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Methanolysis Fractionation and Catalytic Conversion of Poplar Wood toward Methyl Levulinate, Phenolics, and Glucose

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Supporting Information

ABSTRACT: In the present study, methanolysis of poplar biomass was conducted for the selective transformation of hemicellulose and lignin, which leads to methyl glycosides (mainly C5 glycosides) and lignin fragments in the liquefied products that can be separated according to their difference in hydrophilicity. The distribution of methyl glycosides and delignification was dependent on the presence of acid catalysts and reaction temperatures. The obtained lignin fraction was separated into solid lignin fragments and liquid lignin oil according to their molecular weight distribution. Subsequently, directional conversion of methyl C5 glycosides into methyl levulinate was performed with dimethoxymethane/methanol as the cosolvent. A yield of 12−30% of methyl levulinate yield (based on the methyl glycoside) was achieved under these conditions. The remaining cellulose-rich substrate showed enhanced susceptibility to enzymatic hydrolysis, resulting in a yield of glucose of above 70%. Overall, the described strategy shows practical implications for the effective valorization of biomass.

KEYWORDS: *methanolysis, glycoside, phenolics, enzymatic hydrolysis, methyl levulinate*

1. INTRODUCTION

Widely distributed and highly abundant lignocellulosic biomass has been considered as a promising source of energy, biofuels, and biochemicals.[1,2] Lignocellulose has a complex and strongly interlinked structure consisting of three main biopolymers, cellulose, hemicellulose, and lignin, in the plant cell wall. Chemical, biological, or physical constraints and their interrelations must be overcome before the conversion of various feedstocks into valuable chemicals and materials can be achieved.[3] The efficient fractionation of biomass into its individual components and efficient utilization of each component is optimized via biorefinery processes for the valorization of the entire biomass.[4−6] Specifically, cellulose is considered to be the most economical because it can be depolymerized into monosaccharides (glucose) and then converted to bioethanol, biobutanol, or other chemicals using chemical or fermentation strategies.[6,7] The released sugars from hemicellulose can also be used as substrates for fermentation or anaerobic digestion. Alternatively, sugars can be converted into various value-added chemicals such as furfural and levulinic acid/ester by catalytic conversion.[8−10] Lignin, a complex heterogeneous natural polymer with different phenylpropane units linked by various C−O and C−C bonds (i.e., β-O-4, β-β, β-5, and β-1), has received much attention in the production of various phenolics and materials.[11]

Currently, biomass refinery methods have attracted much attention for the efficient fractionation of lignocellulose biomass. Numerous approaches have been investigated for the pretreatment of lignocellulose, such as hydrothermal,[12,13] steam explosion,[14] alkali methods,[15] and solvolysis treatment[16−18] and have been combined with high-pressure, ultrasound-assisted, and microwave-assisted processing. Remarkable progress has been made in the application of solvolysis pretreatment for converting biomass and the facilitation of further upgrading due to an improvement in reactivity. For example, an increasing number of solvolysis studies in supercritical or subcritical methanol have been reported.[18−20] It provides higher solubility for decomposed products from biomass because of the lower dielectric constants in comparison with water. Another advantage is that methanol can be recovered by simple distillation, which reduces the overall energy consumption and separation cost of the process. Furthermore, alcohol-derived decomposition products (e.g., hydrogen, alkoxy moieties) can quench some intermediates during the reaction process.[21,22] The −OH in methanol acts as a nucleophile and can attract the glycosidic

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bonds of (hemi)cellulose and acid-ester bonds of lignin compounds with an acid catalyst and can cause depolymerization of aromatics and polysaccharides in lignocellulose.[23] Previous studies have mostly focused on increasing yields and characterizing properties of bio-oil. The previous studies have typically yielded unstable liquid products, containing various complex components (e.g., aldehydes, organic acids, esters, phenolics, and oligomers); these products mainly contributed to the structural complexity and different properties of biomass components.[16,23] The selective conversion of (hemi)cellulose and lignin toward their respective oligomers produces sugar derivatives and lignin fragments which can be separated by their hydrophilic differences. Due to the similar chemical nature of each stream, the reduced complexity of the chemical components in each product stream will facilitate downstream upgrading processes.

Methyl levulinate (MLA) is one of the most promising derivatives converted from carbohydrates and can be widely used in many fields such as medicines, solvents, organic chemistry, and fragrance.[24,25] Numerous studies have reported that C6 sugars in carbohydrates of biomass can potentially be converted into methyl levulinate/levulinic acid with an acid catalyst.[25−27] However, multiple steps involving both hydrogenation and acid-catalyzed processes for the transformation of C5 sugars to methyl levulinate/levulinic acid are required. Recently, the investigation of direct conversion of C5 sugar or furfural into methyl levulinate/levulinic acid was proposed.[28,29] In this study, to further valorize the hemicellulose, we explored a one-step transformation of C5 sugar (C5 glycoside) derived from biomass into methyl levulinate in a dimethoxymethane/methanol cosolvent with an acid catalyst.

Here, methanolysis treatment was used to release the hemicellulose sugar (methyl glycoside) and lignin fragments from poplar sawdust, leaving behind a cellulose-rich solid substrate (cellulose pulp) (Figure 1). Various acid catalysts and

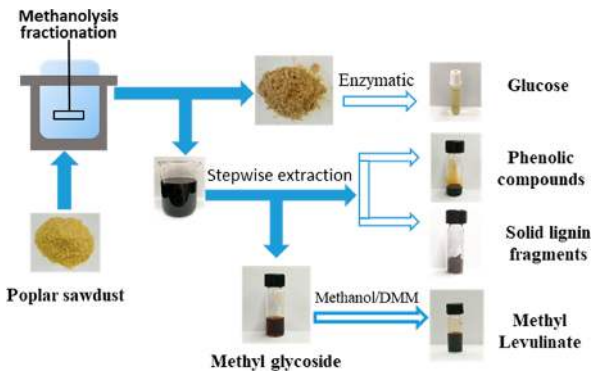

Figure 1. Process scheme for the selective fractionation and catalytic conversion of poplar wood for value-added chemicals.

temperatures were assessed in the methanolysis process to determine the fractionation efficiency, delignification, and product distributions. Pure lignin fragments (including solid lignin fragments and liquid lignin oil) were separated from methyl glycoside in high yield with stepwise fractionation methods based on the hydrophilic differences. Subsequently, the hemicellulose sugar (mainly methyl C5 glycosides) with high purity were directly transformed to levulinic acid/ester in a dimethoxymethane/methanol mixture with an acid catalyst. The cellulose-rich sample was further subjected to enzymatic hydrolysis to provide a high yield of glucose. The described strategy achieved the fractionation and sequential utilization of all the biomass components.

