Supplementary materials for

Fractionation of Lignin from Eucalyptus Bark Using Amine-Sulfonate Functionalized Ionic Liquids

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Experimental

1. Materials

The eucalyptus bark was obtained from China National Pulp and Paper Research Institute. The samples were ground using a mill (SM200 Rostfrei, Retsch, Germany) to pass through a 40 mesh (~1mm) screen. The moisture content of eucalyptus bark was7.72 wt% (in reference to the oven dry material). Cellulase Ctec-2 (150 FPU/ml) was received from Novozymes (Beijing, China). The enzyme activitywas measured according to the NREL standard protocol[1]. Cholinium hydroxide ([Ch][OH]), tetraethylammonium hydroxide([Et_4N][OH]), kraft lignin, ligninsulfonate and xylan were purchased from Sigma–Aldrich. The other reagents and solvents were obtained from commercial suppliers and were used without further purification. Triple-distilled water was used for the preparation of the aqueous solutions in the synthesis of ILs.

2. General procedure for preparation of the ASF-ILs

The ASF-ILs were synthesized by methods analogous to literature procedures (Fig. S1).[2] Typically, an aqueous solution of tetraethylammonium hydroxide or cholinium hydroxide was neutralized with equal molar amounts of an appropriate zwitterion. The ASF-ILs were obtained through the alkylation reaction between sultones and amines. Here we gave a representative synthesis for [Et_4N][n-$BuNHC_3SO_3$].

Zwitterion n-butylamino-propanesulfonate salt [n-$BuNH_2C_3SO_3$]: In a 500 mL round bottomed flask with a magnetic stir bar and fitted, 0.20mol of 1,3-propane sultone is dissolved in 200 mL of reagent grade toluene. An excess amount of n-butylamine was slowly added to the 1,3-propane sultone solution. Within a short time,

the clear, colorless solution begins to turn cloudy. The solution is stirred overnight at room temperature. The white solid product is separated by vacuum filtration and dried vacuum at 85℃ for 8 h. When desired, the product can be recrystallized from hot ethanol.

Tetraethylammoniumn n-butylamino-propanesulfonic IL [Et_4N][n-$BuNHC_3SO_3$]: In a 500 mL flask, the quaternary tetraethylammonium hydroxide aqueous solutions was slowly added to the zwitterion [n-$BuNHC_3SO_3$] solid. The solid zwitterion dissolved instantly, and the resulting solution was stirred for 30 min. The [Et_4N][n-$BuNHC_3SO_3$] IL was obtained after water was removed by rotary evaporation. The product was dried in vacuo for at least 24 h at 85℃. The yields of all ASF-ILs are higher than 98%.

[Et_4N][i-$BuNHC_3SO_3$]. Tetraethylammonium isobutylamino-propanesulfonic IL: ^{1}H NMR (400 MHz, DMSO) δ:3.25 (8 H, q, *J*= 7.2 Hz), 2.50 (2 H, t, *J*= 7.0 Hz), 2.46 – 2.37 (2 H, m), 2.27 (2 H, d, *J*= 6.7 Hz), 1.75 – 1.55 (3 H, m), 1.21 – 1.14 (12 H, m), 0.85 (6 H, d, *J*= 6.7Hz); ^{13}C NMR (100 MHz, DMSO) δ: 58.00, 51.95, 50.24, 49.45, 28.45, 26.34, 21.18, 7.59. FT-IR (ATR, υ_{max}/cm^{-1}): 3306, 2956, 2920, 2880, 2803, 1733, 1716, 1494, 1460, 1443, 1417, 1393, 1366, 1279, 1256, 1217, 1183, 1153, 1128, 1070, 1030, 997, 931, 847, 819, 781, 743.

[Et_4N][n-$BuNHC_3SO_3$]. Tetraethylammoniumn butylamino-propanesulfonic IL: ^{1}H NMR (400 MHz, DMSO) δ: 3.24 (8 H, q, *J*= 7.2Hz), 2.55 – 2.32 (6 H, m), 1.75 – 1.59 (2 H, m), 1.41 – 1.24 (4 H, m), 1.20 – 1.15 (12 H, m), 0.86 (3 H, t, *J*= 7.1Hz). ^{13}C NMR (100 MHz, DMSO) δ: 51.93, 50.26, 49.44, 49.34, 32.37, 26.32, 20.50, 14.41, 7.58. FT-IR (ATR, υ_{max}/cm^{-1}): 3303, 2956, 2927, 2872, 2811, 1733, 1716, 1507, 1460, 1404, 1377, 1273, 1194, 1176, 1079, 1032, 781, 720.

