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Effect of Formic Acid and Furfural on the Enzymatic Hydrolysis of Cellulose Powder and Dilute Acid-Pretreated Poplar Hydrolysates

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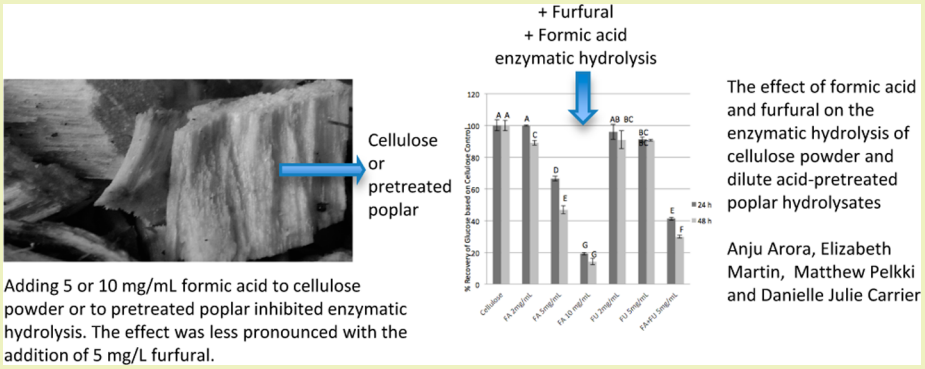

ABSTRACT: Biomass pretreatment often leads to the formation of compounds that are inhibitory to enzymatic hydrolysis. To remove inhibitory compounds prior to enzymatic hydrolysis, pretreated biomass is washed with at least 3 volumes of water. However, this washing step would be difficult to manage in commercial operations because of the unsustainable water consumption. This study reports on the effects of formic acid and furfural on Accellerase 1500 with cellulose powder and dilute acid-pretreated poplar as substrates. Using cellulose powder as the substrate for enzymatic hydrolysis with the addition of 5 or 10 mg/mL formic acid, glucose recovery was reduced by 34% and 81%, respectively, in comparison to the control. The addition of furfural, at 2 or 5 mg/mL, to the enzymatic system reduced glucose recovery by 5% and 9%, respectively. When 5 mg/mL of formic acid was combined with 5 mg/mL of furfural, glucose recovery in the cellulose powder enzymatic system was reduced by 59%. Inhibition of sugar recovery was more pronounced when dilute acid-pretreated poplar was used as a substrate for enzymatic hydrolysis. At 24 h incubation, recovery reductions were 94%, 97%, and 93% in the presence of 5 or 10 mg/mL formic acid or the 5 mg/mL combination.

KEYWORDS: *Cellulase, Enzyme inhibition, Formic acid, Furfural, Enzymatic saccharification*

1. INTRODUCTION

Conversion of cellulosic biomass to biofuels and bioproducts is an attractive proposition because feedstock is abundant. Cellulosic biomass includes forestry and agricultural products and residues, dedicated energy crops, and food and construction wastes.[1] Feedstock can be converted to biofuels and bioproducts through the biochemical conversion route: pretreatment of biomass, which loosens the lignin carbohydrate complex; hydrolyzation of pretreated biomass with cellulase and xylanase preparations; and fermentation of hydrolysates for production of target compounds.[2] Cellulosic biofuels, often termed as second generation, are carbon-neutral and therefore do not contribute to additional CO_2 emissions into the atmosphere.[3] Second-generation liquid biofuels reduce dependence on petroleum. Although advantageous, conversion of cellulosic biomass to biofuels and bioproducts is beleaguered with technical barriers that need to be conquered, such that the process can become economically viable.[4]

Apart from distillation, pretreatment of biomass and enzymatic conversion of carbohydrates to fermentable sugars are two of the most cost-intensive steps in biomass-to-ethanol processes.[5−7] Different pretreatment protocols selectively remove lignin or hemicelluloses. Dilute acid pretreatment, releasing hemicellulose in the hydrolysates, is emerging as one of the leading chemical pretreatment technologies.[8] Unfortunately, dilute acid-pretreatment processes often result in the production of inhibitory byproducts that hinder enzymatic saccharification and fermentation.[9,10] Because of the presence of these inhibitors, enzymatic hydrolysis reactors cannot be

