

ORIGINAL RESEARCH

Maleic acid hydrotropic fractionation of wheat straw to facilitate value-added multi-product biorefinery at atmospheric pressure

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Abstract

Wheat straw was rapidly fractionated using maleic acid (MA) as an acid hydrotrope at atmospheric pressure under a range of conditions. MA hydrotropic fractionation (MAHF) was very selective in dissolving lignin and hemicelluloses resulting in a cellulose-rich water insoluble solids (WIS), which was evaluated for producing fibers through bleaching and cellulosic nanofibrils by fibrillation, as well as glucose through enzymatic saccharification. The residual lignin (RL) within the WIS has a low degree of condensation, which eased bleaching. The RL was also carboxylated through MAHF, which facilitated nanofibrillation to produce lignin-containing cellulose nanofibrils. The carboxylation also aided enzymatic saccharification of WIS by decreasing nonproductive cellulase binding to RL through electrostatic repulsion between carboxylated (charged) RL and the cellulase at elevated pH (>cellulase isoelectric point). The dissolved lignin (DL) from MAHF also has a low degree of condensation, making it favorable for further catalytic conversion. Carboxylation improved DL antioxidant capability. MA is an FDA-approved indirect food additive (21CFR175-177) and is much less corrosive than other acids typically used for cellulosic fractionation. It is also less soluble which is a distinct and very promising advantage for acid recovery. Therefore, MAHF has notable advantages and significant potential for sustainable biorefinery.

KEYWORDS

2D NMR analysis, enzymatic hydrolysis, lignin antioxidant activity, lignin esterification/carboxylation, lignin-containing cellulose nanofibrils (LCNFs), maleic acid hydrotropic fractionation (MAHF), sustainable biorefinery

1 | INTRODUCTION

Lignocellulosic biomass, the most abundant natural material, has become an important feedstock for sustainable production of biofuels, biomaterials, and platform chemicals through the biorefinery concept (Rajinipriya et al., 2018; Rinaldi et al., 2016; Zhu & Pan, 2010). The International Renewable

Energy Agency estimated agricultural residue and waste can contribute 40–60 EJ/year energy by 2030 (Manzanares, 2020). Wheat straw as an agricultural residue is not only renewable but also abundant. Its annual production in United States is approximately 30–40 million tons. Europe, as one of the largest wheat producers, can supply approximately 200 million tons wheat straw annually (Bakker et al., 2013).

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China alone reported the production of over 150 million tons wheat straw in 2008 (Zhou et al., 2011). Furthermore, wheat straw has the lowest cost at approximately \$60/dry ton in the United States compared with other energy crops, such as switchgrass at \$95–130/dry ton (Perlack & Stokes, 2011). Therefore, efficient utilization of this abundant resource can facilitate not only sustainable economic development in rural areas but also address environmental issues associated with its disposal using primitive methods, such as open field burning that is still a common practice in many regions of the world (Bakker et al., 2013; Montero et al., 2017). Although conventional utilization of wheat straw—such as papermaking using existing pulping technologies, animal feed and bedding, soil conditioner, etc.—has achieved some success (Xie et al., 2012, 2017; Zhang et al., 2014), using wheat straw as a feedstock for the future biorefinery through effective fractionation is a high value proposition (Cherubini & Ulgiati, 2010; Zhang et al., 2018), especially with the valorization of lignin (Galkin & Samec, 2016; Ma et al., 2018).

Traditional fractionation technologies such as hydrothermal (Rodríguez-Zúñiga et al., 2015; Thomsen et al., 2008), dilute acid (Chen, Zhao, et al., 2016; Nair et al., 2016), ionic liquid (IL) (Da Costa Lopes et al., 2013, 2018), and organosolv (Snelders et al., 2014) have had limited commercial success to fractionate wheat straw because all existing biorefinery processes are heavily focused on fuel or energy production from the carbohydrate fraction, which requires severe reaction conditions for delignification and/or high temperatures for dissolution of hemicelluloses. In addition to high operating and capital costs, the lignin fraction is condensed with substantial β -O-4 linkage cleavage and C-C bond formation (Rinaldi et al., 2016). The condensed lignin is less reactive and, therefore, less amenable to high value utilization through further processing. Despite recent efforts in using fractionation and membrane separation technologies to upgrade technical lignin (Gillet et al., 2017) along with novel fractionation processes to produce less condensed lignin (Kochepka et al., 2020; Morais et al., 2015), commercial viability, and success of these efforts yet need to be seen.

Acid hydrotropic fractionation (AHF) has demonstrated its advantages for rapid fractionation of wood (Cai, Hirth, et al., 2020; Chen et al., 2017) and herbaceous biomass (Ma et al., 2018; Su et al., 2021) at atmospheric pressure and low temperatures. Compared with existing fractionation processes, AHF has several advantages: (1) Rapid fractionation at low temperatures results in lignin with low degree of condensation which facilitates valorization (Cai, Li, et al., 2020; Wang et al., 2019). (2) Lignin separation can be achieved simply by diluting the fractionation liquor to below the minimal hydrotropic concentration (MHC). (3) High selectivity in preserving cellulose for materials applications such as producing cellulose nanomaterials, as well as for sugars/biofuels production through enzymatic hydrolysis and fermentation

(Bian et al., 2017; Cai, Hirth, et al., 2020). (4) While it is not the aim of the present study to dehydrate the dissolved xylan into furfural—a high value chemical—high furfural yield was reported using the acid hydrotrope in the fractionation liquor without additional catalysts (Cai, Hirth, et al., 2020; Zhu et al., 2019), similar to some acidic IL systems (Peleteiro et al., 2016). (5) Lignin and cellulose are carboxylated using maleic acid (MA) as the hydrotrope, which, in turn, facilitates enzymatic hydrolysis of the fractionated cellulose fraction at low cellulase dosages and aids mechanical fibrillation for producing cellulose nanomaterials with decreased energy input (Cai, Li, et al., 2020; Su et al., 2021). In view of these advantages, this study is aimed at evaluating MA hydrotropic fractionation (MAHF) for fractionating wheat straw.

Previously, we evaluated *p*-Toluenesulfonic acid (*p*-TsOH) as an acid hydrotrope to fractionate wheat straw (Ma et al., 2018). Compared with *p*-TsOH, MA is less acidic and less soluble, both of which are significant advantages for acid recovery (Cai, Li, et al., 2020) and for decreasing capital and operating costs. Furthermore, MA is a US Food and Drug Administration (FDA)-approved indirect food additive (21CFR175-177) with minimal environmental impact. With the goal of improving sustainability, it is worthwhile investigating MAHF of wheat straw for biorefinery applications. Specifically, we characterize MAHF using reaction-kinetics-based severity factors to determine appropriate conditions for process scale-up. We also evaluate the effectiveness of MAHF with focus on exploring potential applications of the resultant fractionated cellulose and lignin from wheat straw, such as for producing materials and/or sugars from the cellulosic solid fraction. We characterize the fractionated dissolved lignin (DL) and enzymatic hydrolysis residual lignin (RL) to identify the extent of condensation, molecular weight (Mw) distribution, degree of carboxylation, and potential application as an antioxidant. Additionally, the impact of condensation of the residue lignin on the cellulosic fraction on bleaching is investigated for fiber production. The importance of this study lies in the fact that MA can serve as a minimal toxicity, recyclable, single-component catalyst to fractionate all three major components of wheat straw at atmospheric pressure through the biorefinery concept.

2 | MATERIALS AND METHODS

2.1 | Materials

The air-dried and lightly hammer-milled wheat straw from a Canadian farm, supplied by Whispering Green Energy Inc., was used as received.

The anhydrous MA, 2,2-diphenyl-1-picrylhydrazyl (DPPH), and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfo nic acid) diammonium salt (ABTS), all ACS HPLC grade,

were purchased from Sigma-Aldrich. A commercial complex cellulase, Cellic CTec3 (abbreviated CTec3) was complementarily provided by Novozymes North America, with cellulase activity of 217 FPU/ml.

2.2 | AHF of wheat straw

Wheat straw fractionation was carried out following the schematic flow diagram shown in Figure 1 using aqueous MA solutions in a range of concentrations, temperatures, and reaction times from 20 to 60 wt%, 40 to 120°C, and 30 to 120 min, respectively. MA solutions were prepared in glass flasks by solubilizing the required amount of MA in deionized (DI) water to make 150 g acid solution at the target concentration. Each flask was placed on a magnetic agitator with a temperature controller (C-MAG HS7DS1; IKA) to aid MA dissolution.

10 g of oven-dried wheat straw was added to 150 g MA solution under constant stirring at a designated temperature and stirred for a preset period of fractionation time. At the end of each run, the water insoluble solids (WIS) were separated from the fractionation liquor through filtration, then washed with DI water until the filtrate was diluted to 10 wt% MA concentration so as to precipitate the DL. After centrifugation, the pelletized DL was dialyzed in DI water for a week and then freeze-dried.