[Et_4N][i-$PrNHC_3SO_3$]. Tetraethylammonium isopropylamino-propanesulfonic IL:^{1}H NMR (400 MHz, DMSO) δ:3.24 (8 H, q, *J*= 7.2Hz), 2.65 (1 H, dt, J_1= 12.4Hz, J_2= 6.2Hz), 2.50 (2 H, t, *J*= 7.1Hz), 2.41 (2 H, dd, J_1= 8.9Hz, J_2= 6.6Hz), 1.73 – 1.59 (2 H, m), 1.21 – 1.14 (12 H, m), 0.94 (6 H, d, *J*= 6.2Hz). ^{13}C NMR (100 MHz, DMSO) δ: 51.95, 50.32, 48.33, 46.74, 26.61, 23.43, 7.59. FT-IR (ATR, υ_{max}/cm^{-1}): 3295, 2961,

2871, 1733, 1716, 1456, 1395, 1378, 1337, 1308, 1264, 1169, 1089, 1031, 1001, 833, 783, 723.

[Et_4N][$Me_2NC_4SO_3$]. Tetraethylammonium dimethylamino-butanesulfonic IL: ^{1}H NMR (400 MHz, DMSO) δ: 3.23 (8 H, q, *J*= 7.3Hz), 2.43 – 2.34 (2 H, m), 2.19 – 2.12 (2 H, m), 2.10 (6 H, d, *J*= 14.3Hz), 1.62 – 1.49 (2 H, m), 1.42 (2 H, dd, J_1= 14.5Hz, J_2= 7.4Hz), 1.23 – 1.11 (12 H, m). ^{13}C NMR (100 MHz, DMSO) δ: 59.53, 51.93, 45.66, 26.93, 23.61, 7.56. FT-IR (ATR, υ_{max}/cm^{-1}): 2974, 2938, 2857, 2812, 2781, 2860, 1733, 1716, 1456, 1405, 1372, 1316, 1271, 1225, 1208, 1191, 1176, 1071, 1031, 967, 889, 850, 808, 786, 769, 723.

[Ch][n-$HeNHC_3SO_3$]. Cholinium hexylamino-propanesulfonic IL: ^{1}H NMR (400 MHz, DMSO) δ: 3.84 (2 H, dd, J_1= 9.8Hz, J_2= 5.2Hz), 3.40 (2H, d, *J*= 4.9Hz), 3.12(9 H, s), 3.27 – 2.38 (6 H, m), 1.80 – 1.60 (2H, m), 1.57 – 1.11 (4 H, m), 1.45 – 1.18 (4 H, m), 0.85 (3 H, t, *J*= 6.7Hz). ^{13}C NMR (100 MHz, DMSO) δ: 67.39, 55.66, 53.61, 50.11, 49.61, 49.01, 31.72, 29.76, 26.98, 25.81, 22.54, 14.36. FT-IR (ATR, υ_{max}/cm^{-1}): 3398, 2955, 2927, 2858, 1733, 1716, 1643, 1608, 1507, 1471, 1416, 1377, 1163, 1085, 1037, 953, 864, 830, 788, 726, 698.

[Ch][n-$BuNHC_3SO_3$]. Cholinium butylamino-propanesulfonic IL: ^{1}H NMR (400 MHz, DMSO) δ: 3.83 (2 H, ddd, J_1= 7.9Hz, J_2 = 5.2Hz,J_3 = 2.8Hz), 3.47 – 3.34 (2 H, m), 3.12 (9 H, s), 2.52 – 2.41 (6 H, m), 1.69 (2 H, dd, J_1= 10.4Hz, J_2 = 4.7Hz), 1.32 (4 H, dd, J_1 =14.7Hz, J_2=7.6Hz), 0.86 (3 H, t, *J*= 7.0Hz).^{13}C NMR (100 MHz, DMSO) δ: 67.39, 55.65, 53.61, 50.20, 49.30, 49.14, 32.13, 26.04, 20.45, 14.40.FT-IR (ATR, υ_{max}/cm^{-1}): 3256, 3033, 2930, 2861, 2808, 1733, 1716, 1484, 1467, 1377, 1352, 1292, 1263, 1250, 1182, 1135, 1089, 1033, 978, 950, 866, 785, 753, 733.

[Ch][i-$BuNHC_3SO_3$]. Cholinium isobutylamino-propanesulfonic IL: ^{1}H NMR (400 MHz, DMSO) δ: 3.83 (2 H, dd, J_1 = 5.3Hz, J_2=4.0Hz), 3.43 (2 H, d, *J*= 5.0Hz), 3.13 (9 H, s), 2.50 (4 H, dd, J_1 = 9.4Hz, J_2 = 6.3Hz), 2.28 (2 H, d, *J*= 6.8Hz), 1.76 – 1.56 (3 H, m), 0.84 (6 H, d, *J*= 6.7Hz). ^{13}C NMR (100 MHz, DMSO) δ: 67.35, 57.72, 55.68, 53.57, 50.13, 28.23, 25.87, 21.13.FT-IR (ATR, υ_{max}/cm^{-1}): 3300, 2951, 2870, 2818, 1733, 1716, 1470, 1416, 1367, 1163, 1086, 1032, 952, 866, 830, 784, 726.