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Table 1. Sugars Recovered from Cellulose Powder by the Action of Accellerase1500 in the Presence of Formic Acid and Furfural

	glucose recovered (mg/g of cellulose)					
time (h)	control: cellulose	formic acid, 5 mg/mL	formic acid, 10 mg/mL	furfural, 2 mg/mL	furfural, 5 mg/mL	furfural + formic acid, each 5 mg/mL
6	245.3 ± 5.2	238.3 ± 8.7	59.9 ± 10.4	220.2 ± 36.8	234.0 ± 1.2	156.6 ± 3.3
24	405.4 ± 14.0	269.2 ± 6.1	77.6 ± 3.2	388.4 ± 19.1	369.5 ± 6.6	167.5 ± 4.0
48	460.8 ± 15.0	216.4 ± 12.0	65.8 ± 9.1	419.5 ± 26.0	417.4 ± 2.7	137.2 ± 4.3

loaded at solids concentrations greater than 10 g/L, a loading lower than 200 g/L, which corresponds to loadings required in an economically viable production system.[11] The profile of these inhibitors varies with the nature of the feedstock. Biomass, such as hardwood, softwood, or herbaceous plants, differs in terms of their hemicellulose and lignin content, resulting in different classes and concentrations of inhibitors. In addition to varying feedstock chemical composition, the severity of the applied dilute acid pretreatment, regulated by temperature, pH, and residence time, also affects the concentration and nature of inhibitors.[9] However, there is agreement as to which inhibitors are common to most biomass.[9−14] Common degradation compounds include hemicellulose-derived oligomers, furfural, formic acid, and acetic acid; cellulose-derived hydroxymethylfurfural; and lignin-derived phenolic compounds. Some of these compounds can be removed by washing the pretreated biomass, while others remain embedded in the biomass and are released during successive bioconversion steps.[12,15] Inhibitors not only reduce glucose conversion during fermentation but also impede enzymatic hydrolysis.[9,10] Thus, it is critical to delineate the identity and corresponding inhibitor concentrations that impede enzymatic hydrolysis. Knowing which compounds need to be avoided could facilitate the design of pretreatment operations that minimize their concentrations, resulting in reduced water usage during biomass rinsing.

Specific inhibitors formed during pretreatment that impede the enzymatic hydrolysis step include (i) lignin derivatives, which cause nonproductive binding of the cellulase/xylanase preparation; (ii) xylose degradation compounds that cause inhibition to the enzymes; and (iii) oligomers and phenolic-derived compounds that cause the deactivation of the enzymes over time.[9,12−14,16] At the bench and pilot scale, inhibitory compounds are removed from dilute acid-pretreated biomass by washing with at least 3 volumes of water.[15] Unfortunately, this water usage would be difficult to replicate at the deployment scale because of the massive amounts of required water. Thus, a clear understanding of the effect that common degradation products, such as formic acid and furfural, have on enzymatic systems is mandatory, such that water usage can be minimized. This present study was conducted to determine the effect of two common degradation compounds, formic acid and furfural, which are readily formed during hemicellulose depolymerization in dilute acid pretreatments, on the commercial enzyme complex Accellerase1500.

2. MATERIALS AND METHODS

2.1. Cellulase Complex. Accellerase1500 (Genencor, Rochester, NY), endoglucanase (2200−2800 CMCase units/g), and β-glucosidase (525−750 pNPG units/g) enzyme were used in this study. The Accelerase1500 cocktail was obtained from a genetically modified microbial strain of *Trichoderma reesei*.