The chemical composition of all WISs from MAHF was determined by the Analytical Chemistry and Microscopy Laboratory at the USDA Forest Products Laboratory, using a conventional two-step acid hydrolysis followed by ion chromatographic analysis, as described previously (Luo et al., 2010). Briefly, fractionated wheat straw WISs along with raw wheat straw were hydrolyzed in two steps for 1 h in each step using sulfuric acid of 72% (v/v) at 30°C and 3.6% (v/v) at 120°C, respectively. The hydrolysates were analyzed for carbohydrates using anion exchange chromatography (Dionex

ICS-5000; ThermoFisher Scientific) using pulsed amperometric detection (HPAEC-PAD). The balance between the unsolubilized solids and the ash after muffle furnace at 560°C for 3 h was considered to be Klason lignin. The chemical composition of the fractionation liquors was analyzed by an HPLC system (Ultimate 3000; ThermoFisher Scientific) equipped with a BioRad Aminex HPX-87H column (300 mm × 7.8 mm) operated at 60°C and a refraction index detector (RI-101, Shodex; Ma et al., 2018; Zhou et al., 2013).

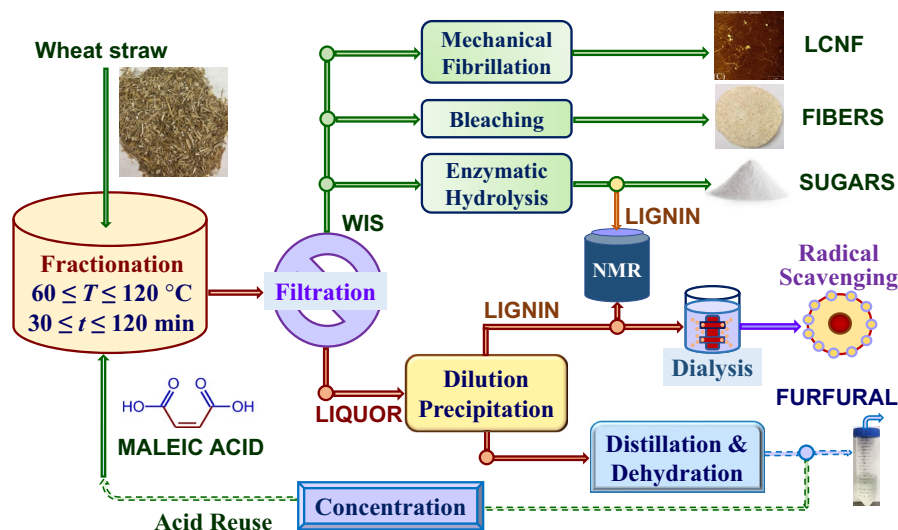
2.3 | Enzymatic sugar production from WISs

Enzymatic hydrolysis of WISs was conducted in 125 ml conical flasks in a temperature-controlled shaking bed incubator at 200 rpm and 50°C (Model 4450; Thermo Scientific). Hydrolysis was carried out at solids loading of 1% (w/v) in 50 mM acetate buffer at pH 5.7 for 96 h; the elevated pH was used to reduce nonproductive binding of cellulase to substrate lignin through electrostatic repulsion (Lan et al., 2013; Lou et al., 2013). The CTec3 cellulase loading was 10 FPU/g glucan. An aliquot of hydrolysate was taken at 2, 4, 6, 8, 10, 24, 48, 72, and 96 h during each hydrolysis run to obtain time-dependent sugar production. The enzymatic hydrolysate samples were centrifuged at 9630 g for 10 min and the supernatant was analyzed in duplicate for glucose using a biochemistry analyzer (YSI 2900D; YSI Inc.).

2.4 | Production and characterization of lignin-containing cellulose nanofibrils

A WIS sample from run M60T120t60 was selected to produce lignin-containing cellulose nanofibrils (LCNFs). The sample was dispersed in DI water with stirring for 10 min and

FIGURE 1 A schematic flow diagram showing fractionation of wheat straw using maleic acid as a hydrotrope; the production of cellulose nanomaterials, fibers, and sugars from the fractionated cellulosic solids; furfural from dissolved xylan; and carboxylated lignin with low degree of condensation for valorization. Processes with dashed lines were not carried out in the present study



then disintegrated using a laboratory disintegrator (TMI) at 0.5 wt% for 15,000 revolutions. Then the fiber suspension at approximately 0.5 wt% was directly fed into a microfluidizer (M-110EH; Microfluidics Corp.) to mechanically fibrillate the fibers at 120 MPa. The suspension passed through two chambers in series with orifice diameters of 200 and 87 μm , respectively, for five passes and nine passes.

Morphologies of the resultant LCNFs were observed by atomic force microscopy (CS-3230; AFM workshop). An aliquot of LCNF sample was diluted to approximately 0.01 wt% and a drop of the diluted suspension was deposited on a clean mica substrate, then air-dried overnight at room temperature. The height distribution of LCNFs was obtained by analyzing the AFM topographic images using Image-Pro Plus software (Media Cybernetics) as described previously (Bian et al., 2017).

The carboxyl group content in the resultant LCNFs was determined using conductometric titration as described previously (Bian et al., 2017b; da Silva Perez et al., 2003). Briefly, the LCNF suspension containing 100 mg LCNFs (in oven dry [OD] weight) was mixed with 100 ml of 1 mM NaCl solution, and then titrated with 10.0 mM NaOH solution added dropwise every 30 s to allow time for equilibration before measuring conductivity. Conductance was measured using a conductivity meter (Model 35; YSI, Inc.).

The surface charge of the LCNFs was measured using LCNF suspensions at approximately 0.05 g/L and room temperature by a zeta potential analyzer (Nanobrook Omni; Brookhaven Instruments). Each sample was measured for five cycles and the averages were reported. The water retention value (WRV) of a LCNF sample was measured according to the standard test method SCAN-C 62:00 (SCAN, 2000) as described previously (Gu et al., 2018; Luo & Zhu, 2011).

2.5 | WIS Fiber bleaching and characterization

5 g of each of the four selected fractionated fiber (WIS) sample was bleached at 10% consistency using oxygen (O), chlorine dioxide (D), and hydrogen peroxide (P). O bleaching was performed in a 100 ml stainless steel can rotating at 2 rpm for 60 min under two sets of conditions: O_2 at 0.62 and 0.75 MPa with 3% and 6% NaOH charge on WIS (OD weight) at 100 °C and 110 °C, respectively, along with 0.5% MgSO_4 on WIS. D bleaching was performed in a sealed plastic bag in a water bath at 70 °C for 120 min with two active chlorine dioxide charges of 2% and 5% on WIS, respectively. P bleaching was performed at 80 °C using two chemical charges of 3% and 6% (w/w) H_2O_2 with 3% and 6% (w/w) NaOH on WIS, respectively, along with 0.5% MgSO_4 charge in a conical flask on a shaker at 300 rpm (Excella 25; Eppendorf North America). When the bleaching was finished, the fibers and bleaching

liquor were separated by vacuum filtration, and then the fibers were washed with DI water until the filtrate was neutral.

Multi-stage bleaching using sequence ODP was also conducted to improve fiber brightness: specifically, O_2 at 0.75 MPa with 6% NaOH and 0.5% MgSO_4 charge on WIS at 110 °C, followed by reaction with ClO_2 at 2% charge at 70 °C for 120 min, and then with 3% H_2O_2 and 3% NaOH and 0.5% MgSO_4 charge at 80 °C for 60 min.

Fiber intrinsic viscosity, η , was determined in a cupri-ethylenediamine solution according to ISO standard 5351:2010, as reported previously (Ma et al., 2020). Briefly, 12.50 ml of 1.00 ± 0.02 mol/l cupri-ethylenediamine solution was used to solubilize 0.125 g (OD weight) of bleached WIS fibers at 1% consistency. The fiber suspension was first stirred for 30 s by a motor-driven copper bar in a tube after purging the tube using nitrogen for 1 min. The fiber cupri-ethylenediamine solution was stirred for 15 min at approximately 400 rpm. The viscosity of the dissolved fiber solution was then measured.

Thick WIS fiber pads of 250 g/m² were prepared according to the TAPPI Standard Test Method T218 sp-97. Diffuse brightness values of the fiber pads were determined using TAPPI Standard Test Method T525 om-92on and a ColorTouch PC instrument (Technidyne Corporation), as described previously.

The Kappa number (KN) of WIS fiber samples was determined according to TAPPI Useful Method 246—Micro-KN because of the limited amount of sample available.

2.6 | Preparation of wheat straw milled wood lignin and WIS RL

Air-dried wheat straw was milled to pass 30 mesh in a Wiley mill (model no. 2; Thomas Scientific). The dried powder was then milled in a vibratory ball mill (PM100; Retsch) for 12 h and then extracted using aqueous dioxane solution of 96% (v/v) with a solid to liquid ratio of 1:20 (g/ml) for 24 h (Holtman et al., 2006). After repeated extraction, the solids were dried at 40 °C to remove dioxane to obtain crude milled wood lignin (MWL). The crude MWL was then purified using acetic acid and 1,2-dichloroethane and ethanol as described previously (Cai, Hirth, et al., 2020; Su et al., 2021).

