

Electronic Supplementary Information

A biomass pretreatment using cellulose-derived solvent Cyrene

Xianzhi Meng¹, Yunqiao Pu², Mi Li³, Arthur J. Ragauskas^{1,2,3,}*

¹ *Department of Chemical & Biomolecular Engineering, University of Tennessee Knoxville, Knoxville, TN 37996, USA*

² *Joint Institute for Biological Sciences, Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN 37831, USA*

³ *Department of Forestry, Wildlife, and Fisheries; Center for Renewable Carbon, The University of Tennessee Knoxville, Institute of Agriculture, Knoxville, TN 37996, USA*

Corresponding Author

**Arthur J. Ragauskas. Fax: 865-974-7076; Tel: 865-974-2042; E-mail: ragauskas@utk.edu.*

Experimental Section

Cellulose characterization. Holocellulose was prepared from the extractive-free raw and pretreated biomass using peracetic acid at room temperature for 24 h to ensure maximum lignin removal. The holocellulose was then filtrated and rinsed with DI water. α -Cellulose was then isolated from the holocellulose and derivatized to cellulose tricarbonylates as described elsewhere.¹ Each cellulose sample was dissolved in THF overnight at a concentration of 1.0 mg/mL followed by being filtered through a 0.45 μ m PTFE filter. The molecular weight was analyzed by an Agilent GPC SECurity 1200 system using THF as the mobile phase at a flow rate of 0.3 mL/min. Cellulose sample for crystallinity test was isolated from holocellulose by acid hydrolysis as described elsewhere.² Pre-wet cellulose was packed into a 4- mm cylindrical ceramic MAS rotor, and the solid-state NMR experiment was carried out on a Bruker Avance DXS-400 spectrometer operating at a spinning speed of 10 kHz. Cellulose accessibility was performed according to Chandra et al. using a modified Simons staining technique.³ ~100 mg never-dried raw and pretreated *Populus* were weighed into six centrifuge tubes with 1.0 mL of phosphate buffered saline solution. A series of orange dye solution at a concentration of ~10 mg/mL were added into each tube at increasing volumes (i.e., 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mL) to create a Langmuir adsorption isotherm. The maximum amount of dye adsorbed by the substrate (mg dye/g substrate) is determined using the Langmuir adsorption isotherm as detailed in literature.³

Lignin NMR characterization. All the NMR experiments were acquired on a Bruker Ascend™ 500 MHz spectrometer. For HSQC experiment, a standard Bruker heteronuclear single quantum coherence pulse sequence (hsqcetgp) was used on a 5-mm N2 cryogenically cooled Broadband Observe (BBO) H&F probe. Lignin samples (~60 mg) were dissolved in ~0.50 mL deuterated dimethyl sulfoxide (DMSO-d₆). To measure the contents of lignin hydroxyl groups, ³¹P NMR spectra were acquired after dissolving lignin (~25 mg) in a pyridine/CDCl₃ (1.5/1.0, v/v) solution and derivatizing with TMDP (75 μ L). Chromium acetylacetonate and *endo*-N-hydroxy-5-norbornene-2,3-dicarboximide (NHND) were also added into the solution as relaxation agent and internal standard, respectively. Quantitative ³¹P NMR spectra were acquired using an inverse-gated decoupling (Waltz-16) pulse sequence with a 25 second pulse delay and 128 scans.

Lignin molecular weight distribution analysis. Lignin molecular weight was measured by a gel permeation chromatography (GPC) after acetylation. ~10 mg of dry lignin was added into a mixture of acetic anhydride/pyridine (1:1, v/v, 2.00 mL) and stirred at room temperature for 24 h. Ethanol (5 mL) was added to the reaction mixture, left for 30 min and then removed with a rotary evaporator. The addition and removal of ethanol was repeated at least 3 times until all traces of acetic acid were removed. Acetylated lignin samples were then dissolved in THF at a concentration of 1mg/mL. The molecular weight distributions of the acetylated lignin samples were analyzed on a PSS-Polymer Standards Service (Warwick, RI, USA) GPC SECurity 1200 system featuring Agilent HPLC 1200 components equipped with four Waters Styragel columns (HR1, HR2, HR4 and HR6) and an UV detector (270 nm). Tetrahydrofuran was used as the mobile phase and flow rate was 0.3mL/min. Data collection and processing were performed using Polymer Standards Service WinGPC Unity software (Build 6807). Standard narrow polystyrene samples were used for calibration.

