



## Reprint



# Comparison of Two Acid Hydrotropes for Sustainable Fractionation of Birch Wood

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This study reports on a comparative study of acid hydrotropic fractionation (AHF) of birch wood using maleic acid (MA) and *p*-toluenesulfonic acid (*p*-TsOH). Under the same level of delignification, lignin dissolved by MA is much less condensed with a higher content of ether aryl  $\beta$ -O-4 linkages. Lignin depolymerization dominated in MA hydrotropic fractionation (MAHF) and resulted in a single lower molecular weight peak, in contrast to the competitive depolymerization and repolymerization in *p*-TsOH AHF with a bimodal distribution. The less condensed MA-dissolved lignin facilitated catalytic conversion to monophenols. Carboxylation of residual lignin in fractionated cellulosic water-insoluble solids (WISs) enhanced enzymatic saccharification by decreasing nonproductive cellulase binding to lignin. At a low cellulase loading of 10 FPU g<sup>-1</sup> glucan, sac-

charification of WIS-M<sub>T120</sub> from MAHF at 120 °C was 95% compared with 48% for WIS-P<sub>T85</sub> from *p*-TsOH AHF at 85 °C under the same level of delignification of 63%. Residual lignin carboxylation also facilitated nanofibrillation of WIS for producing lignin-containing cellulose nanofibrils (LCNFs) through an enhanced lignin lubrication effect, which substantially decreases fibrillation energy. LCNFs from only one pass of microfluidization of WIS-M<sub>T120</sub> have the same morphology as those from WIS-P<sub>T85</sub> after three passes. MA also has a lower solubility and higher minimal hydrotropic concentration, which facilitated acid recovery. MA is U.S. Food and Drug Administration (FDA)-approved as an indirect food additive, affording significant advantages compared with *p*-TsOH for biorefinery applications.

## Introduction

Using lignocellulosic biomass as a renewable feedstock to produce biofuels, biochemicals, and bioproducts through the biorefinery concept can help to achieve a sustainable future. However, economic and sustainable biomass preprocessing methods are needed to fractionate lignocelluloses into easily processable building blocks such as sugars, lignin precursors, and lignocellulosic nanomaterials for efficient downstream conversion to a variety of biobased products. Only limited success has been achieved despite many research efforts in the last several decades. Traditional fractionation processes, including commercial wood pulping, dilute acid,<sup>[1,2]</sup> steam explosion,<sup>[3]</sup> al-

kaline,<sup>[2]</sup> organosolv,<sup>[4–6]</sup> and sulfite,<sup>[7,8]</sup> are primarily focused on obtaining polysaccharides by dissolving hemicelluloses and/or lignin and are conducted at high temperatures resulting in condensed lignin,<sup>[9,10]</sup> which is not suitable for value-added utilization other than as boiler fuel.<sup>[11]</sup> Recent advances such as reductive catalytic fractionation,<sup>[12]</sup> organic solvents,<sup>[5,13–16]</sup> and ionic-liquid-based systems<sup>[17]</sup> have not fully addressed chemical recovery, chemical toxicity, or value-added utilization of all components of the lignocelluloses. Therefore, biorefining lignocellulosic biomass remains a challenge.

Acid hydrotropic fractionation (AHF) was recently developed in our laboratory<sup>[18]</sup> and has several attractive characteristics: (1) rapid ( $\leq 30$  min) fractionation at atmospheric pressure and low temperatures with the potential of substantially decreasing capital cost compared with technologies operated at high pressures with extensive lignin condensation,<sup>[19–21]</sup> (2) high selectivity in dissolution of hemicelluloses and lignin while preserving cellulose for high-value utilization,<sup>[21,22]</sup> (3) easy lignin separation using simple precipitation by diluting the fractionation liquor with water to the minimal hydrotropic concentration (MHC),<sup>[18,23]</sup> (4) no need for an additional catalyst to dehydrate the dissolved hemicellulosic sugars into furfural,<sup>[20,24]</sup> and (5) direct reuse of the acid hydrotrope after lignin separation and reconcentration.

Since the initial discovery of aromatic acids, such as *p*-toluenesulfonic acid (*p*-TsOH), having hydrotropic properties for rapid dissolving lignin,<sup>[18]</sup> we recently found that maleic acid (MA), a nonaromatic dicarboxylic acid, has good hydrotropic

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properties in solubilizing wood lignin and produces esterified lignin with a light color and low degree of condensation.<sup>[20]</sup> MA is a solid at room temperature (20 °C) and has a lower solubility (40 wt% compared with 60 wt% for *p*-TsOH), which eases acid recovery. MA also has a higher MHC (25 wt%) than *p*-TsOH (11.5 wt%), which decreases the amount of water needed for lignin separation. MA is a U.S. Food and Drug Administration (FDA)-approved indirect food additive (21CFR175-177) (Code of Federal Regulations (CFR)) and is less corrosive ( $pK_a=1.9$ ) than *p*-TsOH ( $pK_a=-2.8$ ), which can significantly decrease environmental impact and capital cost of biomass biorefinery.

Concentrated monocarboxylic acids have long been used for pulping<sup>[25–27]</sup> and lignocellulosic biomass fractionation.<sup>[28,29]</sup> Formic acid and acetic acid have a Hildebrand solubility of 12.1 and 10.1 MPa<sup>1/2</sup>, respectively, which is close to that of lignin (11 MPa<sup>1/2</sup>). This accounts for their ability to dissolve lignin in aqueous solutions at high concentrations after lignin cleavage by acidolysis. This lignin dissolution mechanism is different from that of AHF<sup>[18]</sup> using dicarboxylic MA. Furthermore, for effective lignin dissolution, a longer reaction time or a higher temperature is often required when using acetic or formic acid solvent systems than for maleic acid hydrotopic fraction (MAHF). As monocarboxylic acids, they esterify but do not carboxylate cellulose and/or lignin. They are fully miscible with water and have a similar boiling point as water, both of which makes recovery difficult compared with MA.

The objective of this study was to evaluate AHF of birch wood using *p*-TsOH and MA. For meaningful comparison, we chose to use the same acid concentration for both *p*-TsOH and MA and targeted the same level of delignification by properly controlling reaction temperature and time. We measured the resultant fractionated water-insoluble solids (WISs) yield and their utility for producing enzymatic sugars and cellulosic nanomaterials. We also compared the chemical structure of the dissolved lignin by 2D nuclear magnetic resonance (NMR) spectroscopy and the utility of the dissolved lignin for producing aromatics through supercritical methanol dehydration hydrodeoxygenation. Finally, we evaluated the recovery potential of the two acids through crystallization. This study has value

for developing practically implementable and cost-effective acid hydrotopic biorefinery technologies.

## Results and Discussion

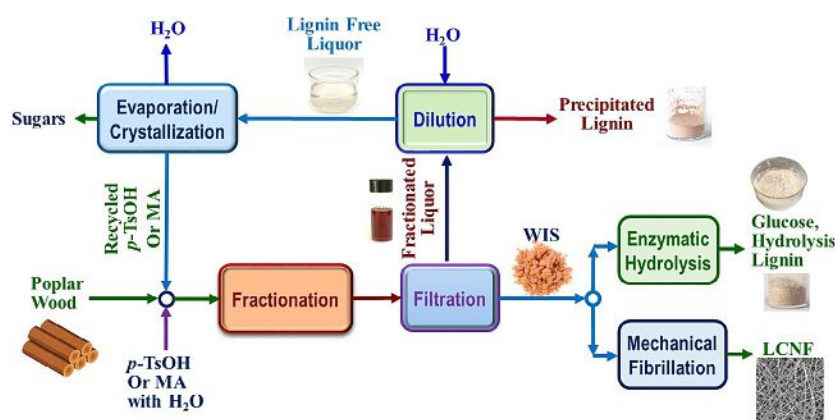
### Wood fractionation by *p*-TsOH and MA

Wood fractionation experiments were carried out according to the schematic flow diagram shown in Figure 1. AHF of birch using *p*-TsOH at 60 wt% and 85 °C for 20 min dissolved 62.5% lignin and 61.0% xylan (Table 1). Using the same 60 wt% MA and 120 °C for 30 min achieved comparable delignification of 63.4% with a slightly higher xylan dissolution of 66.8%. Decreasing MAHF reaction temperature to 110 °C decreased both lignin and xylan dissolution. It should be pointed out that the MA solution was not boiling at 120 °C because MA elevated the boiling point (the experimentally measured boiling point of 60 wt% MA aqueous solution is approximately 130 °C). MAHF at 120 °C resulted in 15% cellulose dissolution compared with 10% using *p*-TsOH at 85 °C. Most of the dissolved xylan was converted into xylose for all runs, as listed in Table 1.

