Electronic supplementary information

Insights into the effect of dilute acid, hot water and alkaline pretreatment on cellulose accessible surface area and overall porosity of *Populus*

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1. Materials and methods

1.1. Biomass feedstock

Baseline *Populus* (*Populus trichocarpa x deltoids)* were harvested in 2012 from area 0800 at Oak Ridge National Laboratory, TN. Samples were shipped to National Renewable Energy Laboratory in Golden, CO for room temperature air drying, debarking, and size reductions. Samples were stored in a freezer to maintain the moisture content and shipped to Georgia Tech upon request. The biomass was then size-reduced in a Wiley mill using a 20-80 mesh screen. Extractives were subsequently removed by adding ~5 g of biomass into an extraction thimble in a Soxhlet extraction apparatus. The extraction flask was filled with dichloromethane and then refluxed at a boiling rate which cycled the biomass for ~8 h.

1.2. Biomass pretreatment

For DAP, lignocellulosic samples were first prepared by presoaking in a 1% wt dilute sulfuric acid solution for 4 h at room temperature. The presoaked slurry was then filtered to remove the solid material and washed with an excess of deionized water. A mass of 5g of the presoaked samples was transferred to a 300 mL mini-Parr reactor with 1% wt sulfuric acid solution at 5% dry solids. Three different pretreatment conditions were applied. The reactor was sealed under ambient atmospheric condition, and heated to 120 ^{o}C and held at this temperature (±2 ^{o}C) for 10 min (±30 s). The other two DAP are both done at 160 ^{o}C for two specified residence time: 10 min and 60 min. HW and dilute alkaline pretreatment were performed in similar procedure by using the corresponding solvent, water and 1% wt sodium hydroxide. HW pretreatment were also performed at 120 ^{o}C for 10 min and 160 ^{o}C for 10 min and 60 min, while alkaline pretreatment were performed at 80 ^{o}C for 10 min and 120 ^{o}C for 10 min and 60 min. After quenching in an ice bath for ~10 min, the pretreated slurry was filtered to remove the solid material and washed with an excess of deionized water. The yield of biomass recovered after pretreatment ranged between 60% and 70% by mass of dry extractive-free lignocellulosic solids. Samples were never dried, sealed in air-tight bags and stored in a freezer for future study.

1.3. Chemical composition analysis

Samples for carbohydrate and acid-insoluble lignin analysis were prepared using a two-state acid hydrolysis protocol based on Tappi method T-222 om-88 with slight modification. 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 G8 glass fiber filter (Fisher Scientific, USA), dried, and weighted 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. 0.2 M and 0.4 M NaOH was used as the eluent and post-column rinsing effluent. The total analysis time was 70 min, with a flow rate 0.4 mL/min. Calibration was performed with standard solutions of Glucose, xylose, arabinose, mannose and galactose, and fucose was used as an internal standard.

1.4. Enzymatic hydrolysis

Cellulase from *Trichoderma reesei ATCC 26921* and Novozyme 188 (β-glucosidase) from *Aspergillus niger* were purchased from Aldrich–Sigma and used as received. Enzymatic hydrolysis of different samples was performed at a consistency of 1% (w/v) in 50 mM citrate buffer (pH 4.8) and with cellulase and β-glucosidase loadings of 20 FPU/g and 40 CBU/g, respectively. The substrate and buffer mixtures were placed on the shaking incubator for 10 min to allow the substrate to disperse uniformly in the buffer prior to the addition of enzymes. The mixture was then incubated at 50 °C under continuous agitation at 150 rpm for 24 h. A sample of hydrolysis liquid (1.00 mL) was withdrawn and the hydrolysis was quenched by submersion for 10 min in a vigorously boiling water bath. The liquid samples were then immediately frozen to -20 °C until analysis on an Agilent 1200 series HPLC system equipped with an auto sampler and an Aminex HPX-87H column and pre-column (Bio-rad Laboratories). The analysis was carried out at 65 °C using 10 mM nitric acid as eluent at a flow rate of 0.6 mL min^{-1} and with refractive index detection. Substrate digestibility is expressed as mg glucose/g dry biomass, and the calculation is based on the dry weight of untreated and pretreated biomass.

1.5. Simons' Stain

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 Blue 1 was used as received. Although the original staining method developed by Simons utilized both the orange and blue dye as received, later studies suggested that only the high molecular weight fraction of the Direct Orange dye was responsible for the increased affinity for cellulose, whereas the low molecular weight part had a very similar affinity for cellulose as the Direct Blue dye did. 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. To calculate the concentration of the Direct Orange dye after ultrafiltration, 1.00 mL of the solution was dried in a 50 °C oven for a week and the weight of the solid residue was measured.