Selected WISs were enzymatically hydrolyzed to remove carbohydrates and enrich lignin. Enzymatic hydrolysis experiments were conducted at 50 °C and buffered at pH = 6.0 using Novozyme CTec3 at 20 FPU/g glucan. The hydrolyzed samples were washed using DI water and collected. The oven-dried residue was ball milled for 12 h using a PM 100 Planetary Ball Mill (Retsch) at 600 rpm in a 50 ml vessel containing ZrO_2 balls (10 mm $\times$ 10 mm). The ball-milled sample was extracted by stirring in 96% dioxane- H_2O solution with a solid-to-liquid ratio of 1:20 (g/ml) for 24 h, then filtered,

and repeated for 3 times. The solid residue was washed with the same solvent until clear. All the washing filtrates were combined. The filtrate was dropped into 100 ml iced DI water to precipitate the enzymatic hydrolysis residue lignin (RL). The precipitated enzymatic hydrolysis RL was collected and washed with DI water until free of acid and then freeze-dried.

2.7 | Lignin characterization

For lignin Mw determination, 0.1 g of the freeze-dried lignin preparation was dissolved in 2 ml of pyridine–acetic anhydride (1:1 by volume) solution placed on a temperature-controlled shaker at 150 rpm and 40°C for 72 h and placed in a box to avoid light. The solution was then added dropwise into 100 ml ice-cold DI water with continuous stirring to precipitate lignin acetate. The acetylated lignin was collected by filtration, then washed with DI water, freeze-dried, and dissolved in tetrahydrofuran (THF, HPLC grade) at 1 mg/ml, and then analyzed for Mw distribution by gel permeation chromatography (Yang et al., 2016) on a liquid chromatograph (LC-20A; Shimadzu) equipped with an organic size-exclusion chromatography column (KF-804, 300 mm × 8 mm i.d., 7 µm). The column temperature was 40°C and eluted with THF at a flow rate of 1 ml/min. Polystyrene spheres of Mws of 1000, 2000, 5000, 10,000, and 12,000 g/mol were used for calibration.

Fourier transform infrared spectral (FTIR) analysis of lignin samples was conducted using an FTIR system (Spectrum Two; PerkinElmer) with a universal attenuated-total-reflection probe. All samples were freeze-dried before analysis.

Both ^{31}P and 2D ^1H - ^{13}C nuclear magnetic resonance (NMR) spectroscopic analyses of lignin were carried out using a Bruker AVANCE 500 MHz spectrometer. For ^{31}P NMR analysis, 20 mg of non-acetylated lignin was dissolved in 0.5 ml anhydrous pyridine and deuterated chloroform (1.6/1, v/v) solution followed by adding 100 µl of cyclohexanol (11.02 mg/ml) as internal standard and 100 µl chromium (III) acetylacetonate (5 mg/ml) as relaxation reagent. 60 µl phosphitylating reagent (2-chloro-4,4,5,5-tetramethyl-1,2,3-dioxaphospholane) was then added. The mixture was well shaken for 30 min at room temperature before transferring to an NMR tube for analysis. For 2D ^1H - ^{13}C NMR experiments, 70 mg of lignin was dissolved in 0.5 ml of dimethyl sulfoxide- d_6 as described previously (Kim & Ralph, 2010). Heterogeneous single quantum correlation (HSQC) of the prepared lignin samples was performed using the Bruker standard pulse program hsqcetgisp2.2. Spectra were acquired using 40 scans and an interscan delay of 1 s for a total time of 3 h and used a 12 ppm sweep width in F2 (1 H) using 1024 data points for an acquisition time of 85 ms and a 215 ppm sweep width in F1 (^{13}C) using 512 increments with

50% nonuniform sampling density. The number of various lignin substructures and lignin carbohydrate complex linkages (LCCs) in lignin was calculated from the NMR spectra according to the reported work (del Rio et al., 2012; Yelle et al., 2008). Topspin 3.5pl7 was used for interactive integration of the cross-peaks as described previously (Su et al., 2021).

3 | RESULTS

3.1 | Fractionation mass balance and severities

Fractionation runs were labeled as MxxTyytzz to represent fractionation condition of MA concentration in xx wt% at yyy°C for zz min. The chemical composition of fractionated WISs from wheat straw along with species composition of the fractionated liquor was analyzed (Table S1). The results clearly indicate that both lignin and hemicellulose dissolution were enhanced with increasing reaction severity; however, cellulose degradation only mildly increased with 75% or more glucan retention in all WISs except at the most extreme fractionation severities. Glucan degradation became significant at combined hydrolysis factor (CHF) ≥ 150 or combined delignification factor (CDF) ≥ 2000 (Table S1) when dissolutions of xylan and lignin already plateaued (Figure 2). Most of the dissolved xylan were in the form of oligomeric sugars at temperatures below 110°C (Table S1). Furfural formation was negligible.

To facilitate process scale-up, kinetics-based reaction severity factors, CHF (Zhu et al., 2012) and CDF (Ma et al., 2018), were developed using a biphasic model (Springer et al., 1963; Zhao et al., 2012) for predicting xylan and lignin dissolution (Equations S1 and S2), respectively, as described previously (Ma et al., 2018; Su et al., 2021; Zhu et al., 2019). As shown in Figure 2, xylan and lignin dissolution are well predicted using CHF and CDF, respectively, as the independent variable determined by concentration, temperature, and fractionation time. Fitting to the experimental data at various CHF and CDF yields the parameters for Equations (S1) and (S2) (Table S2).

3.2 | MAHF WISs for enzymatic sugar production

The MAHF WISs of wheat straw were found readily digestible even at a low enzyme dosage of 10 FPU/g glucan (Figure S1). Previously, we found that lignin carboxylation by MAHF reduced nonproductive cellulase binding (Cai, Hirth, et al., 2020; Cai, Li, et al., 2020) through enhanced electrostatic repulsive interactions between cellulase and carboxylated

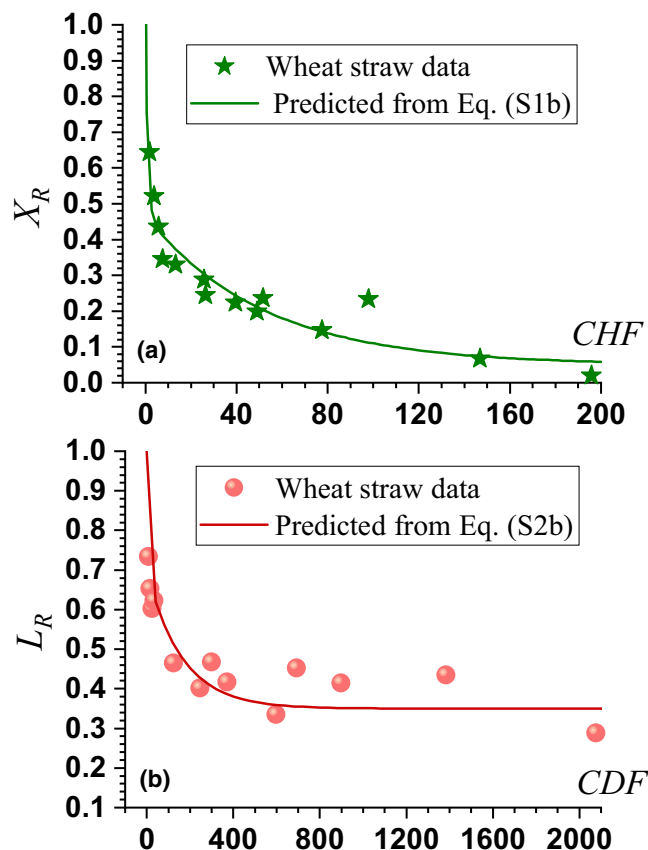


FIGURE 2 Measured xylan (a) and lignin (b) retained in water insoluble solids under various fractionation conditions, fitted using reaction kinetic-based severity factors CHF and CDF, respectively. CDF, combined delignification factor; CHF, combined hydrolysis factor

(charged) lignin at elevated pH of 5.5–6.0 (Lan et al., 2013; Lou et al., 2013). The carboxylation of RL in WIS will also be demonstrated in this study through NMR analyses, as will be discussed later. The advantage of MAHF can be clearly seen from comparing terminal substrate enzymatic digestibilities (SEDs) of MAHF WISs with those from different fractionation methods, as listed in Table 1. Hydrothermal fractionation has been considered most promising for fractionating wheat straw because no chemicals are needed. It relies on significant dissolution of hemicelluloses and depolymerization of cellulose under very high temperatures, such as 190°C, to achieve good enzymatic saccharification of cellulose. However, the application of lytic polysaccharide monooxygenases (LPMO) (Rodríguez-Zúñiga et al., 2015) or a higher cellulase dosage (Thomsen et al., 2008) was required in order for the high severity hydrothermal fractionation runs to produce similar level of SEDs to those from the present study. Notably, despite much higher hemicellulose dissolution and higher cellulase loadings, dilute acid fractionation—that dissolves only xylan either at low (Chen, Zhao, et al., 2016) or high (Nair et al., 2016) temperatures—resulted in lower SEDs than those reported here using MAHF. This is

attributed to both lignin dissolution (in addition to hemicellulose dissolution) in MAHF and lignin carboxylation that decreases nonproductive cellulase binding. Even with an additional sodium chlorite bleaching step to remove over 90% lignin from the dilute acid fractionated substrate (Chen, Zhao, et al., 2016), a higher cellulase loading is still needed to achieve a similar SED of approximately 90% compared with a MAHF WIS with a similar hemicellulose dissolution and a lower level of lignin removal.

