohydrates in Lignocellulose Feedstock for Promotion of Cellulosic L-Lactic Acid Production

Xushen Han,[†] Feng Hong,[‡] Gang Liu,*[,†] and Jie Bao*[,†]

[†]State Key Laboratory of Bioreactor Engineering, East China University of Science and Technology, 130 Meilong Road, Shanghai 200237, China

[‡]College of Chemistry, Chemical Engineering and Biotechnology, Donghua University, Shanghai 201620, China

Supporting Information

ABSTRACT: Agricultural lignocellulose biomass generally contains certain amounts of water-soluble carbohydrates (WSC) such as glucose, fructose, or sucrose. These sugars are generally degraded in pretreatment at high temperature or discharged with wastewater in a detoxification process. This study proposed an approach of utilizing frequently ignored water-soluble carbohydrates for promotion of cellulosic L-lactic acid production. A simple solid state fermentation was performed during a corn stover storage period to convert the sugars into L-lactic acid and then a dry biorefining technology was applied to convert cellulose and hemicellulose fractions into the same L-lactic acid product. The 5-hydroxymethylfurfural (HMF) formation in pretreatment was significantly reduced and the consequent biodetoxification time was shortened. L-Lactic acid production was increased from 130.2 g/L to 139.0 g/L, and the minimum L-lactic acid selling price was reduced by 5.9%. This study provided an important option of biorefinery processing technology for production of value added biochemicals.

KEYWORDS: *lignocellulose, water-soluble carbohydrates, L-lactic acid, solid state fermentation, simultaneous saccharification and cofermentation, techno-economic analysis*

INTRODUCTION

Agricultural residues contain more or less water-soluble carbohydrates (WSC) such as glucose, fructose, or sucrose in the stems and leaves for buffering the grain filling rate.[1] In corn stover, water-soluble carbohydrates account for around 6% of dry feedstock[2] and vary in species, anatomical fractions (cobs, husks, leaves, and stalks), and crop maturity.[1,3−5] As typical fermentable sugars, the utilization of them in lignocellulose biorefinery is frequently ignored, since they are difficult to be preserved after the first pretreatment step. In acid based pretreatments such as dilute acid, steam explosion, and liquid hot water,[6−10] they are easily degraded to 5-hydroxymethylfurfural (HMF)[11] and then negatively affect the consequent hydrolysis yield and fermentability.[12,13] In other pretreatments such as dilute alkaline, organosolv, or ionic liquid, the washing step after pretreatment generates a large quantity of wastewater and most of the water-soluble carbohydrates are washed away.[6,14]

Extraction of the carbohydrates from corn stover is not feasible because the very low sugar titer in the liquid is hard to be utilized cost-effectively. Direct conversion of the sugars in corn stover should meet several concerns: (1) Conversion of water-soluble carbohydrates should be conducted before pretreatment to avoid the degradation of WSC; (2) Wastewater generation should be avoided before the product recovery step to prevent the loss of sugars or products; (3) The converted product from water-soluble carbohydrates should be the same with the major product from cellulose and hemicellulose fractions, or easily separated from the major biorefinery product.

L-Lactic acid is an important monomer chemical for production of biodegradable poly lactic acid (PLA) plastic.[15,16] Several efforts have been made to produce L-lactic acid from lignocellulose feedstocks,[14,17−19] while these studies did not concern the utilization of water-soluble carbohydrates in lignocellulose feedstock. Here we first performed a solid state fermentation during the storage period of corn stover with bioconversion of water-soluble glucose and fructose to L-lactic acid, which was easy and feasible to perform in processes such as ensilage.[20,21] The cellulose and hemicellulose fractions of corn stover were converted to the same L-lactic acid product by dry biorefining technology, in which no wastewater was discharged in dry acid pretreatment, biodetoxification, and simultaneous saccharification and cofermentation (SSCF).

In such a process, the product generated from the water-soluble carbohydrates could be almost completely preserved in pretreatment and biodetoxification and added into the cellulosic L-lactic acid production process, leading to the higher product titer and lower purification cost after SSCF. The biodetoxification period was significantly shortened due to the reduced HMF formation. Also, the cost generated in the solid state fermentation process was relatively low. The techno-economic analysis was performed based on rigorous Aspen Plus process simulation to evaluate and show the improved production economy and decreased minimum L-lactic acid

Received: July 9, 2018
Revised: September 11, 2018
Accepted: September 12, 2018
Published: September 12, 2018

DOI: 10.1021/acs.jafc.8b03592
J. Agric. Food Chem. 2018, 66, 10225−10232

selling price (MLSP) at commercial scale. This study provided a simple, practical, and economic approach to promote cellulosic L-lactic acid production by utilization of water-soluble carbohydrates in lignocellulose feedstocks.

MATERIALS AND METHODS

Raw Materials. Corn stover was harvested from Tongliao, Inner Mongolia, China, in the Fall of 2016. The harvested corn stover was sun-dried and milled using a hammer crusher to pass through the 10 mm (diameter) apertures, and then it was sealed in plastic bags and stored at room temperature until use. The corn stover contained 31.2% of cellulose, 22.3% of xylan, 20.8% of lignin, and 6.2% of ash by weight percentage determined according to NREL protocols LAP-002 and LAP-005.[22,23] Water-soluble carbohydrates in corn stover were extracted by mixing 5 g of corn stover with 100 mL distilled water and shaking vigorously in a 250 mL flask at 30 °C for 1 h, and determined to be 21.9 mg/g of glucose and 20.8 mg/g of fructose on a dry basis. Other water-soluble carbohydrates were negligible in this study due to their low content in corn stover.

Strains and Media. *Pediococcus acidilactici* TY112 was stored in China General Microorganisms Collection Center (CGMCC 8664), Beijing, China, and used for bioconversion of water-soluble carbohydrates into L-lactic acid by solid state fermentation.[19]

Pediococcus acidilactici ZY271 was also stored in CGMCC with the number of 13611 and used for simultaneous saccharification and cofermentation of corn stover feedstock (SSCF) into L-lactic acid (with xylose utilization).[18]

The simplified MRS medium used for seed culture of the two strains 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 $MnSO_4$. In the seed culture step, one vial of the stock was inoculated into 20 mL of the seed medium and cultured for 12 h at 42 °C and then inoculated at 10% (v/v) inoculum into the fresh seed medium for 5 h at 42 °C before the final inoculation for solid state fermentation and SSCF, respectively. 1% (v/v) glucoamylase solution was added into the seeds culture to prevent cells flocculation.[17]

Amorphotheca resinae ZN1 was stored in CGMCC with the registration number of 7452 and used for biodetoxification.[24] *A. resinae* ZN1 was maintained on potato-dextrose-agar (PDA) slant prepared by boiling 200 g of peeled and sliced potatoes in one liter of deionized water for 30 min with the addition of 20 g of glucose and 15 g of agar.