Fiber samples (~100 mg) were weighed into five centrifuge tubes, and 1.00 mL of phosphate buffered saline solution (pH 6, 0.3M PO4, 1.40M NaCl) was also added to each tube. A set of tubes containing 1:1 mixture of DB and DO dyes at increasing concentrations were prepared by adding same amount of DB and DO dyes in a series of increasing volumes (0.25, 0.5, 0.75, 1.00, 1.50 mL), which can be then used to measure the dye adsorption isotherm. Distilled water was added to each tube to make up the final volume to 10.00 mL. All these centrifuge tubes were

incubated at 70 °C for ~6 h with shaking at 200 rpm. After that, the absorbance of the supernatant solution was obtained on a Lambda 35 UV-vis spectrophotometer at 455 nm and 624 nm which represent the wavelength of maximum absorbance for DO and DB, respectively. To calculate the concentration of the dye in the supernatant, two Lamber-Beer law equations were solved simultaneously. The amount of each 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. The maximum amount of either orange dye or blue dye adsorbed to the lignocellulosic substrates was calculated using the following Langmuir adsorption equation:

$$[C]/[A] = 1/(K_{ads}[A]_{max}) + [C]/[A]_{max}$$

where *[C]* (mg/mL) is the free dye concentration, *[A]* (mg/mg) is the amount of dye adsorbed by the substrate, K_{ads} is Langmuir adsorption constant, and $[A]_{max}$ is the maximum amount of dye adsorbed. The Langmuir isotherm plot, which is prepared by plotting *[C]*/*[A]* versus *[C]*, yields a slope = $1/[A]_{max}$.

1.6. Organic solvent exchange drying

Organic solvent exchange drying experiment was done in an effort to avoid irreversible pore collapse during typical biomass drying process. Samples were first soaked in deionized water for 24 h, and were then subsequently transferred to Soxhlet apparatus and solvent exchanged with wet methanol for 24 h, dry methanol for 24 h, and finally with dry toluene for 24 h. Molecular sieves (4 Å) were put in the round flask to adsorb the water diffusing from the biomass during the exchange process with dry methanol. Toluene saturated biomass were then transferred to a vacuum oven at 45 °C and dried for 5 h.

1.7. Mercury porosimetry

Mercury intrusion porosimetry was used for the evaluation of pore size distributions. The measurements were performed with an AutoPore IV 9500 porosimeter using a pressure range from 1×10^{-1} to 6×10^{4} psia (Micromeritics Atlanta, Georgia, USA) on the organic solvent exchange dried sample immersed in the non-wetting mercury. During the analysis, the largest of pores fill at the lowest of the pressures; as pressure increasing, mercury was intruded into smaller voids progressively. The pore volume can be determined based on the quantity of the intruded mercury, and the pore size distribution can be derived according to the Washburn equation, which gives the relationship between pore size and pressure:

$$r = -2\gamma\cos\theta/p$$

r = pore radius, γ = surface tension of mercury (0.485 N/m), θ = wetting angle of mercury (130°).

2. Additional data

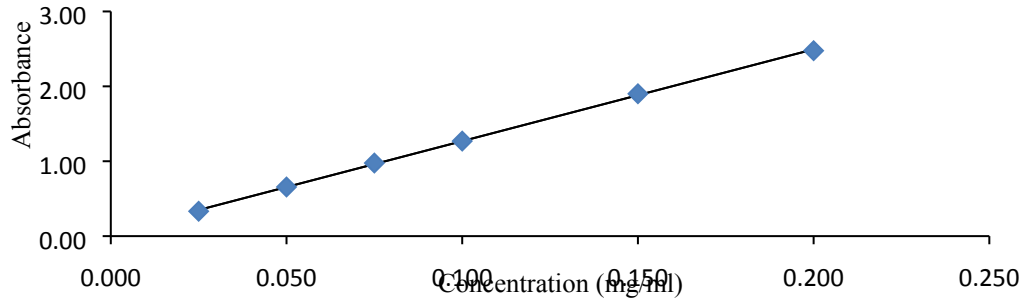
2.1. Determination of extinction coefficient of Simons' stain orange and blue dye at its maximum wavelength

In this modified Simons stain method, the use of UV-Vis spectroscopy for quantifying the remaining dye in the liquid phase eliminates the need for the filtration and dye-stripping steps, therefore the amount of dye absorbed onto the fiber was determined using the difference in the concentration of the initial added dye and the concentration of the left dye in the supernatant. The concentration of the DO and DB dyes (C_O and C_B) in the supernatant from the centrifuge tubes was determined using following two equations (Lambert-Beer law for a binary mixture) that were solved simultaneously:

