Electronic Supplementary Information

An economically viable ionic liquid for the pretreatment of lignocellulosic biomass

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Experimental Details

***Fractionation of Biomass (also see Gschwend et al, 2016, JoVE*,** doi: 10.3791/54246)
An ionic liquid/water master-mix was prepared by adding 20 wt% of water to triethylammonium hydrogen sulfate followed by mixing until a colourless, homogenous, viscous solution was obtained. The water content was confirmed by Karl-Fischer titration in triplicate.

Figure S1 (see ESI) is a schematic representation of the ionoSolv process used for the pretreatments. 10±0.05 g of ionic liquid/water master-mix was weighed into a 15 ml glass pressure tube with silicone front seal (Ace Glass) and the exact weight recorded. Between 1.04 and 1.09 g of ground *Miscanthus* was added, the vials capped and the content mixed with a vortex shaker. The samples were then placed into a preheated convection oven (OMH60 Heratherm Advanced Protocol Oven). After the pretreatment period, they were taken out and immediately cooled under running water. Experiments were carried out in triplicate.

After the pretreatment, 40 mL of ethanol was added to the pretreatment mixture and the suspension transferred into a 50 mL centrifuge tube. The tube was shaken for one minute and the mixture then left at room temperature for at least 1 hour. The tube was mixed again for 30 seconds and then centrifuged at 4000 rpm for 50 minutes. The supernatant was decanted carefully into a round bottom flask. The washing step was repeated three more times. The remaining pulp was then transferred into a cellulose thimble and further washed by Soxhlet extraction with refluxing ethanol (150 mL) for 22 hours. The thimbles were then left on the bench overnight to dry. The ethanol used for the Soxhlet extraction was combined with the previous washes and evaporated under reduced pressure at 40°C, leaving the dried ionic liquid/lignin mixture. To the dried ionic liquid/lignin mixture, 30 mL of water was added in order to precipitate the lignin. The suspension was then transferred into a 50 mL falcon tube, shaken for one minute and then left at room temperature for at least 1 hour. The tube was centrifuged and the supernatant decanted and collected in a round bottom flask. This washing step was repeated twice more.

The air-dried pulp yield was determined by weighing the recovered biomass from the cellulose thimbles. The oven-dried yield was determined as described for the untreated biomass. The lid of the Falcon tube containing the lignin was pierced and the tube put into a vacuum oven overnight to dry at 40°C under vacuum. The dried lignin was weighed the next day.

Compositional Analysis

200-300 mg of air-dry biomass or recovered biomass was weighed out into a pressure tube and the weight recorded. 3 mL of 72% sulfuric acid was added, the samples stirred with a Teflon stir rod and the pressure tubes placed into a preheated water bath at 30°C. The samples were stirred every 15 min for one hour, they were then diluted with 84 mL distilled water and the lids closed. The samples were autoclaved (Sanyo Labo Autoclave ML5 3020 U) for 1 h at 121°C and left to cool to close to ambient temperature. The samples were then filtered through filtering ceramic crucibles of a known weight. The filtrate was filled in two 50 ml centrifuge tubes and the remaining black solid washed with distilled water. The crucibles were placed into a convection oven (VWR Venti-Line 115) at 105°C for 24±2 h. They were then taken out and placed in a desiccator for 15 min before they were weighed and the weight recorded. They were then placed into a muffle oven (Nabertherm + controller P 330) and ashed to constant weight at 575°C. The weight after ashing was recorded. The content of acid insoluble lignin (AIL) was determined according to equation 1. The content of one of the Falcon tubes was used for the determination of acid soluble lignin content (ASL) by UV analysis at 240 nm (equation 2) (Perkin Elmer Lambda 650 UV/Vis spectrometer).

$$\%AIL = \frac{Weight_{crucible + AIR} - Weight_{crucible + ash}}{ODW_{sample}} \cdot 100 \qquad \text{(eq. 1)}$$

$$\%ASL = \frac{A}{l \cdot \varepsilon \cdot c} \cdot 100 = \frac{A \cdot V_{filtrate}}{l \cdot \varepsilon \cdot ODW_{sample}} \cdot 100 \qquad \text{(eq. 2)}$$

where $Weight_{crucibles+AIR}$ is the weight of the oven-dried crucibles plus the acid insoluble residue, $Weight_{crucibles+ash}$ is the weight of the crucibles after ashing to constant temperature at 575°C, A is the absorbance at 240 nm, l is the pathlength of the cuvette in cm (1 cm in this case), ε is the extinction coefficient (12 L/g cm), c is the concentration in mg/mL, ODW is the oven-dried weight of the sample in mg and $V_{filtrate}$ is the volume of the filtrate in mL and equal to 86.73 mL.