### Chemical and physical properties of lignin from AHF

2D-NMR spectroscopy revealed differences in the chemical structure of AHF-dissolved lignin (AHL) using MA and *p*-TsOH. Compared with milled wood lignin (MWL), shown in Figure 2A, the AHL samples (Figures 2B–D) showed higher carbohydrate content because of retained lignin carbohydrate complex (LCC) bonds.<sup>[30]</sup> The spectra for the two AHLs, L-M<sub>T110</sub> (Figure 2B) and L-M<sub>T120</sub> (Figure 2C) from MAHF at 110 and 120 °C, respectively, have peaks at  $\delta_C/\delta_H=127.8/6.2$ ,  $133.2/6.4$ , and  $63.6/(4.03, 4.39)$  that are all characteristic of lignin esterification by MA at the  $\gamma$ -OH position, as verified by our earlier study.<sup>[20]</sup> These signals are absent in the spectrum of the AHL, L-P<sub>T85</sub> (Figure 2D), dissolved by *p*-TsOH at 85 °C because *p*-TsOH does not esterify lignin.

Despite higher temperatures and longer fractionation time, MAHF results in significantly higher retention of  $\beta$ -O-4 linkages than *p*-TsOH AHF because of its weaker acidity ( $pK_a$  of MA =

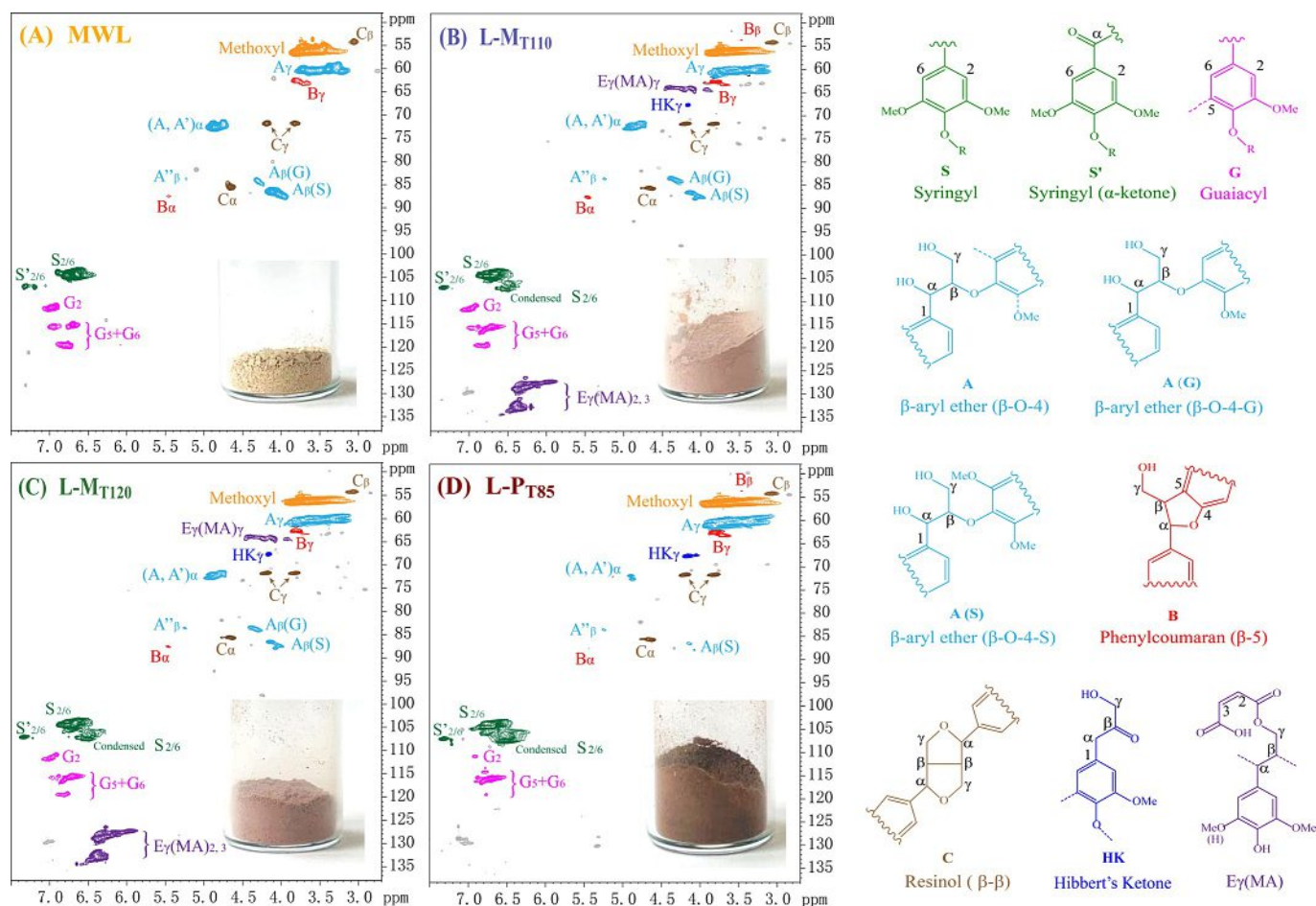


**Figure 1.** Process flow diagram of treating wood biomass with solid-acid hydrotrope for a comprehensive utilization of lignocellulose. LCNF: lignin-containing cellulosic nanofibrils.

**Table 1.** Chemical composition of fractionated WISs and their corresponding fractionation liquors under different conditions. The numbers in the parentheses are percentages of the component retained in WIS or percentages of wood xylan or mannan converted to xylose or mannose in the fractionated liquor on a total untreated dry wood basis.

| Sample <sup>[a]</sup> | Yield of WIS [%] | Content in WIS [%] |             |             |            | Content in fractionation liquor [g kg <sup>-1</sup> ] |           |
|-----------------------|------------------|--------------------|-------------|-------------|------------|-------------------------------------------------------|-----------|
|                       |                  | kraft lignin       | glucan      | xylan       | mannan     | xylose                                                | glucose   |
| birch                 | –                | 19.5               | 40.0        | 23.0        | 1.4        | –                                                     | –         |
| M60T110t30            | 62.9             | 16.2 (52.3)        | 55.2 (86.9) | 15.9 (43.5) | 1.9 (81.2) | 12.4 (53.9)                                           | 0.5 (1.3) |
| M60T120t30            | 55.4             | 12.9 (36.6)        | 61.3 (85.0) | 13.8 (33.2) | 1.5 (57.7) | 14.9 (64.8)                                           | 1.1 (2.8) |
| P60T85t20             | 57.1             | 12.8 (37.5)        | 62.9 (89.9) | 15.7 (39.0) | 2.3 (93.8) | 13.6 (59.2)                                           | 0.4 (1.0) |

[a] M/PxxTyytzz: Mxx or Pxx stands for maleic acid or *p*-TsOH concentration in xx wt%, Tyy stands for temperature in yy °C, tzz stands for fractionation time in zz min.



