

Supporting Information

Physicochemical structural changes of poplar and switchgrass during biomass pretreatment and enzymatic hydrolysis

Xianzhi Meng^{†,‡}, Qining Sun[†], Matyas Kosa[§], Fang Huang[§], Yunqiao Pu^{‡,||}, Arthur J. Ragauskas^{*,†,‡,||}

[†] Department of Chemical and Biomolecular Engineering, University of Tennessee, Knoxville, TN, 37996, USA.

[‡] BioEnergy Science Center, Oak Ridge National Laboratory, Oak Ridge, TN, 37831, USA

[§] School of Chemistry and Biochemistry, Renewable Bioproducts Institute, Georgia Institute of Technology, Atlanta, GA, 30332, USA

^{||} Biosciences Division, Oak Ridge National Laboratory, Oak Ridge, TN, 37831, USA

*Corresponding author: Art J. Ragauskas. Fax: 865-974-7076; Tel: 865-974-2042; E-mail: argauskas@utk.edu

1. Chemical composition analysis

In brief, the extractive-free samples were treated with 72% sulfuric acid for 4 h at 30 °C and then diluted to 3% sulfuric acid using deionized water and subsequently autoclaved at 121 °C for ~1 h. The resulting solution was cooled to room temperature and the precipitate was then filtered through a G8 glass fiber filter (Fisher Scientific, USA), dried, and weighed to get the Klason lignin content. The resulting filtrate was diluted 50-fold, filtered and injected into high-performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD) using Dionex ICS-3000 (Dionex Corp., USA) with an conductivity detector, a guard CarboPac PA1 column (2 × 50 mm, Dionex), a CarboPac PA1 column (2 × 250 mm, Dionex), a AS40 automated sampler and a PC 10 pneumatic controller at room temperature.

2. Cellulose isolation and derivatization for GPC analysis

Holocellulose samples were prepared by delignification via treatment of ~0.6 g samples (untreated, pretreated, and hydrolyzed poplar and switchgrass) with 5% w/w peracetic acid for 24 h at 25 °C. Samples were then washed with DI water and air-dried overnight. α -Cellulose was isolated from holocellulose samples by solubilization in 17.5 wt% NaOH for 2 h and followed by 8.75% NaOH for an additional 2 h. The cellulose was obtained by filtration and washing with 1% acetic acid and an excess of DI water until the pH of the filtrate was close to 7, and then air-dried in fume hood. Cellulose (~15 mg) was then derivatized using anhydrous pyridine (4 mL) and phenyl isocyanate (0.5 mL) over 48 h at 70 °C. The reaction was then quenched by anhydrous methanol (1.00 mL). Methanol and water mixture (7:3, v/v) was added drop-wise to promote precipitation of the cellulose derivative. The solids were collected by filtration and then washed with the methanol and water mixture (50 mL), followed by water (50 mL). The cellulose derivative was then dried overnight under vacuum at 40°C. Prior to GPC analysis, the cellulose derivative was dissolved overnight in tetrahydrofuran (1.0 mg/mL), and the solution was filtered through a 0.45 μ m PTFE syringe filter, and placed in a 2 mL auto-sampler vial. The molecular weight distributions were analyzed by Agilent GPC SECurity 1200 system equipped with four Waters Styragel columns (HR0.5, HR2, HR4, HR6), Agilent refractive index (RI) detector and Agilent UV detector (270 nm).

3. *Simons' stain for accessibility test*

Direct Blue 1 (Pontamine Fast Sky Blue 6BX) and Direct Orange 15 (Pontamine Fast Orange 6RN) dyes were obtained from Pylam Products Co. Inc. (Garden City, NY). Direct Orange dye was responsible for the increased affinity for cellulose binding, whereas the low molecular weight part had a very similar affinity for cellulose as the Direct Blue dye did.¹ Therefore an ultrafiltration of the orange dye to remove the low molecular weight part is necessary, and it is done by filtering a 1% solution of orange dye through a 100 K membrane using an Amicon ultrafiltration apparatus (Amicon Inc., Beverly, MA) under ~200 kPa nitrogen gas pressure.² Orange dye solution was poured into the Amicon container and filtered through until about 20% of the original volume remained. After two round of ultra-filtration, ~1.0 mL of the dye retained on the filter was dried in a 50 °C oven for at least 5 days and the weight of the solid residue was measured to determine the concentration of the top fraction in the filter. The ultrafiltrated dye with known concentration was then used as the concentrated stock solution for further dilution to the concentration required (10 mg/mL) for the staining procedure. The amount of dye adsorbed by the biomass sample was determined using the difference between the concentration of the initial added dye and the concentration of the dye in the supernatant calculated by solving two Lambert-Beer law equations simultaneously. The maximum amount of dye adsorbed to the lignocellulosic substrates was calculated using the Langmuir adsorption equation.

Table S1. Common assignment of FTIR absorption bands for biomass

Band position (cm ⁻¹)	Assignment
3200-3400	O-H stretching
2850-2940	C-H stretching
1735-1750	C=O ester; carbonyl groups in branched hemicellulose
1595	Aromatic ring stretch associated with lignin
1509-1512	C=C related to lignin; guaiacyl ring of lignin
1465	C-H methyl and methylene deformation
1417-1423	C-H deformation (asymmetric) of cellulose
1370-1380	C-H stretching of cellulose
1330	O-H in-plane deformation, syringyl ring breathing deformation
1215	C-C and C-O stretch associated with lignin
1160	C-O-C asymmetrical stretching
1100	C-O vibrations of crystalline cellulose
900	Amorphous cellulose vibration

Table S2 – 8 shows the actual data used to make Figure 1, 2, 4, 5, 6 and 7 in the main manuscript.