[Ch][i-PrNHC$_3$SO$_3$]. Cholinium isopropylamino-propanesulfonic IL: ^{1}H NMR (400 MHz, DMSO) δ: 3.88 – 3.79 (2 H, m), 3.45 – 3.36 (2 H, m), 3.11 (9 H, s), 2.72 – 2.65 (1 H, m), 2.50 (2 H, dd, J_1 = 3.7Hz, J_2 = 2.0Hz), 2.46 – 2.39 (2 H, m), 1.71 – 1.63 (2 H, m), 0.95 (6 H, d, J= 6.2Hz). ^{13}C NMR (100 MHz, DMSO) δ: 67.39, 55.64, 53.62, 50.26, 48.42, 46.54, 26.28, 23.15. FT-IR (ATR, υ_{max}/cm^{-1}): 3278, 3033, 2961, 2869, 1733, 1716, 1478, 1417, 1379, 1364, 1339, 1272, 1162, 1085, 1031, 952, 924, 866, 783, 726.

[Ch][i-PrNHC$_4$SO$_3$]. Cholinium isopropylamino-butanesulfonic IL: ^{1}H NMR (400 MHz, DMSO) δ: 3.84 (2 H, ddd, J_1= 8.0Hz, J_2 = 5.3Hz, J_3 = 2.9Hz), 3.47 – 3.39 (2 H, m), 3.12 (9 H, s), 2.67 (1 H, dt, J_1 = 12.4Hz, J_2 = 6.2Hz), 2.48 – 2.38 (4 H, m), 1.63 – 1.53 (2 H, m), 1.46 – 1.36 (2 H, m), 0.95 (6 H, d, J= 6.2Hz). ^{13}C NMR (100 MHz, DMSO) δ: 67.41, 55.65, 53.59, 52.03, 48.59, 47.17, 29.76, 23.68, 23.34. FT-IR (ATR, υ_{max}/cm^{-1}): 3270, 3029, 2930, 2858, 1733, 1716, 1484, 1455, 1444, 1390, 1372, 1339, 1276, 1248, 1223, 1175, 1145, 1081, 1058, 1033, 964, 950, 917, 894, 862, 831, 781, 732.

[Ch][Et$_2$NC$_3$SO$_3$]. Cholinium diethylamino-propanesulfonicIL: ^{1}H NMR (400 MHz, DMSO) δ: 3.84 (2 H, ddd, J_1 = 7.9Hz, J_2 =5.3Hz, J_3 =2.9Hz), 3.50 – 3.41 (2 H, m), 3.15 (9 H, s), 2.49 – 2.34 (8 H, m), 1.74 – 1.60 (2 H, m), 0.94 (6 H, t, J= 7.1Hz). ^{13}C NMR (100 MHz, DMSO) δ: 67.31, 55.70, 53.51, 51.93, 50.11, 46.75, 23.28, 12.20. FT-IR (ATR, υ_{max}/cm^{-1}): 3033, 2968, 2925, 2832, 1733, 1716, 1492, 1462, 1374, 1349, 1309, 1285, 1225, 1212, 1182, 1142, 1074, 1046, 1033, 1014, 979, 957, 925, 864, 822, 793, 755, 737.

[Ch][Et$_2$NC$_4$SO$_3$]. Cholinium diethylamino-butanesulfonic IL: ^{1}H NMR (400 MHz, DMSO) δ: (400 MHz, DMSO) 3.27 – 3.18 (8 H, m), 2.38 (8 H, ddd, J 15.6, 12.9, 5.9), 1.64 (2 H, t, J 7.5), 1.17 (12 H, ddd, J 7.2, 4.4, 1.6), 0.92 (6 H, t, J 7.1). ^{13}C NMR (100 MHz, DMSO) δ: (100 MHz, DMSO) 52.11, 51.93, 51.90, 50.21, 46.77, 23.49, 12.37, 7.57. FT-IR (ATR, υ_{max}/cm^{-1}): 3284, 3033, 2964, 2932, 2870, 2789, 1481, 1418, 1367, 1291, 1263, 1162, 1139, 1091, 1031, 951, 869, 784, 759, 719.

[Ch][Me$_2$NC$_4$SO$_3$]. Cholinium dimethylamino-butanesulfonic IL: ^{1}H NMR (400 MHz, DMSO) δ: 3.84 (2 H, ddd, J_1 =8.0Hz, J_2 =5.3Hz, J_3 =3.0Hz), 3.43 (2 H, dd, J_1

=5.0Hz, J_2 =3.7Hz), 3.13 (9 H, s), 2.43 (2 H, td, J_1 =7.9Hz, J_2 =2.6Hz), 2.21 – 2.14 (2 H, m), 2.11 (6 H, s), 1.65 – 1.50 (2 H, m), 1.44 (2 H, dd, J_1 =14.5Hz, J_2 =7.4Hz). ^{13}C NMR (100 MHz, DMSO) δ: 67.34, 59.38, 55.68, 53.54, 51.88, 45.56, 26.74, 23.51. FT-IR (ATR, υ_{max}/cm^{-1}): 3301, 3028, 2941, 2870, 2813, 2758, 1733, 1716, 1484, 1467, 1384, 1261, 1162, 1083, 1056, 1031, 960, 893, 862, 840, 789, 734.