Ionic liquids have been widely studied for lignocellulosic biomass fractionation (Brandt et al., 2011; Chen et al., 2020). However, it under performs MAHF in terms of enzymatic saccharification as reported previously (Ma et al., 2020). Here, wheat straw WISs from MAHF are much more enzymatically digestible even under a lower cellulase loading than that from IL fractionation (Da Costa Lopes et al., 2018).

3.3 | MAHF WIS for producing LCNFs

Atomic force microscopy images indicate that very fine LCNFs containing globular-shaped lignin nanoparticles (bright spots) were obtained from a wheat straw MAHF WIS, as shown in Figure 3a and b. LCNFs were visually estimated to be over 1 μm in length. Heights of LCNFs decreased as the extent of fibrillation increased (more passes through the microfluidizer) based on AFM topographic measurements. As shown in Figure 3c, the mean LCNFs height was reduced from 10.8 to 4.4 nm after increasing the numbers of passes through the 87 μm chamber of the microfluidizer from five to nine passes. Increasing the extent of fibrillation also increased the uniformity of LCNFs. The lignin content of the two LCNF samples shown should be the same as that of the WIS of 16.4% (Table S1) because fibrillation did not remove lignin.

Maleic acid can carboxylate cellulose (Chen, Zhu, et al., 2016; Wang et al., 2017) and lignin (Cai, Hirth, et al., 2020) to result in a carboxylated WIS, which logically suggests that LCNFs derived from MAHF WISs are also carboxylated. As listed in Table 2, the carboxyl group content and zeta potential of LCNFs were slightly increased with increasing number of passes in the microfluidizer. WRV, a measure of the interaction between a lignocellulosic material and water, represents fibril pore volume or surface area and was increased with fibrillation. Both LCNFs had a surface charge exceeding 40 mV (absolute value), suggesting they are dispersible—important for aqueous processing.

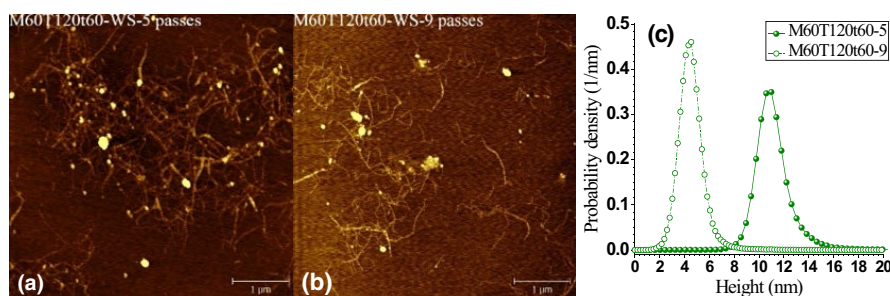
3.4 | Bleaching of MAHF WIS for fiber production

Four WIS samples from MAHF using the same MA concentration of 60 wt% under different fractionation temperatures

TABLE 1 Comparisons of terminal enzymatic digestibilities of wheat straw WISs from MAHF with those from other fractionation methods

Fractionation condition	Xylan; lignin content (removal; %)	Enzyme loading (FPU/g glucan)	Substrate enzymatic digestibility (SED; %)	Source
Maleic acid = 40 wt% @ $T = 80^{\circ}\text{C}$ for $t = 120$ min	11.8 (65.6); 22.4 (37.8)	CTec 3 = 10	41.6	This study; maleic acid hydrotropic fractionation
Maleic acid = 60 wt% @ $T = 100^{\circ}\text{C}$ for $t = 30$ min	9.5 (67.2); 16.0 (53.6)		77.7	
Maleic acid = 60 wt% @ $T = 120^{\circ}\text{C}$ for $t = 60$ min	8.4 (76.1); 16.4 (56.6)		89.4	
Hydrothermal @ $T = 190^{\circ}\text{C}$ for $t = 10$ min Solids = 20 wt%	5.1 (~86); 34.1(~0)	CTec 2 (with LPMO) = 10	76.8	Rodríguez-Zúñiga et al. (2015)
Hydrothermal @ 180 for 15 min then 195 for 3 min	4.8 (79.2); 32.8 (~0)	Cellubrix L = 64	82.3	Thomsen et al. (2008)
Sulfuric acid @ 120°C for 60 min		CTec 2 =		Chen, Zhao, et al. (2016); Dilute acid
Acid = 0.5%	18.4 (46.7); 29.0 (3.7)	21.7	28.8	
Acid = 1.0%	11.3 (70.7); 32.1 (4.7)	19.9	44.3	
Acid = 3.0%	5.9 (86.1); 36.0 (1.7)	17.7	45.9	Dilute acid+bleaching for delignification
Acid = 8.0%	2.5 (94.5); 38.7 (1.8)	17.2	52.6	
+NaClO ₂ @ 75°C for 180 min				
Untreated	28.3 (22.0); 7.9 (75.1)	24.2	77.2	
Acid = 0.5%	17.8 (67.8); 4.6 (90.4)	16.0	84.1	
Acid = 1.0%	14.6 (77.2); 3.2 (94.3)	14.2	89.2	Da Costa Lopes et al. (2018); Ionic liquid
Acid = 3.0%	8.7 (87.2); 3.3 (94.4)	13.4	90.4	
[emin][HSO ₄] = 41.3wt% @ 131°C for 88 min Solids = 10 wt%	6.7 (79.6); 29.3 (~0)	CTec 2≈20	75.8	

Abbreviations: LPMO, lytic polysaccharide monooxygenase; MAHF, maleic acid hydrotropic fractionation; WIS, water insoluble solid.

**FIGURE 3** AFM images of LCNFs from AHF WISs along with AFM topography measured LCNF height probability distributions. (a) M60T120t60-5P; (b) M60T120t60-9P; (c) LCNF height distributions. AFM, atomic force microscope; AHF, acid hydrotropic fractionation; LCNF, lignin-containing cellulose nanofibril; WIS, water insoluble solid**TABLE 2** List of water retention values (WRVs), carboxyl group contents, and surface charges of LCNF samples

Samples	WRV (%)	Carboxyl group content (mmol/g)	Surface charge (mv)
M60T120t60-5WS	758 ± 21	0.141 ± 0.01	-40.70 ± 2.2
M60T120t60-9WS	849 ± 18	0.144 ± 0.08	-43.84 ± 3.4

Abbreviation: LCNF, lignin-containing cellulose nanofibril.

and times were selected for the bleaching study. These WIS samples were labeled Tyytzz, as listed in Table 3, with Tyyy and tzz representing reaction temperature at $yyy^{\circ}\text{C}$ and time

for zz min. The bleached samples are labeled Tyytzz-B_{aa}, with B representing bleaching method, that is, B=O (oxygen), =D (chlorine dioxide), =P (peroxide); subscript _{aa}

TABLE 3 Chemical composition, solid yield, ISO brightness, and kappa number (KN) of AHF cellulosic water insoluble solids (WIS), with and without bleaching