**Figure 2.** 2D <sup>1</sup>H–<sup>13</sup>C HSQC NMR spectra of MWL and AHLs. E<sub>γ</sub>(MA) is the S or G unit lignin with γ-OH maleated. L-M-T<sub>110</sub> and L-M-T<sub>120</sub>: dissolved lignin by maleic acid at 110 and 120 °C for 30 min, respectively; L-P-T<sub>85</sub>: dissolved by *p*-TsOH at 85 °C for 20 min.

1.9;  $pK_a$  of *p*-TsOH = −2.8). L-M-T<sub>110</sub> and L-M-T<sub>120</sub> retained more than 60% and 40% of the β-O-4 linkages, respectively, whereas AHL L-P-T<sub>85</sub> lost almost 95% β-O-4 (Figure 2 and Table 2). L-P-T<sub>85</sub> also has a much darker color than MWL, L-M-T<sub>110</sub>, and L-M-T<sub>120</sub> (Figure 2) because of condensation and the formation of chromophore groups. The pinkish color of the AHL is attributed to the conjugated double bond and aldehyde auxochrome group in coniferyl aldehyde and the ketone auxochrome group in 1-hydroxy-3-(4-hydroxy-3-methoxyphenyl)-2-propanone (HHMP)<sup>[31]</sup>, both formed by depolymerization of Hibbert's

ketones (HK), which are observed at  $\delta_C/\delta_H$  = 67.1/4.2 (γ-position) in Figure 2B–D.<sup>[32]</sup>

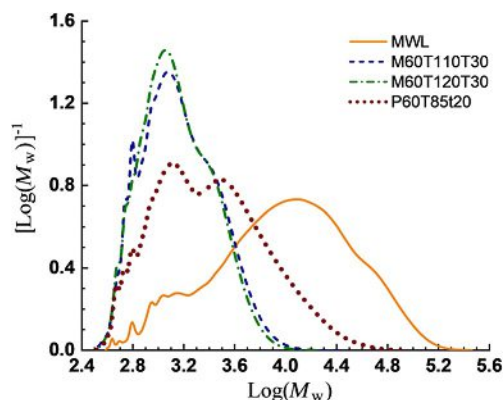
The S/G ratio increased after AHF (Table 2) but much more significantly with the use of *p*-TsOH mainly because of the decreased G signal upon condensation. The decrease in the amount of G subunits in L-P-T<sub>85</sub> compared with MWL, as measured by G<sub>2</sub> signal, was 90%, in contrast to the decrease of 56% and 33% in L-M-T<sub>110</sub> and L-M-T<sub>120</sub>, respectively. This suggests that MAHF more effectively dissolves G subunits than does *p*-TsOH fractionation at equivalent levels of delignification.

**Table 2.** Structural characteristics (interunit linkages, aromatic units, and S/G ratio) of AHL from integrating  $^1\text{H}$ - $^{13}\text{C}$  correlation peaks in the HSQC spectra (the condensed  $\text{S}_{2/6}$  is abbreviated as  $\text{S}_{\text{Cond}}$ ).

| AHL <sup>[a]</sup>          | Aromatic units [%]      |                          |                          |          |          | S/G  | Interunit linkages [%] |         |         | <i>M</i> <sub>n</sub> | <i>M</i> <sub>w</sub> | <i>M</i> <sub>w</sub> / <i>M</i> <sub>n</sub> |
|-----------------------------|-------------------------|--------------------------|--------------------------|----------|----------|------|------------------------|---------|---------|-----------------------|-----------------------|-----------------------------------------------|
|                             | <i>S</i> <sub>2/6</sub> | <i>S'</i> <sub>2/6</sub> | <i>S</i> <sub>Cond</sub> | <i>S</i> | <i>G</i> |      | β-O-4 [%]              | β-5 [%] | β-β [%] |                       |                       |                                               |
| MWL                         | 76.7                    | 4.1                      | –                        | 80.8     | 19.2     | 4.2  | 64.6                   | 1.2     | 11.2    | 3227                  | 14832                 | 4.60                                          |
| L- <i>M</i> <sub>T110</sub> | 74.0                    | 3.3                      | 9.8                      | 87.1     | 12.9     | 6.7  | 41.0                   | 1.0     | 11.1    | 1048                  | 1598                  | 1.52                                          |
| L- <i>M</i> <sub>T120</sub> | 62.9                    | 3.2                      | 25.4                     | 91.5     | 8.5      | 10.7 | 26.7                   | 0.1     | 8.9     | 1035                  | 1516                  | 1.46                                          |
| L- <i>P</i> <sub>T85</sub>  | 40.4                    | 3.2                      | 55.0                     | 98.6     | 1.4      | 72.5 | 3.6                    | 0       | 6.4     | 1346                  | 3290                  | 2.44                                          |

[a] L- $\text{M}_{\text{T}110}$  and L- $\text{M}_{\text{T}120}$ : by maleic acid at 110 and 120 °C for 30 min, respectively; L- $\text{P}_{\text{T}85}$ : by *p*-TsOH at 85 °C for 20 min.

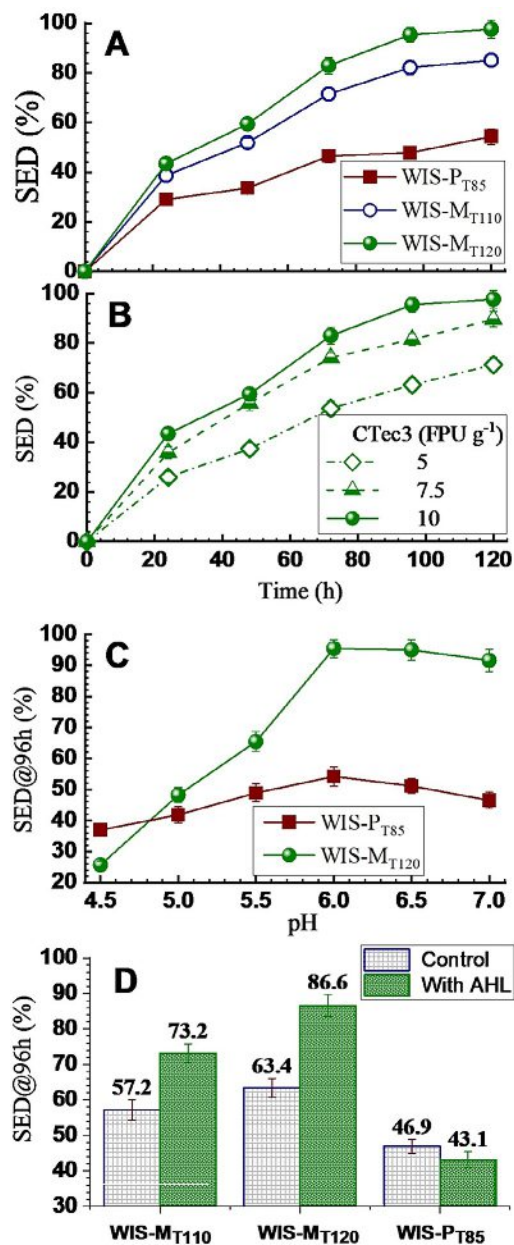
The weight-average molecular weight ( $M_w$ ) and number-average molecular weight ( $M_n$ ) of MWL are 3227 and 14832, respectively, with a broad distribution as shown in Figure 3. AHL substantially depolymerized lignin as well as repolymerized lignin through forming C–C bonds to condense lignin.<sup>[21,31]</sup> L- $\text{M}_{\text{T}110}$  and L- $\text{M}_{\text{T}120}$  had much lower molecular weight and more narrow (or more uniform) distribution than MWL because of increased depolymerization (Figure 3 and Table 2). The fact that the  $M_w$  distribution of L- $\text{M}_{\text{T}120}$  almost completely overlaps that of L- $\text{M}_{\text{T}110}$ , with only a slight decrease in average  $M_w$  suggests that repolymerization starts becoming important as MAHF temperature increases above 110 °C, approaching the boiling point of approximately 130 °C. This is also reflected in the decrease in  $\beta\text{-O-4}$  linkages (Table 2). By comparison, the  $M_w$  of L- $\text{P}_{\text{T}85}$  was substantially greater than that of L- $\text{M}_{\text{T}120}$ , suggesting that repolymerization becomes significant at much lower temperatures using the aromatic acid. This is also reflected in the substantial decrease in  $\beta\text{-O-4}$  linkages to only 3.6% and the bimodal distribution with the first peak very similar to the peak of the distributions of L- $\text{M}_{\text{T}120}$  and L- $\text{M}_{\text{T}110}$ .