3. Characterization of ASF-ILs, lignin and biomass.

FT-IR analysis. A leveled attenuated total reflectance (ATR) accessory with a 3-mm-diameter diamond plate purchased from Pike Technologies was used for the infrared spectroscopy measurement on aThermo Scientific Nicolet iS50 Adv FTIR Spectrometer equipped with a liquid nitrogen cooled MCT detector. The spectra were recorded in the rangefrom 4000 to 650 cm^{-1} at 4 cm^{-1} resolution and 50 scans per sample. A steady flow of nitrogen was purged above the sample to maintain a dry atmosphere during the measurement.

Thermo gravimetric analysis (TGA). Thermo gravimetric analysis was performed on a thermal analyzer (STA 449 F3, NETZSC, Germany). The lignin and biomass samples were heated from room temperature to 800°C at a rate of 10 °C/min in nitrogen. The ionic liquids samples were heated from room temperature to 600°C at a rate of 10°C/min in nitrogen. Prior to the measurement, the samples were extensively dried for 24 h in an oven at 85°C under vacuum to eliminate the presence of water.

NMR spectra of the ASF-ILs. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker Advance III 400 MHz NMR spectrometer at 298K, using the solvent residual peak as internal standard.

Viscosity. Viscosities were measured at 100°C with a GB/T265 Ubbelohde viscometer. Prior to measurement, the samples were extensively dried for 24 h in an oven at 85℃ under vacuum to eliminate the presence of water.

Chemical analysis. C, H, N and S elements of lignin were determined using a vario EL cube elemental analyzer. The percentage of oxygen was calculatedby subtracting the C, H, N and S contents from 100%.

X-ray diffraction (XRD). The crystal structure of all samples were measured using a X-ray diffractometer (X'Pert Powder, PANalytical, Holland) with the scanning rate of 2°/min, ranging from 5° to 50°. The crystallinity of the samples, as expressed by crystallinity index (*CrI*), was determined from XRD data and calculated according to the formula $CrI(\%)=(I_{002}-I_{am})/I_{002}\times 100\%$, where I_{002} is the intensity for the crystalline portion of biomass at 2θ=22.5°, and I_{am} is the peak for the amorphous at 2θ=18.0°.

4. Solubility of lignin, xylan and cellulose

All dissolution experiments were carried out in a heating and stirring module (TS-18821, Thermo Sci-entific, USA).The stirring was set at 500 rpm for a specified time. The dissolution of the kraft lignin, lignosulfonate, xylan and cellulose indifferent ASF-ILs was performed at 100℃. In a typical solubility measurement, 5mg of each samplewas added to 1 g of an ASF-IL until saturation was reached. Because the color of lignin-IL solution was dark, so the lignin dissolution was verified via optical microscopy. A drop of the lignin–ILsolution was transferred to a microscopic slide and investigated with a KEYEN E VHX-2000E optical microscope (magnification=250×).

5. Composition analysis

Compositional analysis of eucalyptus barkbefore and after pretreatment was performed via a two-stepacid hydrolysis according to the National Renewable EnergyLaboratory (NREL) protocol.[3]The sample was treated with 72% (v/v) sulfuric acid at 30℃ for 1 h, followed by dilute acid (4%) at 121℃ for 1 h. The hydrolysis solution was filtered and analyzed for sugar content. The hydrolysis products (glucose and xylose) were quantified by HPLC equipped with are flective index detector (Agilent 1260 series, USA) and a HPX-87H column (300×7.8mm, Bio-Rad, USA). Running temperature was 65℃ and 5mM sulfuric acid solution was used as a mobile phase at a flow rate of 0.6 mL/min. The injection volume was 25μL with a run time of 20 min. The cellulose and xylan contents were calculated from glucose and xylose contents multiplied by conversion factors of 0.90 and 0.88, respectively.

6. Eucalyptus bark pretreatment.

A 500 mg portion of eucalyptus bark was treated with 10g of ASF-ILs in a 100ml flask at 120℃ for 10 h. The mixture of eucalyptus bark and ASF-ILs was stirred with a magnetic stirrer and the reaction temperature was controlled using a silicone oil bath. Then the flask was allowed to cool to room temperature after pretreatment. Hot water (40 mL) was slowly added to the eucalyptus bark/IL slurry with continued stirring. The mixturewas transferred to 50 mL falcon tubes and centrifuged at high speed (8000 rpm) to separate the solid. The solid was washed five times with 40 mL of hot water to make sure the ionic liquid was essentially removed. The regenerated eucalyptus barkwas dried at 95 °C for 24 h before carryingout enzymatic hydrolysis reaction and characterizations.