Sample	Yield (%) ^a	Lignin (%) ^a	Cellulose (%) ^a	Xylan (%) ^a	Brightness (%)	KN
Wheat straw	100	22.3	37.9	21.2	—	—
T110t30	60.7	17.1 (46.7)	49.0 (78.6)	10.0 (28.7)	19.5 ± 0.3	126.1 ± 0.5
T110t60	53.1	14.1 (33.5)	57.0 (79.8)	9.4 (23.6)	20.6 ± 0.3	87.8 ± 0.8
T120t30	58.8	17.1 (45.2)	48.3 (75.0)	7.2 (19.9)	18.6 ± 0.3	121.9 ± 0.3
T120t90	47.5	13.5 (28.8)	33.7 (42.3)	3.0 (6.7)	19.0 ± 0.1	77.8 ± 0.4
Single-stage bleaching						
T110t30-O ₀₆	53.4 (87.9)	13.7 (32.8)	63.4 (89.3)	10.2 (22.4)	20.3 ± 0.1	80.7 ± 0.8
T110t60-O ₀₆	45.9 (86.5)	14.0 (28.8)	65.6 (79.4)	7.4 (14.0)	20.2 ± 0.3	83.3 ± 1.0
T120t30-O ₀₆	50.9 (86.2)	12.5 (28.5)	64.2 (86.2)	9.2 (19.3)	20.9 ± 0.2	74.5 ± 0.7
T120t90-O ₀₆	42.1 (88.6)	10.2 (19.3)	67.4 (74.9)	5.8 (10.0)	20.4 ± 0.2	61.0 ± 0.4
T110t30-O ₀₇	52.4 (86.3)	10.4 (24.4)	62.8 (86.8)	9.3 (20.1)	30.2 ± 0.3	56.3 ± 0.7
T110t60-O ₀₇	45.3 (85.4)	9.2 (20.7)	65.5 (78.3)	8.0 (14.9)	26.8 ± 0.2	44.2 ± 0.9
T120t30-O ₀₇	46.3 (78.8)	8.5 (17.6)	67.2 (82.1)	9.5 (18.1)	33.5 ± 0.1	38.5 ± 0.5
T120t90-O ₀₇	38.2 (80.6)	8.0 (13.7)	71.5 (72.1)	5.9 (9.3)	23.4 ± 0.2	43.8 ± 0.6
T110t30-D ₂	60.0 (98.9)	17.8 (47.9)	57.6 (91.2)	10.0 (24.7)	19.1 ± 0.1	86.7 ± 0.3
T110t60-D ₂	48.7 (91.7)	12.7 (27.7)	55.0 (70.7)	7.7 (15.4)	19.6 ± 0.2	72.6 ± 0.2
T120t30-D ₂	54.2 (92.2)	16.6 (40.3)	58.9 (84.2)	8.8 (19.6)	17.8 ± 0.2	98.0 ± 0.4
T120t90-D ₂	45.4 (95.5)	12.6 (25.7)	62.4 (74.7)	5.0 (9.3)	16.5 ± 0.3	68.2 ± 0.5
T110t30-D ₅	48.9 (80.7)	11.2 (35.5)	59.1 (76.3)	9.9 (19.9)	20.6 ± 0.3	60.2 ± 0.1
T110t60-D ₅	46.6 (87.8)	14.1 (29.5)	61.9 (76.1)	9.2 (17.6)	19.6 ± 0.1	72.8 ± 0.4
T120t30-D ₅	52.4 (89.2)	10.1 (34.8)	61.0 (84.3)	7.1 (15.3)	19.3 ± 0.2	70.9 ± 0.1
T120t90-D ₅	43.7 (92.0)	11.0 (23.5)	65.5 (75.5)	5.6 (10.1)	19.0 ± 0.3	70.3 ± 0.7
T110t30-P ₃	52.4 (86.4)	17.2 (33.4)	62.0 (85.7)	9.3 (20.1)	18.7 ± 0.4	97.3 ± 0.3
T110t60-P ₃	51.1 (96.2)	16.9 (38.7)	62.5 (84.3)	10.4 (21.9)	19.4 ± 0.2	85.4 ± 0.2
T120t30-P ₃	52.4 (89.2)	16.3 (36.0)	63.4 (87.7)	7.9 (17.0)	17.6 ± 0.2	96.3 ± 0.5
T120t90-P ₃	42.1 (88.6)	11.4 (21.5)	68.4 (76.0)	5.8 (10.0)	16.5 ± 0.3	76.7 ± 0.4
T110t30-P ₆	46.7 (76.9)	11.7 (24.5)	58.3 (71.8)	9.5 (18.3)	25.4 ± 0.3	70.8 ± 0.3
T110t60-P ₆	44.0 (82.9)	8.8 (21.9)	64.0 (74.3)	8.4 (15.2)	20.0 ± 0.2	57.4 ± 0.5
T120t30-P ₆	49.1 (83.6)	8.7 (21.4)	64.5 (83.6)	8.3 (16.8)	21.3 ± 0.2	54.6 ± 0.2
T120t90-P ₆	31.7 (66.7)	5.3 (7.5)	70.3 (58.8)	4.5 (5.9)	16.5 ± 0.3	43.9 ± 0.6
Multistage bleaching						
T110t30-O ₀₇	50.2 (82.7)	10.6 (23.9)	62.5 (82.8)	9.5 (19.6)	31.4 ± 0.2	54.1 ± 0.4
T110t30-OD ₂	45.8 (75.5)	9.2 (18.9)	63.2 (76.4)	10.9 (20.5)	28.9 ± 0.1	48.6 ± 0.2
T110t30-ODP ₃	44.2 (72.8)	5.0 (9.9)	71.9 (83.9)	8.4 (15.3)	47.2 ± 0.2	28.2 ± 0.3
T110t60-O ₀₇	44.1 (83.1)	10.2 (20.2)	66.1 (76.9)	8.3 (15.1)	26.3 ± 0.3	45.7 ± 0.5
T110t60-OD ₂	36.7 (69.1)	7.3 (12.0)	72.2 (69.9)	9.1 (13.7)	32.2 ± 0.2	40.0 ± 0.1
T110t60-ODP ₃	32.9 (62.0)	5.2 (7.7)	72.2 (62.7)	8.2 (11.1)	43.3 ± 0.4	27.7 ± 0.3
T120t30-O ₀₇	41.7 (70.9)	8.3 (15.5)	68.0 (74.8)	9.2 (15.8)	33.8 ± 0.3	40.5 ± 0.4
T120t30-OD ₂	37.6 (63.9)	6.8 (11.5)	71.3 (70.7)	8.7 (13.5)	29.7 ± 0.3	36.3 ± 0.2
T120t30-ODP ₃	33.9 (57.7)	4.7 (7.1)	73.6 (65.8)	7.6 (10.6)	44.1 ± 0.2	24.8 ± 0.3
T120t90-O ₀₇	42.4 (89.3)	7.8 (14.8)	71.5 (80.0)	6.5 (11.3)	22.8 ± 0.1	41.7 ± 0.7
T120t90-OD ₂	39.1 (82.3)	6.3 (11.0)	71.8 (74.1)	8.5 (13.7)	23.4 ± 0.3	33.4 ± 0.3
T120t90-ODP ₃	38.7 (81.5)	3.6 (6.2)	74.1 (75.7)	5.7 (9.1)	47.1 ± 0.2	18.3 ± 0.3

^aThe numbers in the parentheses are total solids or solid component yields from fractionation or bleaching based on the total and component mass in wheat straw, respectively.

represents either oxygen pressure (MPa) in O bleaching or chemical charge in wt% on OD weight WIS in chloride dioxide or peroxide bleaching. The KN, an indirect measure of RL content in fibers used in the pulp and paper industry, and ISO brightness values are used to characterize the unbleached and bleached MAHF WISs (Table 3). Bleaching was effective using all three methods, that is, O, D, and P. In general, a higher bleaching severity led to more lignin removal and significantly lower KN. O bleaching was the most effective among the single-stage bleaching processes studied.

Unbleached WISs from mild fractionation runs, T110t30 and T120t30, had KN of 126 and 122 (Table 3), respectively, or lignin content approximately 17%. Under severe O bleaching (oxygen pressure of 0.75 MPa), their KN decreased by approximately $\Delta\text{KN} = -70$ and -80 , respectively; however, mild bleaching of 0.62 MPa oxygen or 3% peroxide was less effective. Unbleached WISs from more severe MAHF runs, T110t60 and T120t90, presented lower starting KN of 87.8 and 77.8, respectively, or lignin content approximately 14% (Table 3). However, mild bleaching conditions did not change their KN and lignin content much either (Table 3). This can be attributed to lignin condensation as will be illustrated by NMR analysis to follow and in accordance with a previous study (Ma et al., 2020), in addition to relatively high lignin content of 14%. Only the most severe bleaching conditions showed substantial delignification. For example, lignin content decreased from 13.5% to 5.9%, or $\Delta\text{KN} = -34$, and from 14.1% to 8.0%, or $\Delta\text{KN} = -44$, for T120t90 and T110t60, respectively, with the most severe O bleaching, compared with $\Delta\text{KN} = -17$ and -5 under mild O bleaching. Similar results

were observed using P and D bleaching, with O being most effective. Despite appreciable delignification from single-stage bleaching, all final KNs remained high (40 or more) because of the high starting lignin content (14% or higher), with their resultant ISO brightness 30% at best (Table 3).

Comparing images of handsheets from unbleached and bleached WISs shows that increasing severe bleaching improved brightness, as shown top to bottom in Figure 4. Notably, however, despite more severe fractionation runs resulting in substantially more delignification as compared to milder fractionations (e.g., M60T120t90: 71% delignification or $\text{KN} = 77.8$ vs. M60T110t30: 54% delignification or $\text{KN} = 126.1$), the handsheet from unbleached, the milder fractionation is visually much brighter than that from more severe fractionation (Figure 4A1–A4, even though this visual difference is not reflected in brightness measurements of the inhomogeneous handsheets (Table 3). This inconsistency between sheet brightness and the extent of delignification is due to lignin condensation. Condensed lignin in WIS from severe fractionation (e.g., M60T120t90) tends to have a darker color than less condensed lignin from mild fractionation (e.g., M60T110t30; Wang et al., 2019). Similar visual differences were also observed with oxygen bleached WISs: T110t30-O₀₇ with 75.6% lignin removal is brighter than T120t90-O₀₇ with 86.3% removal (Figure 4B1–B4).