**Figure 3.** Molecular weight distributions of the AHLs in comparison with that of MWL.

### Enzymatic hydrolysis of fractionated WISs

The fractionated WISs were evaluated for enzymatic sugar production. The substrate enzymatic digestibility (SED) of three WISs were significantly different as shown in Figure 4A. SED of more than 95% was achieved after 96 h hydrolysis for the WIS from M60T120T30 (WIS- $\text{M}_{\text{T}120}$ ) at a commercial cellulase CTec3



**Figure 4.** A) Time-dependent SED of WISs from three AHLs (pH 6.0, cellulase loading: 10 FPU g glucan<sup>−1</sup>); B) time-dependent SED of WIS- $\text{M}_{\text{T}120}$  under different cellulase loadings (pH 6.0); C) comparison of SED@96 h between WIS- $\text{M}_{\text{T}120}$  and WIS- $\text{P}_{\text{T}85}$  at different pH (cellulase loading: 10 FPU g glucan<sup>−1</sup>); D) comparisons of SED@96 h of WISs from three AHLs with and without spiking 1 g L<sup>−1</sup> their corresponding AHL (pH 5.5, cellulase loading: 10 FPU g glucan<sup>−1</sup>).

dosage of only  $10 \text{ FPU g}^{-1}$  glucan. By comparison, the WIS from P60T85t20 (WIS- $P_{T85}$ ) with a similar level delignification of 63% achieved only half of the digestibility or SED of 48%. This is also significantly lower than the 82% SED from the milder M60T110t30 (WIS- $M_{T110}$ ), which has lower levels of delignification (48%) and hemicellulose removal (57%) (Table 1). Furthermore, the two WISs from MAHF had a much faster hydrolysis rate than the WIS- $P_{T85}$ . Notably, this also clearly indicates that factors other than WIS physical porosity (accessibility) created by dissolution of lignin and hemicelluloses affected SED.

The SED of WIS- $M_{T120}$  was also evaluated under significantly decreased cellulase loadings, as shown in Figure 4B. Even with CTec3 loading decreased by 50% to  $5 \text{ FPU g}^{-1}$  glucan, SED@120 h was 71%, significantly higher than the 54% for WIS- $P_{T85}$  achieved at double the CTec3 loading of  $10 \text{ FPU g}^{-1}$  glucan. With extended hydrolysis time beyond 120 h, SED of WIS- $M_{T120}$  could be expected to further increase over 71% because the time-dependent hydrolysis curve has not reached a plateau (Figure 4B). This suggests that using MAHF for enzymatic sugar production can save more than 50% of cellulase compared with using *p*-TsOH fractionation.

The charged lignin surface resulting from carboxylation by MAHF<sup>[20]</sup> decreases nonproductive cellulase binding to lignin under elevated pH of 6.0.<sup>[33,34]</sup> As shown in Figure 4C, we compared enzymatic hydrolysis of WIS- $M_{T120}$  and WIS- $P_{T85}$  at a range of pH between 4.5 and 7.0. At the commonly used pH between 4.8 and 5.0 near the iso-electrostatic point (*pI*) of cellulase, the SED@96 h of WIS- $M_{T120}$  is nearly equal to that of WIS- $P_{T85}$  because of almost identical delignification and xylan dissolution.

Increasing buffer solution pH increased SED to a maximal value at pH 6.0 for both substrates. However, WIS- $M_{T120}$  exhibited a 138% increase (more than double) from approximately 40% at pH 4.8 to 95% at pH 6.0, whereas WIS- $P_{T85}$  had only a 35% increase from 40% to 54%. Elevated pH (> cellulase *pI* of approximately 5.0) not only increases lignin surface charge because of the presence of functional groups on substrate surface (mainly carboxyl for WIS- $M_{T120}$  in this study) but also results in negatively charged cellulase, which produces a repulsion force between lignin and cellulase to decrease nonproductive binding of cellulase to substrate lignin.<sup>[33]</sup> WIS- $P_{T85}$  has a much lower carboxyl group content (native from wood) than WIS- $M_{T120}$ , as can be seen from the comparison of carboxyl group content of lignin-containing cellulose nanofibrils (LCNFs) mechanically derived from these two WISs (Table 3). Above pH 6.0, the loss of cellulase effectiveness outweighs the increased availability from decreased nonproductive binding to result in decreasing SED (Figure 4C).

**Table 3.** Carboxyl content and zeta potential of LCNFs obtained by different pretreatments.

| LCNF              | Carboxyl content [ $\text{mmol g}^{-1}$ ] | Zeta potential [mV] |
|-------------------|-------------------------------------------|---------------------|
| LCNF-3 $M_{T110}$ | $0.218 \pm 0.009$                         | $-40.81 \pm 2.49$   |
| LCNF-3 $M_{T120}$ | $0.216 \pm 0.008$                         | $-42.25 \pm 3.30$   |
| LCNF-3 $P_{T85}$  | $0.063 \pm 0.005$                         | $-32.04 \pm 2.35$   |