7. Enzymatic hydrolysis

All enzymatic hydrolysis experiments were performed in the 50 ml stoppered conical flask at 50°C placed on a rotary shaker at 160 rpm. A 7 mL citrate buffer (50 mM, pH 4.8) containing cellulase in 30 FPU/ g glucan and 20 mg (dry weight) biomass were added. Aliquots (200 μL) of samples were withdrawn at specified time intervals, and treated in boiling water for 5 min to stop the enzymatic reaction. All reactions were carried out induplicate.

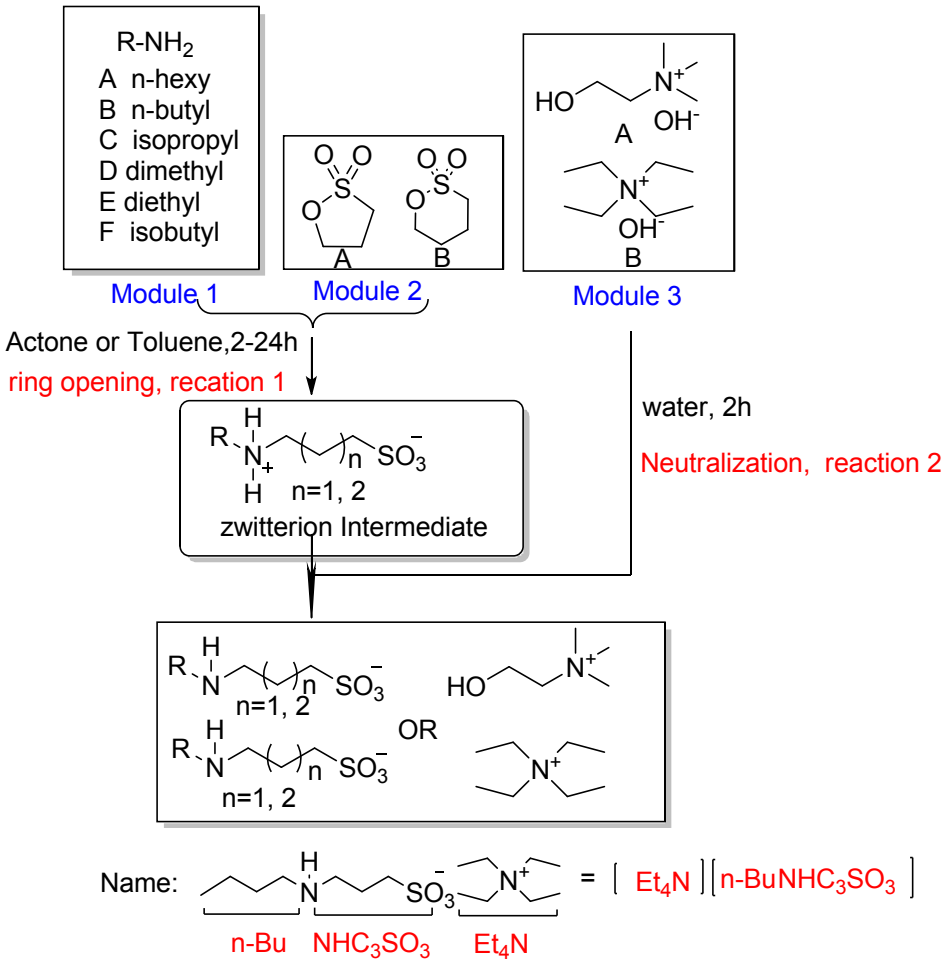

Figure S1 Synthetic route and structures of amine-sulfonate functionalized ILs

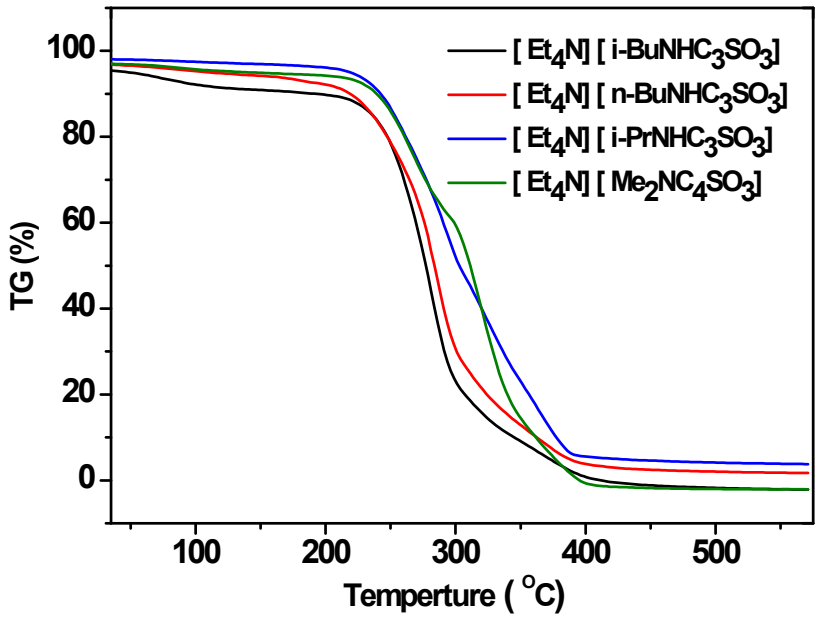

Figure S2 TG analysis for amine-sulfonate functionalized ionic liquids with [Et_4N] cation