A multistage ODP bleaching sequence was also conducted on the four WIS samples. O bleaching was conducted at the more severe condition of 0.75 MPa oxygen pressure to achieve effective delignification for all samples. It is hypothesized that subsequent D and P bleaching can be effectively applied under lower bleaching severity with lower chemical charges of 2% and 3% on OD WIS, respectively, so

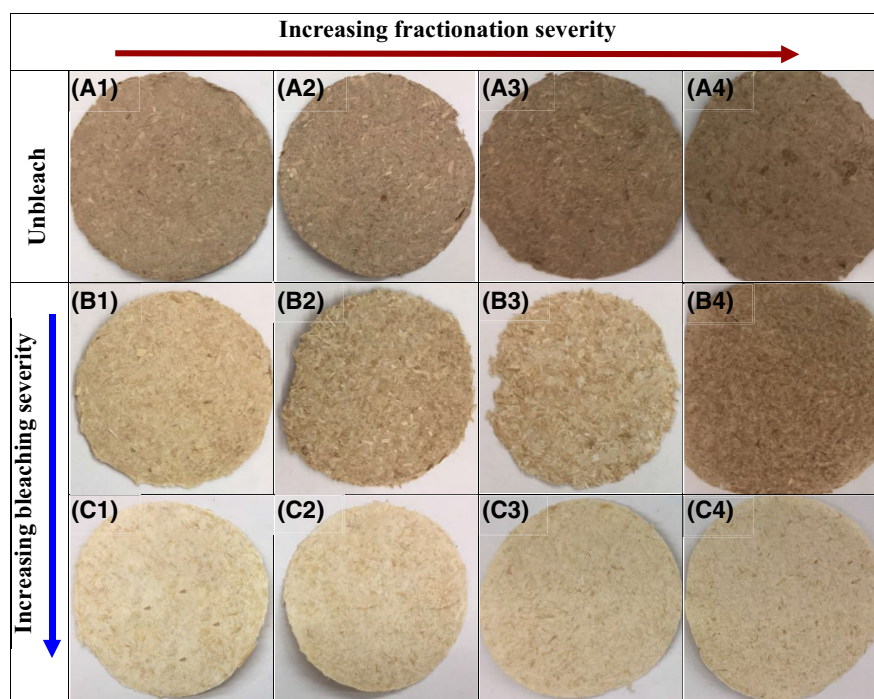


FIGURE 4 Images of handsheets made of fractionated cellulosic water insoluble solids (WISs) before and after bleaching. (A1)–(A4): unbleached; (B1)–(B4): O₀₇ bleached; (C1)–(C4): ODP bleaching sequence. Column 1 (most left column): from M60T110t30; Column 2: from M60T110t60; Column 3: from M60T120t30; Column 4 (most right column): M60T120t90

as to conserve chemicals. The results demonstrate that ODP bleached WIS from the most severe MAHF have the same brightness of 47% as those from the mildest MAHF (i.e., both T120t90-ODP₃ with 3.6% lignin (KN = 18) and T110t30-ODP₃ with 5.0% lignin (KN = 28) have the same ISO brightness of 47%). Additionally, the most severe MAHF resulted in the lowest yield of cellulose of 75.7% and xylan of 9.1% for T120t90-ODP₃ compared with 83.9% and 15.3%, respectively, for T110t30-ODP₃. As a result, the bleached WIS, T110t30-ODP₃, from the mildest run had a much greater bleached fiber yield of 44.2% than 38.7% for T120t90-ODP₃ from the most severe run M60T120t90. Fiber yield has critically practical importance to process economics.

Cellulose degree of polymerization (DP) is an important parameter for producing fibers. DP is often measured by the intrinsic viscosity ($[\eta]$) of the solution of solubilized fibers. As shown in Figure 5, the results indicate that multistage bleached WIS samples have approximately the same viscosity of 640 ml/g, or DP approximately 900, except for the WIS from the most severe fractionation M60T120t90 with a low viscosity of only 540 ml/g, or DP of 750 ml/g, due to cellulose degradation and lignin depolymerization during AHF. Considering high brightness, high yield, and good DP, mild fractionation such as M60T110t30 should be considered for fiber production.

3.5 | MAHF lignin chemical structure

2D ¹H-¹³C HSQC NMR spectral analysis was utilized to compare structural features of selected MAHF DL and RL with those of MWL. Peak assignments (Table S3) and quantification of important internal structure per 100 monomeric lignin units (100Ar) are according to literature (Capanema et al., 2004; Del Rio et al., 2016; Mansfield et al., 2012). As

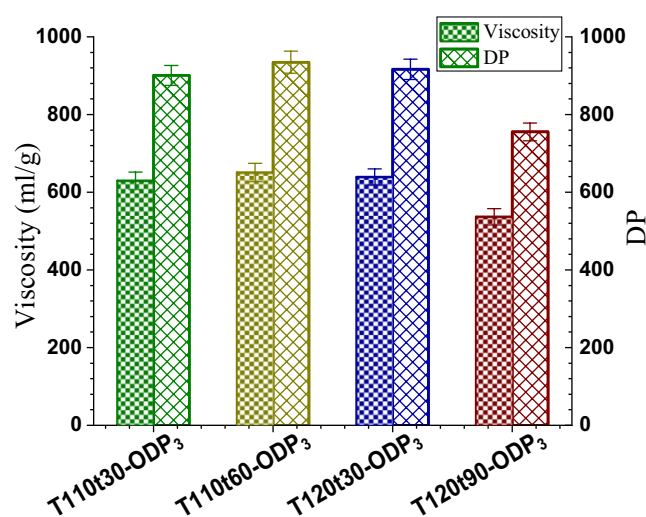


FIGURE 5 Relative viscosity and degree of polymerization of multistage bleached WISs. WIS, water insoluble solid

shown in Figure 6 and Figure S2, spectra were divided into the side-chain (δ_C/δ_H 110–40/6.0–2.5 ppm) and aromatic regions (δ_C/δ_H 150–90/8.0–6.0 ppm). The prominent aliphatic cross-peaks corresponding to methoxyl groups (OMe), ether aryl β -O-4' linkages (A), phenylcoumarans (β -5, B), and resins (β - β , C) substructures were observed in all lignin samples. In addition, signals of associated carbohydrates, such as β -D-xylopyranoside units (X), arabinofuranoside units (Ara), and 4-O-methyl- α -D-glucuronic acid units (U), could also be clearly seen in the side-chain region of all spectra.

Under mild fractionation conditions, lignin condensation was minimal: the amount of β -O-4' linkages for DL-T80t120 (Figure 6d) was approximately the same as that for MWL (Figure 6a) of 50% (Table 4). Increasing MAHF severity increased the extent of lignin condensation and reduced the amount of β -O-4' linkages (Table 4). The β -O-4' linkages in DL-T120t90 decreased to 22.5%, compared with 40.1% for DL-T110t30 (Figure 6b; Table 4). Its corresponding RL RL-T120t90, however, still retained approximately 30% β -O-4' linkages, suggesting that DL was subjected to more severe degradation than RL.

Lignin condensation can also be seen by comparing the signal intensities of B_α and C_α at δ_C/δ_H 87.2/5.46 and 85.0/4.66 ppm, respectively, in the side-chain region of DL-T110t30 and DL-T120t90. The abundance of B_α and C_α increased from 2.3% to 4.8% and 1.2% to 1.7%, respectively. For runs with 60 wt% MA concentration and a short reaction time of 30 min, increasing reaction temperature from 100 to 120°C had a minimal effect on lignin condensation with β -O-4' linkages remaining approximately 38% (Table 4). By contrast, increasing reaction time had a substantial effect on lignin condensation. The amount of β -O-4' linkages was decreased from 40% to 33%, or by 17%, when reaction was extended from 30 to 60 min at 110°C. The decrease is much more drastic from 38% to 25%, or by 34% at 120°C (Figure S2).

Signals for Hibbert's ketones (HK: δ_C/δ_H 44.4/3.65 ppm, α -position; δ_C/δ_H 66.8/4.16 ppm, γ -position) were observed in the lignin samples from more severe runs (Figure 6e,g,i; CDF $\geq$ 598 in Table 4), but were absent from MWL (Figure 6a) and lignin samples from mild fractionation runs (Figure 6b–d; CDF < 300 in Table 4). HK are formed during acidic delignification because of acid-catalyzed formation of a benzylic cationic intermediate (Cheng et al., 2019; Li et al., 2018).