Enzyme and Reagents. Commercial cellulase Cellic CTec 2.0 was purchased from Novozymes, Beijing, China. The filter paper activity was determined by NREL protocol LAP-006,[25] the cellobiase activity was assayed by the method of Ghose,[26] and the protein concentration was determined by the Bradford method.[27] Glucoamylase GA-L NEW was purchased from Genencor, Wuxi, China and the enzymatic activity was 103900 WU/mL.

Glucose, $MgSO_4{\cdot}7H_2O$, diammonium hydrogen citrate, sodium acetate, $K_2HPO_4{\cdot}3H_2O$, $MnSO_4{\cdot}H_2O$, $Ca(OH)_2$, and H_2SO_4 were analytical pure and obtained from Lingfeng Chemical Reagent Co., Shanghai, China. Yeast extract and peptone were purchased from Oxoid Ltd., Basingstoke, Hampshire, UK.

Solid State Fermentation. The seed culture of *P. acidilactici* TY112 and fresh water were sprayed on corn stover feedstock and thoroughly mixed and then filled into airtight plastic bags, and the air was squeezed out for anaerobic solid state fermentation according to the silage production procedure.[20,21] Samples were withdrawn and mixed with deionized water into slurries at the solid−liquid ratio of 1:20 and shaken vigorously in a 250 mL flask at 30 °C for 1 h. The supernatant of the slurry was collected by centrifugation at 11,167*g* for 5 min and used for analysis of lactic acid and sugars.

The solids loading (33%, 50%, 67%), nutrients (with or without), inoculum (2.5%, 5%, 10%), and temperature (25 °C, 37 °C, 42 °C) of solid state fermentation were optimized in small plastic bags filled with 30 g wet materials. Samples were withdrawn from the sealed bags every day, and the opened bags were discarded. The fermentation time shown here was the minimum day to reach the constant content of lactic acid and residue sugars. The experiments were carried out in duplicate, and the data here was the average of two parallel experiments.

The solid state fermentation was then cost-effectively scaled up in large airtight plastic bags filled with 15 kg wet materials at 67% (w/w) solids loading, 2.5% (w/w) *P. acidilactici* TY112 inoculum, no nutrients addition, 25 °C for 6 days.

Dry Acid Pretreatment and Biodetoxification. Dry acid pretreatment of corn stover feedstock was conducted according to Zhang et al.[28] Briefly, the feedstock and sulfuric acid solution were fed into the 20 L pretreatment reactor and maintained for 5 min at 175 °C with helically stirring mixing of 50 rpm. The initial and final solids loading was 60% and 45% (w/w, dry base), respectively, with no wastewater generation. The pretreatment efficiency was assayed by measuring the hydrolysis yield according to NREL LAP-009.[29] Briefly, 2.5% (w/w) of the pretreated feedstock solids was hydrolyzed using 26 mg cellulase protein/g cellulose (20 FPU/g dry feedstock matter) at pH 4.8, 50 °C for 72 h. The experiments of pretreatment and hydrolysis assay were all carried out in duplicate.

The pretreated feedstock was biodetoxified to remove the inhibitors.[12,24] Briefly, the pretreated feedstock was neutralized using 20% (w/w) $Ca(OH)_2$ to pH 5−6 and then disk milled. *A. resinae* ZN1 was inoculated at 10% (w/w) of the solid feedstock and maintained for 48−72 h at 28 °C and 0.8 vvm of aeration in a 15 L bioreactor. No wastewater was generated from the biodetoxification operation.

Simultaneous Saccharification and L-Lactic Acid Cofermentation (SSCF). Simultaneous saccharification and L-lactic acid cofermentation (SSCF for L-lactic acid, with xylose utilization) was carried out in a 5 L helical agitated bioreactor using the pretreated and biodetoxified corn stover at 30% (w/w) solids loading, 5 mg cellulase protein/g DM.[18,30] After 6 h prehydrolysis at 50 °C, 150 rpm, SSCF was started with 10% (v/v) of *P. acidilactici* ZY271 inoculation and lasted for 72 h at 42 °C, pH 5.5, 150 rpm. The ingredients of the simplified MRS medium excluding glucose, sodium acetate, $MgSO_4$, and K_2HPO_4 were added. The pH was maintained by addition of 50% (w/w) $Ca(OH)_2$ slurry.

Analysis of Sugars, L-Lactic Acid, and Inhibitors. Cellulose and xylan contents were analyzed using a two-step sulfuric acid hydrolysis method.[23] Oligomers of glucan and xylan were measured by a one-step sulfuric acid hydrolysis method.[31] Sugars, lactic acid, and inhibitors were measured using HPLC (LC-20AD pump, RID-10A refractive index detector, Shimadzu, Kyoto, Japan) with a Bio-Rad Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) operated at the column temperature 65 °C and 0.6 mL/min of 5 mM H_2SO_4. The chiral purity of lactic acid was assayed using the D/L Lactic Acid Kit (Megazyme International, Bray, Wicklow, Ireland), and all assays were conducted in triplicate.

Process Model Establishment and Techno-economic Analysis. The techno-economic analysis in the study used the "*n*th-plant" economics for the evaluation at mature technology stage. The flow sheet model of the L-lactic acid production from corn stover was established on the Aspen plus platform (AspenTech Co., Cambridge, MA, USA) based on the NREL model[32] and our previous model of cellulosic L-lactic acid production.[17] The general flow sheet is shown in Figure S1 composed of ten process areas: feedstock handling (A100), pretreatment (A200), detoxification (A250), hydrolysis and fermentation (A300), cellulase enzyme production (A400), product recovery (A500), wastewater treatment (A600), storage (A700), combustor-boiler-turbogenerator (A800), and utilities (A900). The main parameters input for the model was based on the actual experiments in the area of solid state fermentation, dry acid pretreatment, biodetoxification, and simultaneous saccharification and L-lactic acid cofermentation. The operation parameters in other areas were cited from the literature[17] and the NREL report.[32] The main changes compared to the previous model were shown as follows:

(1) The sulfuric acid usage in dry acid pretreatment (A200) was increased from 2.5 to 3.7 g per 100 g dry biomass, because the ash content of this batch corn stover (6.2%, w/w) was much

DOI: 10.1021/acs.jafc.8b03592
J. Agric. Food Chem. 2018, 66, 10225−10232

Table 1. Components and Hydrolysis Yield of the Pretreated Feedstocks[a]