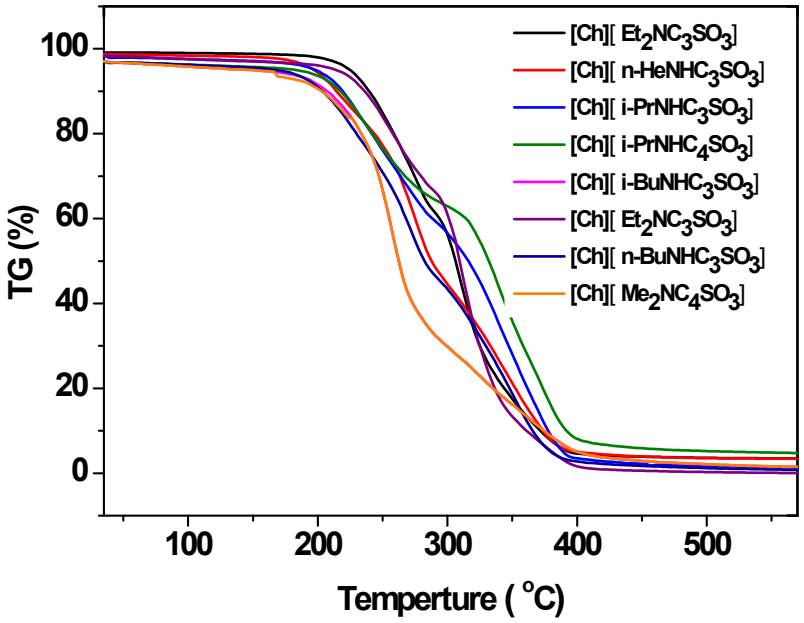

Figure S3 TG analysis for amine- sulfonate functionalized ionic liquids with [Ch] cation