2. EXPERIMENTAL SECTION

2.1. Material. Poplar wood (*Populus* L) was obtained from Nanjing, China, passed through a 250−425 μm sieve (60−80 mesh), and then dried overnight at 105 °C. The composition (wt %) of the poplar wood was determined following NREL/TP-510-42618, and the cellulose, hemicellulose, and lignin contents were 47.14, 22.86, and 23.73 wt %, respectively. Methanol (99%), dimethoxymethane (99%), methyl levulinate, H_2SO_4 (98%), H_3PO_4 (85%), HCl (37%), p-TsOH (98%), HPW (phosphotungstic acid), and sulfamic acid (99.3%) were purchased from Nanjing Kermel Chemical Reagent Co., Ltd. Methyl-α-D-glucopyranoside (≥99%), methyl-β-D-glucopyranoside (≥99%), methyl-α-D-xylopyranoside (≥99%), and methyl-β-D-xylopyranoside (≥99%) were purchased from Sigma-Aldrich. Amberlyst 70 catalyst (a solid acidic resin catalyst) was purchased from Dow Chemical.

2.2. Methanolysis Fractionation of Poplar Biomass. The methanol solvothermal fractionation was carried out in a high-pressure reactor. In each run, 10 g of poplar sawdust, 100 mL of solvent methanol (solid/liquid ratio of poplar to methanol was 1/10 (w/w)), and a certain amount of an acid catalyst mixture were loaded into the reactor. The reactor was sealed and flushed three times with N_2 to remove air. The mixtures were heated to 140−200 °C, and then the temperature was kept constant for 40 min. After the reaction, the reaction vessel was cooled to room temperature. Then, the insoluble solid residues were separated by a filtration step from the liquid mixture and dried overnight at 105 °C.

The liquid mixture consisted sugars and lignin fragments along with the methanol solvent. The stepwise separation of the liquid mixture was as follows: deionized water was added to the filtrate, and the mixture was evaporated under a negative pressure at 40 °C to remove half of the methanol solvent, leading to the precipitation of solid lignin 1# from the mixture. The previous step was repeated and then all methanol solvent in the mixture was completely distilled, to precipitate solid lignin 2#. Next, ethyl acetate was added to the remaining deionized water and product mixture, which was separated into an ethyl acetate soluble phase and a water soluble phase using a separatory funnel. Finally, methyl glycoside and liquid lignin oil products could be obtained from the aqueous solution phase and ethyl acetate phase, respectively.

$$\text{conversion (wt \%)} = \left(1 - \frac{m_s}{m_0}\right) \times 100\%$$

$$\text{yield of methyl glycoside (wt \%)} = \left(\frac{m_{gly}}{m_0}\right) \times 100\%$$

$$\text{yield of lignin oil (wt\%)} = \left(\frac{m_{oil}}{m_0}\right) \times 100\%$$

$$\text{yield of methyl xyloside (wt \%)} = \left(\frac{m_{xyl}}{m_0}\right) \times 100\%$$

$$\text{delignification (wt \%)} = \left(1 - \frac{m_{L_D}}{m_{L_0}}\right) \times 100\%$$

$$\text{(hemi)cellulose retention (wt \%)} = \left(\frac{m_{H_D}}{m_{H_0}}\right) \times 100\%$$

where m_0 is the original mass of the poplar, m_s is the mass of cellulose-rich substrate after the methanolysis process, m_{gly} and m_{oil} are the masses of the methyl glycoside and the lignin oil, respectively, m_{xyl} is the mass of the methyl xyloside based on the HPLC result., m_{C_0}, m_{H_0}, and m_{L_0} are the masses of the original cellulose, hemicellulose, and lignin in the feedstock, respectively, and m_{C_D}, m_{H_D}, and m_{L_D} are the

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Table 1. Catalytic Performance of Various Acid Catalysts for the Conversion of Poplar in Methanol Medium

		yield of sugars (%)			yield of lignin (%)			carbohydrate retention (%)	
acid catalyst[a]	conversn (%)	methyl xyloside[b]	methyl glucoside	total[c]	solid lignin[d]	lignin oil	delignification (%)	cellulose	hemicellulose
no acid	18.24	2.25	0.14	5.31	4.74	0	18.04	95.25	87.54
HCl	40.21	9.57	4.92	16.77	10.22	2.59	50.25	73.34	38.39
H_3PO_4	37.06	8.93	4.07	15.64	9.72	2.2	46.93	78.83	42.51
H_2SO_4	52.25	12.32	3.77	24.85	13.68	4.09	74.21	82.63	14.33
HPW	45.35	11.01	4.32	18.42	12.45	3.54	52.32	76.34	23.51
$C_7H_7SO_3H$	49.36	13.74	5.65	21.48	12.34	2.09	61.32	70.34	16.51
p-TSOH	51.71	12.23	4.13	25.92	13.34	3.73	69.56	78.53	18.12

[a]Reaction conditions: temperature, 160 °C; pressure, about 1.7 MPa at 160 °C; catalyst, 1.0 g; poplar, 20 g; methanol, 200 mL; time, 30 min. The acid dose is based on the amount of H_2SO_4: 1 equiv of p-TsOH and $C_7H_7SO_3H$, 10 equiv of H_3PO_4 and HCl, and 0.3 equiv of HPW. [b]Methyl xyloside contains methyl α-D-xylopyranoside, and methyl β-D-xylopyranoside; methyl glucoside contains methyl-a-D-glucopyranoside and methyl-β-D-glucopyranoside. [c]The total yield of glycoside was calculated on the basis of the original poplar wood. [d]The solid lignin contained lignin 1# and lignin 2#.

masses of cellulose, hemicellulose, and lignin, respectively, in the cellulose-rich substrate.

2.3. Catalytic Conversion of Methyl Glycoside. Further conversion of methyl glycoside (mainly methyl C5 glycoside) was conducted as described below. The mixtures comprising 1.5 g of methyl glycoside reactants, 3.0 g of Amberlyst 70, and 30 g of cosolvent methanol/DMM (w/w, 15/15) were loaded into a 50 mL high-pressure reactor. The reactor was sealed and flushed three times with pressurized N_2 to remove air. Afterward, the reactor was heated to 140−170 °C, and the temperature was held constant for 120 min with 350 rpm stirring. Once the reaction time was achieved, the autoclave was cooled using flowing water until the temperature dropped below 30 °C. The solid products were separated with filter paper from the liquid mixture, washed three times with methanol, then dried at 105 °C overnight. The weight of the coke (solid polymer) was the weight of the solid insoluble matter minus the weight of the catalyst (2 g).

$$\text{yield of methyl levulinate (wt \%)} = \left(\frac{m_{\text{mla}}}{m_{\text{gly}}}\right) \times 100\%$$

where m_{mla} is the mass of the methyl levulinate based the GC result.