	Cellulose (%)	Xylan (%)	Oli-glu (mg/g DM)	Oli-xyl (mg/g DM)	Xylose (mg/g DM)	Acetate (mg/g DM)	Furfural (mg/g DM)	Hydrolysis yield (%)
Sugar free CS	36.43 ± 1.19	3.43 ± 0.18	26.31 ± 0.09	52.42 ± 4.77	145.95 ± 0.90	19.45 ± 0.12	3.92 ± 0.08	94.10 ± 0.88
Sugars resupplemented CS	35.65 ± 0.37	3.24 ± 0.12	23.47 ± 5.27	59.13 ± 3.38	134.10 ± 4.49	18.46 ± 0.60	3.79 ± 0.11	93.42 ± 3.34
Raw CS	33.31 ± 0.28	3.35 ± 0.09	11.88 ± 1.22	53.38 ± 4.20	115.38 ± 0.71	17.54 ± 1.57	3.77 ± 0.03	92.05 ± 0.46

[a]The composition was defined as milligrams components in the per gram dry material. The experiments were carried out in duplicate and the data used here was taken from the average of two parallel experiments. CS, corn stover. Oli-glu, oligomer of glucan. Oli-xyl, oligomer of xylan.

higher than the previous corn stover (3.5%, w/w), and more sulfuric acid was needed to neutralize the extra ash.[33−36]

(2) The operation time of biodetoxification (A250) was extended to 72 h because more inhibitors were generated in this batch of corn stover after pretreatment due to the higher water-soluble carbohydrates content compared to the previous batch.

(3) The pH regulator was changed from NaOH to $Ca(OH)_2$ for forming calcium salt (A300), which can obtain the L-lactic acid product easier. The enzyme loading of cellulase was decreased from 28 mg protein to 15 mg protein per g cellulose, while the L-lactic acid yield improved a lot based on the previous experiments.

(4) The biggest difference was in the product recovery (A500), in which electrodialysis was replaced by sulfuric acid hydrolysis. The fermented mash was heated to near boiling for improving solid−liquid separation efficiency, as well as sterilization. The heated fermentation mash was separated into fermented liquid and lignin solid residual with 35% moisture. After decolorization by activated carbon, the fermented liquid was concentrated to 30% calcium lactate solution by double effect evaporation. The crude L-lactic acid was obtained by sulfuric acid hydrolysis and solid−liquid separation. Then it was decolorized again and triple effect evaporated to obtain the industrial grade L-lactic acid product with a mass concentration of 88%. The overall L-lactic acid yield in the product recovery process was about 80%, the same as for the mature organic acid separation process.

The biorefining plant size was 900 tons processing capacity of corn stover each day (300,000 tons annually) with an annual operation of 8,000 h. To determine the minimum L-lactic acid selling price (MLSP, $/kg), a discounted cash flow rate of return (DCFROR) analysis was performed, which required a net present value of zero for 8% internal rate of return after taxes. The important assumptions for the DCFROR analysis are shown in Table S1. All costs were indexed to 2013 with the exchange rate from dollar to Chinese Yuan 6.2 in this analysis. The prices of pretreatment reactors, fermentation tanks, and helical agitators, chemical prices, and staff wages were calculated according to the actual situations of China. The raw feedstock composition and the cost of the main reactors and materials in the process are shown in Table S2, Table S3, and Table S4.

RESULTS AND DISCUSSION

Conversion of Water-Soluble Carbohydrates in Dry Acid Pretreatment. The loss of water-soluble carbohydrates in pretreatment was evaluated on several different corn stover feedstocks: (1) the sugar free corn stover (CS) by thoroughly water washing of raw corn stover (no glucose and fructose detected), (2) the sugars containing corn stover by resupplementing sugars onto the sugar free feedstock (21.9 mg/g of glucose and 20.8 mg/g of fructose), and (3) the raw corn stover (21.9 mg/g of glucose and 20.8 mg/g of fructose). Essentially the same pretreatment efficiency was acquired by the adjustment of "Base pH Approaching method",[36] with the similar cellulose hydrolysis yield (94.10% ± 0.88%, 93.42% ± 3.34%, and 92.05% ± 0.46%) and composition (cellulose, xylan, oligo-glucan, oligo-xylan, xylose, acetate, and furfural) of the three pretreated feedstocks (Table 1).

The major difference appeared on the content of glucose, fructose, and HMF (Figure 1). The pretreated sugar free corn

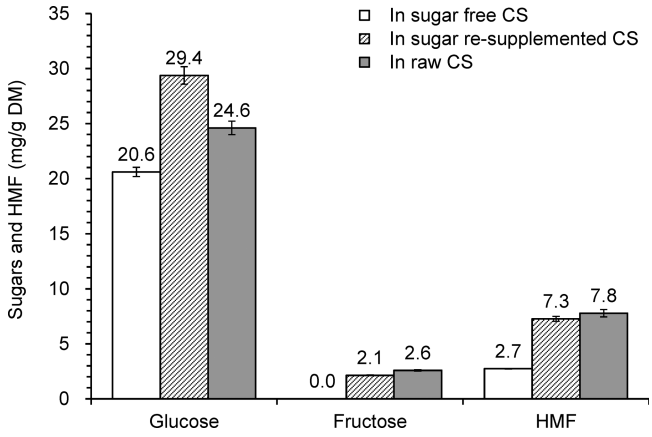

Figure 1. Loss of water-soluble glucose and fructose in dry acid pretreatment. Sugar free corn stover and sugar resupplemented corn stover were pretreated at 175 °C for 5 min with the acid usage of 25 mg H_2SO_4/g DM. Raw corn stover was pretreated at 175 °C for 5 min with the acid usage of 37 mg H_2SO_4/g DM according to the "Base pH Approaching method".[36] Glucose shown here was originated from cellulose hydrolysis in pretreatment and residue water-soluble glucose, while fructose only originated from residue water-soluble fructose. CS, corn stover.

stover contained 20.6 mg/g of glucose from the partial hydrolysis of cellulose in pretreatment, while the pretreated sugars resupplemented corn stover contained 43% more glucose (29.4 mg/g) and the pretreated raw corn stover contained 19% more glucose (24.6 mg/g), due to the existence of the original glucose in the feedstocks. For the pretreated sugars resupplemented corn stover, the residual water-soluble glucose was only 8.8 mg/g (the total glucose in the feedstock, 29.4 mg/g, subtracted the generated glucose from the cellulose hydrolysis in pretreatment, 20.6 mg/g), indicating that 60% of the water-soluble glucose was degraded during the pretreatment. Meanwhile the loss of water-soluble fructose was more than 90% (the residual 2.1 mg/g from the original 20.8 mg/g). For the raw corn stover, 82% and 88% loss of the original water-soluble glucose and fructose were found. HMF is the direct overdegradation product of glucose and fructose during pretreatment. Only 2.7 mg/g of HMF was generated from the sugar free corn stover, while almost three times of HMF was generated from the sugars containing feedstocks (7.3 mg/g and 7.8 mg/g for the sugars resupplemented and the raw corn stover, respectively).

