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Understanding the Pine Dilute Acid Pretreatment System for Enhanced Enzymatic Hydrolysis

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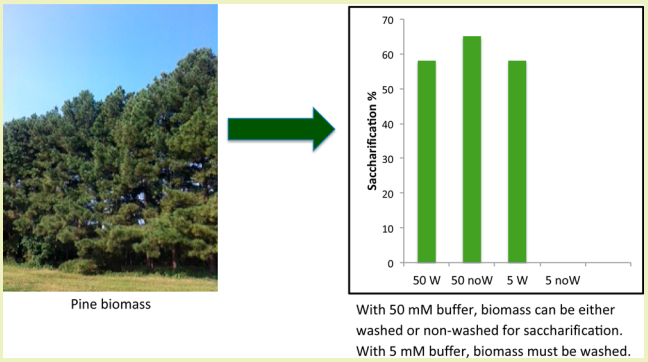
ABSTRACT: In the United States, loblolly pine is touted as a potential feedstock for biobased product manufacturing, as it is fast growing and abundant in the southeast. To release the sugar content from pine biomass, pretreatment must occur. Dilute acid pretreatment is, among others, a pretreatment method that can be used. Unfortunately, this pretreatment results in the production of inhibitory byproducts, which need to be removed prior to enzymatic hydrolysis and fermentation; the main inhibitors include organic acids predominantly formic and acetic. Rinsing dilute acid pretreated biomass is a way to remove inhibitory byproducts. Buffering capacity of the enzymatic hydrolysis reaction mixture alleviates inhibition due to organic acids, while maintaining a pH environment that is conducive to enzymatic activity. However, the use of buffer salts at commonly prepared concentrations, such as 50 mM, may not be economically viable at production scale and could hinder fermentation due to their chelating capacity. In this study, two enzymatic hydrolysis citrate buffer strengths, 5 mM and 50 mM, were investigated for their effect on the level of saccharification of rinsed (30X) and unrinsed (0X) acid pretreated pine biomass. Buffer strength did not affect saccharification as long as the pretreated biomass was rinsed. Saccharification was still possible in unwashed biomass as long as the buffer concentration was 50 mM, where 65% saccharification was obtained. Although formic acid, acetic acid, HMF, and furfural concentrations were higher in unrinsed hydrolyzates than in their rinsed counterparts, saccharification could still occur, indicating that these compounds are not the sole culprits in conferring inhibition. No hydrolysis was observed when unrinsed biomass was subjected to enzymatic hydrolysis in 5 mM buffer.



KEYWORDS: *Loblolly pine, Dilute acid pretreatment, Saccharification, Buffer strength, Biomass rinsing*

INTRODUCTION

Pine forests, located throughout the southeastern states of the U.S., including the southern regions of Arkansas are an attractive source of biomass.[1] Arkansas is one of the most forested states in the US, with 18.0 million acres of timberland that can support sustained wood production, corresponding to 57%, of the state's total land area.[2] Loblolly pine, which is a type of softwood, represented 22% of the total standing tree volume in 2005 and was cultivated in managed forests, where the trees were harvested at 25−35 years of age.[2,3] Softwoods, including loblolly pine, are attractive as feedstock for biofuel production because of their high cellulose content, which can reach up to 45%. Unfortunately, pine remains one of the most recalcitrant species for saccharification due to its high lignin content.[4−7] If pine's recalcitrance could be overcome, it could become an interesting feedstock, as its forest to gate price is one of the lowest for bioenergy feedstock.[8]

To release sugars, pine biomass must first be pretreated to make cellulose accessible to enzymatic hydrolysis. A number of studies have reported on sugar release from pine cell wall via steam explosion, alkaline, dilute acid, hot water, ammonia, organosolv, and SPORL (sulfite pretreatment to overcome recalcitrance of lignocellulose). Unfortunately, softwood biomass saccharification remains complex.[9−12] Among pretreatment options, dilute acid can be used because of its low cost and high sugar yields.[13,14] Pretreatment temperatures between 158 to 180 °C yielded between 20% to 35% sugar recovery. Additionally, pretreatment in 2.2% w/w acid at 180 °C for 30 min resulted in 18% enzymatic conversion of cellulose present in pine biomass,[10] while 35% enzymatic hydrolysis efficiency was reported by pretreating loblolly pine biomass in 1.8% w/w acid at 158 °C for 5 min.[13] Normark et al. reported 20 to 35% combined glucose yield after dilute acid pretreatment and enzymatic saccharification of different pine biomass plant parts.[5]