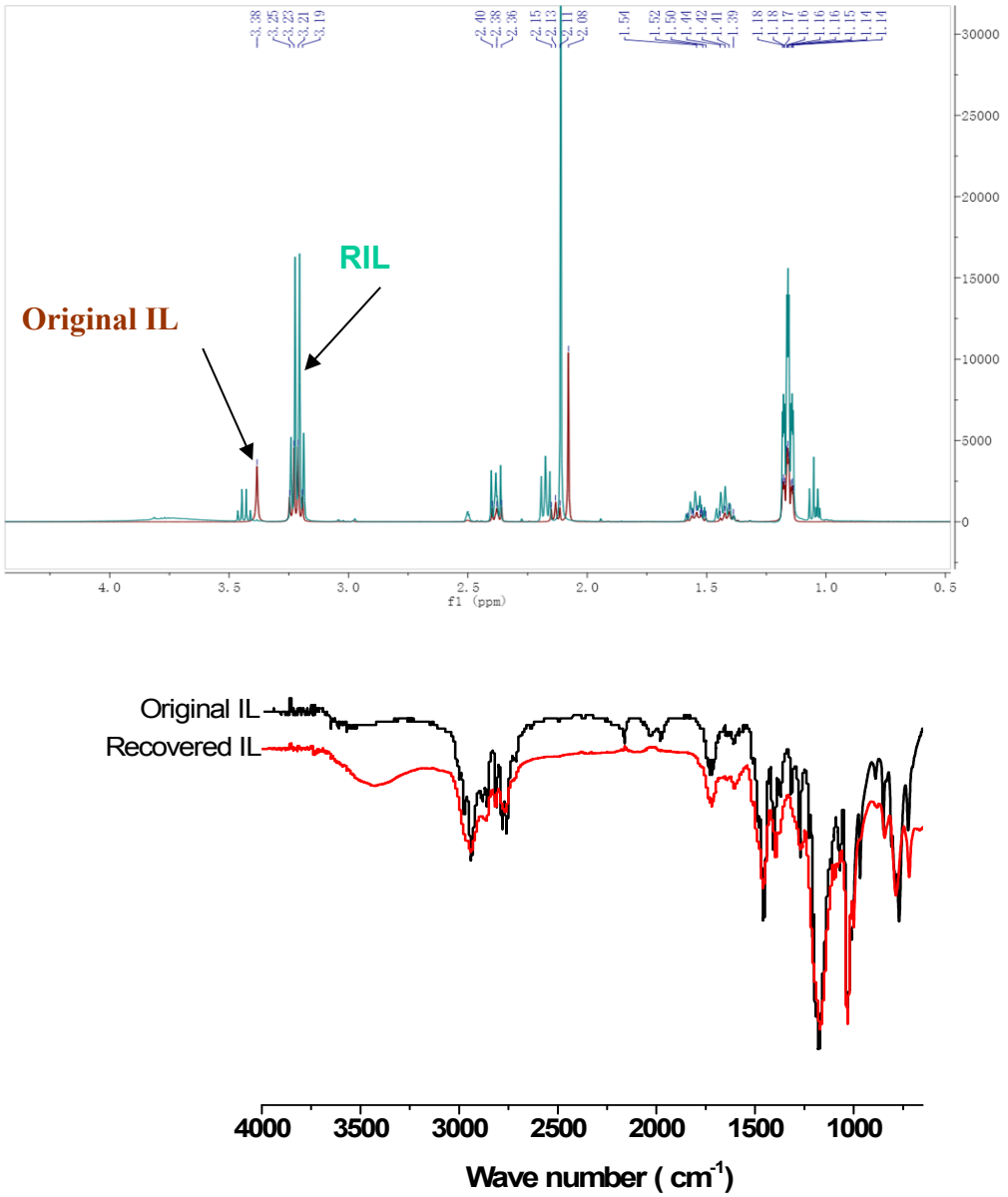

Figure S4 IR and NMR of original and recovered $[Et_4N][Me_2NC_4SO_3]$

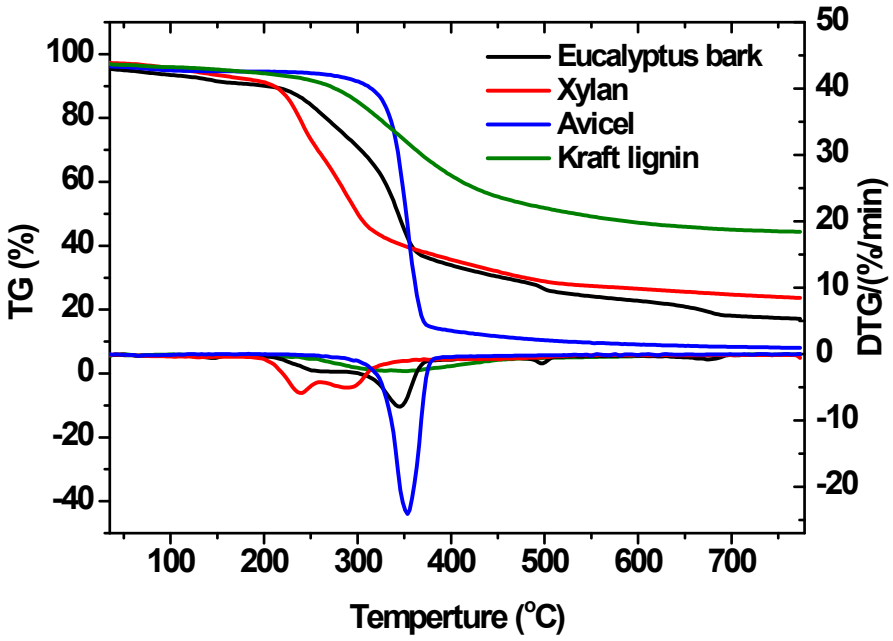

Figure S5 TGA thermo grams of avicel, kraft lignin, xylan from beech wood and eucalyptus bark

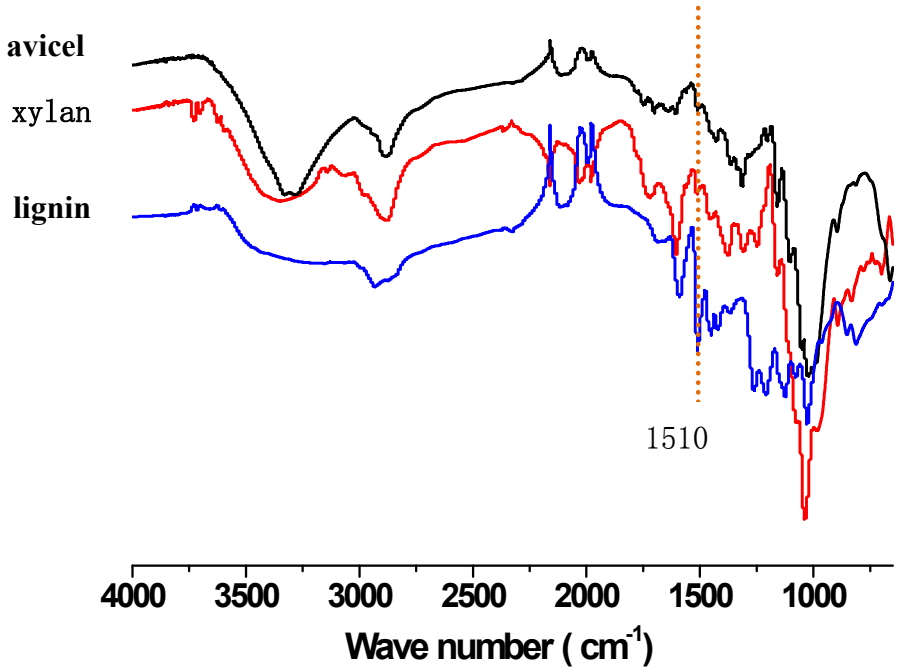

Figure S6 FTIR spectrum of cellulose (Avicel), xylan from beech wood and kraft lignin