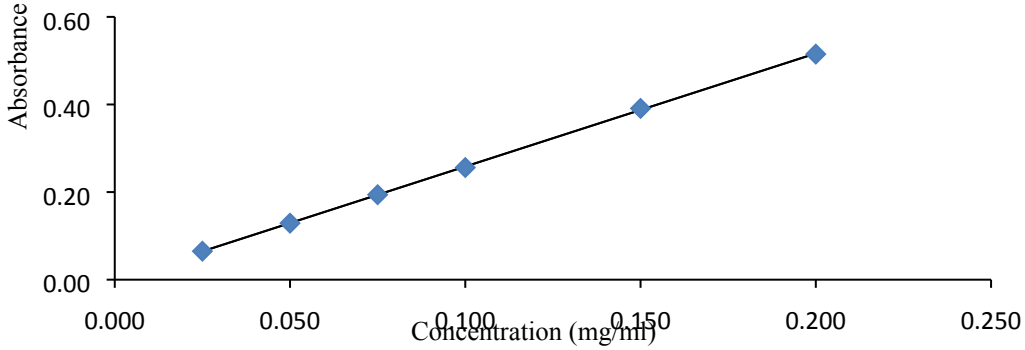
$$A_{455nm} = \varepsilon_{O/455}\, L\, C_O + \varepsilon_{B/455}\, L\, C_B$$
$$A_{624nm} = \varepsilon_{O/624}\, L\, C_O + \varepsilon_{B/624}\, L\, C_B$$

Where A is the absorption of the mixture, ε is the extinction coefficient, and L is the path length. Obviously, in order to calculate dye adsorbed by the biomass, the extinction coefficient of each dye must be calculated at each of the wavelength, 455nm and 624nm, which are the wavelengths of maximum absorbance. According Beer-Lambert law, absorbance is direct proportional to the concentration via extinction coefficient ε at a specific wavelength. Therefore, absorbance of a series of dye solutions was measured using UV spectroscopy and plotted against concentration with the resulting slope being equal to the extinction coefficient. The calibration curves with linear fit equations are shown in **Figures S1**. The values calculated in this study were $\varepsilon_{B/624}$ = 12.26 L g^{-1} cm^{-1}, $\varepsilon_{B/455}$ = 2.58 L g^{-1} cm^{-1}, $\varepsilon_{O/624}$ = 0.23 L g^{-1} cm^{-1}, $\varepsilon_{O/455}$ = 30.18 L g^{-1} cm^{-1}.

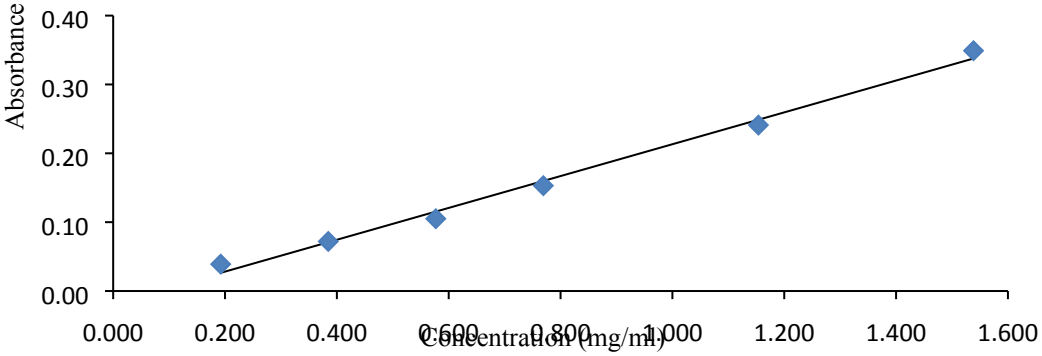
a).



b).



c).



d).

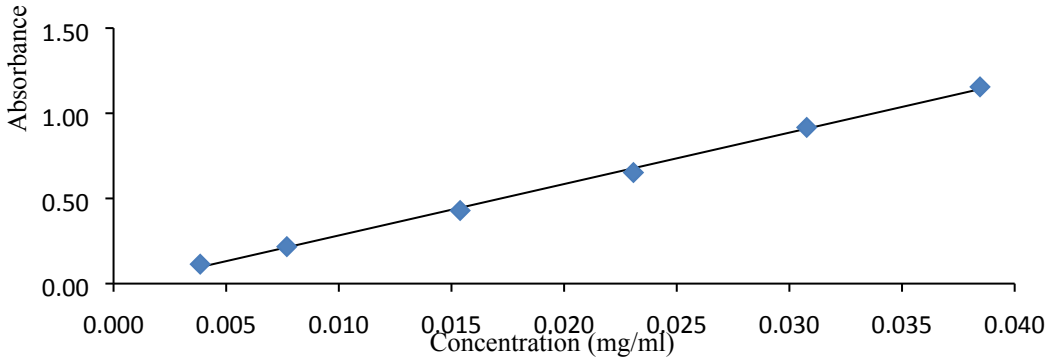


Figure S1. Measured absorbance versus concentration for a series of dye solution at wavelength 624nmand 455nm. (a), Direct Blue 1 at 624nm, $\varepsilon_{B/624}$ = 12.261 L g^{-1} cm^{-1}. (b), Direct Blue 1 at 455nm, $\varepsilon_{B/455}$ = 2.58 L g^{-1} cm^{-1}. (c), Direct Orange 15 at 624nm, $\varepsilon_{O/624}$ = 0.23 L g^{-1} cm^{-1}. (d), Direct Orange 15 at 455nm, $\varepsilon_{O/455}$ = 30.18 L g^{-1} cm^{-1}.