2.2. Substrates and Inhibitory Compounds. Microcrystalline cellulose powder (Sigma-Aldrich, Inc., St. Louis, MO) and dilute acid-pretreated wood from *Populus deltoides* low specific gravity clones were used as substrates for cellulase complex. The wood was from Eastern Texas cottonwood that was harvested after 14 years of growth, from the University of Arkansas Pine Tree Branch Station. The wood biomass, ground to 20 mesh, was pretreated with 1% v/v dilute acid at 160° for 60 min as described earlier.[18] Pretreated biomass was filtered from the slurry and washed with at least 10 volumes of water. Standards of formic acid and furfural (Sigma-Aldrich Co., St. Louis, MO) were used to study inhibition of enzyme activity. Stocks were prepared in Millipore water (resistivity of 18 MΩ) and added to the enzyme reaction mixture to give final concentrations 2, 5, and 10 mg/mL. Formic acid and furfural were applied alone or in combination.

2.3. Enzymatic Saccharification Experiments. Enzymatic saccharification studies were carried out essentially as outlined by Hodge et al.[15] Two series of experiments were set up to study cellulase complex inhibition with cellulose powder or dilute acid-pretreated poplar wood as substrates. One gram of substrate, 500 μL Accellerase 1500, 5 mL 0.1 M citrate buffer, pH 4.8, and water were added to give a total volume of 10 mL in 50 mL amber bottles. The bottles were placed in a shaking water bath (100 rpm) at 55 °C for 48 h. Samples were taken at timed intervals, boiled for 2 min to denature the enzymes, and analyzed for glucose concentrations. Percent activity was calculated as the ratio of sugars present in each treatment sample with and without inhibitory compounds. Glucose recoveries from cellulose powder or poplar slurries without inhibitors were used as controls. The inhibition effect was established by calculating the difference of glucose recovery, using the controls as maximum. The concentrations of formic acid and furfural used in this work were within the ranges of what Canterella et al. and Panagiotou and Olsson tested.[12,13]

2.4. Analysis of Sugars, Aromatic Aldehyde, and Aliphatic Acids by High-Performance Liquid Chromatography. Samples were centrifuged at 600*g* for 5 min; the pH of supernatants was adjusted to neutral and analyzed for sugar content by high-performance liquid chromatography (HPLC) as described by Martin et al.[18] Briefly, aliquots were filtered through a 0.2 μm syringe filter and analyzed for carbohydrate content using a Shodex (Waters, Milford, MA) SP-G precolumn and SP0810 column with water as the eluent, flowing at 0.2 mL/min, using a refractive index detector. A Waters 2695 (Milford, MA) HPLC system combined with a Waters 2996 UV detector was used to detect and quantify furfural and formic acid. The system was equipped with an Aminex (Bio-Rad Laboratories, Inc., Hercules, CA) HPX-87H ion-exchange column, heated at 55 °C, with 0.01 M H_2SO_4 flowing at 0.6 mL/min; UV detection was at 280 and 210 nm for furfural and formic acid, respectively.

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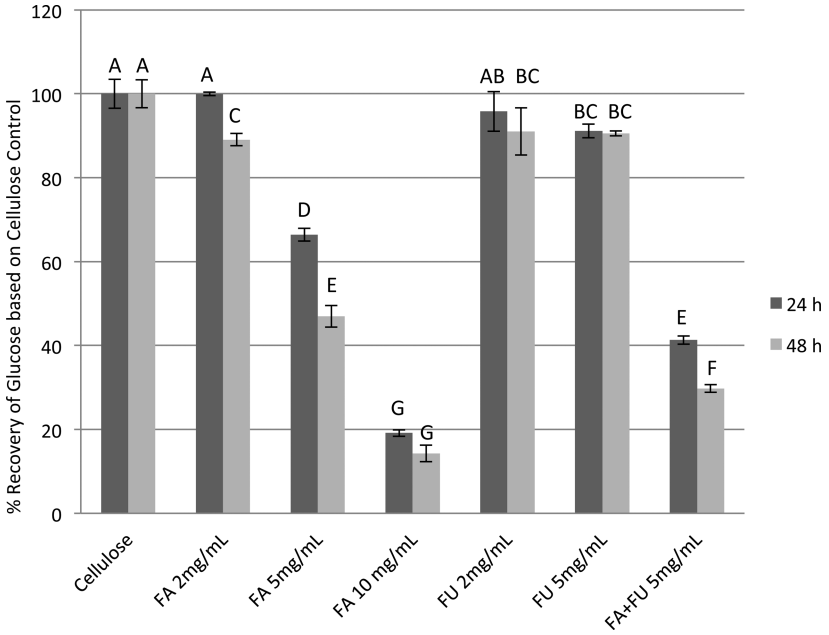