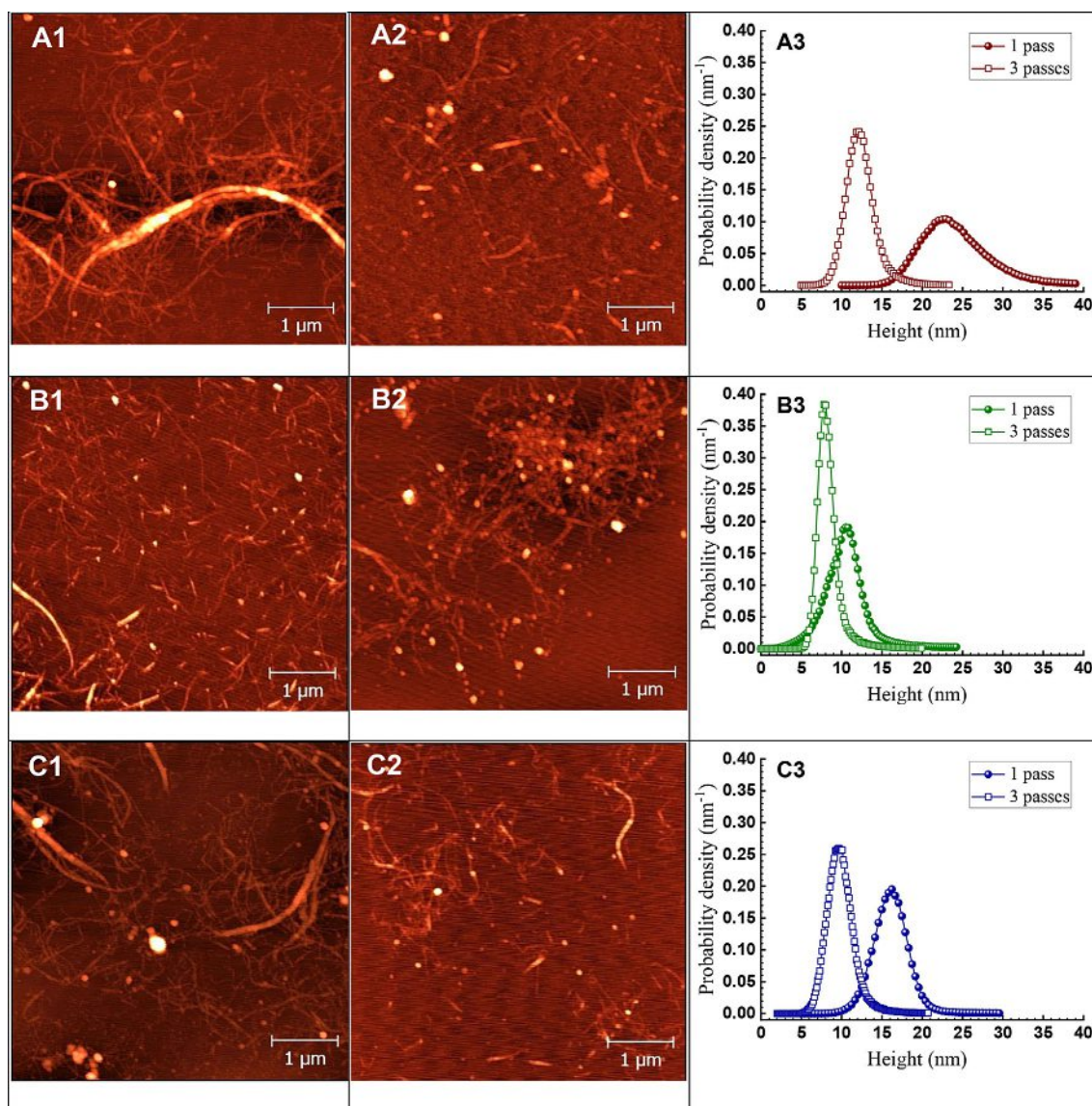
We also compared the affinities of dissolved AHLs to cellulase. Spiking AHL from MA and *p*-TsOH fractionation to their respective WIS suspension resulted in increasing SED for WIS- $M_{T110}$  and WIS- $M_{T120}$  from MAHF but decreased the SED for WIS- $P_{T85}$  from *p*-TsOH as shown in Figure 4D; that is, with the addition of corresponding AHL at  $1 \text{ g L}^{-1}$  and under pH 5.5, the SED@96 h of WIS- $M_{T120}$  and WIS- $M_{T110}$  substantially increased from 57% and 63% to 73% and 87%, respectively, whereas the SED@96 h of WIS- $P_{T85}$  decreased from 47% to 43%. This indicates that AHF-dissolved lignin from MA promoted the hydrolysis of its corresponding substrate, whereas AHF lignin from *p*-TsOH inhibited hydrolysis of its corresponding substrate. The carboxylated AHL from MA has a lignin hydrophobic site as well as a carboxyl group hydrophilic site similar to lignosulfonate and acts as a surfactant to enhance enzymatic hydrolysis of cellulose<sup>[35,36]</sup> by preventing nonproductive cellulase binding to residual lignin in WISs, which is less carboxylated and more hydrophobic with a stronger affinity to cellulase than AHL.

To illustrate the lower hydrophobicity of AHL from MAHF, AHL aqueous suspensions at 0.1 wt% were sonicated for 5 min and allowed to stand at room temperature. After 1 h, the AHL dissolved by *p*-TsOH was almost completely precipitated to the bottom of the bottle, whereas the AHL dissolved by MA was still well-dispersed to form a stable suspension after 1 month (Figure S1). This indicates that lignin dissolved by MA is more hydrophilic.

### Producing LCNFs from fractionated WISs

Our early studies demonstrated that WISs from AHF are excellent sources for producing LCNFs<sup>[20,37]</sup> Here, we compare LCNFs prepared from MA- and *p*-TsOH-fractionated WISs. The results indicate that with only one pass through microfluidization, the three WISs were all easily fibrillated into nanoscale fibrils, as shown by the AFM images and AFM topography-measured fibril height distributions in Figure 5. The bright spots in the AFM images are lignin nanoparticles from the residual lignin in WISs. Under one pass of microfluidization, LCNF-1 $P_{T85}$  (top row, Figures 5A1 and A3) from WIS- $P_{T85}$  by *p*-TsOH had a greater mean diameter/height of approximately 23 nm than the 10 nm of LCNF-1 $M_{T120}$  (middle row, Figure 5B1 and B3) from WIS- $M_{T120}$  by MAHF under the same level of delignification. Furthermore, LCNF-1 $P_{T85}$  had a broader height distribution, or less uniform diameter, than LCNF-1 $M_{T120}$ . Increasing the extent of fibrillation to three passes decreased the fibril diameter and/or height with a more uniform distribution for both LCNF samples, as expected. However, even with three passes of microfluidization, the mean height of LCNF-3 $P_{T85}$  was still greater at 12 nm than the 10 nm from single pass LCNF-1 $M_{T120}$ , and with approximately the same height distribution (comparing Figure 5A2 with B1 and A3 with B3). This implies that applying MAHF requires as much as two-thirds less mechanical energy for adequate fibrillation compared with *p*-TsOH fractionation.

Even decreasing the extent of delignification to 48% in MAHF under M60T110t30 (Table 1), the resultant LCNF-1 $M_{T110}$  had a thinner diameter/height of 16 nm compared with the



**Figure 5.** AFM images and AFM topographic measured height distributions of LCNFs prepared from different WISs: WIS- $P_{T85}$  (top row A); WIS- $M_{T120}$  (middle row B); WIS- $M_{T110}$  (bottom row C). Left and middle columns: LCNFs from one and three passes of microfluidization, respectively; right column: LCNF height distributions.

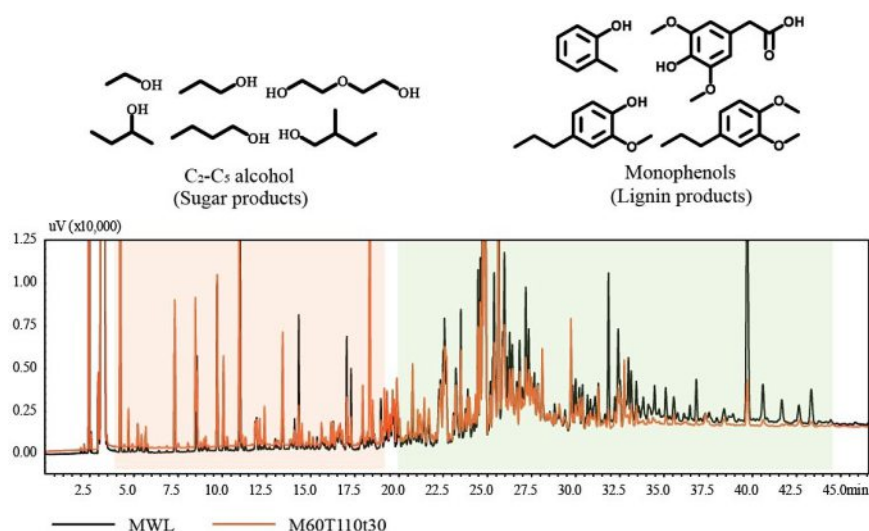
23 nm of 63% delignified LCNF-1 $P_{T85}$  from  $p$ -TsOH fractionated WIS- $P_{T85}$  (comparing Figure 5A1 with C1 and Figure 5A3 with C3).