To the contents of the other Falcon tube calcium carbonate was added until the pH reached 5. The liquid was passed through a 0.2 µm PTFE syringe filter and subsequently submitted to HPLC analysis (Shimadzu, Aminex HPX-87P from Bio-Rad, 300 x 7.8 mm, purified water as mobile phase at 0.6 ml/min, column temperature 85°C) for the determination of total sugar content. Calibration standards with concentrations of 0.1, 1, 2 and 4 mg/mL of glucose, xylose, mannose, arabinose and galactose were used. Sugar recovery standards were made as 10 mL aqueous solutions close to the expected sugar concentration of the samples and transferred to pressure tubes. 278 µL 72% sulfuric acid was added, the pressure tube closed and autoclaved and the sugar content determined as described above. The sugar recovery coefficient (SRC) was determined according to equation 3 and the sugar content of the analysed sample using equation 4:

$$SRC = \frac{c_{HPLC} \cdot V}{initial\ weight} \qquad \text{(eq. 3)}$$

$$\%Sugar = \frac{c_{HPLC} \cdot V \cdot corr_{anhydro}}{SRC \cdot ODW_{sample}} \cdot 100 \quad \text{(eq. 4)}$$

where c_{HPLC} is the sugar concentration detected by HPLC, V is the initial volume of the solution in mL (10.00 mL for the sugar recovery standards and 86.73 mL for the samples), initial weight is the mass of the sugars weighed in, $corr_{anhydro}$ is the correction for the mass increase during hydrolysis of polymeric sugars (0.90 for C_6 sugars glucose, galactose and mannose and 0.88 for C_5 sugars xylose and arabinose) and ODW is the oven-dried weight of the sample in mg.

Saccharification Assay

100±10 mg (calculated on an ODW basis) air-dried biomass was placed into a Sterilin tube and the weight recorded. Three blanks were run with 100 µL of purified water in order to correct for sugar residues present in the enzyme solutions. 9.9 mL solution made from 5 mL 1M sodium citrate buffer at pH 4.8, 40 µL tetracycline solution (10 mg/mL in 70% ethanol), 30 µL cycloheximide solution (10 mg/mL in purified water), 4.71 mL purified water, 60 µL cellulase from *Trichoderma reesei* ATCC 26921 solution and 60 µL cellobiase from *Aspergillus niger* solution was added, the tubes closed and placed into an Stuart Orbital Incubator (S1500) at 50°C and 250 rpm.

Time point samples were taken after 4, 18, 48, and 96 hours and an end point sample after 168 hours. For time point samples, 500 µL of the saccharification mixture was removed from the Sterilin tubes using a 1 ml adjustable pipette with the tip cut-off (representative amount of solids and liquids) and transferred to a microcentrifuge tube. The samples were centrifuged in a table top centrifuge at 4°C and 13.3 G for 10 min. The supernatant was pipetted into another microcentrifuge tube and frozen until analysis. Prior to analysis, samples in the tubes were shaken with a vortex shaker and centrifuged once more at 4°C and 13.3 G for 5 min and then transferred into HPLC vials. End point samples were obtained by filtering 1 mL of the saccharification mixture though a PTFE syringe filter. Samples were analysed on Shimadzu HPLC system with RI detector and an Aminex HPX-87P column (BioRad, 300 x 7.8 mm) with purified water as mobile phase (0.6 mL/min). The column temperature was 85°C and acquisition time was 40 min. Calibration standards with concentrations of 0.1, 1, 2 and 4 mg/mL of glucose, xylose, mannose, arabinose and galactose and 8 mg/mL of glucose were used.

Raw Spectra and Numerical Values

Table S1: Pulp yield and composition as well as lignin precipitate yield after pretreatment at 120 °C at a 1:10 solid:liquid ratio (80% [TEA][HSO_4] with 20% water).

	Pulp Composition							Total pulp yield	Lignin Precip. yield
	Glucan	Xylan	Arabinan	AI lignin	AS lignin	Ash	Extractives		
Untreated *Miscanthus*	47.3 ±0.8	20.1±0.5	2.2±0.5	22.4±0.1	4.0±0.1	0.6±0.4	2.8±0.1	100	N/A
1 h	42.7±0.1	13.6±0.1	-	12.1±0.1	4.9±0.1	0.2±0.1	N/A	73.5±0.2	7.1±0.1
2 h	45.0±0.0	10.8±0.1	-	7.4±0.7	1.5±0.0	-	N/A	64.8±0.5	10.5±0.0
4 h	45.8±0.2	6.6±0.1	-	3.8±0.2	2.0±0.0	0.5±0.0	N/A	58.8±0.4	15.4±0.1
8 h	42.6±0.6	4.9±0.0	-	3.2±0.3	0.6±0.0	-	N/A	51.3±0.8	19.8±0.7
12 h	43.1±0.2	3.5±0.6	-	3.8±0.2	0.6±0.0	-	N/A	51.0±0.7	20.8±2.9
16 h	40.8±0.5	2.7±0.5	-	3.22±0.7	1.1±0.0	0.2±0.0	N/A	48.0±0.6	21.5±2.0
24 h	42.2±0.4	-	-	3.41±0.0	0.8±0.0	0.1±0.0	N/A	46.4±0.4	19.1±0.2