2.4. Enzymatic Hydrolysis. Enzymatic hydrolysis of the cellulose-rich substrate was conducted at 50 °C on a shaking bed incubator at 250 rpm. A 2 g portion of cellulose-rich substrate, 10 mL of acetate buffer (pH 5), and 10 FPU/g glucan was loaded into a 15 mL glass vial. In addition, 1 mL of a tetracycline chloride solution (40 mg/mL) was added to prevent microbial growth. In some cases, a stir bar was added and vortexed with the substrate to facilitate the defibrillation and dispersion of cellulose. A 200 μL portion of liquid was sampled at specific intervals and diluted to 1 mL to determine the sugar yield by HPLC.

$$\text{yield of glucose (wt \%)} = \left(\frac{m_{\text{glu}}}{m_{\text{C}_\text{D}}}\right) \times 100\%$$

where m_{glu} is the mass of the glucose based the result of the HPLC analysis.

2.5. Analytical Methods. The quality of the cellulose-rich substrates was analyzed by chemical composition analysis, scanning electron microscopy (SEM), and X-ray diffraction (XRD) using the two-step hydrolysis method for the chemical composition analysis based on NREL/TP-510-42618. Each sample was tested at least three times, and the mean values are reported. The SEM (5−10 kV accelerated voltage) was used to observe the morphology of the samples with different acid treatment. The SEM specimens were gold-plated before analysis. The crystallinity analysis was conducted by XRD with a XRD-6000 X-ray diffractometer (monochromatic Cu Kα radiation, the diffracted intensity (2θ) varied from 10 to 60° with a step of 0.02°). The crystallinity index (CrI) was determined by the formula[30]

$$\text{CrI (\%)} = (I_{002} - I_{\text{am}})/I_{002} \times 100\%$$

where I_{002} is the peak intensity of the 002 plane at about 2θ = 22.4° and I_{am} represents the peak intensity for the amorphous cellulose portion at about 2θ = 18.0°.

The released sugars (methyl glycoside) were analyzed via GC-MS and HPLC for characterization. A quantitative analysis of the methyl glycosides (methyl α-D-xylopyranoside, methyl β-D-xylopyranoside, methyl α-D-glucopyranoside, and methyl β-D-glucopyranoside) was conducted using an HPLC instrument equipped with a Bio-Rad Aminex HPX-87H (300 × 7.8 mm) column and a refractive index detector. H_2SO_4 (5 mM) was used as the mobile phase at a flow rate of 0.5 mL/min. A quantitative analysis of the methyl glycosides was based on an external standard. The standard curves of concentration of methyl xyloside and methyl glucoside are shown in Figure S1. The composition analysis of methyl glycoside, lignin oil, and methyl levulinate was performed using a GC-MS instrument equipped with an HP-5 column with a flame ionization detector (FID). The sugars (containing methyl glycoside and methyl levulinate) and lignin were dissolved in methanol and tetrahydrofuran, respectively. The carrier gas, He, was used with a flow rate of 2.0 mL/min, and the injection port temperature was 250 °C. The oven temperature program settings were as follows: the column temperature was heated to 90 °C at a rate of 5 °C/min and then heated to 250 °C at a rate of 10 °C/min and held for 20 min. The detector temperature was set to 250 °C. A quantitative analysis of methyl levulinate and furfural was determined using GC on a capillary column with a flame ionization detector. Helium was used as the carrier gas with a flow rate of 1.0 mL/min. The internal standard was *n*-octanol (Figure S2) (standard curve y = 2.06543x − 0.02107, R^2 = 0.9998).

The fractioned lignin fragments containing lignin 1#, lignin 2#, and lignin oil were analyzed by GPC, TG, and 2D NMR HSQC. The molecular weights of lignin fragments were measured using a Waters 2695 GPC instrument with a manually packed column. The mobile phase was tetrahydrofuran with an injection volume of 20 μL and a flow rate of 1 mL/min. Polystyrene was used as an internal standard. The thermal properties of lignin fragments were studied using TGA (PerkinElmer, Waltham, MA). Appropriately 10 mg of the sample was placed into the pan of the instrument and heated from room temperature to 700 °C at a heating rate of 10 °C/min. The lignin fragments (90 mg) were dissolved in DMSO-d_6 (0.5 mL) for 2D HSQC NMR characterization to analyze the specific structures of the complex compounds. The 2D-HSQC NMR spectra were measured by a Bruker DRX 500 NMR spectrometer. The spectral widths were 8.5 and 120 ppm for the ^{1}H and ^{13}C dimensions, respectively.

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Table 2. Methanolysis of Poplar Sawdust for Directional Conversion of Hemicellulose and Lignin at Various Temperatures

		yield of sugars (%)				carbohydrate retention (%)	
temp (°C)[a]	conversn (%)	methyl xyloside	methyl glucoside	total	delignification (%)	cellulose	hemicellulose
140	25.87	5.76	0.97	7.45	46.49	93.21	56.7
160	52.25	12.32	3.77	24.85	76.21	82.63	11.33
180	69.40	15.35	9.83	36.87	85.55	64.79	7.35
200	80.45	5.51	15.43	42.16	91.34	24.81	2.67

[a]Reaction conditions: catalyst, 1.0 g; poplar, 20 g; methanol, 200 mL; time, 30 min; pressure, 0.9, 1.6, 2.5, and 3.2 MPa at 140, 160, 180, and 200 °C, respectively.

3. RESULTS AND DISCUSSION

3.1. Selective Transformation of Hemicellulose and Lignin Fractions in Poplar Wood. Solvolysis fractionation of poplar sawdust was evaluated as a method to extract the structural components of hemicellulose and lignin from biomass. In this study, the methanolysis pretreatment of poplar sawdust was performed for directional fractionation and conversion of hemicellulose and lignin to methyl glycosides (mainly C5 glycoside) and lignin fragments. The methyl glycoside and lignin fragments were separated stepwise on the basis of their difference in hydrophilicity. We investigated the catalytic performance of various liquid acids (H_2SO_4, HCl, and H_3PO_4) and solid acid (HPW, $C_7H_7SO_3H$, and *p*-TsOH) to evaluate their abilities of delignification and the transformation of the target products. Table 1 summarizes the lignin fragments and methyl glycoside yield, product distributions, and delignification of the reactions involving different acids in 160 °C for 30 min (the amount of acid catalyst was optimized as in Table S1).