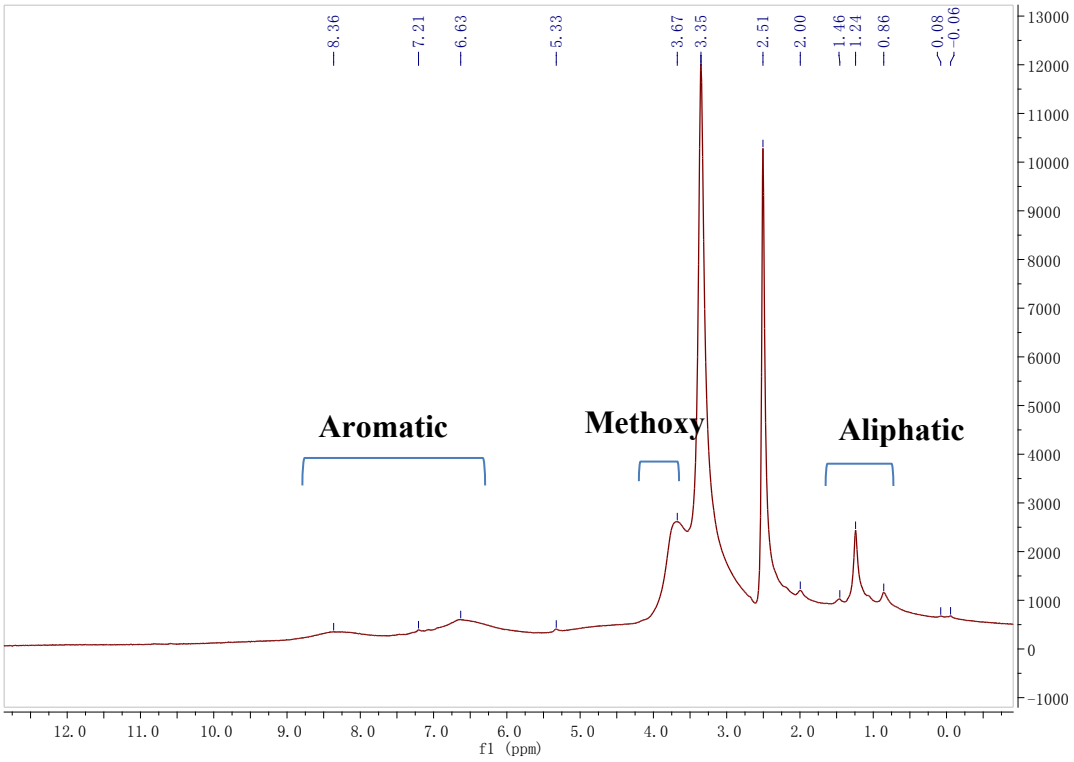

Figure S7 1H NMR spectrum of lignin extract part from eucalyptus bark

Table S1 Elemental analysis of kraft lignin and ligninsulfonate.

Name	N [%]	C [%]	H [%]	S [%]	O [%][a]
Kraft Lignin	1.12	63.61	5.73	1.99	27.55
Kraft Lignin -repeat	1.04	63.60	5.72	1.49	28.15
Ligninsulfonate	0.99	52.26	4.68	4.74	37.33
Ligninsulfonate-repeat	1.01	52.28	4.70	4.47	37.54

[a] O content was calculated by subtracting the C, H, N and S contents from 100%.

Table S2 The crystallinity of eucalyptus bark with and without ASF-ILs pretreatment.

	Raw eucalyptus bark	$[Et_4N][Me_2NC_4SO_3]$	$[Et_4N][i\text{-}PrNHC_3SO_3]$	$[Ch][Et_2NC_4SO_3]$	$[Ch][n\text{-}BuNHC_3SO_3]$
CrI (%)	32.11	45.82	41.54	43.31	38.23

Supporting References

S1 B. Adney and J. Baker, 2008, LAP, NREL/TP-510-42628.

S2 M. D. Soutullo, C. I. Odom, B. F. Wicker, C. N. Henderson, A. C. Stenson and J. H. Davis Jr., *Chem. Mater*., 2007, **19**, 3581.

S3 A. Sluiter, B. Hames, R. Ruiz, C. Scarlata, J. Sluiter, D. Templeton and D. Crocker, 2008, LAP, NREL/TP-510-42618.