N/A: not applicable; - not detected; standard deviation calculated for triplicate; AI: acid insoluble; AS: acid soluble

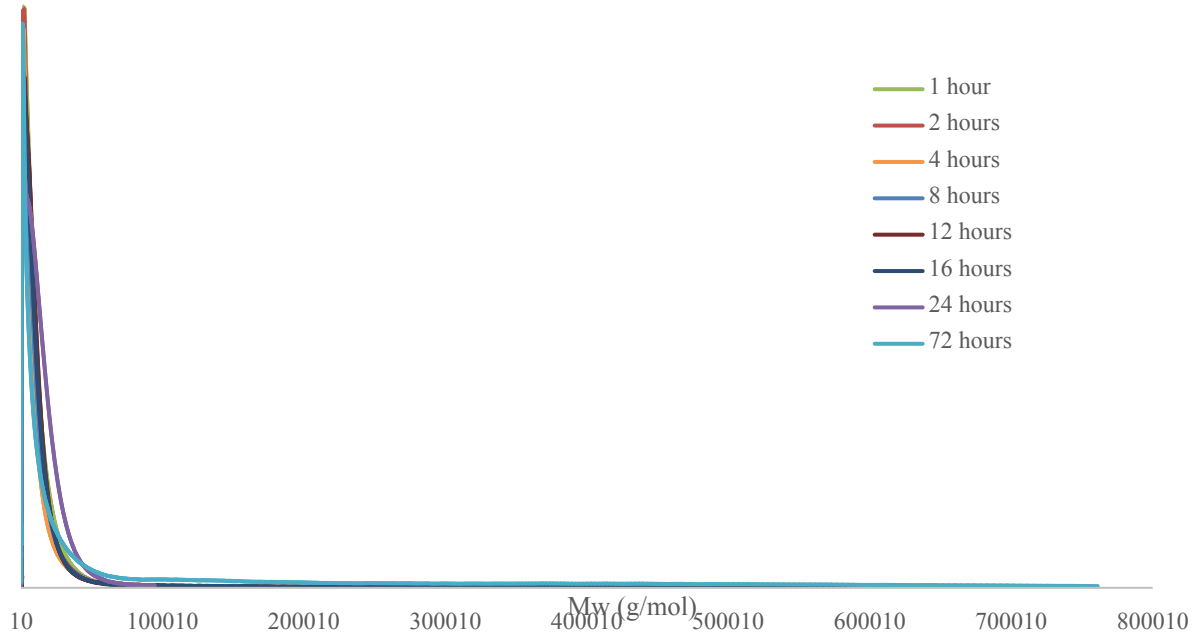

Figure S1: GPC profiles of isolated lignin (time course).

Table S2: Molecular weight parameters of time course lignin (1:1 acid base ratio) determined by GPC

Hours of pretreatment	Mw	Mn	PDI
1	4587	158	2.90
2	3921	1316	2.99
4	3492	1040	3.36
8	3770	1033	3.65
12	4209	1226	3.43
16	4226	1181	3.59
24	5359	1263	4.24
72	5421	924	5.87

Mn: Average molecular weight, Mw number average weight, PDI: Polydispersity index