The reaction without acid failed to convert poplar into the expected products and yielded a low delignification degree of 18.04%. The use of sulfuric acid extracted 74.21% of lignin and obtained a greater amount methyl glycosides (mainly C5 sugars) with a yield of 24.85% (based on the entire poplar feedstock). In comparison, HCl and H_3PO_4 performed poorly for the degradation of poplar. HCl and H_3PO_4 were less effective in extracting lignin and hemicellulose; the delignification was 50.25% and 46.93%, respectively, and the yields of methyl glucosides were 16.77% and 15.64%, respectively, with 10 times the concentration of sulfuric acid equivalent. The use of HPW, a heteropolyacid, has been proven to be an efficient catalyst for the depolymerization of cellulose in an alcohol medium.[31] However, the delignification with HPW was low, which may be attributed to the complex and compact structure of the substrate to hinder its acid hydrolysis under relatively mild conditions. $C_7H_7SO_3H$ efficiently catalyzed the conversion of carbohydrate fractions with 22.48% of methyl glycoside yield, and the effect of delignification was not notable. The presence of p-TsOH showed efficient extraction of lignin and the transformation of hemicellulose, with a higher delignification degree (69.56%) and amount of methyl glycosides (25.92%). Furthermore, it was found that the retention of cellulose and hemicellulose was largely dependent on the acid used. Characterization results showed that 82.63% of cellulose and 11.33% of the hemicellulose were retained with H_2SO_4 as the catalyst. Most of the hemicellulose was dissolved and converted to methyl C5 glycoside. In comparison, the use of HCl and $C_7H_7SO_3H$ caused a more obvious conversion of cellulose components. For example, the use of HCl resulted in 38.39% of hemicellulose and 73.34% of cellulose retained in the cellulose-rich substrate after the methanolysis reaction. These results show that H_2SO_4 was more effective in releasing hemicellulose sugars and depolymerizing lignin during the methanolysis process under relatively mild conditions. We speculate that the stronger acidity of sulfuric acid gives it higher catalytic efficiency. The highly reactive protons in H_2SO_4 can effectively activate the oxygen atoms in the structural bonds of cellulose, hemicelluloses, and lignin and break the glycosidic bonds in the units of holocellulose and C−O in the units of lignin.

To further optimize methanolysis conditions, the four temperatures 140, 160, 180, and 200 °C were tested, all using H_2SO_4 as the catalyst (Table 2). The goal of the methanolysis step was to maximize the conversion of hemicellulose and lignin fractions to release methyl C5 glycoside and lignin fragments while retaining the maximum cellulose fraction. In general, hemicellulose is the most susceptible to thermal degradation and was expected to release C5 sugars via a solvent thermal treatment. As expected, the yield of methyl glycoside significantly increased with increasing reaction temperature from 140 to 200 °C. As evidenced by the HPLC and GC-MS analysis of the products (Figure 2a,b), methyl C5 glycosides were the dominant products below the reaction temperature of 180 °C; this can be seen according to the relative higher peak area of C5 sugars. However, a remarkable increase in cellulose conversion was observed at 180 °C. Methyl C6 glycosides became the main products, and more than 80% of poplar was converted at 200 °C. These results indicate that, in terms of the methanolysis of carbohydrates, the transformation of hemicellulose mainly occurred below 160 °C and that 88.67% of hemicellulose was transformed at 160 °C. Meanwhile, the yield of methyl glycoside was 24.85 wt % with 13.32 wt % of methyl xyloside and 3.77% of methyl glucoside on the basis of the HPLC analysis. The conversion of cellulose started at 180 °C, and 65.19% of cellulose was fractionated from the substrate at 200 °C. Furthermore, various sugar derivatives, such as furfural, 5-methoxymethylfurfural (MMF), and methyl levulinate, were detected in the methyl glycoside compounds, which were derived from further reactions of methyl glycoside.

An investigation of the stability of the methyl C5 sugar was conducted, which can provide a reference for choosing more suitable reaction conditions due to the tendency of hemicellulose to thermally decompose into multiple oxygenated compounds. Methyl α-D-xylopyranoside was used as a representative to investigate the thermal stability of methyl C5 sugar (Table S2). At 160 °C, 26% of pure methyl β-D-xylopyranoside was converted. However, the degradation of methyl α-D-xylopyranoside significantly increased at >180 °C. More than 65% of methyl β-D-xylopyranoside was converted into various chemicals, such as furfural, β-methoxy-2-furanethanol, and 3-ethyl-2-pentenoic acid methyl ester at 180 °C. Therefore, when the alcoholysis conversion efficiency

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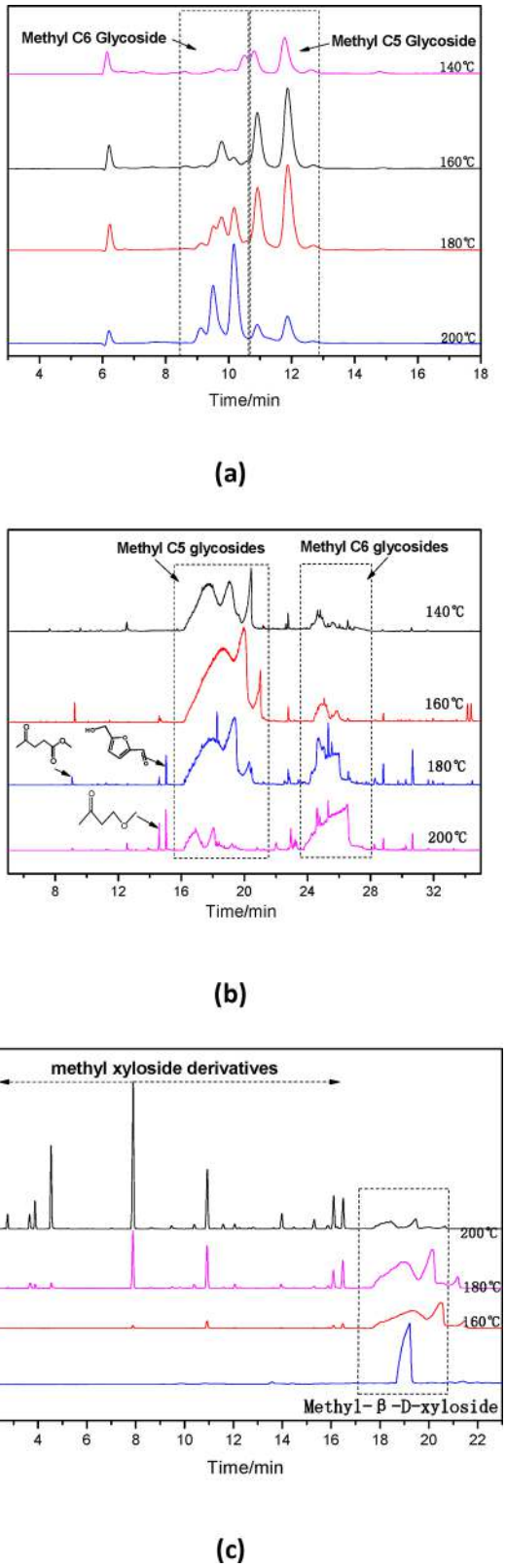

Figure 2. (a) HPLC and (b) GC-MS results of the sugar products from the methanolysis of poplar at various temperatures. (c) Stability analysis of methyl-β-D-xyloside in methanol at different temperatures.

of poplar wood and the stability of methyl xyloside are taken into account, the methanolysis at 160 °C was selected as the reaction temperature for further study.