2.2. Effect of lignin removal by dilute alkaline pretreatment on xylose release

Figure S2 demonstrated the glucose and xylose release for dilute alkaline pretreatment of *Populus* at different severities followed by enzymatic hydrolysis and their relationship to lignin content. The sugar releases are displayed in grams sugars per grams of raw biomass. There is a better linear relationship between lignin content and xylose release compared to glucose release.

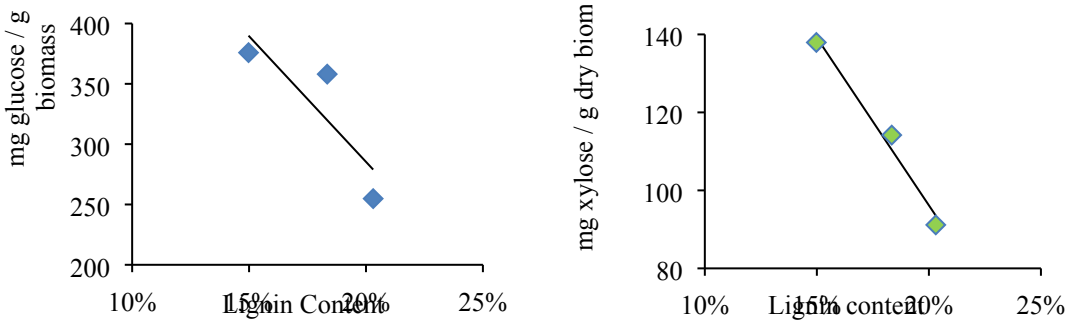


Figure S2. Effect of lignin removal by dilute NaOH pretreatment on glucose and xylose release for *Populus* substrates.

2.3. Relation between total surface area (m^2/g) measured by mercury porosimetry and substrate digestibility (mg glucose/g dry biomass) for a serious of alkaline, HW and DA pretreated *Populus*.

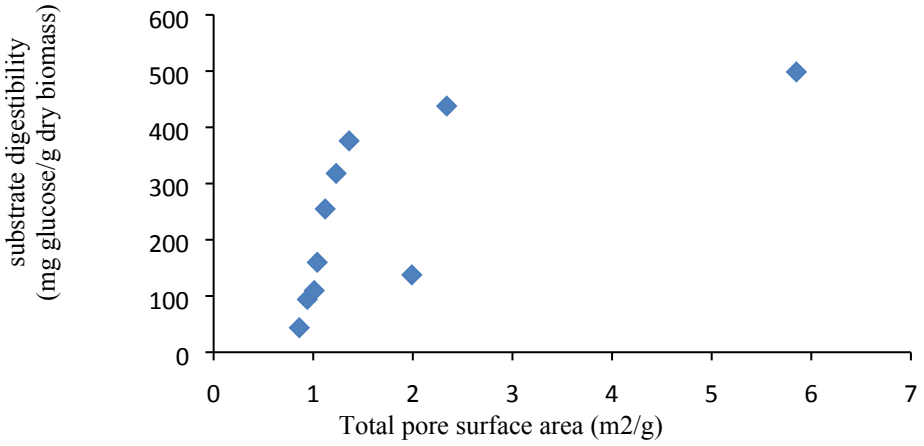


Figure S2. Relation between total surface area (m^2/g) measured by mercury porosimetry and substrate digestibility (mg glucose/g dry biomass) for a serious of alkaline, HW and DA pretreated *Populus*.

2.4. Determination of cellulose accessible surface area by Simons' stain.

Substrate (*Populus*)	**Maximum Adsorbed Orange Dye (mg/g cellulose)**	**Maximum Adsorbed Blue Dye (mg/g cellulose)**	**Total adsorbed Dye (mg/g cellulose)**	**O/B Ratio**
Untreated	19.0	27.5	46.5	0.689
NaOH_80_10m	30.4	31.0	61.5	0.981
NaOH_120_10m	35.0	26.1	58.1	1.342
NaOH_120_60m	43.5	27.6	71.2	1.576
H_2O_120_10m	38.4	36.8	75.2	1.043
H_2O_160_10m	53.6	48.5	102.1	1.107
H_2O_160_60m	61.4	48.1	109.5	1.275
DAP_120_10m	49.7	38.2	87.9	1.301
DAP_160_10m	77.8	54.2	132.1	1.435
DAP_160_60m	102.5	52.3	154.9	1.960

Table S1. Simons' stain results for biomass accessible surface area represented by the amount of adsorbed dye (mg dye/g of cellulose) and relative biomass porosity represented by ratio of adsorbed large orange dye to small Blue dye (O/B).