Figure 1. Inhibition of cellulase activity on cellulose powder in the presence of formic acid (FA) and furfural (FU) at 24 and 48 h. Percentages based on cellulose control to be 100%. Levels not connected by same letters are significantly different. JMP 9.0, Student t α = 0.050, t = 2.16037.

Table 2. Sugars Recovered from Washed Dilute Acid-Pretreated Poplar (Control) by the Action of Accellerase1500 in the Presence of Formic Acid and Furfural [Numbers in Parentheses Show the Percentage of Recovered Glucose Based on Compositional Analyses (463 mg/g of Material)]

	glucose recovered (mg/g of cellulose)			
time (h)	control: washed pretreated poplar	formic acid, 5 mg/mL	formic acid, 10 mg/mL	furfural + formic acid, each 5 mg/mL
24	254.4 ± 0.8 (54.9%)	15.7 ± 5.8 (3.5%)	7.9 ± 0.4 (1.7%)	16.6 ± 11.9 (3.7%)
48	208.8 ± 16.3 (45.1%)	11.6 ± 0.5 (2.6%)	5.9 ± 1.2 (1.3%)	13.7 ± 3.1 (3.0%)

2.5. Statistical Analysis. Analysis of the variance (ANOVA) was determined using JMP 9.0, LSMeans Differences Student t, with α = 0.050.

3. RESULTS AND DISCUSSION

3.1. Sugars Released from Cellulose and Inhibition of Cellulase Activity. The effect of Accellerase1500 on cellulose powder is presented in Table 1. The control glucose recoveries for 6, 24, and 48 h of incubation time were 245, 405, and 461 mg of glucose/g of cellulose. Glucose recovery increased by 160 and 56 mg of glucose/g of cellulose from 6 to 24 and from 24 to 48 h, respectively; incubation times past 24 h raised its concentration by 12%, indicating that the majority of the conversion occurred within 24 h. Initially, the addition of furfural to the cellulose powder system resulted in decreased glucose recovery. Samples incubated for 6 h with 2 mg/mL furfural released 220 mg of glucose/g of cellulose; however, as the incubation time increased to 48 h, the amount of recovered glucose, 420 mg of glucose/g of cellulose, approached that of the control. The addition of 5 mg/mL formic acid for 6, 24, or 48 h resulted in glucose concentrations of 238, 269, and 216, respectively, as compared to 461 mg of glucose/g of cellulose in the control. After 48 h of incubation in 10 mg/mL formic acid, only 66 mg of glucose/g of cellulose was recovered. By incubating in the presence of a combination of 5 mg/mL formic acid and 5 mg/mL furfural, 137 mg of glucose/g of cellulose were recovered, which was higher than with 10 mg/mL formic acid but less than with 5 mg/mL of furfural.