3.2. Conversion of Methyl Glycoside in Methanol/DMM Cosolvent. The methyl C5 glycoside from the methanolysis of carbohydrate at 160 °C was further converted in DMM/methanol cosolvent with an acid catalyst. The effect of the reaction temperature on the transformation of methyl glycosides (mainly C5 glycoside) into methyl levulinate was investigated (Figure 3). Methyl levulinate was the main

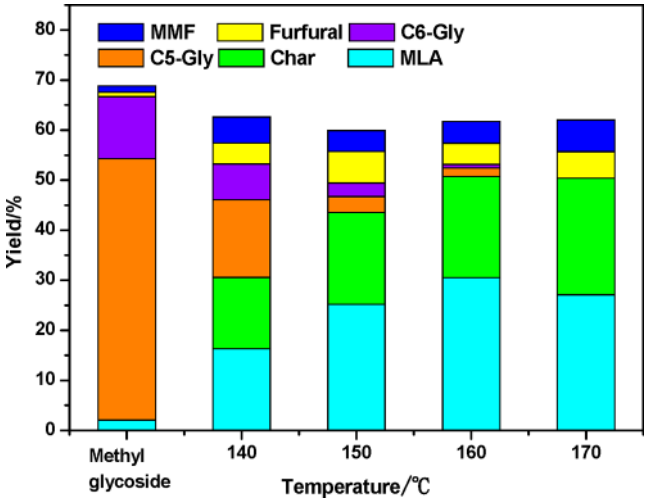

Figure 3. Directional transformation of methyl glycoside into methyl levulinate at various temperatures at 160 °C.

product, and MMF, furfural, and other derivatives were formed as intermediate products. The conversion of methyl glycoside increased with the increase in temperature. Over 90% of methyl xyloside was transformed at 160 °C. It was found that the increase in temperature to <160 °C improved the yield of methyl levulinate; however, a slight decrease was observed at 170 °C due to the aldol condensation of methyl levulinate.[26] Char or other methanol-insoluble substances were significantly increased because of polymerization of the byproducts: for example furfural, which is susceptible to polymerization.[29] The yields of char were 14.24%, 18.33%, 20.21%, and 23.36% from 140 to 170 °C, respectively. The obtained products were analyzed by GC-MS (Figure S3). Various chemicals, such as methyl levulinate, furfural, 5-methoxymethylfurfural, methyl 3-methylene-4-oxopentanoate (MMO), and 5-hydroxymethylfurfural were detected. The transformation of furfural to 5-MMF via electrophilic substitution with DMM as the electrophile was the key step during the reaction. The next step was the further conversion of MMF or its derivatives to methyl levulinate via acid catalyst. Methanol as a reactant can suppress both the polymerization of the byproducts (sugars/furans) and the aldol condensation of levulinic acid. However, coke formation did occur during the reaction, which mainly contributed to the polymerization of both furfural and methyl levulinate.[29] The yield of methyl levulinate (based on methyl glycoside) was up to 27 wt % at 160 °C for 120 min holding time, and the purity of the methyl levulinate reached 84.17% with GC analysis, achieving the valorization of the hemicellulose fraction for value-added chemicals.

3.3. Characteristics of the Lignin Fragments. To gain more insight into chemical properties of the lignin fragments, the degraded lignin was sequentially precipitated by stepwise removal of methanol to provide two types of solid lignin fragments (lignin1# and lignin 2#). Further extraction with

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ethyl acetate extraction with sugar products yielded the liquid lignin oil (phenolic compounds). The weight-average molecular weights (MWs) of all lignin fragments were studied (Figure 4). The average molecular weight (M_w and M_n) and

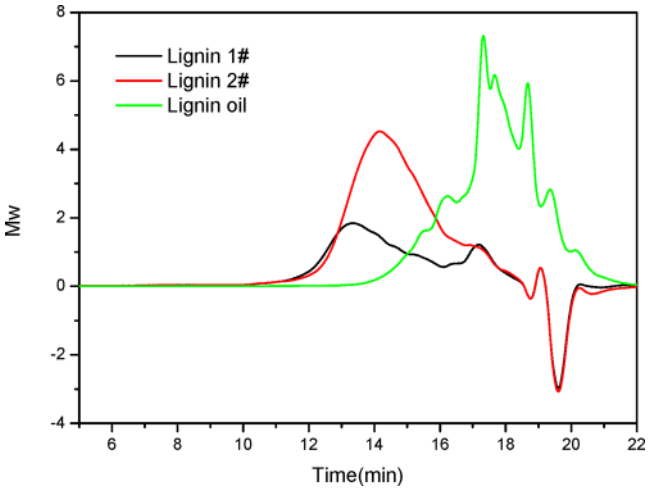

Figure 4. GPC chromatogram of three lignin fragments obtained from the methanolysis of poplar wood at 160 °C.

polydispersity index (PDI) of the lignin fragments are given in Table S3. The results show that lignin 1#, lignin 2#, and lignin oil had MW values of 2376, 1316, and 368 g/mol, which accounted for 20.1, 37.5, and 17.2% of total lignin, respectively. In addition, the lignin stepwise extraction strategy also led to a very narrow molecular weight distribution (PDI = 1.47−1.65).

The thermal properties of the lignin fragments were studied by TGA within a temperature range of 50−700 °C (Figure 5). The thermal decomposition of lignin is related to its functional groups and chemical structure. Decomposition largely consisted of cleavage of the C−O bond and aryl ether bonds, followed by cleavage of side chain oxidation decomposition, such as the carboxylation of an aliphatic hydroxyl group below 400 °C. Afterward, the aromatic ring and C−C bonds began to cleave above 400 °C under nitrogen. Then, char formation occurred due to demethoxylation and recondensation of volatile products as the temperature increased to above 500 °C.[32,33] For lignins 1# and 2#, the residual mass retained a higher value of ∼30% and 40%, respectively at 700 °C, which might contribute to the high content of C−C bonds due to the recondensation of lignin. Lignin oil showed a sharp decrease in mass loss at 150−250 °C, and approximately 90% of lignin oil residual mass was lost at 700 °C, which contributed to its lower molecular weight range. Furthermore, the sharp decrease in mass loss of the three lignin fragments indicated that their respective composition and inherent structures were very similar.

The GC-MS analysis of lignin oil was conducted to identify the low molecular compositions (Figure 6). Various types of phenolic components were detected (all of the components are relatively determined) (Table S4), including 2-methoxy-4-vinylphenol 2-methoxy-4-propylphenol, 2-(4-hydroxy-3-methoxyphenyl)acetic acid, 3-methoxy-4-hydroxybenzoic acid methyl ester, 4-(2-hydroxyethyl)-2,6-dimethoxyphenol, and 4-hydroxy-3,5-dimethoxybenzaldehyde, the information on which mainly contributed to the cleavage of β-O-4 and 4-O-5 units in lignin during the methanolysis process. Meanwhile, few sugars or sugar derivatives (e.g., methyl levulinate, 5-