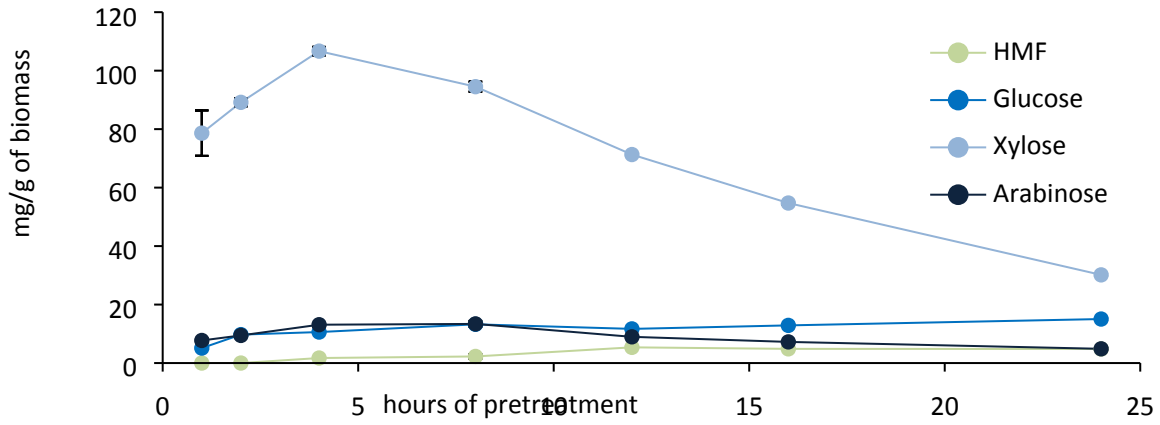

Figure S2: Concentration of solutes in the recycled ionic liquid solution after fractionation and drying. *Miscanthus* was pretreated at a 1:10 solids loading at 120 °C.

Table S3: Molecular weight parameters of time course lignin (excess acid)

Hours of pretreatment	Mw	Mn	PDI
0.5	7788	1772	4.40
1	4548	1336	3.40
2	3842	1228	3.00
4	5686	1306	4.35

Mn: Average molecular weight, Mw number average weight, PDI: Polydispersity index

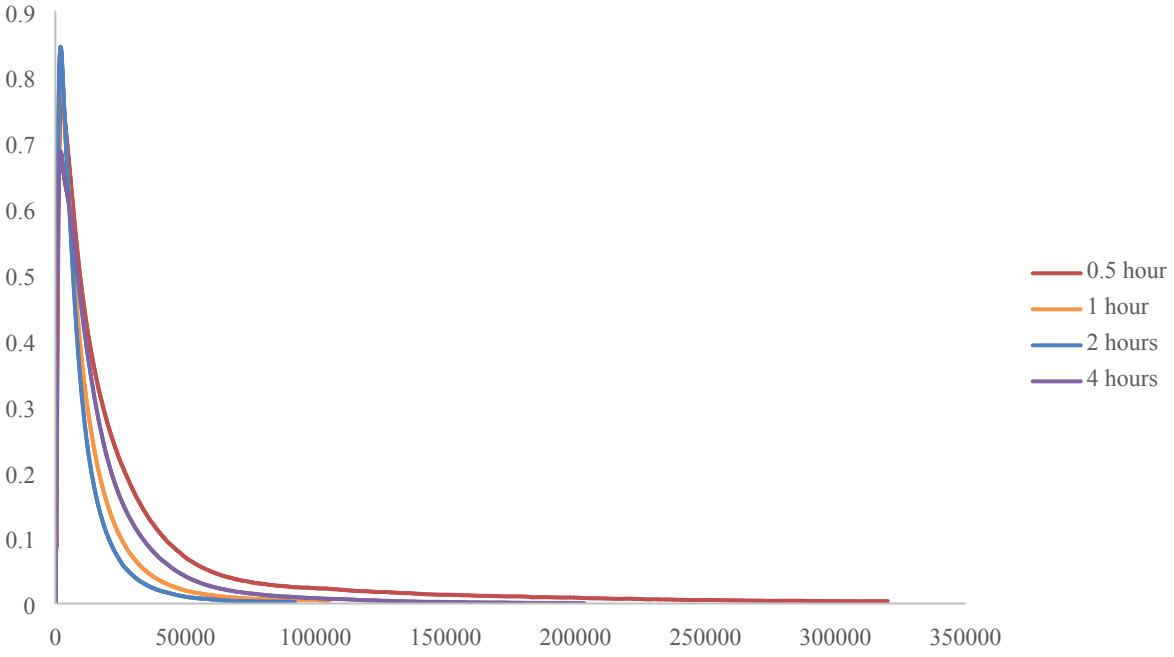

Figure S3: GPC profiles of lignins isolated from IL with excess acid

Table S4: Pulp yields and recovery of lignocellulose components in these pulps as determined by compositional analysis. Miscanthus was pretreated at a 1:10 solids loading in 80% [TEA][HSO4] with 20% water at 120 °C over multiple cycles.