Figure 1 presents a statistical analysis of glucose recovery normalized with the control. When incubating for 24 h, the addition of formic acid and furfural at 2 mg/mL did not significantly affect the hydrolysis; however, after 48 h, this addition adversely affected the recovery. Formic acid at 5 or 10 mg/mL, furfural at 5 mg/mL, or the combination significantly lowered the sugar yields after 24 and 48 h of incubation. At 48 h, 47%, 14%, and 30% of glucose were obtained with 5 or 10 mg/mL formic acid or the combination, respectively. Formic acid (10 mg/mL) was determined to have the most severe effect on hydrolysis, resulting in glucose recovery below 20% at both 24 and 48 h. Panagiotou and Olsson reported the effects of 4 and 15 mg/mL on the enzymatic hydrolysis of Celluclast 1.5 FG and Novozymes 188 on filter paper to also be reduced to 20% glucose recovery.[13]

3.2. Activity of Cellulase on Pretreated Poplar Wood. Although inhibitor studies conducted with cellulose powder are informative, they do not provide the complex matrix that is characteristic of pretreated biomass. Pretreatments, such as dilute acid or steam explosion, lead to the generation of, among others, furfural and formic acid in the hydrolyzate.[12] Tengborg et al. reported that the addition of steam-pretreated softwood hydrolyzates to their enzymatic hydrolysis system reduced cellulose conversion by 36%.[17] Cantarella et al. tested the effect of adding formic acid and furfural to steam-exploded pretreated poplar hydrolysates.[12] They showed that raising the formic acid concentration by 7.8 mg/mL in pretreated poplar hydrolyzates inhibited Novozymes cellulase cocktails, thereby reducing glucose concentration by 83%.

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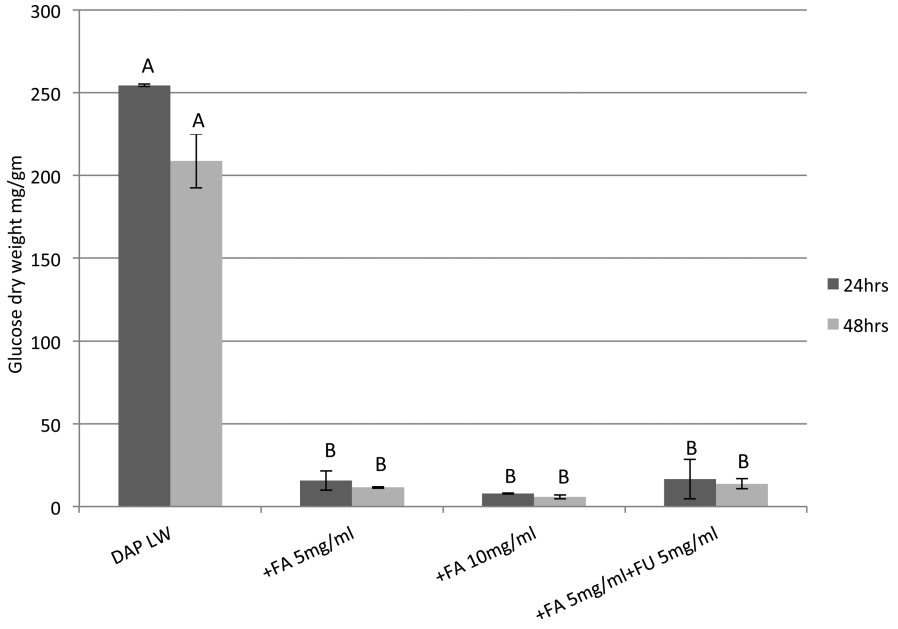

Figure 2. Inhibition of cellulase activity in the presence of formic acid (FA) and furfural (FU) with dilute acid-pretreated poplar (DAP) low specific gravity poplar wood as a substrate, after 24 and 48 h incubation. Levels not connected by same letters are significantly different. Statistical analysis by JMP 9.0, Student t α = 0.050, t = 3.18245.