Unfortunately, dilute acid pretreatment of pine biomass is associated with the generation of several undesired byproducts, such as organic acids, furans and lignin monomers, which

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inhibit enzymes and fermenting organisms, reducing the efficiency of further downstream steps.[15] It is current practice to rinse pretreated biomass with several volumes of water prior to enzymatic hydrolysis. Standard procedures for biomass hydrolysis and cellulase activity are conducted in 50 mM buffer and pH 4.8. In the work of Normark et al., saccharifcation was conducted after rinsing the pretreated biomass with at least two volumes of water and one volume of citrate buffer.[5] Regrettably, rinsing the biomass did not remove all inhibitory compounds. After rinsing, organic acids, such as formic and acetic acids, were detected in the enzymatic hydrolysis hydrolyzate,[5] indicating that biomass washing did not remove all inhibitory products. At demonstration scale, large volumes of rinsewater combined with the use of full strength buffers, such as in the work of Normark et al.,[5] could possibly hinder the commercial feasibility of cellulosic operations. In an ideal situation, enzymatic hydrolyzates would be devoid of organic acids and would be made up of buffer concentrations that are less than full strength, as reductions in buffer concentration and degradation products could facilitate yeast cultivation,[16] adding cost effectiveness to commercial scale applications. Furthermore, buffer concentration could have important effects on growth and physiology of fermenting yeasts.[17,18]

Elimination or reduction of degradation by products combined with reduced buffer concentrations of enzymatic hydrolyzates could facilitate growth and physiology of fermenting organism. In this study, unrinsed and rinsed dilute acid pretreated pine biomass were subjected to enzymatic hydrolysis under diluted buffer, 5 mM, and full strength buffer, 50 mM, to delineate the interaction effect of biomass preparation and buffer strength for enzymatic hydrolysis of glucan.

MATERIALS AND METHODS

Pine Biomass. A 15 year old loblolly pine was harvested from the University of Arkansas at Monticello Teaching and Research Forest in Drew County, Arkansas (latitude 34°03′83″ and longitude 92°22′22″) on January 18, 2014. Mean annual precipitation in this area is 46−63 in., with temperatures ranging from 23.4 and 11.0 °C. The soil profile in the area is characterized as predominantly a Calloway silt loam. The wood was chipped with a Troy-Bilt Chipper and ground in a Wiley Mini-Mill to 20 mesh (Thomas Scientific, Swedesboro, NJ). Material was kept at 4 °C until further use.

Compositional Analysis of Biomass. Compositional analysis of raw, as well as, pretreated pine biomass was performed following National Renewable Energy Laboratory (NREL) protocols.[19] Briefly, ground material was extracted in successive water and ethanol in a Soxhlet apparatus, hydrolyzed in acid at 121 °C, and analyzed for carbohydrate, lignin and ash content. Moisture/solid percentage of the pinewood was analyzed, using a MB45 Moisture Analyzer (Ohaus Corporation, Pine Brook, NJ).

Dilute Acid Pretreatment of Pine Biomass. Pine biomass was pretreated in dilute acid, as previously reported.[20] Briefly, 25 g of ground loblolly pine were placed in a Parr reactor with 250 mL of 1% w/v concentrated sulfuric acid, heated to 160 °C, held at constant temperature for 30 min, and stirred at 144 rpm. Samples were filtered through a Buchner funnel, and filtrate was collected for further sugar and inhibitory byproducts analysis. One half of recovered solids were rinsed with Millipore-Direct-Q water at ratio of and 1:30 (30X) and other was not rinsed. Wash water was collected for inhibitor analysis. Glucan content in acid pretreated biomass was determined as done for compositional analysis of raw biomass using standard NREL protocol. Pretreatment hydrolyzates, washates and enzyme hydrolyzates were analyzed for sugar and inhibitory byproducts content.