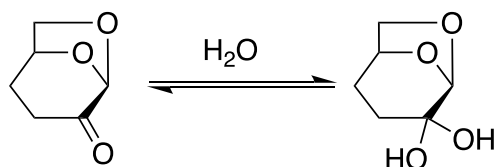


Fig. S4. Formation of geminal diol in Cyrene/water co-solvent.⁴

Table S1. Solubility properties of Cyrene

Solubility parameters	Cyrene
$\delta_D/\text{Mpa}^{0.5}$	18.8
$\delta_P/\text{Mpa}^{0.5}$	10.6
$\delta_H/\text{Mpa}^{0.5}$	6.9
RED	0.89
α (hydrogen bond donator capacity)	0.00
β (hydrogen bond acceptor capacity)	0.61
π^* (polarizability)	0.93

Table S2. Biomass composition analysis based on dry initial raw biomass

Substrates	Initial biomass dry weight (g)	Pretreatment residue (g)	Glucan (%)	Xylan (%)	Lignin (%)	Solid recovery yield (%)
Raw poplar	9.16	N/A	46.7%	16.4%	23.5%	N/A
60min_4:1	9.16	4.25	31.2%	2.0%	3.5%	46.4%
10min_4:1	9.16	6.25	42.3%	3.5%	11.6%	68.3%
60min_1:1	9.16	3.97	30.1%	1.8%	5.1%	43.4%
10min_1:1	9.16	7.39	42.6%	10.0%	16.9%	80.7%
60min_1:2	9.16	4.97	36.3%	2.7%	8.5%	54.3%
10min_1:2	9.16	6.84	38.8%	9.6%	16.3%	74.7%
DAP	9.16	7.01	42.0%	7.8%	19.3%	76.6%

Table S3. Composition analysis of alkaline incubated Cyrene pretreated biomass and lignin yield

Substrates	Glucan (%)	Xylan (%)	Lignin (%)	Lignin yield (%)
60min_4:1	78.2%	2.8%	6.2%	59.1%
10min_4:1	68.4%	3.3%	12.9%	21.0%
60min_1:1	76.5%	3.2%	11.2%	19.8%
10min_1:1	67.5%	6.0%	21.2%	7.0%
60min_1:2	78.0%	3.8%	15.0%	19.3%
10min_1:2	67.5%	6.8%	20.3%	6.1%

Table S4. Assignments of lignin ¹³C-¹H correlation signals observed in the HSQC spectra of lignin samples.

$\delta C/\delta H$ (ppm)	Assignment
53.3/3.48	C _β /H _β in phenylcoumaran (β-5)
55.6/3.73	C/H in methoxyl group
59.9/3.65	C ₇ /H ₇ in β-aryl ether (β-O-4)
70.9/4.19	C ₇ /H ₇ in resinol (β-β)
71.1/4.74	C _α /H _α in β-O-4 linked to a G unit
71.9/4.88	C _α /H _α in β-O-4 linked to a S unit
83.5/4.31	C _β /H _β in β-O-4 linked to a G unit

85.9/4.12	C _β /H _β in β-O-4 linked to a S unit
85.0/4.67	C _α /H _α in resinol (β-β)
86.7/5.48	C _α /H _α in phenylcoumaran (β-5)
103.9/6.71	C _{2,6} /H _{2,6} in syringyl (S) unit
110.9/7.01	C ₂ /H ₂ in guaiacyl (G) unit
114.9/6.80	C ₅ /H ₅ in guaiacyl (G) unit
119.1/6.81	C ₆ /H ₆ in guaiacyl (G) unit

Table S5. Lignin S/G ratio, and content of p-hydroxybenzoate (as percent of S+G) and interunit linkages (as percent of total linkages).

Substrates	S/G	p-hydroxybenzoate	β-O-4	β-β	β-5
CEL	2.8	11.0%	90.3%	7.8%	2.0%
60min_4:1	2.1	21.0%	77.5%	21.2%	1.3%
10min_4:1	1.8	16.0%	83.5%	13.1%	3.5%
60min_1:1	1.9	17.9%	78.3%	17.3%	4.4%
10min_1:1	1.9	14.5%	90.0%	8.2%	1.8%
60min_1:2	2.2	18.6%	82.3%	13.4%	4.3%
10min_1:2	1.9	19.7%	88.1%	7.9%	4.0%

Table S6. Typical chemical shifts and integration regions for lignin samples in ³¹P NMR spectra.

δ (ppm)	Hydroxyl groups
133.6-136.0	Carboxylic acid OH
~137.8	p-hydroxyphenyl OH
139.0-140.2	Guaiacyl OH
140.0-144.5	C5 substituted OH
145.4-150.0	Aliphatic OH

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