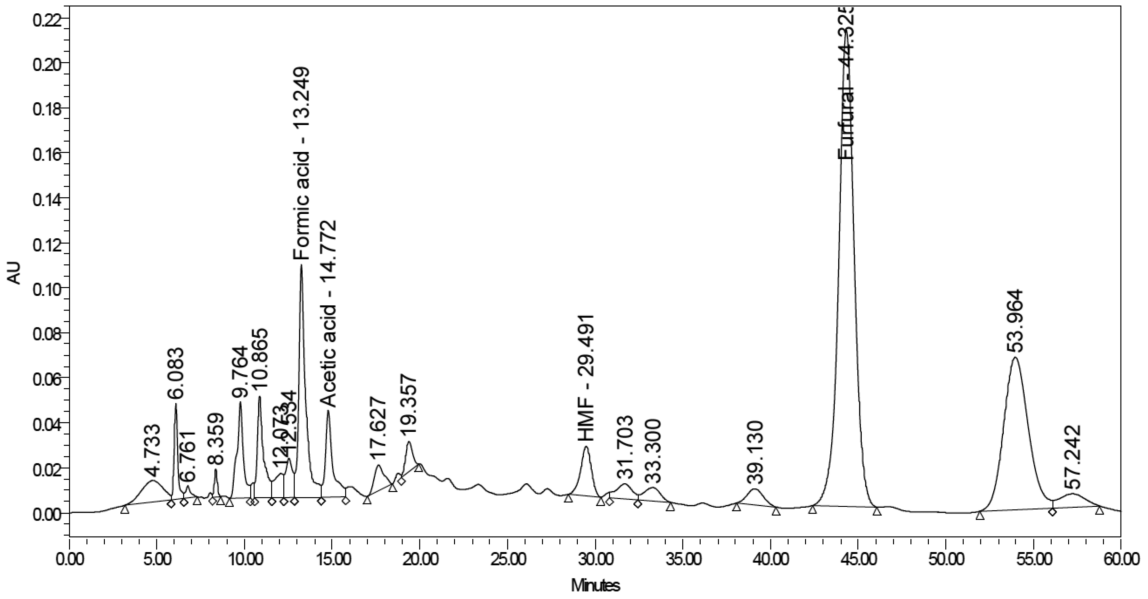

Figure 3. Chromatogram of pretreated wood low specific gravity clone, analyzed by HPLC. Retention times of formic acid, acetic acid, hydroxymethylfurfural, and furfural were 13.3, 14.8, 29.5, and 44.3 min, respectively. Peak at retention 54.0 min remains unidentified. Separation was obtained with an Aminex HPX-87H ion-exchange column, heated at 55 °C, with 0.01 M H_2SO_4 flowing at 0.6 mL/min. Results are presented with UV detection at 210 nm.

Poplar, a potential woody energy crop, is an interesting system in which to test enzymatic hydrolysis inhibitors. Table 2 presents glucose concentrations, as a function of hydrolysis time, of washed dilute acid-pretreated poplar incubated with Accellerase1500 for 24 and 48 h, respectively. Results show that the inhibitory effect did not subside with incubation time. Compositional analysis of wood from low specific gravity poplar clone heartwood determined that glucose content was 463 ± 26 mg/g of material. The recovered glucose yields from washed pretreated poplar were calculated as 55% and 45% for 24 and 48 h hydrolysis, respectively. Addition of 5 mg/mL formic acid reduced 24 h glucose recovery by 94%. The addition of 10 mg/mL formic acid to the poplar enzymatic system resulted in the release of less than 7.9 mg of glucose/g of material. Figure 2 presents, in a graphical fashion, the inhibition effect of formic acid. For all tested formic acid concentrations, no more than 3.5% of glucose was recovered, and the inhibition was significant for all tested conditions. The addition of the combination of each 5 mg/mL formic acid and furfural resulted in 59% and 93% reductions in glucose recovery for cellulose and poplar enzymatic hydrolysis systems, respectively, indicating that addition of aliphatic acid has a marked effect in the pretreated poplar system.