2.5. Determination of pore size distribution by mercury porosimetry.

Pressure (psia)	Pore Diameter (nm)	dv/dx_Native
0.50	359915.20	0.00E+00
1.00	181474.21	3.02E-06
1.50	120824.41	2.29E-05
1.75	103520.32	2.21E-05
2.00	90566.28	3.48E-05
2.25	80462.18	2.16E-05
2.50	72379.38	2.30E-05
2.75	65845.04	2.02E-05
3.00	60320.34	2.00E-05
3.25	55719.53	1.90E-05
3.50	51705.71	1.87E-05
3.75	48264.25	1.75E-05
4.00	45250.51	1.84E-05
4.19	43191.47	1.80E-05
4.38	41336.58	2.00E-05
4.74	38117.66	1.61E-05
5.13	35276.11	1.79E-05
5.49	32916.77	1.80E-05
5.99	30175.06	1.80E-05
7.49	24153.27	1.77E-05
8.49	21311.17	1.82E-05
10.48	17261.29	1.91E-05
12.96	13952.32	2.08E-05
15.99	11309.52	3.10E-05
19.99	9049.05	4.92E-05
38.72	4671.00	2.84E-05
48.55	3724.95	8.05E-05
58.36	3099.31	7.78E-05
73.38	2464.78	7.57E-05
88.91	2034.34	6.89E-05
113.53	1593.03	6.19E-05
138.92	1301.88	5.76E-05
173.57	1042.05	5.39E-05
218.73	826.86	5.53E-05
268.13	674.53	5.60E-05
328.46	550.64	5.04E-05
418.30	432.37	4.51E-05
518.25	348.99	4.83E-05
638.53	283.25	4.43E-05
798.50	226.50	4.13E-05
988.12	183.04	3.88E-05

Table S2. Tabular report for pore size distribution of native *Populus* determined by mercury porosimetry.

Pressure (psia)	Pore Diameter (nm)	dv/dx_NaOH_80_10m
0.50	361349.40	0.00E+00
1.00	181414.03	2.45E-06
1.50	120771.50	2.99E-05
1.75	103523.95	2.78E-05
2.00	90485.58	2.57E-05
2.25	80435.55	2.22E-05
2.50	72420.34	2.05E-05
2.75	65842.43	1.97E-05
3.00	60340.62	1.86E-05
3.25	55668.18	1.80E-05
3.50	51688.61	1.76E-05
3.75	48291.57	1.76E-05
4.00	45239.17	1.72E-05
4.19	43198.44	1.69E-05
4.38	41324.81	1.68E-05
4.75	38101.84	1.72E-05
5.13	35281.24	1.71E-05
5.50	32911.15	1.67E-05
5.99	30173.13	1.70E-05
7.49	24154.81	1.71E-05
8.49	21306.12	1.73E-05
10.47	17266.51	1.79E-05
12.97	13948.47	2.00E-05
15.96	11332.92	2.49E-05
38.51	4696.79	1.97E-05
48.66	3717.21	6.78E-05
58.43	3095.32	6.45E-05
73.28	2468.19	6.17E-05
88.60	2041.26	5.45E-05
113.64	1591.50	4.88E-05
137.98	1310.79	4.49E-05
173.13	1044.67	3.93E-05
218.14	829.12	3.56E-05
268.68	673.15	3.44E-05
328.70	550.23	2.81E-05
418.60	432.07	3.64E-05
518.39	348.90	3.49E-05
638.21	283.39	2.76E-05
798.40	226.53	2.89E-05

988.46	182.97	2.37E-05
1198.00	150.97	1.07E-05
1498.04	120.73	9.46E-06

Table S3. Tabular report for pore size distribution of NaOH pretreated Populus (80 °C, 10min) determined by mercury porosimetry.

Pressure (psia)	Pore Diameter (nm)	dv/dx_NaOH_120_10m
0.50	359957.43	0.00E+00
1.00	181030.54	2.39E-06
1.50	120780.49	2.51E-05
1.75	103480.34	4.19E-05
2.00	90595.05	3.53E-05
2.25	80488.81	2.85E-05
2.50	72413.37	2.56E-05
2.75	65810.61	2.33E-05
3.00	60329.03	2.32E-05
3.25	55658.86	2.10E-05
3.50	51743.72	2.12E-05
3.75	48255.49	2.05E-05
4.00	45251.29	2.04E-05
4.19	43206.44	1.99E-05
4.38	41325.57	2.03E-05
4.75	38102.22	1.98E-05
5.12	35291.81	2.03E-05
5.49	32921.59	1.96E-05
6.00	30167.73	1.94E-05
7.49	24154.03	1.97E-05
8.49	21306.64	1.99E-05
10.48	17262.92	2.01E-05
12.96	13950.43	2.16E-05
15.99	11310.16	2.58E-05
38.52	4694.87	3.07E-05
48.79	3706.81	9.62E-05
58.59	3086.71	9.86E-05
73.55	2458.92	9.41E-05
88.30	2048.30	8.36E-05
113.11	1599.05	7.64E-05
138.41	1306.76	6.66E-05
173.23	1044.10	3.45E-05
218.30	828.52	5.39E-05
268.07	674.68	5.16E-05
328.33	550.85	5.25E-05