Analysis of Sugars, Aromatic, Aldehyde, and Aliphatic Acids by HPLC. Pretreatment hydrolyzate and washates were centrifuged at 600*g* for 5 min; the pH of supernatants was adjusted with $CaCO_3$ to neutral and analyzed for sugar content by high-performance liquid chromatography (HPLC), as previously described.[20] Briefly, aliquots were filtered through a 0.2 μm syringe filter and analyzed on a Shodex (Waters, Milford, MA) SP-G precolumn and SP0810 column with water as the eluent, using a refractive index detector. Furfural, hydroxymethylfurfural (HMF), acetic acid, and formic acid were analyzed by HPLC with an Aminex (Bio-Rad Laboratories, Inc., Hercules, CA) HPX-87H ion-exchange column or by ultra performance liquid chromatography (UPLC), as previously described.[21]

Enzymatic Hydrolysis of Solids. For enzymatic hydrolysis of 0X and 30X rinsed pretreated biomass was loaded to 10% solids on a wet basis corresponding to ∼1% glucan (cellulose). For the 50 mM or 5 mM buffer enzymatic hydrolysis experiments, both unrinsed and rinsed solids were hydrolyzed with CTec2, and HTtec enzymes (Novozymes, North America Inc., Franklinton, NC); 1 g biomass (wet basis) was placed in a 50 mL amber bottle to which 540 μL of CTec2 (33 FPU) and 60 μL of HTtec (1.2 IU), 5 mL of citrate buffer (either 100 mM or 10 mM, corresponding to final concentrations of 50 mM or 5 mM), and sufficient water to bring up the reaction volume up to 10 mL were added. The reaction mixtures were incubated at 50 °C in a shaking water bath 180 rpm at 50 °C. Aliquots of 500 μL were withdrawn periodically and centrifuged at 14 000*g* to separate biomass from supernatants. Glucose levels in supernatants were analyzed with YSI 2900 (YSI Incorporated, Yellow Springs, OH) glucose analyzer. The supernatants from enzymatic hydrolysis were also analyzed by HPLC for carbohydrates, furfural, HMF, and acetic and formic acid, as described above.[21] Saccharification efficiency was calculated as shown below in eq 1, where the glucose released was calculated by YSI, 0.9 accounts for the conversion factor from glucan to glucose, where 1 g of glucan upon hydrolysis yields 1.1 g glucose and conversely 1 g glucose is obtained from 0.9 g of glucan.

$$\%\ \text{saccharification efficiency} = \frac{\text{glucose released} \times 0.9 \times 100}{\text{glucan content in the pretreated biomass}} \quad (1)$$

Statistical Analysis. All treatments were carried out in duplicates and statistical analyses, such as mean standard error and critical difference, were performed using SPSS16.0 software package.

RESULTS AND DISCUSSION

Compositional analysis of pine heartwood used in this work contained, among others, 38.2% cellulose, 5.1% xylose, 4.1% galactose, and 17.7% mannose, as presented in Table 1. It

Table 1. Determined Composition of Pine Biomass

component	percent composition
cellulose	38.2 ± 0.4
xylan	5.1 ± 0.4
galactose	4.1 ± 0.3
mannose	17.7 ± 0.2
ash	0.3 ± 0.0
extractives (EtOH)	5.1 ± 0.1

should be noted that lignin results were not reported due to inaccuracies that were encountered during gravimetric estimations, as previously reported.[5,22] Cellulose content in the material used in this work was between 3.7% and 7.3% lower than previously reported.[10,23,24]

In this study, pine biomass was pretreated in 1% w/v H_2SO_4 at 160 °C for 30 min, and then subjected to enzymatic hydrolysis. When rinsing the pretreated biomass with 30 volumes of water (30X) and subjecting rinsed biomass to enzymatic hydrolysis, 57% of available glucose in initial biomass (or 22 mg of sugar per g of wet biomass) was recovered, as

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shown in Figure 1. The recovery presented in Figure 1 was higher than previously reported recoveries of 20 to 35% from