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Rinsing poplar hydrolysates may not remove all inhibitory compounds. The nature of poplar hydrolysates is likely to be more complex than that of cellulose. Figure 3 presents a HPLC/UV chromatogram of low specific gravity dilute acid hydrolyzates where the retention times of formic acid, acetic acid, hydroxymethylfurfural, and furfural were 13.3, 14.8, 29.5, and 44.3 min, respectively; the compound eluting at 54.0 min remains unidentified. On the basis of hydrolyzate analyses, hydroxymethylfurfural, furfural, formic acid, and acetic acid were quantified as 0.13 ± 0.24, 1.90 ± 0.21, 15.01 ± 2.67, and 4.46 ± 0.92 g, respectively, per 100 g of biomass. These analyses indicate that when 1 g of dilute acid-pretreated poplar is used as a substrate for enzymatic hydrolysis, 150 mg of formic acid will be introduced into the system, necessitating removal or dilution prior to enzymatic hydrolysis.

Elevated inhibitory compound concentrations in prehydrolyzates justify why pretreated biomass must be detoxified prior to enzymatic hydrolysis.[12,15] Recent studies in our laboratory showed that the hydrolysis of xylotetraose, a four xylose hemicellulose-derived oligomer, in 160 °C water for 30 min led to the production of 1.1 mg/mL of formic acid, contributing to the increase of formic acid pools. Xylotetraose is only one of many hydrolyzate components, indicating that there could be a plethora of cell-wall-derived compounds adding to formic acid pools. To remove inhibitors formed during pretreatment, detoxification processes are conducted. Zhang et al. evaluated an activated carbon detoxification system to be used to remove 4 mg/mL furfural from hydrolyzates prior to fermentation.[19] Hodge et al. removed, among others, 3.9 mg/mL furfural that was generated while pretreating corn stover in dilute acid by washing the pretreated biomass with 3 volumes of water prior to enzymatic hydrolysis.[15] Cantarella et al. washed 1 g of steam-explosion poplar pulp with either 12.5 or 66.7 mL of water.[12] At the bench scale, these inhibitor removal strategies can be effective; however, a thorough understanding of formic acid generation during pretreatment would provide a better approach, decreasing the need for additional detoxification unit operations.

This study bridges those of Cantarella et al. and Panagiotou and Olsson where side-by-side testing of the effect of adding formic acid to enzymatic hydrolysis systems of cellulose and dilute acid-pretreated poplar hydrolyzates was evaluated; this aliphatic acid proves to be a potent inhibitor of poplar systems.[12,13]

4. CONCLUSION

This study demonstrated that 5 and 10 mg/mL formic acid inhibited the recovery of glucose from cellulose powder and from dilute acid-pretreated poplar biomass, using the Accellerase1500 complex. The comparison between cellulose powder and dilute acid-pretreated poplar as substrates in enzymatic hydrolysis emphasized that, although rinsed, the addition of 5 mg/mL formic acid to poplar hydrolyzates annulled glucose recovery. These results indicate that there are remaining inhibitory compounds in washed hydrolysates. A better understanding of the effect of pretreatment processing parameters on inhibitor generation will reduce their production, eventually minimizing water usage.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) U.S. Department of Energy. U.S. Billion-Ton Update: Biomass Supply for a Bioenergy and Bioproducts Industry; Perlack, R. D., Stokes, B. J., Eds.; ORNL/TM-2011/224; Oak Ridge National Laboratory: Oak Ridge, TN, 2011; p 227.

(2) Mosier, N. S.; Wyman, C. E.; Dale, B. E.; Elander, R. T.; Lee, Y. Y.; Holtzapple, M. T.; Ladisch, M. R. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2005**, *96* (6), 673−686.

(3) McKendry, P. Energy production from biomass (part 1): Overview of biomass. *Bioresour. Technol.* **2002**, *83* (1), 37−46.

(4) Lynd, L. R.; Laser, M. S.; Bransby, D.; Dale, B. E.; Davison, B.; Hamilton, R.; Himmel, M.; McMillan, J. D.; Sheehan, J.; Wyman, C. E. How biotech can transform biofuels. *Nat. Biotechnol.* **2008**, *26* (2), 169−172.