	Pulp yield	Glucan	Xylan	Arabinan	AI lignin	AS lignin	Ash	Extract.	Mass balance [a]
Untreated *Miscanthus*	N/A	47.3 ±0.8	20.1±0.5	2.2±0.5	22.4±0.1	4.0±0.1	0.6±0.4	2.8±0.1	105.0±2.4
1st use	44.1 ±1.6	37.4 ±1.0	3.0 ±1.2	-	2.6 ±0.2	0.8±0.1	0.3±0.1	N/A	103.2±2.1
2nd use	46.5 ±1.4	39.6 ±1.6	2.6 ±0.6	-	3.2 ±0.1	0.7±0.0	0.3±0.1	N/A	104.0±0.3
3rd use	50.7 ±0.7	41.4 ±1.0	4.1 ±0.9	-	4.0 ±0.3	0.8±0.0	0.4±0.1	N/A	105.9±3.5
4th use	50.3 ±0.7	41.6 ±0.6	3.2 ±0.6	-	4.2 ±0.4	0.8±0.0	0.4±0.1	N/A	101.7±1.6

[a] Sum of compositional analysis mass fractions prior to normalisation, averaged over 3 samples; N/A: not applicable; -: not detected; standard deviation calculated for triplicate measurement, ash is water-insoluble inorganics only

Table S5: Change of IL solution acidity with recycling. *Miscanthus* was pretreated at a 1:10 solids loading in 80% [TEA][HSO_4] with 20% water at 120 °C over multiple cycles.

	pH[a]	H^+ concentration in IL[b]
Virgin IL	1.00 +/- 0.01	1.25 mmol/g
Recycled IL (4th use)	1.04 +/- 0.02	1.14 mmol/g

[a]diluted 1:10 in water, [b]ionic liquid solution (80 wt% IL + 20 wt% water)

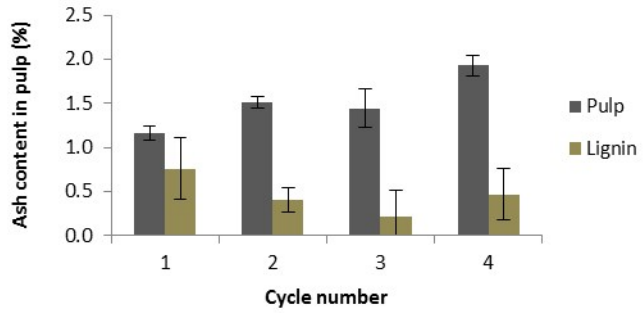

Figure S4: Ash content in recycled pulps and lignins determined by TGA. *Miscanthus* was pretreated at a 1:10 solids loading in 80% [TEA][HSO_4] with 20% water at 120 °C over multiple cycles.

Table S6: Elemental analysis of pulp and lignins isolated during IL recycling experiment. *Miscanthus* was pretreated at a 1:10 solids loading in [TEA][HSO_4] with 20% water at 120 °C.

	C%	H%	N%	S%
Pulp cycle 1	41.8±0.3	6.0±0.1	0.1±0.1	1.2±0.2
Pulp cycle 4	42.4±0.1	5.8±0.1	0.2±0.0	1.3±0.1
Lignin cycle 1	60.8±0.9	5.7±0.2	0.8±0.1	1.8±0.2
Lignin cycle 1 S*	60.1±0.1	6.0±0.1	0.5±0.0	1.0±0.1
Lignin cycle 4	62.7±0.1	5.4±0.0	0.5±0.0	1.0±0.1

C: carbon, H: hydrogen, N: nitrogen, S sulfur content, * lignin was Soxhlet-extracted with water overnight

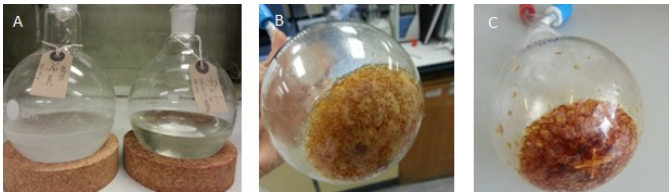

Figure S5: (A) [TEA][HSO_4] after synthesis (dry and with 20 wt% water); (B) after first use, (C) and after four uses. *Miscanthus* was pretreated at a 1:10 solids loading in 80% [TEA][HSO4] with 20% water at 120 °C over multiple cycles.

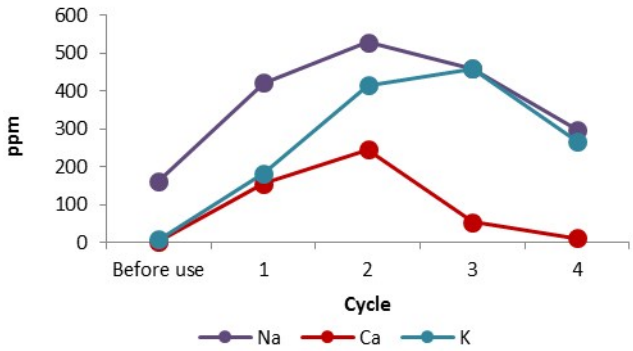

Figure S6: Abundance of selected inorganic cations commonly present in biomass, detected in the ionic liquid solution before pretreatment and after multiple reuses by ICP-OES. *Miscanthus* was pretreated at a 1:10 solids loading in 80% [TEA][HSO_4] with 20% water at 120 °C.