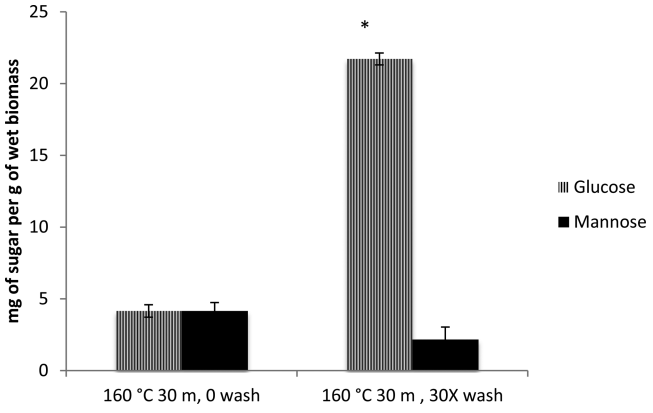

Figure 1. Glucose (vertical lines) or mannose (solid) recovery after enzymatic hydrolysis of pretreated pine heartwood rinsed with 0 or 30 (30X) volumes of water. Pretreatment conditions consisted of the following: 30 min at 160 °C in 1% wt sulfuric acid. Significant differences are denoted by *.

pine after dilute acid pretreatment with acid concentrations of 1.8% to 4.0% w/w, temperatures between 158 to 180 °C, and reaction times from 5 to 12 min.[5,13] Results presented in Figure 1 show that it was critical to rinse pretreated biomass prior to enzymatic hydrolysis to maximize glucose recovery. When pretreated biomass was not rinsed, less than 11% of available glucose was released. It was noted that pine pretreated hydrolyzates needed at least a rinse volume of 5X (results not shown) in order to form a solution that could be filtered, unlike pretreated poplar, which could be filtered with a 3X rinsewater volume.[21] The differences in minimal rinse volume requirements of pretreated pine and poplar in order to obtain flowability signaled inherent differences in their chemical makeup.

Furthermore, it was calculated that more than 10.5% of glucose present in raw pine biomass was lost when pretreated in 1% H_2SO_4, at 160 °C for 30 min. In softwoods, hemicellulose glucan can contribute up to 8% of glucose losses, while additional glucose content can originate from cellulose degradation during pretreatment.[25] Moreover, dilute acid pretreatment temperatures greater than 200 °C resulted in the complete dissolution of hemicellulose and nearly all cellulose present in pine biomass,[26] with monomers originating from hemicellulose and cellulose converted to both primary and secondary degradation products.[27] Conversely, dilute acid pretreatment temperatures at temperatures of 150 °C resulted in maximum conversion of hemicellulose and cellulose with minimization of HMF and furfural.[24,27,28] In the present study, the wet unrinsed biomass obtained after pretreatment at 160 °C for 30 min had a moisture content of 66.9%, while that of the rinsed biomass was 68.6%. Glucan contents of 30.0 and 30.3% on a dry weight basis were obtained from 30X rinsed and unrinsed solids, respectively, as compared to 38% glucose and 3% moisture recorded from starting raw material.

The amount of inhibitors formic acid, acetic acid, HMF, and furfural generated by treating 25 g pine biomass was quantified in acid hydrolyzates recovered after pretreatment and washates after rinsing, as shown in Table 2. For the pretreatment hydrolyzate, the volume recovered was 212 mL at pH 1.66, while the pH of washates was 2.19 and recovered volume was 560 mL. The level of inhibitors formation by acid pretreatment in this study was lower than those reported by van der Pol et al.,[26] where the acid pretreatment of pine wood resulted in detection of furfural and HMF content that was greater than the 875 mg furans formed per 25 g of initial dry softwood biomass. Several reports attest to the fact that organic acids and carbohydrate degradation products result in unfavorable conditions for enzyme action and growth of fermenting organisms.[15,29] The acidity resulting from organic acid byproducts due to dilute acid pretreatment could potentially inhibit the action of cellulases, which optimally proceed at pH 4.8. In enzymatic hydrolyzates, a pH in the range of 4.8 is maintained by conducting the enzyme reaction in 50 mM pH 4.8 citrate buffer. However, carrying out enzyme hydrolysis at 50 mM citrate buffer strength is not commercially feasible when the process is to be carried out at industrial scale. Also, it could be of importance to determine if enzymatic hydrolysis could be conducted in a lower strength buffer, as enzyme hydrolyzates are used for growing yeast, critical for ethanol production. Fermenting organism are deterred by growth media that contain 50 mM citrate buffer, exhibiting reduced growth rates due to citrate buffers that chelate trace elements, which are essential for optimum growth.[16,17,30] The rinsed and unrinsed solids in this study were subjected to enzymatic hydrolysis in 5 mM or 50 mM citrate buffer to investigate the effect of buffering capacity on the level of sugar release and resilience of the system toward inhibitors. Table 3 presents the glucose concentrations of the rinsed and unrinsed samples hydrolyzed in 5 or 50 mM citrate buffer. For washed biomass, analysis of