(5) Wooley, R.; Ruth, M.; Glassner, D.; Sheehan, J. Process design and costing of bioethanol technology: A tool for determining the status and direction of research and development. *Biotechnol. Prog.* **1999**, *15* (5), 794−803.

(6) Lynd, L. R.; Elander, R. T.; Wyman, C. E. Likely features and costs of mature biomass ethanol technology. *Appl. Biochem. Biotechnol.* **1996**, *57*−*58*, 741−761.

(7) Wyman, C. E.; Dale, B. E.; Elander, R. T.; Holtzapple, M. T.; Ladisch, M. R.; Lee, Y. Y. Coordinated development of leading biomass pretreatment technologies. *Bioresour. Technol.* **2005**, *96* (18), 1959−1966.

(8) Sannigrahi, P.; Kim, D. H.; Jung, S.; Ragauskas, A J. Pseudo-lignin and pretreatment chemistry. *Energy Environ. Sci.* **2011**, *4*, 1306−1310.

(9) Kumar, R.; Wyman, C. E. The impact of dilute sulfuric acid on the selectivity of xyloligomer depolymerization to monomers. *Carbohydr. Res.* **2008**, *343*, 290−300.

(10) Palmqvist, E.; Hahn-Hagerdal, B. Fermentation of lignocellulosic hydrolysates. II: Inhibitors and mechanisms of inhibition. *Bioresour. Technol.* **2000**, *74* (1), 25−33.

(11) Ximenes, E.; Kim, Y.; Mosier, N. S.; Dien, B. S.; Ladisch, M. R. Inhibition of cellulases by phenols. *Enzyme Microb. Technol.* **2010**, *46* (3−4), 170−176.

(12) Cantarella, M.; Cantarella, L.; Gallifuoco, A.; Spera, A.; Alfani, F. Effect of inhibitors released during steam explosion treatment of poplar wood on subsequent enzymatic hydrolysis and SSF. *Biotechnol. Prog.* **2004**, *20* (1), 200−206.

(13) Panagiotou, G.; Olsson, L. Effect of compounds released during pretreatment of wheat straw on microbial growth and enzymatic hydrolysis rates. *Biotechnol. Bioeng.* **2007**, *96* (2), 250−258.

(14) Ximenes, E.; Kim, Y.; Mosier, N.; Dien, B. S.; Ladisch, M. R. Deactivation of cellulases by phenols. *Enzyme Microb. Technol.* **2011**, *48* (1), 54−60.

(15) Hodge, D. B.; Karim, M. N.; Schell, D. J.; Macmillan, J. D. Soluble and insoluble solids contributions to high-solids enzymatic hydrolysis of lignocellulose. *Bioresour. Technol.* **2008**, *99* (18), 8940−8948.

(16) Berlin, A.; Balakshin, M.; Gilkes, N.; Kadla, J.; Maximenko, V.; Kubo, S.; Saddler, J. Inhibition of cellulase, xylanases and β-glucosidase

activities by softwood lignin preparations. *J. Biotechnol.* **2006**, *125* (2), 198−209.
(17) Tengborg, C.; Galbe, M.; Zacchi, G. Reduced inhibition of enzymatic hydrolysis of steam-pretreated softwood. *Enzyme Microb. Technol.* **2001**, *28* (9−10), 835−844.
(18) Martin, E. M.; Bunnell, K. A.; Lau, C. S.; Pelkki, M. H.; Patterson, D. W.; Clausen, E. C.; Smith, J. A.; Carrier, D. J. Hot water and dilute acid pretreatment of high and low specific gravity *Populus deltoides* clones. *J. Ind. Microbiol. Biotechnol.* **2011**, *38* (2), 355−361.
(19) Zhang, K.; Agrawal, M.; Harper, J.; Chen, R.; Koros, W. J. Removal of the fermentation inhibitor using activated carbon in cellulosic-ethanol production. *Ind. Eng. Chem. Res.* **2011**, *50*, 14055−14060.