DOI: 10.1021/acs.jafc.8b03592
J. Agric. Food Chem. 2018, 66, 10225−10232

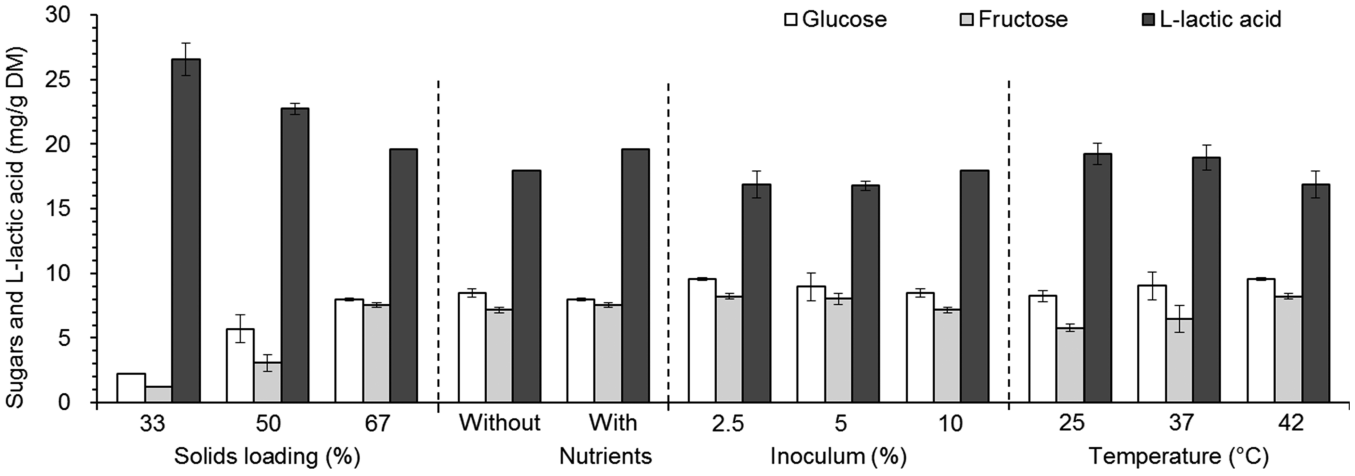

Figure 2. Bioconversion of water-soluble carbohydrates to L-lactic acid by solid state fermentation using *P. acidilactici* TY112. Fermentation conditions: Solids loading at 33, 50, and 67% (w/w), 10% (w/w) inoculum, with nutrients addition, 42 °C for 1 day; With nutrients addition or not, 5 g/kg YE, 5 g/kg peptone, 1 g/kg triammonium citrate, and 2.5 g/kg sodium acetate based on the dry corn stover, 67% (w/w) solids loading, 10% (w/w) inoculum, 42 °C for 1 day; Inoculum size at 2.5, 5, and 10% (w/w), 67% (w/w) solids loading, no nutrients addition, 42 °C for 1 day; Temperature, 25, 37, and 42 °C for 5, 2, and 1 days, respectively, 67% (w/w) solids loading, 2.5% (w/w) seed inoculum, no nutrients addition.

The results indicate that a large proportion of water-soluble carbohydrates were degraded and converted into some toxic compounds such as HMF under such pretreatment intensity, and less than 20% water-soluble carbohydrates were preserved for following L-lactic acid fermentation.

Feasibility of Solid State Fermentation in Different Storage Conditions. Since it is hard to preserve water-soluble carbohydrates in dilute acid pretreatment, we first performed a solid state fermentation at storage period of corn stover to make conversion of these sugars to L-lactic acid before pretreatment. No contamination was observed in solid state fermentation due to the well-controlled anaerobic culture and low pH (about 4.0) generated by lactic acid production. Another important reason was that the lactic acid fermenting bacterium, *P. acidilactici* TY112, could secrete a unique antibacterial peptide and effectively prevented the contamination.[37] The varying process parameters of water-soluble carbohydrates conversion in solid state fermentation were tested including solids loading, nutrients, inoculum size, and temperature (Figure 2).

The results show that higher moisture (lower solids loading) led to increased L-lactic acid generation, but the maximum moisture of the feedstock should be restricted to zero wastewater generation in the consequent pretreatment. The nutrient addition into corn stover and the inoculum size gave limited effect on the final lactic acid yield because of the existence of sufficient nitrates, trace elements, and vitamins in corn stover[36,38] and also the high inhibition of low pH (about 4.0) to the lactic acid conversion.[20,21] Moreover, the bioconversion could be applied in a relatively wide temperature range, indicating the ambient temperature change during the corn stover storage was acceptable. As the temperature reduced below 42 °C (optimal temperature for this strain), longer storage time was needed to convert water-soluble carbohydrates into lactic acid.

Therefore, lactic acid containing feedstock was produced simply by scaling up solid state fermentation to large plastic bags (15 kg wet materials) at relatively higher solids loading (67%) and lower inoculum (2.5%), without nutrients addition, 25 °C for 6 days. 18.6 mg/g of L-lactic acid, 6.6 mg/g of glucose, and 4.0 mg/g of fructose were found in the obtained L-lactic acid containing corn stover feedstock with almost 69.9% of glucose and 80.8% of fructose utilization. The reason for the unthorough conversion was that the solid state fermentation was conducted at high solids loading without well-controlled environment such as insufficient mass and heat transfer, declined pH by the lactic acid generation, poorly dispersed seeds inoculation, etc. Further increase of the conversion yield certainly led to the cost increase such as supplement of neutralizer (ammonia); therefore, natural condition without extensive modification was chosen.

Promoting Cellulosic L-Lactic Acid Production by Utilization of Water-Soluble Carbohydrates. For the purpose of integrated utilization of cellulose and hemicellulose fractions for L-lactic acid production in SSCF, lactic acid containing corn stover was dry acid pretreated first with no wastewater discharged.[28] The L-lactic acid in the feedstock was found to be stable during pretreatment and almost completely preserved with an increased titer from 18.6 to 21.9 mg/g due to dry matter loss. Also, partial sulfuric acid catalyst was replaced by the produced L-lactic acid (35 mg/g of sulfuric acid +18 mg/g of L-lactic acid), compared to the regular sulfuric acid usage of 37 mg/g. The cellulose hydrolysis yield of the pretreated L-lactic acid containing feedstock was essentially the same as that of the raw corn stover (92.80% ± 1.20% vs 92.05% ± 0.46%). The compositions of cellulose, xylan, xylo-oligomer, xylose, acetic acid, and furfural were also essentially the same, but HMF was reduced from 7.8 to 3.2 mg/g due to the reduced water-soluble carbohydrates in feedstock (Figures 3A, 3B).