Previously, we stated that lignin esterification enhanced the lignin lubrication effect,<sup>[38,39]</sup> which facilitated mechanical fibrillation in producing LCNFs from MA-fractionated WIS via electrostatic repulsion from the negatively charged carboxyl groups.<sup>[20]</sup> The carboxyl content and zeta potential of the three LCNFs with three passes of microfluidization were measured, as listed in Table 3. The results clearly show substantial carboxylation of the two MAHF-derived LCNFs of more than  $0.2 \text{ mmol g}^{-1}$  by MA, whereas minimal carboxylation of LCNF-3 $P_{T85}$  from  $p$ -TsOH fractionation of only  $0.06 \text{ mmol g}^{-1}$  inherited from native wood. Accordingly, the LCNF-3 $M_{T110}$  and LCNF-3 $M_{T120}$  each have greater surface charge of more than 40 mV compared with LCNF-3 $P_{T85}$  of only 32 mV (Table 3). The greater

surface charge of LCNFs from MAHF resulted in a more stable suspension of LCNF-3 $M_{T120}$  compared with that of LCNF-3 $P_{T85}$  from  $p$ -TsOH fractionation even after a month of standing (Figure S2).

#### AHL aromatics by supercritical methanol dehydration hydrodeoxygenation

Supercritical methanol dehydration hydrodeoxygenation (SCM-DHDO) is a catalytic technology that converts both the lignin and holocellulose portion of biomass into alcohols using a CuAlMgO<sub>x</sub> catalyst in supercritical methanol.<sup>[10,40,41]</sup> To demonstrate the utility of AHLs produced in this study, a CuAlMgO<sub>x</sub> catalyst was used to produce monophenols. CuAlMgO<sub>x</sub> has been shown to be effective at converting a variety of biomass feedstock including lignin dissolved by  $\gamma$ -valerolactone,<sup>[42]</sup> or



**Figure 6.** GC-FID analysis of products from SCM-DHDO of lignin (reaction conditions: batch reactor, 300 °C for 4 h, 100 mg lignin samples, 100 mg CuAlMgO<sub>x</sub> catalyst, and 2.4 g MeOH).

ganosolv lignin,<sup>[43]</sup> maple wood,<sup>[44]</sup> and cellulose<sup>[45]</sup> into C<sub>2</sub>–C<sub>5</sub> alcohols from cellulose and hemicelluloses, and monophenols from the lignin portion of the biomass. Figure 6 shows the gas chromatograph with flame ionization detection (GC-FID) spectra of SCM-DHDO of MWL and dissolved lignin L-M<sub>T110</sub> from MAHF under M60T110t30. More than 60 products are detected by GC–MS (Table S1). The lighter products with retention times of 2.5–20 min are primarily C<sub>2</sub>–C<sub>5</sub> alcohols (ethyl alcohol, 1-propanol, 2-butanol, 3-pentanol, 1-butanol, 2-ethyl-, etc.), which came from residual sugars and had low yields of less than 5% (Table 4) because the lignin samples had a very low carbohydrate content, as qualitatively shown by 2D NMR analysis (Figure 2). The compounds with retention times between 20 and 45 min in the GC-FID chromatograms (Figure 6) are primarily monophenols from lignin. The peak assignments in the GC-FID spectra were verified by GC–MS (Table S1). These SCM-DHDO lignin products include lignin monophenols (cyclohexanol 2-methyl, cyclohexanemethanol 2-methyl-, 4-ethylcyclohexanol, 2-propylcyclohexanol, etc.) with retention times of 20 to 45 min. CuAlMgO<sub>x</sub> promoted lignin C–O bond ( $\beta$ -O-4 and  $\alpha$ -O-4) cleavage to produce phenol compounds. These phenol compounds were hydrodeoxygenated to cyclic alcohols in the presence of methanol at 300 °C. Methanol reformation at 300 °C provided needed H<sub>2</sub> for hydrogenation. The gas phase has previously been analyzed and shown to contain primarily CO, CO<sub>2</sub>, and H<sub>2</sub>.<sup>[46]</sup>

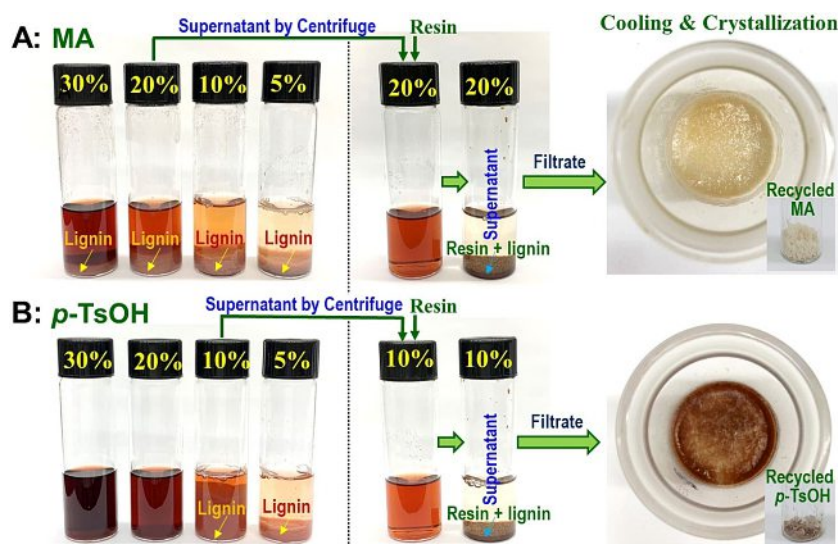
**Table 4.** Product yields of supercritical methanol dehydration hydrideoxy-genation of lignin catalyzed by CuAlMgO<sub>x</sub>.

| Lignin              | Monophenol yield [C%] | C <sub>2</sub> –C <sub>5</sub> alcohol yield [C%] |
|---------------------|-----------------------|---------------------------------------------------|
| MWL                 | 35.9                  | 4.7                                               |
| L-M <sub>T110</sub> | 25.8                  | 4.8                                               |
| L-M <sub>T120</sub> | 20.2                  | 4.1                                               |
| L-P <sub>T85</sub>  | 16.9                  | 3.3                                               |

The monophenol yields, defined as the percentage of total carbon in a lignin sample converted into monophenols, from MAHF lignin L-M<sub>T110</sub> and L-M<sub>T120</sub> were 25.8% and 20.2%, respectively, compared with 35.9% for MWL. However, monophenol yield of L-P<sub>T85</sub> from *p*-TsOH fractionation was only 16.9%. The differences in lignin reactivity were caused by condensation as reflected in the decrease of  $\beta$ -O-4 content (Table 2). The comparative yields from SCM-DHDO experiments (Table 4) between AHL and MWL demonstrate that L-M<sub>T110</sub> and L-M<sub>T120</sub> are promising lignin precursors for producing platform aromatic compounds.

### Acid recovery

As mentioned in the Introduction, MA has a higher MHC of 25 wt% than the 11.5 wt% for *p*-TsOH. We compared the recovery of MA and *p*-TsOH for the two runs under the same level of delignification of 63% (Table 1), M60T120t30 and P60T85t20, to demonstrate the advantage of greater MHC of MA for acid recovery. After diluting the fractionation liquors to 30 wt% acid, approximately 37% of the dissolved lignin in the MAHF M60T120t30 liquor (Liq-M) was precipitated compared with negligible precipitation in the *p*-TsOH fractionation P60T85t20 liquor (Liq-P). Figure 7A and B show the liquor samples from MA and *p*-TsOH treatment diluted to different concentrations. After further diluting to 20 wt% acid, nearly 83% of the dissolved lignin in the MA fractionation liquor, Liq-M, was precipitated compared with 16% from *p*-TsOH fractionation liquor, Liq-P. Only after diluting the acid to 10 wt% did lignin precipitation from Liq-P reach 81%. These results indicate that MAHF requires only half the dilution for lignin precipitation than *p*-TsOH fractionation. Here, the amounts of precipitated lignin were quantified gravimetrically after separation through centrifugation, filtration, and freeze drying (Supporting Information). The percentage of lignin precipitation listed in Table 5 was based on the total amount of lignin dissolved in



**Figure 7.** Fractionation liquor dilution to precipitate lignin, followed by resin adsorption of residual dissolved lignin for recovering acid after evaporation by crystallization through cooling.