418.32	432.36	4.67E-05
518.47	348.84	4.19E-05
638.12	283.43	4.45E-05
798.68	226.45	3.56E-05
988.15	183.03	3.27E-05
1198.31	150.93	0.00E+00
1498.37	120.71	0.00E+00
1897.92	95.30	9.12E-06
2347.79	77.04	7.52E-05

Table S4. Tabular report for pore size distribution of NaOH pretreated *Populus* (120 °C, 10min) determined by mercury porosimetry

Pressure (psia)	Pore Diameter (nm)	dv/dx_NaOH_120_60m
0.50	361103.20	0.00E+00
1.00	181406.20	2.14E-06
1.50	120598.85	2.82E-05
1.75	103446.10	4.07E-05
2.00	90615.87	3.19E-05
2.25	80459.31	2.66E-05
2.50	72384.79	2.38E-05
2.75	65806.08	2.23E-05
3.00	60325.16	2.15E-05
3.25	55699.39	2.10E-05
3.50	51706.49	2.07E-05
3.75	48274.55	2.01E-05
4.00	45253.53	2.01E-05
4.19	43201.03	1.99E-05
4.38	41327.40	2.01E-05
4.75	38112.09	2.01E-05
5.12	35292.54	2.05E-05
5.50	32912.62	2.02E-05
5.99	30179.49	2.12E-05
7.49	24149.39	2.16E-05
8.49	21305.41	2.22E-05
10.48	17264.84	2.33E-05
12.97	13950.02	2.52E-05
15.95	11337.21	2.86E-05
38.57	4688.99	3.95E-05
48.25	3748.43	9.51E-05
58.57	3088.13	9.56E-05
73.32	2466.78	9.06E-05
88.29	2048.49	8.00E-05

113.78	1589.63	7.23E-05
138.86	1302.49	6.77E-05
173.31	1043.60	5.87E-05
219.12	825.42	4.20E-05
269.09	672.13	3.69E-05
328.25	550.99	4.66E-05
418.23	432.45	3.96E-05
518.51	348.81	2.82E-05
637.96	283.50	3.96E-05
798.30	226.56	2.82E-05
988.14	183.03	0.00E+00
1198.41	150.92	0.00E+00
1498.30	120.71	0.00E+00
1897.81	95.30	0.00E+00
2348.41	77.02	4.21E-06
2897.23	62.43	3.31E-05

Table S5. Tabular report for pore size distribution of NaOH pretreated *Populus* (120 ^{o}C, 60min) determined by mercury porosimetry.

Pressure (psia)	Pore Diameter (nm)	dv/dx_H_2O_120_10m
0.50	360914.90	0.00E+00
1.00	181514.96	3.69E-06
1.50	120819.23	3.48E-05
1.75	103564.87	1.25E-05
2.00	90477.85	2.36E-05
2.25	80495.97	1.94E-05
2.50	72404.88	2.00E-05
2.75	65803.27	1.82E-05
3.00	60348.35	1.81E-05
3.25	55688.79	1.74E-05
3.50	51716.11	1.73E-05
3.75	48291.05	1.73E-05
4.00	45248.88	1.67E-05
4.19	43197.53	1.65E-05
4.38	41333.94	1.66E-05
4.75	38107.89	1.58E-05
5.12	35290.48	1.64E-05
5.50	32908.82	1.61E-05
5.99	30180.22	1.56E-05
7.49	24150.43	1.59E-05

8.49	21305.50	1.59E-05
10.48	17265.03	1.69E-05
12.97	13948.74	2.29E-05
15.95	11338.77	3.71E-05
38.75	4666.99	4.16E-05
48.45	3732.61	7.17E-05
58.68	3081.95	6.26E-05
73.39	2464.52	5.99E-05
88.69	2039.28	5.30E-05
113.12	1598.83	4.96E-05
138.55	1305.39	4.25E-05
173.20	1044.24	3.98E-05
218.20	828.90	2.30E-05
268.30	674.11	1.02E-05
328.53	550.52	3.83E-05
418.22	432.46	3.81E-05
518.31	348.95	3.59E-05
638.92	283.08	2.90E-05
798.68	226.45	3.20E-05
987.91	183.08	3.36E-05
1198.26	150.94	1.34E-05
1498.34	120.71	9.07E-06

Table S6. Tabular report for pore size distribution of hot water pretreated *Populus* (120 ^{o}C, 10min) determined by mercury porosimetry.