Biodetoxification by *A. resinae* ZN1was conducted at solid state to degrade the inhibitors until no furfural and HMF were detected, and biodetoxification time was significantly shortened from 72 to 48 h in the pretreated L-lactic acid containing corn stover due to less HMF generation. No wastewater was discharged in biodetoxification,[24] and most of the lactic acid was preserved (16.3 mg/g) in the solid material with partial degradation by *A. resinae* ZN1. Xylose and glucose released during the pretreatment were preserved without observable loss because of the priority of inhibitors as substrates for *A. resinae* ZN1. No cellulose degradation was observed during the biodetoxification period.

DOI: 10.1021/acs.jafc.8b03592
J. Agric. Food Chem. 2018, 66, 10225−10232

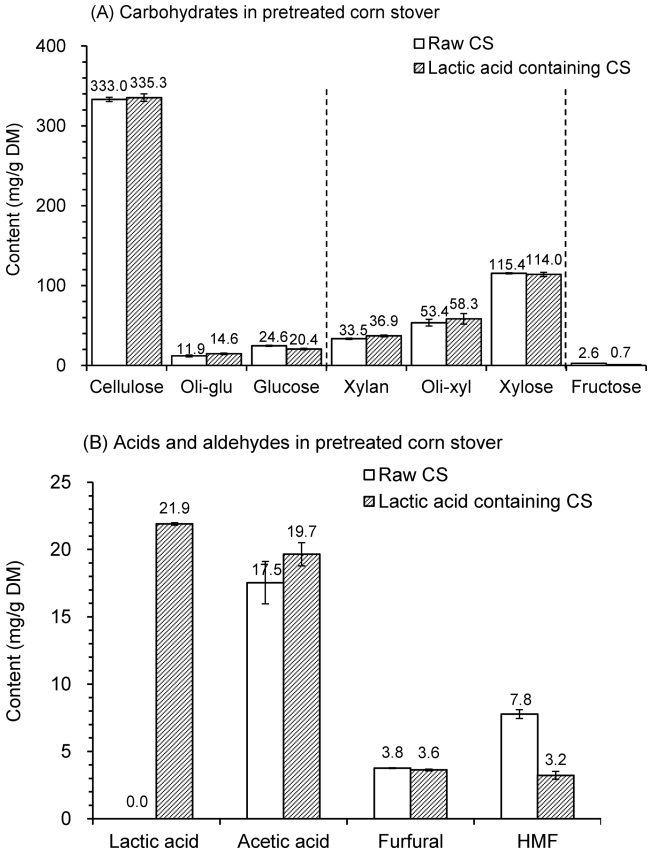

Figure 3. Composition of pretreated L-lactic acid containing corn stover. (A) Carbohydrates in the pretreated corn stover; (B) Acids and aldehydes in the pretreated corn stover. The raw corn stover and the L-lactic acid containing corn stover were dry acid pretreated at 175 °C for 5 min with sulfuric acid usages of 35 and 37 mg/g DM, respectively. The composition was defined as milligrams components in the per gram dry material. CS, corn stover. Oli-glu, oligomer of glucan. Oli-xyl, oligomer of xylan.

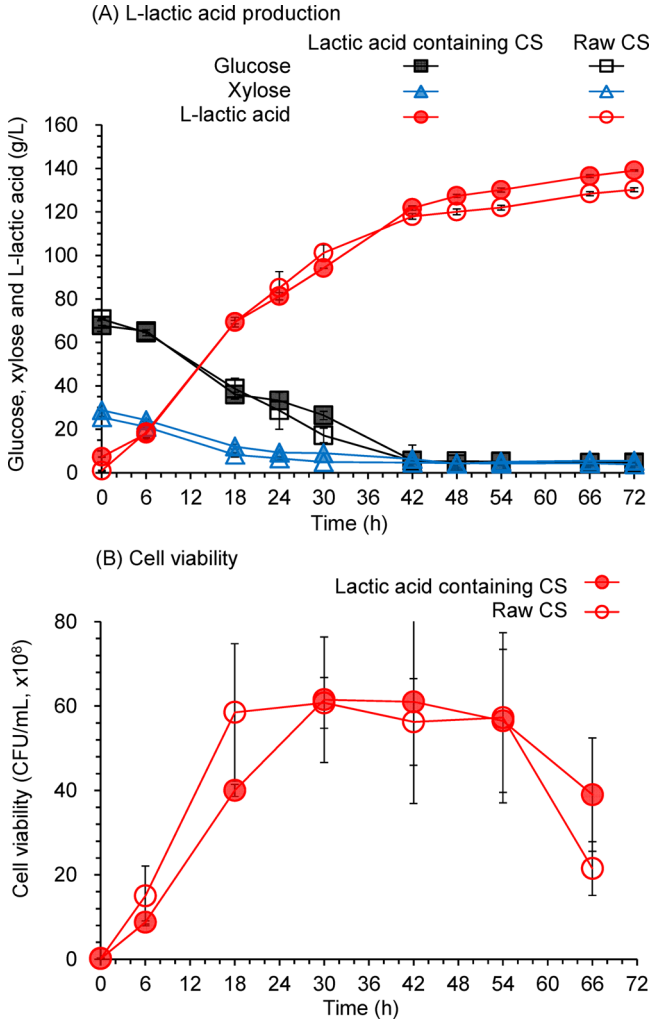

Figure 4. Simultaneous saccharification and cofermentation (SSCF) by *P. acidilactici* ZY271 using L-lactic acid containing corn stover. (A) L-Lactic acid production; (B) Cell viability. The SSCF was conducted using the pretreated and biodetoxified corn stover at 30% (w/w) solids loading, 5 mg cellulase protein/g DM, 50 °C, pH 5.5, 150 rpm for 6 h in the prehydrolysis stage, and 42 °C, pH 5.5, 150 rpm for 72 h by *P. acidilactici* ZY271 in the SSCF stage. pH was maintained by automatic feeding of 50% (w/w) $Ca(OH)_2$. CS, corn stover.