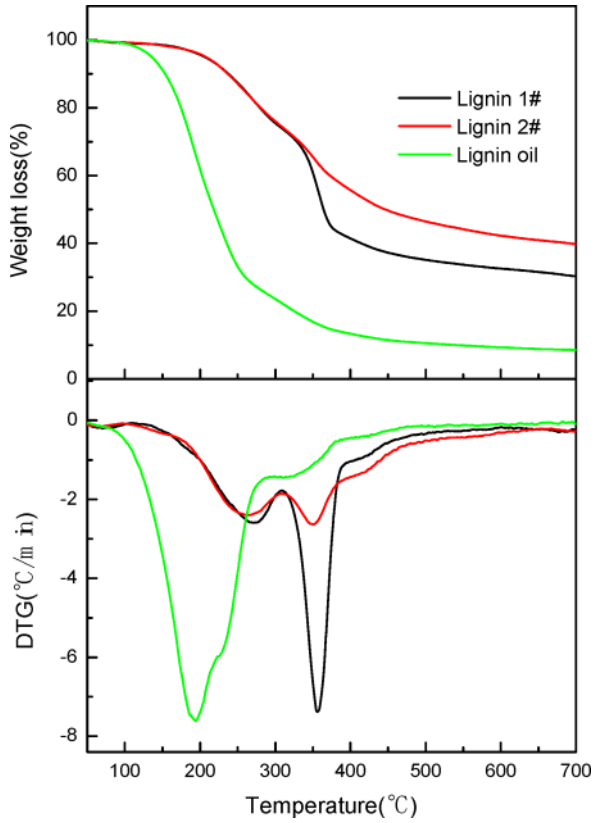

Figure 5. TG curves of solid lignin fragments and lignin oil.

hydroxymethylfurfural) were detected due to the incomplete separation with the methyl glycoside compounds.

To better understand the conversion of lignin and the comprehensive structural features of lignin fragments, 2D HSQC NMR technology was used to characterize the extracted lignin fragments and the HSQC spectra are shown in Figure 7. The side-chain (δ_C/δ_H 45−115/2.5−5.5 ppm) and aromatic regions (δ_C/δ_H 100.0−140.0/5.5−8.5) were the two main independent regions and provided the main signal information. The cross signals in the spectra were annotated on the baiss of previous studies.[19,34−36] Signals in the carbohydrate regions (δ_C/δ_H 90−105/4.0−5.5) in solid lignin fragment samples indicated that the lignin−carbohydrate complex (LCC) structure was cleaved during the methanolysis process.[37] Few signals appear in the carbohydrate regions of lignin oil spectra, which may be due to the incomplete separation with a few sugar products, and this was confirmed by the GC-MS analysis of lignin oil.

Syringyl (S) and guaiacyl (G) are basic components of the lignin structures and provide a correlation for the aromatic C−H group. In addition, their cross signals can be easily observed in the spectra of the aromatic regions. The prominent signal for $C_{2,6}/H_{2,6}$ correlations of S units was found at δ_C/δ_H 107.0/6.50 ppm, and the C_α-oxidized (C_α=O) structure in oxidized S units was observed at 107.5/7.25−7.35 ppm. The C_5/H_5 and C_6/H_6 correlations in the G unit were shown at δ_C/δ_H 114.5/6.70−6.80 and 115.5/6.65 ppm, respectively. The cross-signals at δ_C/δ_H 130.0/7.5 contributed to the cross-link characteristic signals of $C_{2,6}$−$H_{2,6}$ in PCA. The spectrum of the solid lignin fragments exhibited a higher ratio and border signals in G and S units in comparison to the lignin oil, which contributed to the higher molecular weight and slower molecular motion due

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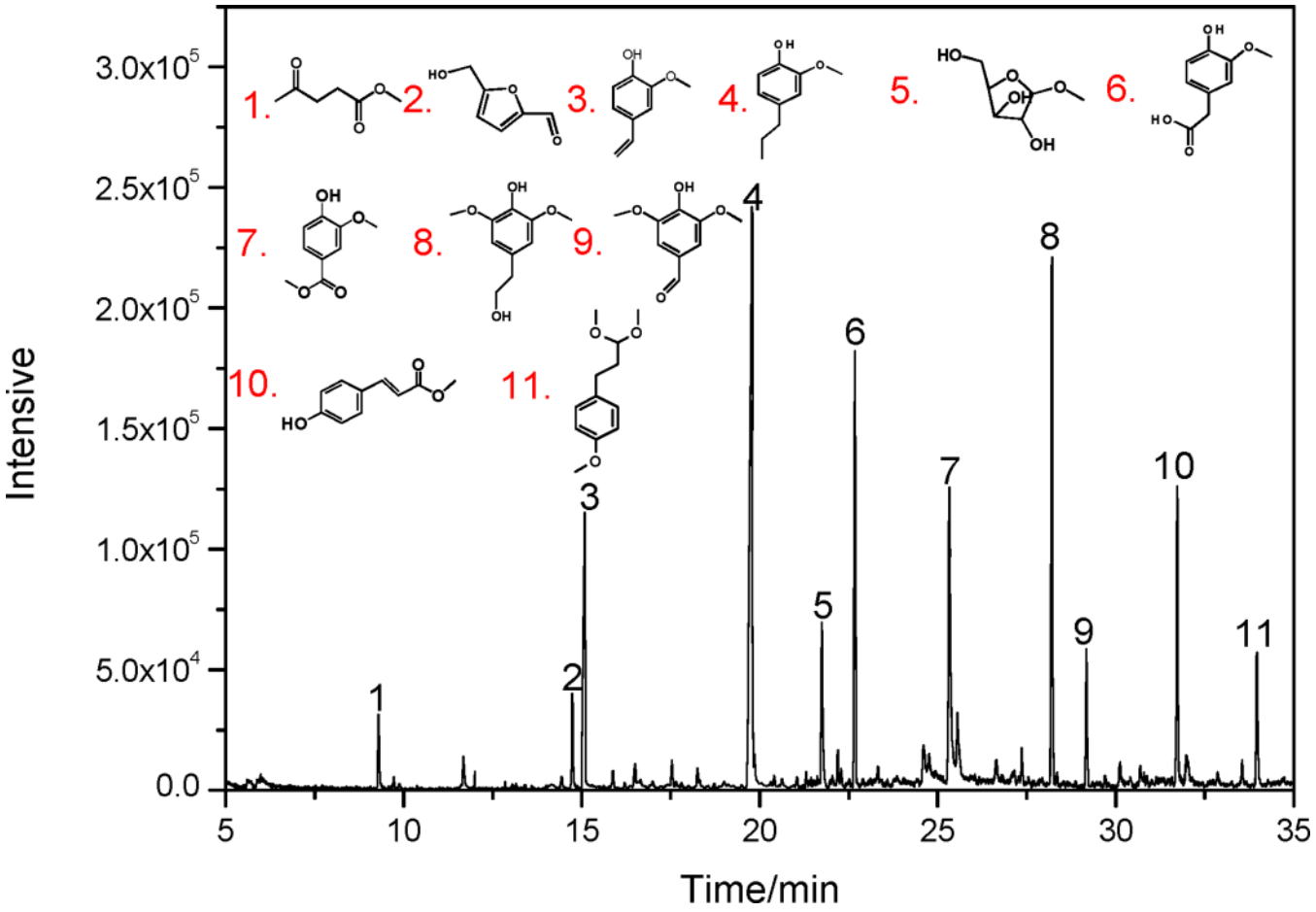

Figure 6. GC-MS analysis of the lignin oil derived from the methanolysis of poplar at 160 °C.

to lignin condensation.[19] The signals in the side-chain region primarily provide structural information about the interunit linkage of the lignin fragments. The methoxy group signals (δ_C/δ_H 53−58/3.15−3.95 ppm) are some of the most prominent cross signals in this region. However, the methanolysis of poplar with H_2SO_4 resulted in the disappearance of the β-O-4 and β-β' signals. Also, the phenylcoumaran (β-5′) substructures were very weak and were even not detected in the lignin fragments, indicating that most ether bonds have been cleaved during delignification. According to the result of 2D HSQC NMR and GPC, it can be inferred that the LCC bonds of the aryl ethers and phenyl glycosides were cleaved, which resulted in the release of a lignin fraction with the acid catalyst in the methanolysis process.