Table 3. Concentrations of Glucose Released after Enzymatic Hydrolysis of Acid Pretreated Rinsed and Unrinsed Biomass in 5 and 50 mM Buffer Strength[a]

		glucose (mg/mL)	
biomass	buffer (mM)	24 h	48 h
washed	50	5.90	10.35
	5	5.80	9.85
unwashed	50	7.23	6.07
	5	N.D.	N.D.
SE(m) ±	0.15	0.23	
CD@5%	0.44	0.66	

[a]"SE" is for standard error, "CD" is for critical difference, and "N.D." is for non-detectable.

Table 2. Presence of Inhibitors in Acid Hydrolyzates and Rinse Water (Washates) after Dilute Acid Pretreatment of 25 g of Ground Heartwood Pine

		(mg)			
	recovered vol (mL)	formic acid	acetic acid	HMF	furfural
acid hydrolyzates	212	154.8 ± 0.5	195.0 ± 0.3	84.8 ± 0.6	186.6 ± 0.4
rinse water (washate)	560	280.0 ± 0.3	162.4 ± 0.3	89.6 ± 0.4	123.2 ± 0.1

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results showed that glucose concentrations were not significantly different when using either buffer strength. On the other hand, when hydrolyzing unwashed biomass in 5 mM buffer, no glucose was detected. Saccharification efficiency at 1% glucan loading, was calculated by taking into account moisture and glucan content, and is presented in Figure 2. Complete glucan

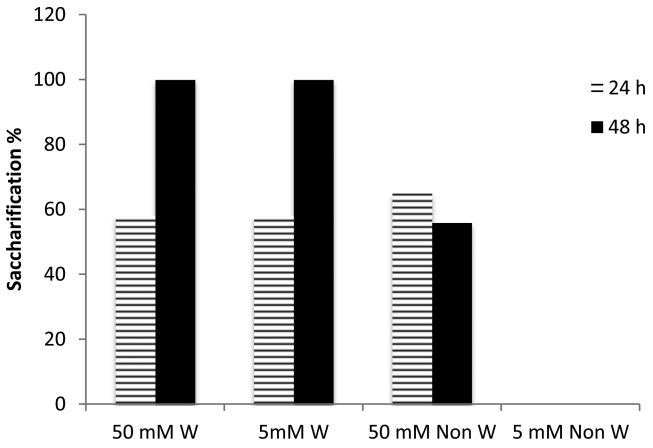

Figure 2. Dilute acid pine biomass pretreated in 1% H_2SO_4 at 160 °C for 30 min was either unrinsed or rinsed with 30X volumes water. Enzymatic hydrolysis was conducted in 50 mM or in 5 mM citrate buffer for either 24 or 48 h.

saccharification was obtained when enzymatic hydrolysis was done with rinsed biomass in either buffer, indicating that the enzyme cocktail could also perform in lowered buffer concentration. Enzymatic hydrolysis could still be carried out in unrinsed biomass as long as the buffer concentration was 50 mM, where 65% saccharification was calculated. Overall, the results showed that stronger buffering strength of the enzymatic hydrolysis system, such as that of the 50 mM, could possibly alleviate inhibitory effects of organic acids and other sugar degradation products that are generated during pretreatment. The enzymatic hydrolysis efficiency achieved in this study, with the use of rinsed biomass with either buffer, was higher than previously reported by.[13,10] In contrast, the use of 5 mM citrate buffer with unrinsed biomass resulted in virtually no glucose release.