Simultaneous saccharification and cofermentation (SSCF) was finally conducted at 30% (w/w) solids loading, 5 mg cellulase protein/g DM to produce cellulosic L-lactic acid using pretreated and biodetoxified L-lactic acid containing corn stover. Cellulase Novozyme Cellic CTec 2.0 was used in SSCF with filter paper activity of 203.2 FPU/mL, cellobiase activity of 4900 CBU/mL and protein concentration of 87.3 mg/mL. The results show that initial titer of L-lactic acid (7.2 g/L) was evidently higher compared to the control (0.9 g/L) at the start of SSCF, with essentially the same titer of glucose (67.7 and 70.7 g/L) and xylose (28.7 and 25.5 g/L) in the slurry. Cell growth was relatively lower in the first 18 h using the L-lactic acid containing corn stover, and the corresponding sugars utilization rate and L-lactic acid productivity were relatively slower. This might be due to the slight inhibition of the cell growth by the L-lactic acid in the feedstock. However, as almost all the glucose and xylose were utilized, higher titer of L-lactic acid gradually emerged, and the final production of L-lactic acid in 72 h was 139.0 g/L compared to the control (130.2 g/L) with the same optical purity of 99.7% (Figures 4A, 4B). The experiments were repeatable and had been demonstrated twice with low error bar. The convincing result clearly reveals that L-lactic acid generated from water-soluble carbohydrates maintained stable in dry acid pretreatment and biodetoxification process, and then added into the final product to elevate cellulosic L-lactic acid titer. Although the elevated proportion is not much higher (only 6.8%), the new process will significantly promote cellulosic lactic acid production in an industrial-scale production process.

By applying the simple solid state fermentation on the water-soluble carbohydrates in corn stover, the total L-lactic acid yield was increased; meanwhile, the sulfuric acid catalyst was partially reduced, the harmful HMF generation was reduced, and the biodetoxification time was significantly shortened. This approach could also be applied to other high solids loading pretreatment processes such as steam explosion and ammonia fiber expansion (AFEX) and to the products other than L-lactic acid such as citric acid, gluconic acid, and itaconic acid.

Techno-economic Analysis of Two Cellulosic L-Lactic Acid Production Scenarios. The techno-economic analysis of the regular cellulosic L-lactic acid production process (Scenario 1) and the cellulosic L-lactic acid production process with water-soluble carbohydrates utilization (Scenario 2) were calculated based on the differences in the two processes:

DOI: 10.1021/acs.jafc.8b03592
J. Agric. Food Chem. 2018, 66, 10225−10232

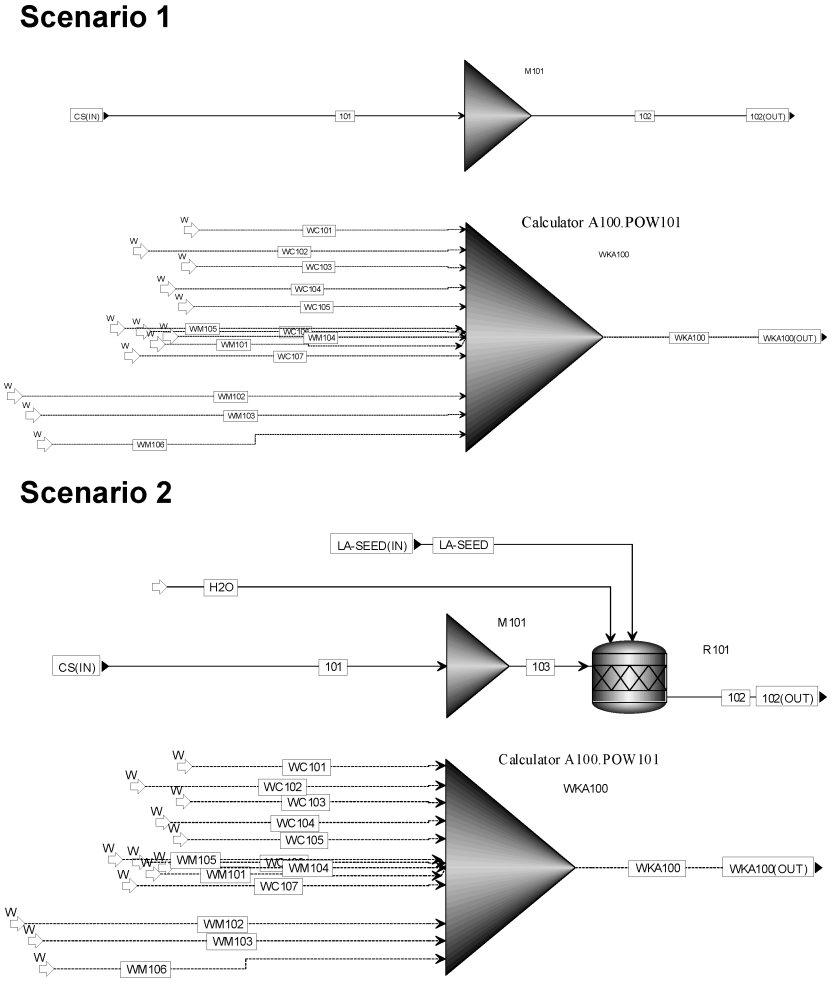

Figure 5. Major difference of the two scenarios in the Prehandling Area (A100) in Aspen Plus flow sheet simulation process. Scenario 1, regular process without water-soluble carbohydrates utilization. Scenario 2, process with water-soluble carbohydrates utilization. The additional lactic acid fermentation seed culture reactor R101 is added.

In prehandling Area 100: The moisture of the corn stover feedstock is adjusted from 15% (w/w) (Scenario 1) to 33% (w/w) (Scenario 2) by spraying water and the lactic acid bacteria seeds in the prehandling area. The difference of the flow sheet in the two scenarios was shown in Figure 5. The fermentation reactor (R101) is just a virtual block with no special needs and controls during the practical operation. Since the feedstock storage capacity of the biorefining plant is at least 1 week,[32] it just satisfies the regular storage period for the 6 days' solid state fermentation with conversion of water-soluble carbohydrates into L-lactic acid. The increased cost in this area mainly comes from the additional fermentation seed culture section used for the solid state fermentation, cover material for anaerobic condition, and additional operators. The capital cost (equipment and installation) increases from $2,885,000 to $2,921,000, about a 1% increase, and the operating cost increases by $0.08 million per year for additional seed culture. The bulk density of raw corn stover is about 0.12 t per cubic meter; namely, the volume of one metric ton of corn stover is about 8.333 m^3.[39] Thus, corn stover of one metric ton can be piled into a cube hayrick with edge length of 2.03 m and surface area of about 25 m^2. Agricultural PE plastic film will be used to cover the cubic hayrick for anaerobic condition of solid fermentation. The surface area of PE plastic film is estimated 50 m^2 for one metric ton corn stover because of overlap and tailor. The price of agricultural PE plastic film is $1.80 per kg with area of 120 m^2 (www.1688.com). The cover material cost for one metric ton corn stover is about 50 × $1.80/120 = $0.75. Therefore, the variable operating cost increases $0.23 million per year caused by cover material. In addition, at least 20 extra operators are needed to inoculate and package the 900 t corn stover every day. The fixed operating costs will increase $0.22 million per year caused by the labor increase.