3.5. Physical and Chemical Analysis of Cellulose-Rich Substrate. Effective enzymatic hydrolysis of cellulose to glucose is an important process in the valorization of cellulose. In general, cellulose in biomass is resistant to enzymatic digestion mainly due to the presence of lignin, which makes it difficult for enzymes to contact cellulose, particularly with high crystallinity. Therefore, the structural characteristics of cellulose-rich substrates with different acid treatments were studied to understand the suitability of cellulose for enzymatic hydrolysis.

The cellulose-rich substrates were subjected to XRD analysis to study changes in the crystal structure and crystallinity of the cellulose (Figure 8). The signal peaks at ∼16, 22, and 35° observed in all samples conform that cellulose I was present in raw poplar and the cellulose-rich substrate.[38] The poplar feedstock and sample without acid treatment showed CrI values of 55.61% and 59.34%, respectively. The sample treated with H_2SO_4 showed the highest CrI value, exhibiting a 12.31% enhancement from the sample not treated with acid. The CrI values of the samples with different acid catalysts were in the order H_2SO_4 > p-TsOH> $C_7H_7SO_3H$ > HPW > HCl > H_3PO_4. Undoubtedly, the increasing CrI values of the samples with acid treatment contributed to the extraction of the noncrystalline region such as hemicellulose and lignin from biomass.

The morphology of recovered cellulose-rich fractions was characterized by SEM (Figure 9). Raw poplar showed a highly intact morphology with a dense fibrillar structure, and the structure of the sample treated in methanol without acid remained almost unchanged in comparison to the feedstock. This is because the environment was unfavorable to the penetration of cellulolytic enzymes. The use of acid catalyst resulted in noticeable changes (loose and highly corroded) in the morphological structure of samples. These changes were due to the removal of hemicellulose and lignin, especially with sulfuric acid and p-TsOH. A rough surface can still be observed at the edge of the fiber, which was mainly formed by the repolymerization of lignin during the methanolysis reaction. The significant morphological changes might allow hydrolase to more easily penetrate and hydrolyze the cellulose-rich substrate after methanolysis treatment.

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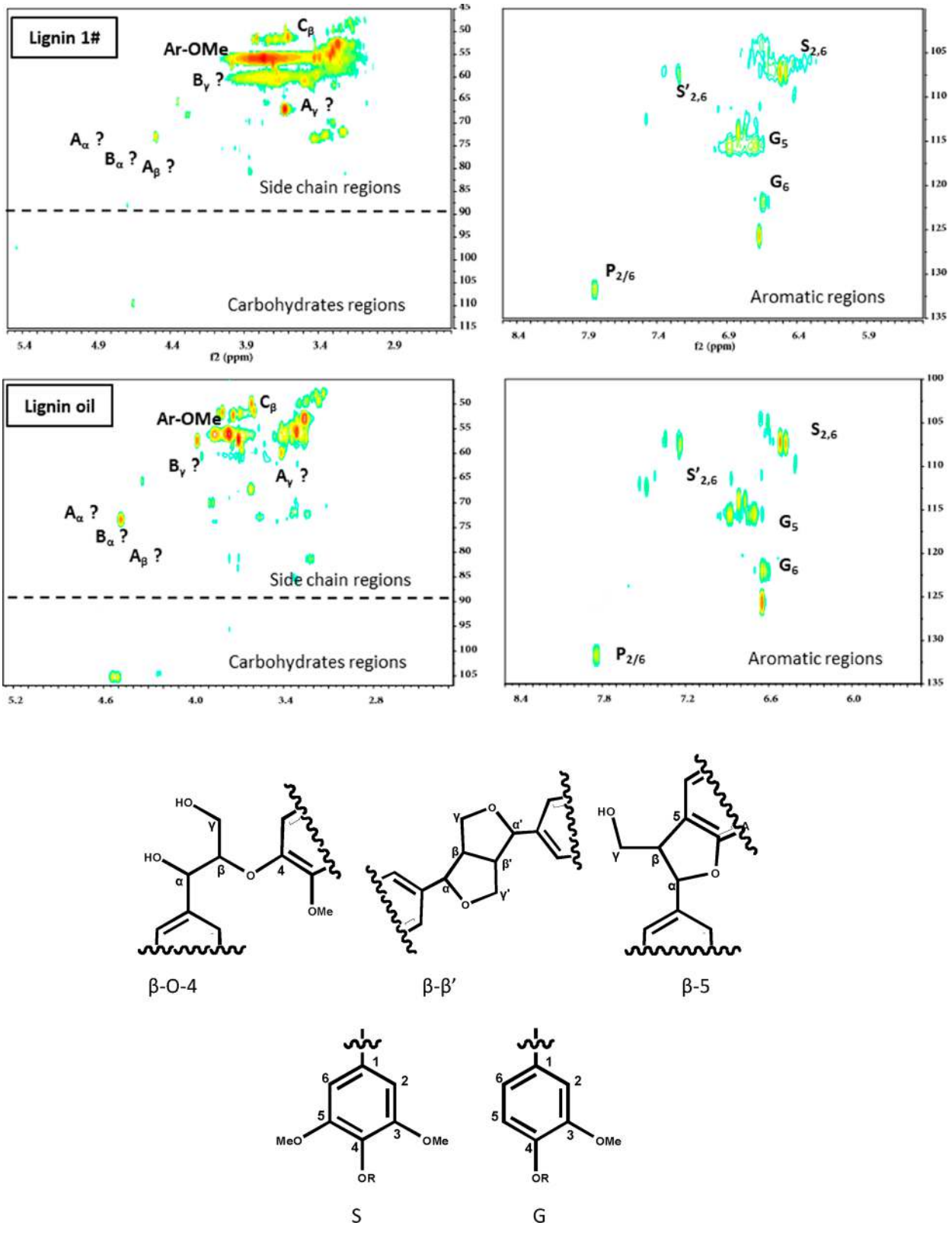

Figure 7. 2D HSQC NMR spectra of solid lignin and lignin oil obtained from the methanolysis reaction.