Table S2. Mass balance on ~20 g basis of untreated poplar and switchgrass during dilute acid and alkaline pretreatment.^a

Sample	Initial mass (g)	Solid recovery (g)	Arabinan (g)	Galactan (g)	Glucan (g)	Xylan (g)	Mannan (g)	Lignin (g)	Total mass closure (g)
Poplar	20.0	N/A	0.07	0.13	10.3	3.2	0.48	4.68	18.9
Switchgrass	20.0	N/A	0.74	0.26	7.64	5.1	0.05	4.10	17.9
DAP-poplar	20.8	12.6	N/A	N/A	8.44	0.006	N/A	4.34	12.8
Alkaline-poplar	20.6	15.3	N/A	0.05	9.58	2.0	0.005	3.09	14.7
DAP-switchgrass	20.5	10.8	N/A	N/A	5.55	0.04	N/A	5.23	10.6
Alkaline-switchgrass	20.8	8.39	0.28	0.04	5.32	1.69	0.02	0.58	7.93

^a Expressed as Mean value.

Table S3. Experimental glucose yield during 72 h enzymatic hydrolysis of untreated, DAP, and alkaline pretreated poplar and switchgrass.

Sample Name	Glucose yield (%) at different time intervals (h)						
	2 h	4 h	8 h	12 h	24 h	48 h	72 h
Untreated poplar	7.00	8.68	9.51	9.92	11.2	12.6	13.1
Untreated switchgrass	13.8	16.4	18.1	19.9	23.6	25.8	25.9
DAP-poplar	18.8	24.8	32.2	35.9	42.7	46.5	50.0
Alkaline-poplar	17.6	23.2	29.1	31.7	38.9	43.9	50.3
DAP-switchgrass	34.9	44.8	54.5	59.8	68.5	73.6	78.2
Alkaline-switchgrass	44.6	57.6	67.2	74.0	83.9	87.5	92.2

Table S4. Theoretical glucose yield during 72 h enzymatic hydrolysis results simulated by AKE kinetic model.

Sample Name	Glucose yield (%) at different time intervals (h)						
	2 h	4 h	8 h	12 h	24 h	48 h	72 h
Untreated poplar	7.36	8.28	9.31	9.96	11.2	12.6	13.4
Untreated switchgrass	14.2	16.2	18.4	19.8	22.4	25.4	27.2
DAP-poplar	20.4	25.0	30.3	33.8	40.5	48.0	52.6
Alkaline-poplar	18.4	22.8	28.0	31.4	38.1	45.6	50.3
DAP-switchgrass	37.1	44.5	52.7	57.7	66.5	75.1	79.8
Alkaline-switchgrass	46.7	56.6	66.6	72.4	81.6	89.2	92.7

Table S5. Weight average degree of polymerization of cellulose isolated from untreated, DAP, and alkaline pretreated poplar and switchgrass.

Sample Name	Weight average degree of polymerization
Untreated poplar	5405
Untreated switchgrass	4639
DAP-poplar	582
Alkaline-poplar	3950
DAP-switchgrass	560
Alkaline-switchgrass	3467

Table S6. Changes of degree of polymerization of cellulose isolated from untreated and pretreated poplar and switchgrass during enzymatic hydrolysis.

Sample Name	Cellulose DP during enzymatic hydrolysis (h)						
	2 h	4 h	8 h	12 h	24 h	48 h	72 h
Untreated poplar	4996	4113	3959	3623	3969	4164	4034
Untreated switchgrass	3945	4079	3514	3197	3390	3501	3802
DAP-poplar	307	155	268	258	219	279	231
Alkaline-poplar	3013	2776	2320	2227	2201	2179	2124
DAP-switchgrass	440	417	341	337	325	259	277
Alkaline-switchgrass	2268	2030	1880	1850	1638	1203	1341

Table S7. The adsorption of orange dye during Simons' staining (mg dye/g cellulose) of untreated and various pretreated poplar and switchgrass.

Sample Name	Orange dye adsorption (mg/g cellulose)
Untreated poplar	21.6
Untreated switchgrass	27.8
DAP-poplar	69.5
Alkaline-poplar	50.9
DAP-switchgrass	86.3
Alkaline-switchgrass	138.4

Table S8. Changes of cellulose accessibility measured by Simons' stain (mg dye/g cellulose) of untreated, DAP and alkaline pretreated poplar and switchgrass during 72 h enzymatic hydrolysis.

Sample Name	Cellulose accessibility (mg/g cellulose) during enzymatic hydrolysis (h)						
	2 h	4 h	8 h	12 h	24 h	48 h	72 h
Untreated poplar	22.2	28.0	23.5	22.4	23.3	25.0	28.6
Untreated switchgrass	26.1	31.4	31.2	27.8	29.4	26.0	27.8
DAP-poplar	81.7	85.4	89.8	75.1	62.0	40.7	45.0
Alkaline-poplar	57.9	73.1	89.8	70.1	92.3	76.7	55.9
DAP-switchgrass	98.4	103	113	112	151	123	114
Alkaline-switchgrass	229	266	242	378	332	279	161

Reference

- (1). Yu, X.; Atalla, R. H. A staining technique for evaluating the pore structure variations of microcrystalline cellulose powders. *Powder Technol.* **1998**, 98, 135-138.
- (2). Esteghlalian, A. R.; Bilodeau, M.; Mansfield, S. D.; Saddler, J. N. Do enzymic hydrolyzability and Simons' stain reflect the changes in the accessibility of lignocellulosic substrates to cellulase enzymes? *Biotechnol. Prog.* 2001, 17, 1049-1054.