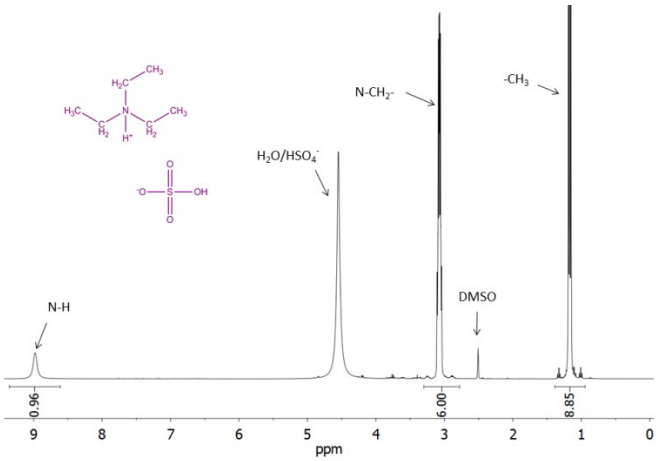

Figure S7: ^{1}H-NMR spectrum of an 80% [TEA][HSO_4] 20 wt% water solution after 4 uses in *Miscanthus* pretreatment at a 1:10 solids loading at 120 °C.

HSQC-NMR raw spectra:

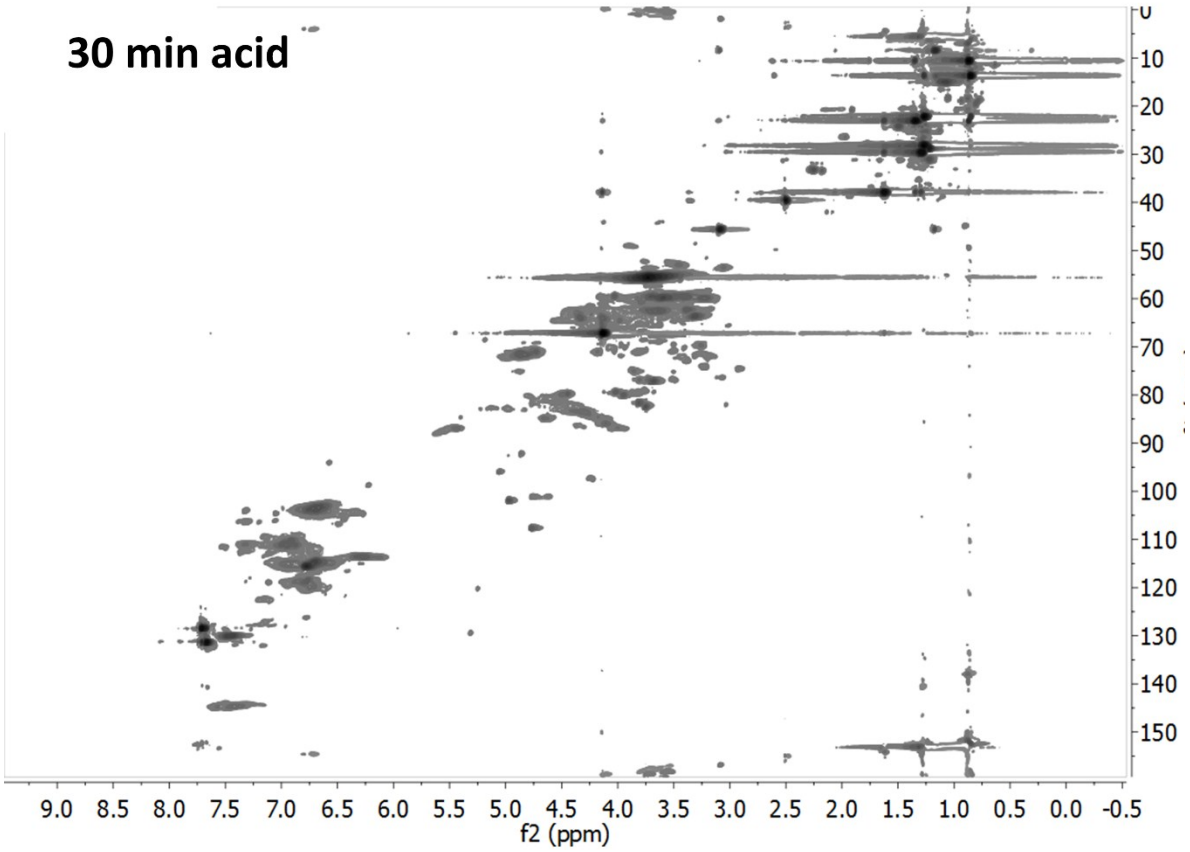

Figure S8: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution and excess acid for 30 min.