All DLs and RLs (Figure 6b–i) have distinct peaks at δ_C/δ_H 128.4/6.37, 131.9/6.41 in the aromatic region (Figure 6b–i) associated with lignin esterification by MA at the γ -OH position ($E_\gamma(\text{MA})_{2,3}$), as verified by our earlier study (Cai, Hirth, et al., 2020). These two peaks are absent in the MWL spectra. This lignin esterification signal at δ_C/δ_H 63.3/(3.85, 4.32) in the aliphatic region overlapped with the signal from A'_γ -Est (Figure 6a) which is known as acylated γ -position by *p*-coumarate of gramineous lignins (Balakshin et al., 2011; Wen et al., 2013b) and also observed previously in

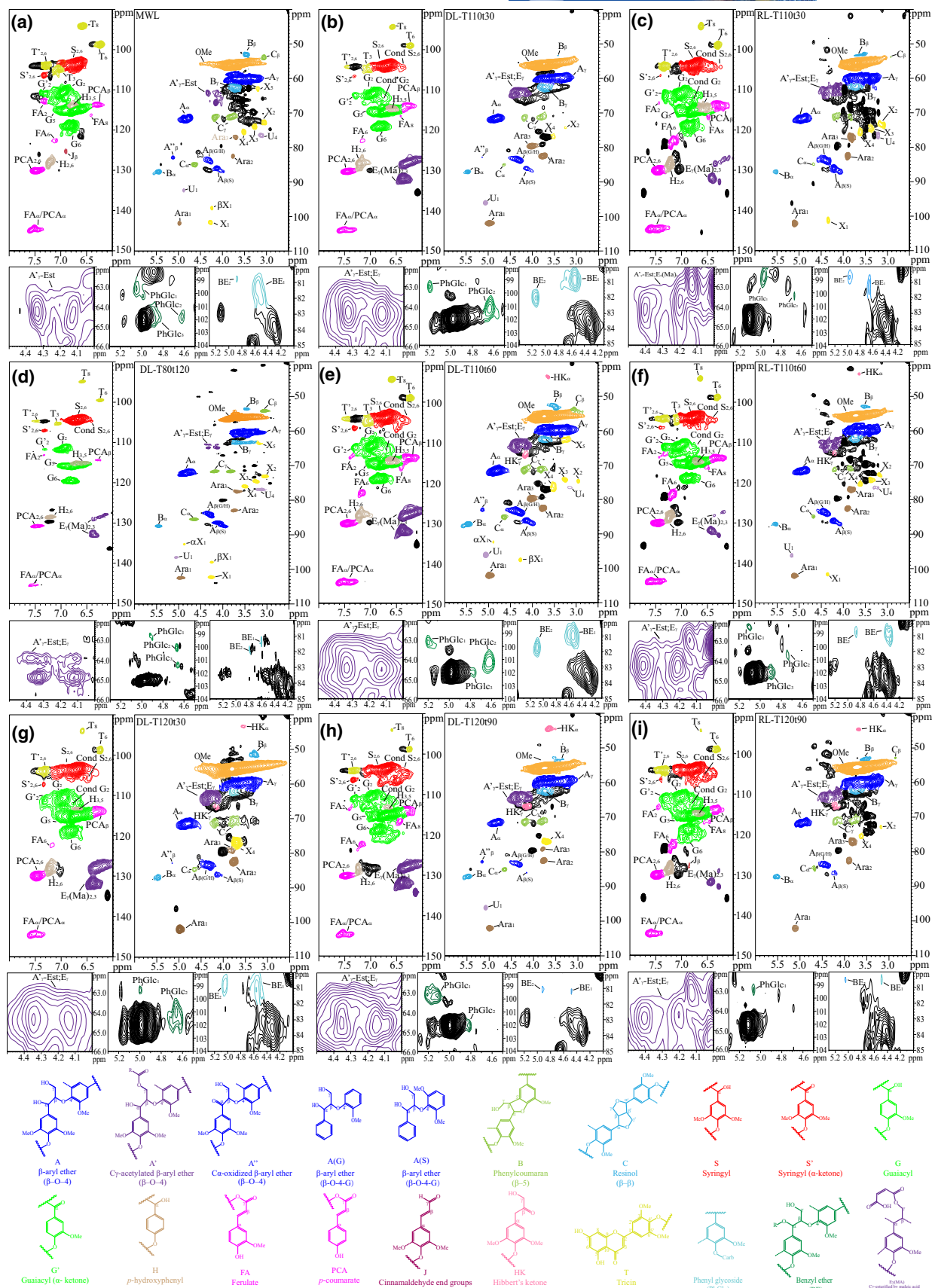


TABLE 4 Amount of lignin substructure and lignin carbohydrate complex (LCC) linkages along with mean molecular weight (Mw), DLs, and RLs samples (/100 Ar)

RUN	MWL	M40T80t120	M60T100t30	M60T110t30	M60T110t60	M60T120t30	M60T120t60	M60T120t90	M60T110t60	M60T120t30	M60T110t60	M60T120t30	M60T120t90
CDF	0	33	124	299	598	692	1385	2077	598	692	598	692	2077
Delignification (%)	37.8	53.6	53.3	53.3	66.5	54.8	56.6	71.2	53.3	54.8	66.5	54.8	71.2
Lignin	MWL	DL-T80t120	DL-T100t30	DL-T110t30	DL-T110t60	DL-T120t30	DL-T120t60	DL-T120t90	DL-T110t60	DL-T120t30	DL-T110t60	DL-T120t30	DL-T120t90
Mw	13206	8096	7009	8113	6903	7811	5727	5047	8514	7417	8194	6302	6302
Mn	4396	3645	3708	3744	3481	3693	3024	3157	3415	3317	3552	3079	3079
Mw/Mn	3.0	2.2	1.9	2.2	2.0	2.1	1.9	1.6	2.5	2.2	2.1	2.0	2.0
Interunit linkages ^a													
β -O-4' (A)	50.5	50.3	38.1	40.1	33.3	37.5	25.1	22.5	40.5	35.4	39.0	30.1	30.1
β -5' (B)	5.0	3.4	5.9	2.3	4.2	2.2	6.3	4.8	2.5	2.9	2.6	3.8	3.8
β - β' (C)	4.7	7.9	9.6	1.2	2.0	1.1	10.2	1.7	1.1	1.5	1.2	2.5	2.5
Cond degree ^c	15.7	18.3	28.9	8.0	15.6	8.1	39.7	23.2	8.2	11.1	8.9	17.3	17.3
γ -esterif	11.0	14.7	26.8	19.1	33.4	21.4	37.2	45.8	12.7	26.2	17.1	35.7	35.7
HK α	ND	ND	2.4	ND	2.9	1.3	7.7	6.4	ND	1.6	1.0	4.1	4.1
Aromatic units ^b													
S	36	50	53	43	48	44	34	46	40	43	42	44	44
S _{cond}	ND	1	5	1	6	2	2	12	1	5	2	8	8
G	61	47	44	54	50	53	63	51	56	53	55	52	52
G _{cond}	ND	ND	ND	2	14	2	1	23	1	11	2	16	16
H	3	3	3	3	3	4	3	4	4	4	3	4	4
S/G ratio	0.60	1.11	1.20	0.80	0.96	0.83	0.54	0.90	0.71	0.81	0.76	0.85	0.85
<i>p</i> -Hydroxycinnamates ^b													
<i>p</i> -coumarates	8.0	3.3	4.3	9.2	7.7	8.5	14.8	6.8	7.3	6.5	6.3	5.0	5.0
Ferulates	2.5	2.8	0.9	2.1	1.5	1.1	1.5	1.0	4.7	1.0	1.5	2.5	2.5
Flavonoid ^b													
Tricin	9.1	2.7	1.0	2.7	2.3	2.7	0.4	3.3	3.0	2.9	3.5	3.6	3.6
LCC linkages													
PhGlc	4.8	3.3	2.2	1.9	1.5	2.1	1.7	1.1	1.3	1.4	1.7	1.3	1.3
BE	7.2	3.4	1.5	2.6	2.1	2.3	1.2	1.6	3.2	2.8	2.9	1.9	1.9
Total	12.1	6.7	3.7	4.5	3.6	4.4	2.9	2.7	4.5	4.2	4.6	3.2	3.2

Abbreviations: BE, benzyl ether; CDF, combined delignification factor; DL, dissolved lignin; MWL, milled wood lignin; ND, non-detected; RL, residual lignin.

^aMolar percentage (H + G + S = 100).^bInterunit linkages, *p*-coumarate, ferulate, and tricinn molar contents as percentages of lignin content (H + G + S).^cCondensed degree, % = $100 \times (I_{Ba} + I_{Ca}) / (I_A + I_{Ba} + I_{Ca})$, which *I* referred to the integral value of each signal in 2D HSQC NMR.

switchgrass (Su et al., 2021). Increasing fractionation severity increased the extent of esterification as can be seen from the signal intensities at δ_C/δ_H 128.4/6.37, 131.9/6.41 by comparing Figure 6d (DL-T80t120 being the mildest with a lower MA concentration of 40 wt%, CDF = 33 in Table 4) with Figure 6b,e,g,h (DL-T120t90, being the most severe under MA concentration of 60 wt% with CDF = 2077 in Table 4). The calculated extent of esterification for DL-T80t120 was 14.7% compared with 45.8% for DL-T120t90 (Table 4).

When comparing DLs with their corresponding RLs from the same run, for example, DL-T110t30 with RL-T110t30 (Figure 6b with c), DL-T110t60 with RL-T110t60 (Figure 6e with f), and DL-T120t90 with RL-T120t90 (Figure 6h with i), DLs have a stronger $E_\gamma(\text{MA})_{2,3}$ signal than their corresponding RLs, suggesting DLs have a greater extent of esterification. The calculated extents of esterification were 19.1% and 12.7%, 45.8% and 35.7% for DLs and RL, respectively, for the mildest and most severe run M40T80t120 and M60T120t90.