**Table 5.** Lignin precipitation percentage and acid recovery from liquor.

| Liquor | Lignin precipitation [%] @ acid conc. |      |      |      | Acid recovery |            |
|--------|---------------------------------------|------|------|------|---------------|------------|
|        | 30%                                   | 20%  | 10%  | 5%   | yield [%]     | purity [%] |
| Liq-M  | 37.1                                  | 82.6 | 86.3 | 92.8 | 87.2 ± 7.1    | 96.1       |
| Liq-P  | 0                                     | 16.4 | 81.4 | 91.7 | 75.6 ± 7.2    | 94.2       |

fractionation calculated from the balance of the amount of lignin retained in fractionated washed cellulosic solids, WISs, listed in Table 1.

The diluted Liq-M at 20 wt % (83 % precipitated) and diluted Liq-P at 10 wt % (81 % precipitated) were used to compare MA and *p*-TsOH recovery. After centrifugation, the remaining suspended lignin in each supernatant was removed using resin adsorption (Sepabeads SP825L, Alfa Aesar, Ward Hill, MA, USA), as shown in Figure 7. After 2 h of agitation, the resin and adsorbed lignin were allowed to settle and the liquid phase became nearly colorless. The liquid phase was separated from the solids through filtration. Each filtrate was then concentrated through evaporation in a conventional oven (no vacuum) at a low temperature of 60 °C to avoid sugar degradation. When initial acid precipitation was observed, that is, acid concentration in the liquor reached the solubility limit at 60 °C, the liquor was cooled to 20 °C to crystallize the acid. The process was repeated twice. More MA than *p*-TsOH precipitation was observed in each cycle, consistent with the lower solubility of MA. The purity of the crystallized acid was analyzed by high-performance liquid chromatography (HPLC). The total net amounts of crystallized acid in the two evaporation–crystallization cycles were used to determine acid recovery based on the amount of acid applied for fractionation. Acid recovery was determined to be 87.2 % and 75.6 % for MA and *p*-TsOH, respectively (Table 5), ignoring the acid remaining with the precipitated lignin and remaining in the resin-adsorbed lignin.

Evaporative reconcentration of the diluted acid solution can be energy intensive. However, the energy used is low quality steam and the amount needed is no more than that used for concentrating dilute pulping spent liquor at commercial pulp mills.

Because of the strong acidity of *p*-TsOH, its fractionation liquor gradually turned brownish-black due to the degradation of dissolved sugars when concentrated at 60 °C. As a result, the recovered *p*-TsOH was brown-blackish, as shown in Figure 7B. In contrast, the recovered MA from diluted Liq-M remained pale yellowish through 60 °C concentration as shown in Figure 7A. The purity of the recovered MA and *p*-TsOH was 96.1 % and 94.2 % (Table 5), respectively, as measured by HPLC.

We also compared the overall mass balance for these two fractionations, M60T120t30 and P60T85t20. The two runs had approximately the same level of delignification of 63 %, based on lignin retained in the fractionated cellulosic solids of approximately 37 % (Table 1). MA dissolved more xylan at 67 % and preserved slightly less cellulose (glucan) at 85 % compared with 61 % and 90 %, respectively, for *p*-TsOH. The dissolved xylans were mostly in the form of xylose for both fractionations as the xylan mass balances were approximately 98 % for both runs by accounting for the xylose in the liquor and the xylan retained in the fractionated cellulosic solids. By contrast, most dissolved cellulose was not in the form of glucose, with very low glucose concentrations in both fractionation liquors (Table 1), resulting in total cellulose mass balances of 88 % and 91 % for MA and *p*-TsOH fractionations, respectively. By accounting only for the precipitated lignin (Table 5) from water dilution to 20 wt % acid concentration and the lignin retained on the WIS, the lignin mass balance was 90 % and 48 % for MA and *p*-TsOH fractionations, respectively. By decreasing the acid concentration to 10 wt %, lignin mass balance increased to 91 % and 88 % for MA and *p*-TsOH fractionations, respectively. Acid recovery from crystallization by repeated (twice) evapora-

tion at 60 °C and cooling to 20 °C was 87% and 76% for MA and *p*-TsOH fractionations, respectively. The added estimated loss of acid, of approximately 7% to 11% in the unwashed fractionated cellulosic solids (based on an earlier washing study),<sup>[21]</sup> brings the mass balance to ≈ 95%.

## Conclusions

Both maleic acid (MA) and *p*-toluenesulfonic acid (*p*-TsOH) can effectively fractionate lignin from birch wood at atmospheric pressure and low temperatures. *p*-TsOH is more effective at achieving near-complete dissolution of lignin than MA at lower temperatures. However, acid-hydrotropic-dissolved lignin (AHL) dissolved by MA was less condensed with greater  $\beta$ -O-4 linkages, resulting in higher monophenol yields through subsequent supercritical methanol dehydration hydrodeoxygenation, and also had a more uniform  $M_w$  distribution. Lignin esterification by MA decreased nonproductive cellulase binding to lignin during enzymatic saccharification of water-insoluble solids (WISs) through pH mediation, resulting in a 50% decrease in cellulase loading compared with WIS from *p*-TsOH. Lignin esterification also facilitated nanofibrillation of WIS to produce LCNFs through an enhanced lignin lubrication effect, decreasing fibrillation energy by as much as two-thirds compared with WIS from *p*-TsOH. The lower solubility and higher MHC of MA facilitated acid recovery. Furthermore, MA is much less corrosive than *p*-TsOH, and its inclusion in 21CFR175-177 (CFR: Code of Federal Regulations) as an indirect food additive offers significant advantages for biorefinery applications.

## Experimental Section

### Materials

Anhydrous maleic acid (MA) and *p*-TsOH-H<sub>2</sub>O were both purchased from Sigma-Aldrich (purity ≥ 98%, St. Louis, MO, USA). Synthetic adsorbent resin (Sepabeads SP825L, high-porosity cross-linked polystyrene-divinylbenzene (PS-DVB) copolymer, specific surface area of 930 m<sup>2</sup> g<sup>-1</sup>, specific pore volume of 1.4 mL g<sup>-1</sup>) was obtained from Alfa Aesar (Ward Hill, MA, USA). Methanol was purchased from Fisher Chemical (HPLC grade, A452, Thermo Fisher Scientific, Inc., Waltham, MA, USA). All chemicals were used as received.

Birch wood chips were hammer-milled using a 4.8 mm screen and were air-dried at ambient condition for 24 h to approximately 10% moisture content. The air-dried material was stored in a refrigerator for later use. Commercial complex cellulase, Cellic CTec3, was complementarily provided by Novozymes North America (Franklinton, NC, USA).

### Wood fractionation by MA and *p*-TsOH

800 g aqueous MA or *p*-TsOH solutions at 60 wt% were prepared in 2 L glass flask by solubilizing the required amounts of acid in de-ionized (DI) water. The flask was placed on a temperature-controlled shaker (Model 4450, Thermo Fisher Scientific, Inc.) at 250 rpm to promote acid dissolution. 80 g in oven-dry (OD) weight of the hammer-milled birch wood fibers were placed into the prepared MA or *p*-TsOH solution with continuous shaking at a designated temperature for a preset reaction time. At the end of each

fractionation, the fractionation liquor was separated by filtration using a membrane filter (0.45  $\mu$ m pore size, Millipore, Bedford, MA, USA). Near the end of filtration, 800 g hot (≈ 80 °C) DI water was added to wash additional acid and lignin from the WIS, resulting in a diluted fractionation liquor with acid concentration of approximately 30 wt%, which was set aside. The WIS was further washed using 1600 g hot DI water, and the filtrate was discarded. The 1st wash filtrate (diluted fractionation liquor) was used for the acid recovery study. Lignin precipitation was carried out by further diluting the fractionation liquor to 20 wt%, 10 wt%, and 5 wt%. The precipitated lignin was washed with DI water to remove acid and was used for 2D NMR analysis and the catalytic conversion study. The washed WISs were analyzed for chemical compositions and used for enzymatic sugar and LCNF productions (see the Supporting Information for additional details).