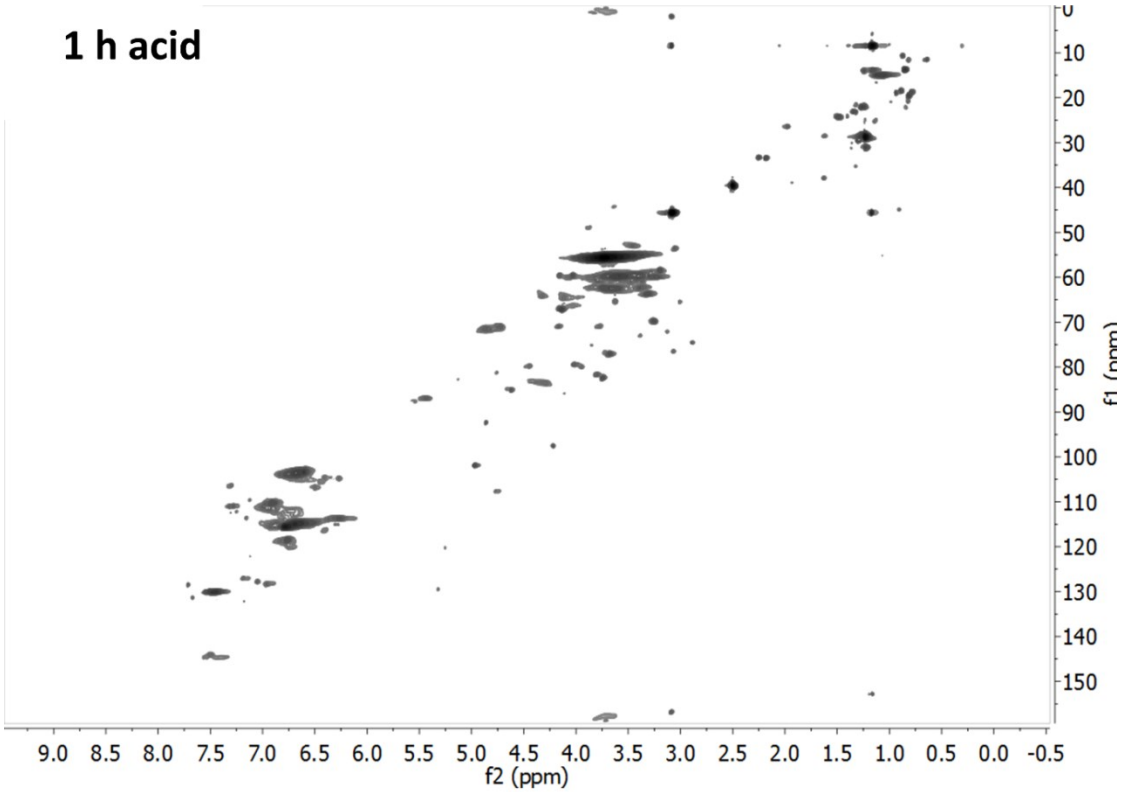

Figure S9: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution and excess acid for 1 h.