The corresponding concentrations of organic acids and carbohydrate degradation products in enzymatic hydrolyzates of rinsed and unrinsed biomass are presented in Figure 3. Rinsing pretreated biomass reduced the concentrations of organic acids in enzymatic hydrolysis mixtures. Barring a 35% decrease in glucose release, the enzymatic hydrolysis reaction could still be carried out with unrinsed biomass in 50 mM citrate buffer, indicating that aliphatic acids and carbohydrate degradation compounds are not the sole culprits in conferring inhibition. The use of the 50 mM citrate buffer may be important to alleviate the nonspecific binding of lignin residues to cellulase cocktail enzymes and also not allow the lignin fragments to repolymerise under acidic conditions and inhibit cellulase, while a 5 mM buffer may not provide similar effect.[31] Citrate buffer may counteract the pH lowering effect of organic acids, produced during pretreatment, contributing to maintaining favorable conditions that are necessary for enzyme action. Jing et al. reported that pretreatment conditions, 165 °C and pH 1.3, resulted in the generation of compounds, such as, acetic acid, HMF, and furfural, at concentrations above the threshold that are inhibitory to both enzymes and fermentation bioprocesses.[32] On the other hand, a higher buffer concentration may play a role in impeding the repolymerization of polysaccharide degradation products formed during dilute acid pretreatment by scavenging carbonium ions.[33] Removal of lignin and/or repolymerized material prior to enzymatic hydrolysis is critical for enzyme bounding, which is critical for cellulose and hemicellulose hydrolysis.[34]

CONCLUSION

In conclusion, this study highlighted that the enzymatic hydrolysis step can tolerate lower citrate buffer concentrations, as long as the biomass is rinsed. Aliphatic acids and carbohydrate degradation compounds are adverse to enzymatic action, but do not seem to be the only deterrents to enzymatic hydrolysis.

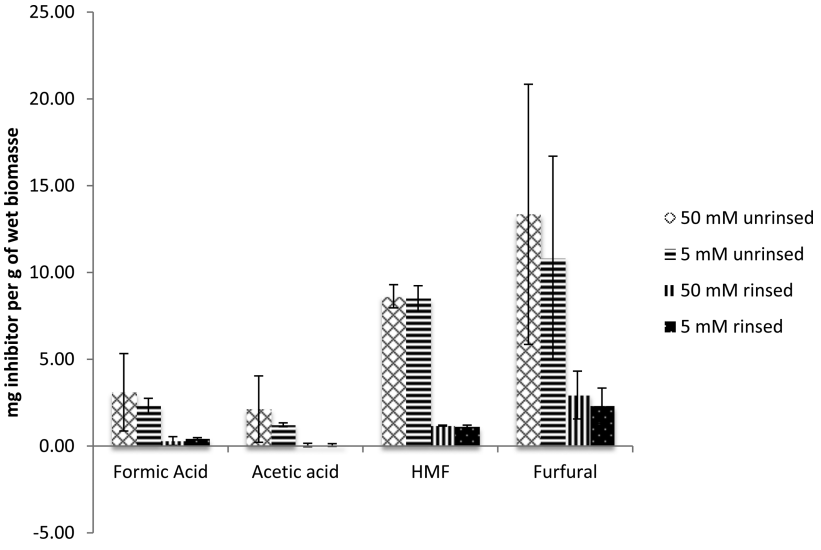

Figure 3. Dilute acid pine biomass pretreated in 1% H_2SO_4 at 160 °C for 30 min was either unrinsed or rinsed with 30X volumes of water. Enzymatic hydrolysis was conducted in 50 mM or in 5 mM citrate buffer. Formic acid, acetic acid, HMF, and furfural concentrations in enzymatic hydrolysis reaction mixture are presented. Results for the 24 h reaction are presented.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Baker, J. B.; Balmer, W. E. Loblolly pine. In *Silvicultural Systems for the major Forest types of the United States*; Burns, R. M., Ed.; United States Department of Agriculture: Washington, D.C., 1983; pp 148−152, Forest Service Agriculture Handbook 455.

(2) Rosson, J. F.; Rose, A. K. *Arkansas' forests, 2005*; U.S. Department of Agriculture, Forest Service: Asheville, NC, 2010; Southern Research Station Technical Report SRS-166.

(3) Cunningham, K.; Barry, J.; Walkingstick, T. *Managing loblolly pine stand. From A to Z*; University of Arkansas, Division of Agriculture, Cooperative Extension Service, FSA5023, 2008.

(4) Wyman, C. E., Ed. *Aqueous pretreatment of plant biomass for biological and chemical conversion to fuels and chemicals*; John Wiley & Sons, 2013.