Pressure (psia)	Pore Diameter (nm)	dv/dx_H_2O_160_10m
0.50	360972.53	0.00E+00
1.00	181427.58	1.77E-06
1.50	120709.59	1.51E-05
1.75	103479.56	4.74E-05
2.00	90448.89	4.43E-05
2.25	80487.37	3.57E-05
2.50	72432.70	3.04E-05
2.75	65809.01	2.74E-05
3.00	60327.42	2.62E-05
3.25	55698.39	2.48E-05
3.50	51697.19	2.34E-05
3.75	48264.25	2.34E-05
4.00	45246.17	2.23E-05
4.19	43193.41	2.13E-05
4.38	41327.90	2.17E-05

4.74	38124.60	2.15E-05
5.13	35277.27	2.08E-05
5.50	32900.68	2.04E-05
5.99	30178.60	2.00E-05
7.49	24147.07	1.97E-05
8.49	21307.11	1.96E-05
10.48	17265.29	2.03E-05
12.97	13948.05	2.52E-05
15.96	11334.22	3.83E-05
38.81	4660.69	4.68E-05
48.36	3740.05	7.48E-05
58.46	3093.80	6.72E-05
73.25	2469.16	6.12E-05
88.52	2043.14	5.73E-05
113.87	1588.40	5.18E-05
138.55	1305.37	4.61E-05
173.64	1041.62	4.33E-05
218.55	827.58	4.13E-05
268.30	674.10	4.15E-05
328.37	550.80	4.03E-05
418.66	432.00	3.48E-05
518.12	349.08	3.57E-05
638.15	283.42	0.00E+00
798.51	226.50	0.00E+00
989.00	182.87	2.34E-05
1198.18	150.95	3.11E-05
1498.41	120.70	0.00E+00
1898.36	95.27	5.61E-06

Table S7. Tabular report for pore size distribution of hot water pretreated *Populus* (160 ^{o}C, 10min) determined by mercury porosimetry

Pressure (psia)	Pore Diameter (nm)	dv/dx_H_2O_160_60m
0.50	359984.28	0.00E+00
0.99	181786.28	2.46E-06
1.50	120776.61	2.66E-05
1.75	103412.34	4.35E-05
2.00	90511.16	3.27E-05
2.25	80480.49	2.66E-05
2.50	72386.33	2.47E-05
2.75	65853.85	2.32E-05
3.00	60335.14	2.19E-05
3.25	55694.54	2.16E-05

3.50	51702.63	2.08E-05
3.75	48265.28	2.09E-05
4.00	45254.68	2.03E-05
4.19	43185.74	1.96E-05
4.38	41331.68	1.96E-05
4.75	38101.86	1.98E-05
5.13	35286.74	1.95E-05
5.49	32917.82	1.92E-05
5.99	30176.43	1.90E-05
7.49	24153.26	1.91E-05
8.49	21309.24	1.90E-05
10.48	17263.55	1.97E-05
12.97	13948.97	2.24E-05
15.95	11338.86	3.06E-05
38.31	4720.67	3.77E-05
48.59	3722.53	7.95E-05
58.91	3070.36	7.32E-05
73.65	2455.57	7.06E-05
88.74	2038.17	6.36E-05
113.50	1593.52	5.94E-05
138.41	1306.74	5.43E-05
173.98	1039.55	4.93E-05
218.38	828.22	4.67E-05
269.12	672.05	4.38E-05
328.54	550.51	4.07E-05
418.06	432.63	3.97E-05
518.53	348.80	2.98E-05
638.23	283.38	2.88E-05
798.52	226.50	2.88E-05
988.81	182.91	2.36E-05
1198.20	150.95	9.98E-06
1498.28	120.71	0.00E+00
1898.25	95.28	0.00E+00
2348.19	77.02	0.00E+00
2897.71	62.42	1.23E-05
3597.54	50.27	2.11E-05

Table S8. Tabular report for pore size distribution of hot water pretreated *Populus* (160 ^{o}C, 60min) determined by mercury porosimetry.

Pressure (psia)	Pore Diameter (nm)	dv/dx_DAP_120_10m
360435.40	0.00	0.00E+00

181578.16	0.31	1.75E-06
120845.08	1.29	1.60E-05
103417.24	1.83	3.12E-05
90556.36	2.21	2.92E-05
80431.56	2.45	2.46E-05
72410.29	2.64	2.33E-05
65811.25	2.78	2.09E-05
60335.47	2.89	2.10E-05
55696.79	2.99	1.98E-05
51718.09	3.06	1.91E-05
48257.56	3.13	1.92E-05
45230.18	3.19	1.85E-05
43212.57	3.22	1.81E-05
41332.49	3.26	1.89E-05
38103.94	3.32	1.81E-05
35283.64	3.37	1.84E-05
32925.27	3.41	1.82E-05
30176.99	3.46	1.83E-05
24150.68	3.57	1.81E-05
21306.58	3.62	1.80E-05
17259.76	3.70	1.90E-05
13947.99	3.77	2.07E-05
11338.85	3.84	2.75E-05
9064.79	3.93	4.14E-05
4692.25	4.18	5.70E-05
3714.95	4.26	8.08E-05
3085.98	4.31	8.26E-05
2465.73	4.36	7.45E-05
2043.07	4.39	6.85E-05
1598.66	4.42	6.47E-05
1309.34	4.43	5.68E-05
1045.20	4.45	5.26E-05
827.60	4.46	5.05E-05
673.87	4.46	4.43E-05
550.71	4.47	2.26E-05
432.04	4.47	0.00E+00
348.62	4.47	7.84E-06
283.09	4.47	9.84E-06
226.51	4.47	7.84E-05
182.94	4.47	3.07E-05
150.98	4.47	2.32E-05
120.71	4.47	2.48E-06
95.30	4.47	9.17E-06