In pretreatment Area 200: The solid−liquid ratio is changed from 2:1 (67% of the solids loading) to 3:2 (60% of the solids loading) in the pretreatment operation, which results in the increased cost on equipment and installation from $3,300,000 to $3,597,000, about 9% increase. The sulfuric acid usage is reduced from 3.7% to 3.5% (from 37 to 35 kg per ton of dry feedstock), leading to the decreased variable operating cost ($0.03 million per year). In biodetoxification Area 250: The biodetoxification time is shortened from 72 to 48 h, causing the decreased equipment and installation cost from $8,854,000 to $6,548,000, approximately 26% reduction. In saccharification and fermentation Area 300: The final L-lactic acid titer after SSCF increases from 130.2 g/L to 139.0 g/L and the total L-lactic acid yield from raw corn stover feedstock increases from 424 to 453 kg per ton of dry feedstock. The costs based on per kg product of each area in Scenario 2 are lower than the regular Scenario 1 as shown in Figure 6.

DOI: 10.1021/acs.jafc.8b03592
J. Agric. Food Chem. 2018, 66, 10225−10232

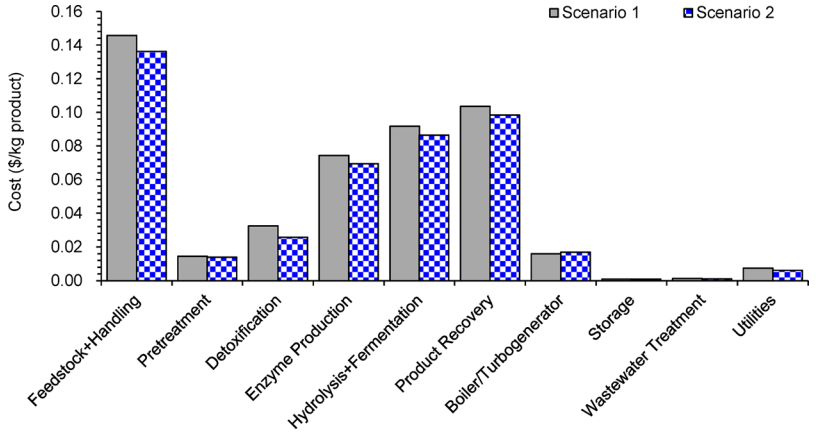

Figure 6. Cost contribution details from each process area (per kg L-lactic acid product) in two scenarios. Scenario 1, regular process without water-soluble carbohydrates utilization. Scenario 2, process with water-soluble carbohydrates utilization.

The techno-economic analysis shows that the total capital investment is decreased from $111.3 million of Scenario 1 to $107.4 million of Scenario 2, mainly caused by the significant reduction of biodetoxification time. The total variable and fixed operating cost increases from $48.82 million to $49.42 million per year. Actually, the variable and fixed operating cost of unit production is reduced from $0.387 to $0.366 per kg L-lactic acid production because of the elevation of L-lactic acid yield. The minimum L-lactic acid selling price (MLSP) of Scenario 2 is $0.459/kg, approximately 5.9% reduction compared to the $0.488/kg of Scenario 1, by utilization of water-soluble carbohydrates in raw corn stover feedstock.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.8b03592.

Flow charts of cellulosic L-lactic acid production on Aspen plus platform, discounted cash flow analysis parameters, composition of corn stover, prices provided by the vendors, and raw material unit cost (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*Gang Liu, Tel/fax: +86 21 64251799, gangliu@ecust.edu.cn.
*Jie Bao, Tel/fax: +86 21 64251799, jbao@ecust.edu.cn.

ORCID

Feng Hong: 0000-0001-5120-3519
Jie Bao: 0000-0001-6521-3099

Funding

This research was supported by the National Key Research and Development Program of China (2017YFB0309302).

Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) Chen, S.; Mowery, R. A.; Scarlata, C. J.; Chambliss, C. K. Compositional analysis of water-soluble materials in corn stover. *J. Agric. Food Chem.* **2007**, *55*, 5912−5918.

(2) Niu, W.; Han, L.; Liu, X.; Huang, G.; Chen, L.; Xiao, W.; Yang, Z. Twenty-two compositional characterizations and theoretical energy potentials of extensively diversified China's crop residues. *Energy* **2016**, *100*, 238−250.

(3) Galletti, G. C.; Reeves, J. B. I.; Bocchini, P.; Muscarella, C. I. Compositional differentiation of maize hybrid stovers using analytical pyrolysis and high-performance liquid chromatography. *J. Agric. Food Chem.* **1997**, *45*, 1715−1719.

(4) Montross, M. D.; Crofcheck, C. L. Effect of stover fraction and storage method on glucose production during enzymatic hydrolysis. *Bioresour. Technol.* **2004**, *92*, 269−274.

(5) Pordesimo, L. O.; Hames, B. R.; Sokhansanj, S.; Edens, W. C. Variation in corn stover composition and energy content with crop maturity. *Biomass Bioenergy* **2005**, *28*, 366−374.

(6) Alvira, P.; Tomas-Pejo, E.; Ballesteros, M.; Negro, M. J. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresour. Technol.* **2010**, *101*, 4851−4861.

(7) Kabel, M. A.; Bos, G.; Zeevalking, J.; Voragen, A. G. J.; Schols, H. A. Effect of pretreatment severity on xylan solubility and enzymatic breakdown of the remaining cellulose from wheat straw. *Bioresour. Technol.* **2007**, *98*, 2034−2042.

(8) Liu, Z.; Chen, H. Xylose production from corn stover biomass by steam explosion combined with enzymatic digestibility. *Bioresour. Technol.* **2015**, *193*, 345−356.

(9) Michelin, M.; Teixeira, J. A. Liquid hot water pretreatment of multi feedstocks and enzymatic hydrolysis of solids obtained thereof. *Bioresour. Technol.* **2016**, *216*, 862−869.

(10) Wyman, C.; Dale, B.; Elander, R.; Holtzapple, M.; Ladisch, M.; Lee, Y. Coordinated development of leading biomass pretreatment technologies. *Bioresour. Technol.* **2005**, *96*, 1959−1966.

(11) Newth, F. H. The formation of furan compounds from hexoses. *Adv. Carbohydr. Chem.* **1951**, *6*, 83−106.

(12) He, Y.; Zhang, J.; Bao, J. Acceleration of biodetoxification on dilute acid pretreated lignocellulose feedstock by aeration and the consequent ethanol fermentation evaluation. *Biotechnol. Biofuels* **2016**, *9*, 19.

(13) Wang, X.; Gao, Q.; Bao, J. Enhancement of furan aldehydes conversion in Zymomonas mobilis by elevating dehydrogenase activity and cofactor regeneration. *Biotechnol. Biofuels* **2017**, *10*, 24.