(5) Normark, M.; Winestrand, S.; Lestander, T. A.; Jonsson, L. J. Analysis, pretreatment and enzymatic saccharification of different fractions of scots pine. *BMC Biotechnol.* **2014**, *14*, 20.

(6) Mabee, W. E.; Gregg, D. J.; Arato, C.; Berlin, A.; Bura, A.; Gilkes, N.; Mirochnik, O.; Pan, X.; Pye, E. K.; Saddler, J. N. Updates on softwood-to-ethanol process development. *Appl. Biochem. Biotechnol.* **2006**, *129−132*, 55−70.

(7) Galbe, M.; Zacchi, G. Pretreatment of lignocellulosic materials for efficient bioethanol production. *Adv. Biochem. Biochem. Eng. Biotechnol.* **2007**, *108*, 41−65.

(8) Lu, X.; Withers, M. R.; Seifkar, N.; Field, R. P.; Barrett, S. R. H.; Herzog, H. J. Biomass logistic analysis for large scale biofuel production: Case study of loblolly pine and switchgrass. *Bioresour. Technol.* **2015**, *183*, 1−9.

(9) Cotana, F.; Cavalaglio, G.; Gelosia, M.; Coccia, V.; Petrozzi, A.; Nicolini, A. Effect of double − step steam explosion pretreatment in bioethanol production from softwood. *Appl. Biochem. Biotechnol.* **2014**, *174*, 156−167.

(10) Li, X.; Luo, X.; Li, K.; Zhu, J. Y.; Fougere, J. D.; Clarke, K. Effects of SPORL and dilute acid pretreatment on substrate morphology, cell physiology and chemical cell wall structures, and subsequent enzymatic hydrolysis of lodgepole pine. *Appl. Biochem. Biotechnol.* **2012**, *168*, 1556−1567.

(11) Frederick, W. J., Jr.; Lien, S. J.; Courchene, C. E.; DeMartini, N. A.; Ragauskas, A. J.; Iisa, K. Production of ethanol from carbohydrates from loblolly pine: A technical and economic assessment. *Bioresour. Technol.* **2008**, *99*, 5051−5057.

(12) Wang, Z.; Lan, T.; Zhu, J. Lignosulfonate and elevated pH can enhance enzymatic saccharifiction of lignocelluloses. *Biotechnol. Biofuels* **2013**, *6*, 9.

(13) Treasure, T.; Gonzalez, R.; Jameel, H.; Phillips, R. B.; Park, S.; Kelley, S. Integrated conversion, financial, and risk modeling of cellulosic ethanol from woody and non-woody biomass via dilute acid pre- treatment. *Biofuels, Bioprod. Biorefin.* **2014**, *8*, 755−769.

(14) Kazi, F. K.; Fortman, J. A.; Anex, R. P.; Hsu, D. D.; Aden, A.; Dutta, A.; Kothandaraman, G. Techno-economic comparison of process technologies for biochemical ethanol production from corn stover. *Fuel* **2010**, *89*, S20−S28.

(15) Panagiotou, G.; Olsson, L. Effect of compounds released during pretreatment of wheat straw on microbial growth and enzymatic hydrolysis rates. *Biotechnol. Bioeng.* **2007**, *96*, 250−258.

(16) Casey, E.; Mosier, N. S.; Adamec, J.; Stockdale, Z.; Ho, N.; Sedlak, M. Effect of salts on the Co-fermentation of glucose and xylose by a genetically engineered strain of *Saccharomyces cerevisiae*. *Biotechnol. Biofuels* **2013**, *6*, 83.

(17) Hahn-Hagerdal, B.; Karhumaa, K.; Larsson, C. U.; Gorwa-Grauslund, M.; Gorgens, J.; van Zyl, H. Role of cultivation media in the development of yeast strains for large scale industrial use. *Microb. Cell Fact.* **2005**, *4*, 31.

(18) Cortez, D. V.; Roberto, I. C. Effect of phosphate buffer concentrationon the batch xylitol production by *Candida guilliermondii*. *Lett. Appl. Microbiol.* **2006**, *42*, 321−325.

(19) Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D. *Determination of structural carbohydrates and lignin in biomass. Laboratory Analytical Procedure*; National Renewable Energy Laboratory: Golden, CO, 2011; Technical Report NREL/ TP-510-42618.