Table S9. Tabular report for pore size distribution of dilute acid pretreated *Populus* (120 °C, 10min) determined by mercury porosimetry

Pressure (psia)	Pore Diameter (nm)	dv/dx_DAP_160_10m
0.50	360473.85	0.00E+00
1.00	181248.19	1.32E-06
1.50	120793.40	5.70E-06
1.75	103423.55	1.57E-05
2.00	90558.77	2.20E-05
2.25	80434.41	2.61E-05
2.50	72438.89	3.02E-05
2.75	65824.02	2.86E-05
3.00	60327.41	3.04E-05
3.25	55680.78	2.87E-05
3.50	51710.59	2.72E-05
3.75	48269.92	2.69E-05
4.00	45248.58	2.64E-05
4.19	43180.87	2.48E-05
4.38	41339.92	2.59E-05
4.75	38102.22	2.63E-05
5.13	35284.10	2.49E-05
5.50	32913.92	2.45E-05
5.99	30176.19	2.53E-05
7.49	24156.87	2.54E-05
8.49	21304.63	2.47E-05
10.48	17263.71	2.52E-05
12.96	13953.35	2.63E-05
15.96	11335.68	2.83E-05
38.61	4684.69	3.27E-05
48.31	3743.94	1.09E-04
58.54	3089.49	1.33E-04
73.20	2470.95	1.44E-04
88.48	2044.18	1.45E-04
113.56	1592.65	1.48E-04
138.52	1305.66	1.46E-04
173.86	1040.27	1.34E-04
218.42	828.06	1.34E-04
268.05	674.74	1.24E-04
328.14	551.17	1.20E-04
418.14	432.54	1.11E-04
518.03	349.14	1.07E-04

637.92	283.52	1.08E-04
798.28	226.57	1.05E-04
988.56	182.96	1.05E-04
1198.12	150.96	9.37E-05
1497.72	120.76	1.15E-04
1897.86	95.30	8.76E-05
2348.04	77.03	7.88E-05
2896.84	62.43	4.85E-05

Table S10. Tabular report for pore size distribution of dilute acid pretreated *Populus* (160 °C, 10min) determined by mercury porosimetry

Pressure (psia)	Pore Diameter (nm)	dv/dx_DAP_160_60m
0.50	360351.50	0.00E+00
1.00	181535.23	5.72E-07
1.50	120926.41	1.58E-06
1.75	103471.01	3.94E-06
2.00	90477.11	3.83E-06
2.25	80511.43	5.32E-06
2.50	72363.17	5.71E-06
2.75	65886.85	6.62E-06
3.00	60332.56	8.59E-06
3.25	55693.99	1.01E-05
3.50	51714.21	1.23E-05
3.75	48263.22	1.28E-05
4.00	45241.27	1.27E-05
4.19	43214.91	1.62E-05
4.38	41325.26	1.50E-05
4.75	38104.93	1.83E-05
5.12	35291.75	1.91E-05
5.50	32912.74	1.87E-05
5.99	30170.30	2.06E-05
7.49	24151.19	2.15E-05
8.49	21313.01	2.31E-05
10.48	17265.31	2.53E-05
12.97	13949.00	2.80E-05
15.99	11309.81	3.17E-05
38.42	4707.60	2.31E-05
48.38	3738.17	6.08E-05
58.34	3099.99	7.95E-05
73.50	2460.86	1.04E-04
88.48	2044.10	1.38E-04

113.12	1598.83	1.85E-04
138.35	1307.32	2.31E-04
173.16	1044.47	2.82E-04
218.78	826.68	3.14E-04
268.19	674.37	3.38E-04
328.17	551.13	3.43E-04
418.18	432.50	3.51E-04
519.27	348.30	3.36E-04
638.91	283.08	3.49E-04
798.09	226.62	3.22E-04
988.03	183.05	3.38E-04
1198.00	150.97	3.39E-04
1498.37	120.71	3.43E-04
1898.41	95.27	3.40E-04
2347.42	77.05	3.54E-04
2896.73	62.44	3.54E-04
3596.81	50.28	3.27E-04
4495.98	40.23	3.17E-04
5595.43	32.32	2.36E-04
6894.49	26.23	1.19E-04

Table S11. Tabular report for pore size distribution of dilute acid pretreated *Populus* (160 ^{o}C, 60min) determined by mercury porosimetry