(14) Hu, J.; Zhang, Z.; Lin, Y.; Zhao, S.; Mei, Y.; Liang, Y.; Peng, N. High-titer lactic acid production from NaOH-pretreated corn stover by *Bacillus coagulans* LA204 using fed-batch simultaneous saccharification and fermentation under nonsterile condition. *Bioresour. Technol.* **2015**, *182*, 251−257.

(15) Nampoothiri, N. K.; Nair, N. R.; John, R. P. An overview of the recent developments in polylactide (PLA) research. *Bioresour. Technol.* **2010**, *101*, 8493−8501.

(16) Farah, S.; Anderson, D. G.; Langer, R. Physical and mechanical properties of PLA, and their functions in widespread applications- A comprehensive review. *Adv. Drug Delivery Rev.* **2016**, *107*, 367−392.

(17) Liu, G.; Sun, J.; Zhang, J.; Tu, Y.; Bao, J. High titer L-lactic acid production from corn stover with minimum wastewater generation

DOI: 10.1021/acs.jafc.8b03592
J. Agric. Food Chem. 2018, 66, 10225−10232

and techno-economic evaluation based on Aspen plus modeling. *Bioresour. Technol.* **2015**, *198*, 803−810.
(18) Qiu, Z.; Gao, Q.; Bao, J. Engineering *Pediococcus acidilactici* with xylose assimilation pathway for high titer cellulosic L-lactic acid fermentation. *Bioresour. Technol.* **2018**, *249*, 9−15.
(19) Yi, X.; Zhang, P.; Sun, J.; Tu, Y.; Gao, Q.; Zhang, J.; Bao, J. Engineering wild-type robust *Pediococcus acidilactici* strain for high titer L- and D-lactic acid production from corn stover feedstock. *J. Biotechnol.* **2016**, *217*, 112−121.
(20) Fitzsimons, A.; Duffner, F.; Curtin, D.; Brophy, G.; Okiely, P.; O'Connell, M. Assessment of *Pediococcus acidilactici* as a potential silage inoculant. *Appl. Environ. Microbiol.* **1992**, *58*, 3047−3052.
(21) Richter, K.; Träger, A. L(+)-Lactic acid from sweet sorghum by submerged and solid-state fermentations. *Acta Biotechnol.* **1994**, *14*, 367−378.
(22) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *Determination of ash in biomass*; NREL: Golden, CO, 2005; NREL/TP-510-42622.
(23) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *Determination of structural carbohydrates and lignin in biomass*; NREL: Golden, CO, 2012; NREL/TP-510-42618.
(24) Zhang, J.; Zhu, Z.; Wang, X.; Wang, N.; Wang, W.; Bao, J. Biodetoxification of toxins generated from lignocellulose pretreatment using a newly isolated fungus, *Amorphotheca resinae* ZN1, and the consequent ethanol fermentation. *Biotechnol. Biofuels* **2010**, *3*, 26.
(25) Adney, B.; Baker, J. Measurement of cellulase activities. Laboratory Analytical Procedure (LAP)-006. NREL: Golden, CO, 1996; Report NREL/TP-510-42628.
(26) Ghose, T. K. Measurement of cellulase activities. *Pure Appl. Chem.* **1987**, *59*, 257−268.
(27) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* **1976**, *72*, 248−254.
(28) Zhang, J.; Wang, X.; Chu, D.; He, Y.; Bao, J. Dry pretreatment of lignocellulose with extremely low steam and water usage for bioethanol production. *Bioresour. Technol.* **2011**, *102*, 4480−4488.
(29) Brown, L.; Torget, R. Enzymatic saccharification of lignocellulosic biomass. Laboratory Analytical Procedure (LAP)-009. NREL: Golden, CO, 1996; Report NREL/TP-510-42629.
(30) Zhang, J.; Chu, D.; Huang, J.; Yu, Z.; Dai, G.; Bao, J. Simultaneous saccharification and ethanol fermentation at high corn stover solids loading in a helical stirring bioreactor. *Biotechnol. Bioeng.* **2010**, *105*, 718−728.
(31) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *Determination of sugars, byproducts, and degradation products in liquid fraction process samples*. NREL: Golden, CO, 2008; NREL/TP-510-42623.
(32) Humbird, D.; Davis, R.; Tao, L.; Kinchin, C.; Hsu, D.; Aden, A.; Schoen, P.; Lukas, J.; Olthof, B.; Worley, M.; Sexton, D.; Dudgeon, D. *Process design and economics for biochemical conversion of lignocellulosic biomass to ethanol*; NREL: Golden, CO, 2011; NREL/TP-5100-47764.
(33) Lloyd, T. A.; Wyman, C. E. Predicted effects of mineral neutralization and bisulfate formation on hydrogen ion concentration for dilute sulfuric acid pretreatment. *Appl. Biochem. Biotechnol.* **2004**, *115*, 1013−1022.
(34) Shi, S.; Guan, W.; Kang, L.; Lee, Y. Y. Reaction kinetic model of dilute acid-catalyzed hemicellulose hydrolysis of corn stover under high-solid conditions. *Ind. Eng. Chem. Res.* **2017**, *56*, 10990−10997.
(35) Canettieri, E. V.; Rocha, G. J.; Carvalho, J. A.; Silva, J. B. Evaluation of the kinetics of xylose formation from dilute sulfuric acid hydrolysis of forest residues of Eucalyptus grandis. *Ind. Eng. Chem. Res.* **2007**, *46*, 1938−1944.
(36) Han, X.; Bao, J. General method to correct the fluctuation of acid based pretreatment efficiency of lignocellulose for highly efficient bioconversion. *ACS Sustainable Chem. Eng.* **2018**, *6*, 4212−4219.
(37) Qureshi, A. S.; Zhang, J.; Sousa, L. D.; Bao, J. Antibacterial peptide secreted by *Pediococcus acidilactici* enables efficient cellulosic open L-lactic acid fermentation. *ACS Sustainable Chem. Eng.* **2017**, *5*, 9254−9262.
(38) Wen, J.; Xiao, Y.; Liu, T.; Gao, Q.; Bao, J. Rich biotin content in lignocellulose biomass plays the key role in determining cellulosic glutamic acid accumulation by *Corynebacterium glutamicum*. *Biotechnol. Biofuels* **2018**, *11*, 132.
(39) Ma, S.; Eckhoff, S. R. Economy of scale for biomass refineries: bulk densities, transportation cost, and producer incentives. *T. ASABE* **2014**, *57*, 85−91.

DOI: 10.1021/acs.jafc.8b03592
J. Agric. Food Chem. 2018, 66, 10225−10232