(20) Rajan, K.; Carrier, D. J. Characterization of rice straw prehydrolyzates and their effect on the hydrolysis of model substrates, using a commercial endo-cellulase, β-glucosidase and cellulase cocktail. *ACS Sustainable Chem. Eng.* **2014**, *2*, 2124−2130.

(21) Frederick, N.; Zhang, N.; Ge, X.; Xu, J.; Pelkki, M.; Martin, E.; Carrier, D. J. Poplar (*Populus deltoides* L.): The effect of washing pretreated biomass on enzymatic hydrolysis and fermentation to ethanol. *ACS Sustainable Chem. Eng.* **2014**, *2*, 1835−1842.

(22) Burkhardt, S.; Kumar, L.; Chandra, R.; Saddler, J. How effective are traditional methods of compositional analysis in providing an accurate material balance for a range of softwood derived residues. *Biotechnol. Biofuels* **2013**, *6*, 90.

(23) Ewanick, S. M.; Bura, R.; Saddler, J. N. Acid-catalyzed steam pretreatment of lodgepole pine and subsequent enzymatic hydrolysis and fermentation to ethanol. *Biotechnol. Bioeng.* **2007**, *98*, 737−746.

(24) Huang, F.; Ragauskas, A. J. Dilute H_2SO_4 and SO_2 pretreatments of Loblolly pine wood residue for bioethanol production. *Ind. Biotechnol.* **2012**, *8*, 22−30.

(25) Nguyen, Q. A.; Tucker, M. P.; Boynton, B. L.; Keller, F. A.; Schell, D. J. 1998. Dilute acid pretreatment of softwoods. *Appl. Biochem. Biotechnol.* **1998**, *70-72* (70−72), 77−87.

(26) van der Pol, E. C.; Bakker, R. R.; Baets, P.; Eggink, G. By-products resulting from lignocellulose pretreatment and their inhibitory effect on fermentations for (bio)chemicals and fuels. *Appl. Microbiol. Biotechnol.* **2014**, *98*, 9579−9593.

(27) Marzialetti, T.; Olarte, M. B. V.; Sievers, C.; Hoskins, T. J. C.; Agrawal, P. K.; Jones, C. W. Dilute acid hydrolysis of Loblolly pine: A comprehensive approach. *Ind. Eng. Chem. Res.* **2008**, *47*, 7131−7140.

(28) Sannigrahi, P.; Ragauskas, A. J.; Miller, S. J. Effects of two-stage dilute acid pretreatment on the structure and composition of lignin and cellulose in loblolly pine. *BioEnergy Res.* **2008**, *1*, 205−214.

(29) Palmqvist, E.; Hahn-Hagerdal, B. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. *Bioresour. Technol.* **2000**, *74*, 25−33.

(30) Garcia, M. J.; Rios, G.; Ali, R.; Belles, J. M.; Serrano, R. Comparative physiology of salt tolerance in *Candidatropicalis* and *Saccharomyces cerevisiae*. *Microbiology* **1997**, *143*, 1125−1131.

(31) Wang, Z.; Lan, T.; Zhu, J. Lignosulfonate and elevated pH can enhance enzymatic saccharifiction of lignocelluloses. *Biotechnol. Biofuels* **2013**, *6*, 9.

(32) Jing, X.; Zhang, X.; Bao, J. Inhibition performance of lignocellulose degradation products on industrial cellulase enzymes during cellulose hydrolysis. *Appl. Biochem. Biotechnol.* **2009**, *159*, 696−707.

(33) Li, J.; Henriksson, G.; Gellerstedt, G. Lignin depolymerization/ repolymerization and its critical role for delignification of aspen wood by steam explosion. *Bioresour. Technol.* **2007**, *98*, 3061−3068.

DOI: 10.1021/acssuschemeng.5b00417
ACS Sustainable Chem. Eng. 2015, 3, 2423−2428

(34) Luterbacher, J.; Morán-Mirabel, J.; Burkholder, E.; Walker, J. Modeling enzymatic hydrolysis of lignocellulosic substrates using fluorescent confocal microscopy II: Pretreated biomass. *Biotechnol. Bioeng.* **2015**, *112*, 32−42.

DOI: 10.1021/acssuschemeng.5b00417
ACS Sustainable Chem. Eng. 2015, 3, 2423−2428