3.6. Enzymatic Digestion of Cellulose-Rich Substrate to Glucose. Enzymatic digestion is an efficient means for the saccharification of cellulose to glucose. The cellulose-rich substrate obtained by the methanolysis process is suitable for enzymatic hydrolysis due to the efficient extraction of lignin and hemicellulose and high C6-sugar retention. The cellulose-rich substrates pretreated by various catalysts were hydrolyzed using enzymatic saccharification. As shown in Figure 10, raw poplar exhibited a higher resistance to enzymatic hydrolysis, yielding only 6.8% glucose. After methanolysis, the yield of

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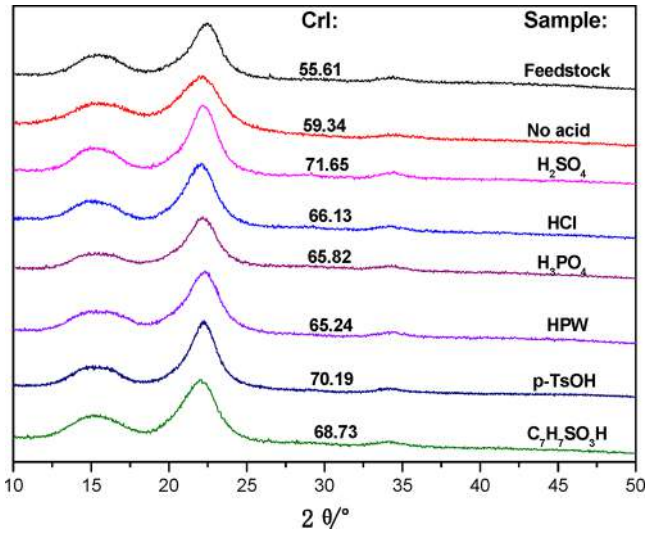

Figure 8. X-ray diffraction patterns of raw feedstock and cellulose-rich substrate obtained using various acid catalysts.

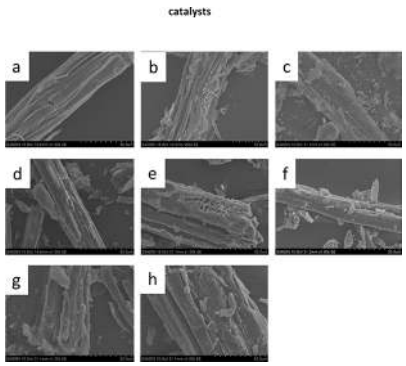

Figure 9. SEM images of (a) poplar and cellulose pulp obtained (b) without acid and (c) with H_2SO_4, (d) with HCl, (e) with H_3PO_4, (f) with HPW (g) with p-TsOH, and (h) with $C_7H_7SO_3H$ at 160 °C.

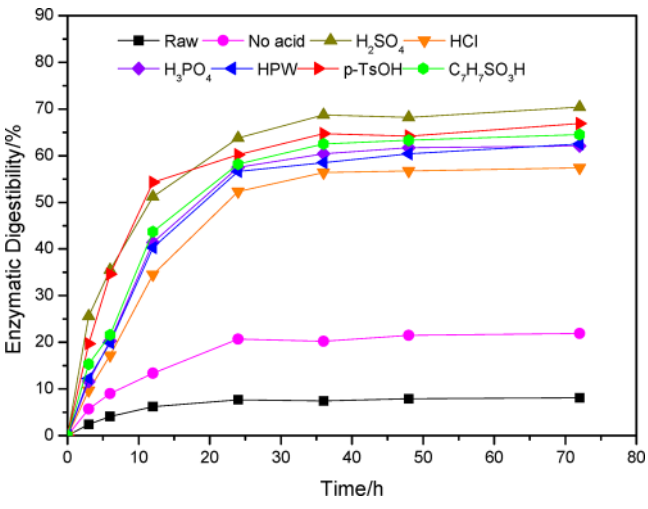

Figure 10. Enzymatic digestibility of raw feedstock and cellulose-rich substrate derived from the methanolysis using various acid catalysts.

glucose was above 70% with H_2SO_4. Similarity, p-TsOH-catalyzed samples showed a higher enzymatic yield (67.1%). HCl, H_3PO_4, HCOOH, HPW, and $C_7H_7SO_3H$ yielded less glucose. The amounts of glucose yield with different acid catalysts in the order of greatest to least were H_2SO_4 > p-TsOH > $C_7H_7SO_3H$ > HPW > HCl > H_3PO_4 > no acid. In general, the presence of hemicellulose and lignin makes cellulose less susceptible to coming in contact with enzymes under mild conditions, and nonproductive adsorption of lignin on the enzyme resulted in the formation of a lignin−enzyme complex. The produced lignin−enzyme contributed to the high resistance to enzymatic hydrolysis of lignocellulose.[39] It was found that the enzymatic glucose yield of cellulose pulp after treatment with different acids was consistent with its delignification.

CONCLUSION

In this study, we developed an efficient method for the valorization of lignocellulosic biomass toward value-added platform chemicals: namely, levulinate ester, phenolics, and glucose. It was found that delignification, fractionation efficiency, and production distributions were highly dependent on the acid catalysts and the temperatures in the methanolysis process. The level of delignification with different acid catalysts was in decreasing order of H_2SO_4 > p-TsOH > $C_7H_7SO_3H$ > HPW > HCl > H_3PO_4 at 160 °C for 30 min at an operating pressure of 1.6 MPa at 160 °C. These conditions can fractionate more than 74% and 85% of total lignin and hemicellulose, respectively, with H_2SO_4. The yield of methyl glycoside was 24.85 wt % with 13.32 wt % of methyl xyloside and 27.08% of methyl glucoside on the basis of the HPLC analysis. Methyl glycoside could be converted into methyl levulinate in dimethoxymethane/methanol medium with acid catalyst. The obtained lignin was separated into solid lignin fragments and liquid lignin oil (phenolic compounds) on the basis of their different molecular weight distributions, and various lignin monomers were detected in lignin oil. A high-purity cellulose stream can be directly degraded to glucose by enzymatic saccharification. It was found that the enzymatic glucose yield of the cellulose pulp treated with different acids was consistent with its delignification. Overall, the present study achieved a comprehensive utilization of all biomass components (cellulose, hemicellulose, and lignin) for value-added chemicals.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jafc.9b03806.

Standard curve of concentration of methyl xyloside and methyl glucoside, GC chromatograms for quantitative analysis of methyl levulinate by an internal standard method, GC-MS analysis of product distributions of the conversion of methyl glycoside in methanol/DMM, methanolysis of poplar wood with different acids and acid concentrations, decomposition behavior of the methyl-β-D-xylopyranoside at various temperatures, molecular distribution (M_w and M_n) and polydispersity (M_w/M_n) of the lignin fragments, and composition analysis of the lignin oil based on the GC-MS analysis (PDF)

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Notes

The authors declare no competing financial interest.

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