The LCC linkages of phenyl glycoside (PhGlc) and benzyl ether (BE) were also identified, as shown in the zoomed-in 2D NMR spectra in Figure 6. The PhGlc has three correlation signals shown in the MWL spectrum (Figure 6a) at δ_C/δ_H 100.2–98.4/5.09–4.90 ppm (PhGlc1), 100.9–100.3/4.85–4.63 ppm (PhGlc2), and 102.0–101.5/4.92–4.79 ppm (PhGlc3; Balakshin et al., 2011; Du et al., 2014). However, some carbohydrate signals disappeared in RLs or DLs spectra due to cleavage of the glycosidic bonds between lignin and carbohydrates under high fractionation severities. DL-T120t90 had the least amount of PhGlc of 1.1%. There are two types of BE: (1) benzyl ethers connecting C α of lignin to carbohydrate primary hydroxyl groups (BE₁, δ_C/δ_H 82–80/4.7–4.5 ppm) and (2) benzyl ethers connecting the C α of lignin to carbohydrate secondary hydroxyl groups (BE₂, δ_C/δ_H 82–80/5.1–4.9 ppm; Huang et al., 2019). With increasing reaction severity, the abundance of BE decreased rapidly. Under the same fractionation severity, RLs showed a less pronounced decrease in BE, that is, from 7.1% for MWL to 1.1% for DL-T120t90 compared with 1.9% for RL-T120t90.

³¹P NMR spectra of lignin samples were assigned according to previous literature (Granata & Argyropoulos, 1995; Pu et al., 2011). MWL has the highest aliphatic OH content of 3.78 mmol/g (Table 5; Figure S3). MAHF significantly decreased aliphatic OH especially under more severe fractionation conditions due to oxidation, acetylation, and acid-catalyzed elimination reactions (Wen et al., 2013c). MAHF increased phenolic OH content especially under more severe fractionation compared with MWL (Table 5), due to the cleavage of β -O-4' linkages by MA, as revealed in literature (Cai, Hirth, et al., 2020; El Hage et al., 2009; Wen et al., 2013c). In general, RLs have a greater aliphatic OH but a lower total phenolic OH than their corresponding DLs have (Table 5).

No significant variations in the p-hydroxyphenyl OH (H-type lignin unit) content among all lignin samples analyzed, suggesting that MA delignification did not result in the demethoxylation reaction as compared to other acid delignification, for instance, formic acid (Wen et al., 2013c; Zhang et al., 2017).

Lignin carboxylation by MA increased with fractionation severity as observed previously from wood lignin (Cai, Hirth, et al., 2020). A similar trend to total phenolic OH, DL-T120t90 from the most severe fractionation has the highest carboxyl content of 0.66 mmol/g. DLs also have a more carboxyl groups content than their corresponding RLs, for example 0.52 mmol/g for DL-T110t30 compared with 0.35 mmol/g for RL-T110t30. A carboxylated lignin with low condensation is desirable in terms of a value-added advantage due to its ability to be modified for a variety of applications (Figueiredo et al., 2017; Sun et al., 2016).

3.6 | MAHF lignin physical properties

Weight-average Mws of DLs and RLs decreased with increasing fractionation severity (Table 4) due to depolymerization by MA. Compared with MWL with Mw of over 13,000 Da, Mw for the mildest fractionation M40T80t120 was over 8000 Da and decreased to approximately 5000 Da

TABLE 5 Quantification of the functional groups in lignin by ³¹P NMR spectroscopy

Samples	Phenolic OH				Total phenolic OH	COOH
	Aliphatic OH	Syringyl OH	Guaiacyl OH	p-Hydroxyphenyl OH		
MWL-WS	3.78	0.30	0.39	0.32	1.01	0.26
DL-T110t30	1.95	0.39	0.40	0.30	1.09	0.52
DL-T120t90	1.26	0.57	0.49	0.29	1.35	0.66
RL-T110t30	1.83	0.37	0.40	0.30	1.07	0.35
RL-T110t60	1.49	0.44	0.43	0.28	1.15	0.38
RL-T120t90	1.37	0.50	0.48	0.28	1.26	0.42

Note: All OH group content in mmol/g lignin.

Samples	MWL-WS	DL-T110t30	DL-T120t90	RL-T110t30	RL-T110t60	RL-T120t90
T _m (°C) ^a	368	357	364	362	352	349
Char (%) ^a	40.9	37.1	41.9	38.1	35.0	41.1
T _g (°C) ^c	171	154	173	158	155	168

Abbreviations: DL, dissolved lignin; MWL, milled wood lignin; RL, residual lignin; T_g, glass transition temperature; T_m, maximal thermal degradation temperature.

^aNonvolatile residue of lignin at 900°C.

TABLE 6 T_m, Residue Char, and T_g of MWL, DLs, and RLs preparations

for the most severe run M60T120t90. RLs have higher Mw than their corresponding DLs; however, the polydispersity indices (PDIs) of all MAHF lignin samples were approximately the same of approximately 2.0. That is, Mw distribution of MAHF lignin is much more uniform than MWL with PDI of 3.0 (Table 4), as illustrated in lignin Mw distribution curves (Figure S4).

In general, increasing fractionation severity slightly decreased lignin thermal stability (Figure S5) as reflected in the maximal degradation temperature, T_m, corresponding to the maximal (peak) value in the dW/dT curve. This is perhaps due to decreasing Mw (or DP) as listed in Table 4 and is consistent with MWL having the highest Mw and highest thermal stability (Faravelli et al., 2010; Wen et al., 2013a). On the other hand, the thermal stability of the lignin samples is dictated by two competing phenomena—depolymerization and repolymerization (condensation)—taking place during fractionation. Table 6 lists the maximal thermal degradation temperature T_m for the lignin samples evaluated.

Lignin is an amorphous solid that allows transition from glassy to rubbery state at the glass transition temperature T_g (Ibrahim et al., 2011), which is an important property for composite application through compounding. In general, lignin T_g is also affected by its Mw (DP) and the extent of condensation (carbon–carbon bond formation) (Cheng et al., 2019). MWL had a significantly higher Mw and much greater T_g of approximately 170°C than the MAHF lignin samples. Accordingly, comparing DL-T110t30 with DL-T120t90 and RL-T120t90, lignin samples with more condensation (lower β–O–4' linkages, Table 4) have higher T_g (Table 6).

3.7 | MA Fractionated lignin radical scavenging capability

All DL and RL lignin also exhibited excellent radical scavenging capacity toward ABTS and DPPH radicals (Figure S8).

4 | DISCUSSION

Maleic acid hydrotropic fractionation can rapidly fractionate wheat straw with great selectivity in solubilizing lignin

and hemicelluloses while preserving cellulose. Dissolution of xylan and lignin reached asymptotic residual values for both soon after the initial rapid reaction phase, and can be fitted well by reaction kinetics-based severity factors. This suggests that the desired extent of xylan dissolution or degree of delignification can be achieved provided that the required overall fractionation severity CHF or CDF is attained. That is, the two severity factors described can be used to deal with any practical constraints of process scale-up particular to an individual facility. For example, prolonged reaction time or elevated temperature can be used to compensate for a low acid loading to reduce acid recovery demand without detriment to fractionation results.

Maleic acid hydrotropic fractionation also showed robust performance for enzymatic sugar production from the fractionated WISs due to (1) simultaneous dissolution of a substantial amount of hemicelluloses and lignin (Leu & Zhu, 2013) even at atmospheric pressure; and (2) lignin carboxylation by MA which facilitated the substantially reduced nonproductive cellulase binding to substrate lignin through pH-mediated electrostatic repulsive interactions (Lou et al., 2013). Lignin carboxylation also facilitated nanofibrillation of the fractionated WISs for producing LCNFs through electrostatic repulsion, which enhanced lignin lubrication during fibrillation (Bian et al., 2017a; Rojo et al., 2015). Lignin carboxylation is also advantageous in improving lignin antioxidant capabilities.

Maleic acid hydrotropic fractionation resulted in minimal lignin condensation due to the rapid fractionation at relatively low temperatures and atmospheric pressure. Our previous work showed that less condensed lignin is favorable for catalytic conversion to lignin aromatics (Cai, Hirth, et al., 2020; Wang et al., 2019). The RL in the fractionated WISs was also minimally condensed, which facilitated bleaching for fiber production. MAHF produced lignin has relatively greater Mw and low glass transition temperature, favorable for composite applications.

Our previous work also showed that the dissolved xylan can be efficiently dehydrated into furfural at high yield using the acid hydrotrope, that is, MA, in the fractionated liquor without additional catalyst (Cai, Hirth, et al., 2020; Cai, Li, et al., 2020; Zhu et al., 2019). Furthermore, the low solubility and high MHC of MA (25 wt%) is advantageous

in acid recycling (Cai, Li, et al., 2020). Considering MA has low toxicity (US FDA-approved indirect food additive [21CFR175-177]) and can valorize all three major components of plant biomass without additional chemicals, MAHF has notable potential for sustainable plant-biomass-based biorefinery.

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DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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