### Chemical compositional analyses

Chemical composition of the WISs was determined by a conventional two-step acid hydrolysis method, described previously, performed by the Analytical Chemistry and Microscopy Lab at the Forest Products Laboratory using ion chromatography.<sup>[47]</sup> Sugar and acid concentrations were analyzed by an HPLC system (Ultimate 3000, ThermoFisher Scientific, Inc.), as described previously.<sup>[48]</sup> Specifically, xylose, glucose, and solid acid were chromatographically separated using an HPX-87H column (Bio-Rad Aminex, 300 mm × 7.8 mm, Bio-Rad Laboratories, Inc., Hercules, CA, USA) operated at 50 °C and detected by a refraction index detector (RI-101, Shodex, Showa Denko America, Inc., New York, USA).

### LCNF production and characterizations

The collected WIS from each run was first placed in a disintegrator (TMI, Ronkonkoma, NY, USA) at 1 wt% for 20 000 revolutions. The suspension was then fed into a microfluidizer (M-110EH, Microfluidics Corp., Westwood, MA, USA) with two chambers in series, orifice diameters 200 and 87  $\mu$ m, at 120 MPa to produce LCNFs.

The morphologies of the LCNFs were observed by an atomic force microscope (AFM) (CS-3230, AFM Workshop, Signal Hill, CA, USA). Aqueous LCNF suspensions of about 0.0025 wt% were sonicated and dispersed onto a mica plate and then air dried at 20 °C. The LCNFs were imaged by AFM under vibration tapping mode. Image-Pro Plus software (Media Cybernetics, Silver Spring, MD, USA) was used to analyze the measured image topography for determining LCNF height distributions.

The carboxyl group content of LCNFs was determined by conductometric titration described in our previous study.<sup>[49]</sup> The zeta potential of LCNFs was measured by diluting LCNF suspensions to 0.5 g L<sup>-1</sup> and processing in a zeta-potential analyzer (Nanobrook Omni, Brookhaven Instruments, Holtsville, NY) at room temperature and pH 7. Reported data were the averages of six measurements.

### Enzymatic hydrolysis

Enzymatic hydrolysis of WISs was conducted at 2 wt% solids with acetate buffer (50 mM) in a shaker at 50 °C and 150 rpm. Three Cel-lulase CTec3 (activity 217 FPU mL<sup>-1</sup>) loadings of 5, 7.5, and 10 FPU g<sup>-1</sup> glucan were used. Aliquots of 0.7 mL of hydrolysate were taken periodically to obtain time-dependent saccharification. Glucose in hydrolysates was measured by a commercial glucose

analyzer (YSI 2700S, YSI, Inc., Yellow Springs, OH, USA). The reported data were the average of three measurements.

## 2D $^1\text{H}$ - $^{13}\text{C}$ NMR

2D  $^1\text{H}$ - $^{13}\text{C}$  NMR analysis of lignin was performed using a Bruker (Billerica, MA, USA) 500-MHz Avance III HD spectrometer equipped with a Prodigy (liquid  $\text{N}_2$ -cooled) 5-mm gradient TCI (inverse configuration)  $^1\text{H}/^{13}\text{C}/^{15}\text{N}$  CryoProbe. Approximately 55 mg of purified lignin was dissolved in 0.5 mL of  $[\text{D}_6]\text{DMSO}$ , referenced at 39.5/2.49 ppm. Heterogeneous single quantum correlation (HSQC) experiments were performed using a Bruker standard pulse programs hsqcetgpsisp 2.2, with nonuniform sampling of 50%. HSQC spectra were acquired using 40 scans and an interscan delay (D1) of 1 s for a total experiment time of 3 h, with a 12 ppm sweep width in F2 ( $^1\text{H}$ ) using 1024 data points for an acquisition time (AQ) of 85 ms and a 215 ppm sweep width using 512 increments for AQ of 9.74 ms in F1 ( $^{13}\text{C}$ ). Data processing used squared cosine-bell in F1 and F2, resulting in a  $1024 \times 1024$  data matrix. Topspin 3.7p17 was used for interactive integration of 2D crosspeaks. Calculations of  $\beta$ -O-4,  $\beta$ - $\beta$ , and  $\beta$ -5 amounts were normalized by total integrated area of C9 signals detected as described in the Supporting Information.<sup>[21]</sup>

## Preparation of MWL

MWL was prepared similar to a procedure described in Holtman et al.<sup>[50]</sup> Birch wood powder was Wiley milled to pass 30 mesh and then vibratory ball milled (Retsch, PM 100) for 24 h. 4 g ball-milled wood was dissolved in 100 mL dioxane/water (v/v = 9:1) and mixed on a shaking bed at 50 °C for 48 h. The solution was then filtered and the filtrate was dried in a rotary vacuum evaporator to obtain crude MWL. The crude MWL was purified in 90% acetic acid solution and precipitated by adding water. After filtration, the solid was dissolved in a mixture of 1,2-dichloroethane and ethanol (volume ratio is 2:1) and precipitated by adding diethyl ether, followed by washing and drying.

## Lignin molecular weight by gel permeation chromatography

The procedure described by Li et al.<sup>[32]</sup> was followed for sample preparation. 0.05 g lignin was first acetylated by dissolving in 2 mL of pyridine-acetic anhydride (1:1 by volume) solution and later added dropwise into 100 mL of ice-cold DI water for precipitation. The molecular weight of the acetylated lignin sample was measured by an ICS-3000 system (Dionex, Sunnyvale, CA, USA) with three  $300 \times 7.8$  mm Phenogel 5U columns (10000, 500, and 50 Å). 3 mg of acetylated lignin was dissolved in 4 mL tetrahydrofuran (THF). 70  $\mu\text{L}$  of the resulting solution was injected into the gel permeation chromatography columns at 30 °C with THF as eluent. Lignin was analyzed by UV absorption at 278 nm. Polystyrene was used as the standard for calibration.

## Supercritical methanol dehydration hydrodeoxygenation of lignin

Supercritical methanol dehydration hydrodeoxygenation experiments were carried out in a batch reactor at 300 °C using  $\text{CuMgAlO}_x$  as a catalyst.  $\text{CuMgAlO}_x$  was synthesized following the co-precipitation method described by Galebach et al.<sup>[51]</sup> For each experiment, 0.1 g  $\text{CuMgAlO}_x$  catalyst, 0.1 g lignin, and 2.4 g MeOH were added into a batch reactor. The reactor was submerged in a fluid-

ized sand bath at 300 °C. After 4 h, the batch reactor was placed into cold water ( $\approx 20$  °C) to cool down. The liquid product was then drawn out using a syringe and filtered through a 0.22  $\mu\text{m}$  filter. Lignin monomer was quantified by a GC-FID (Shimadzu (Kyoto, Kyoto, Japan) GC-2010) after separation by an RTX-VMS column at 40 °C for 5 min. The column was then ramped to 240 °C at  $7.5$  °C  $\text{min}^{-1}$  and held for 15 min (Figure 6 and Table S1). Standards for many lignin monomers were unavailable; therefore, GC-FID response factors were estimated using 4-propylguaiacol calibrations.

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## Conflict of interest

*Zhu is a co-inventor of acid hydrotropic fractionation.*

**Keywords:** acid recovery • cellulose nanofibrils • esterification • lignin • maleic acid

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