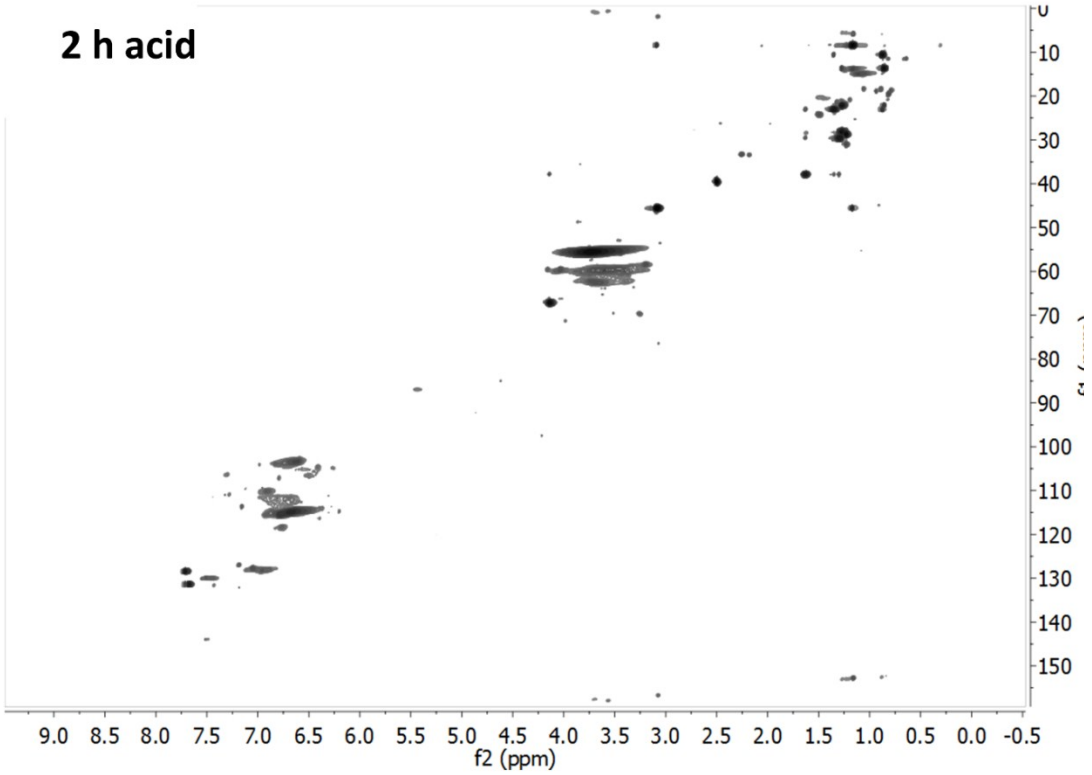

Figure S10: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution and excess acid for 2 h.

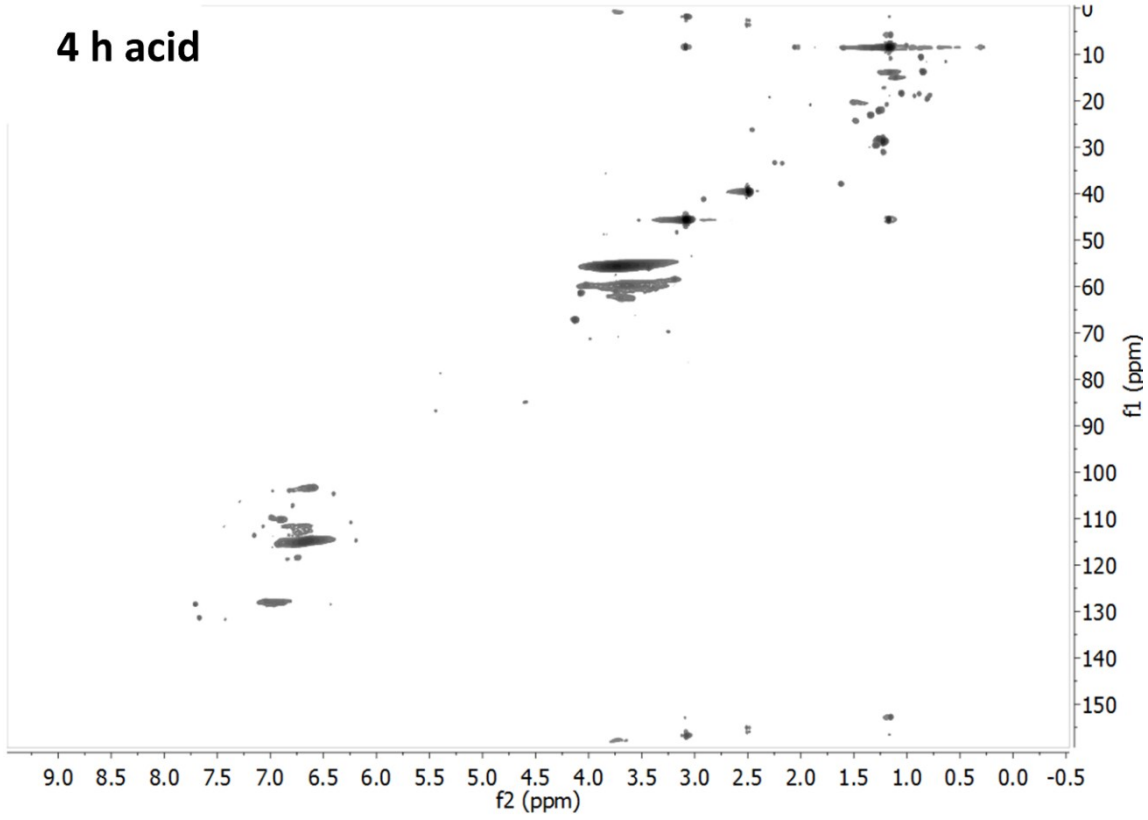

Figure S11: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution and excess acid for 4 h.

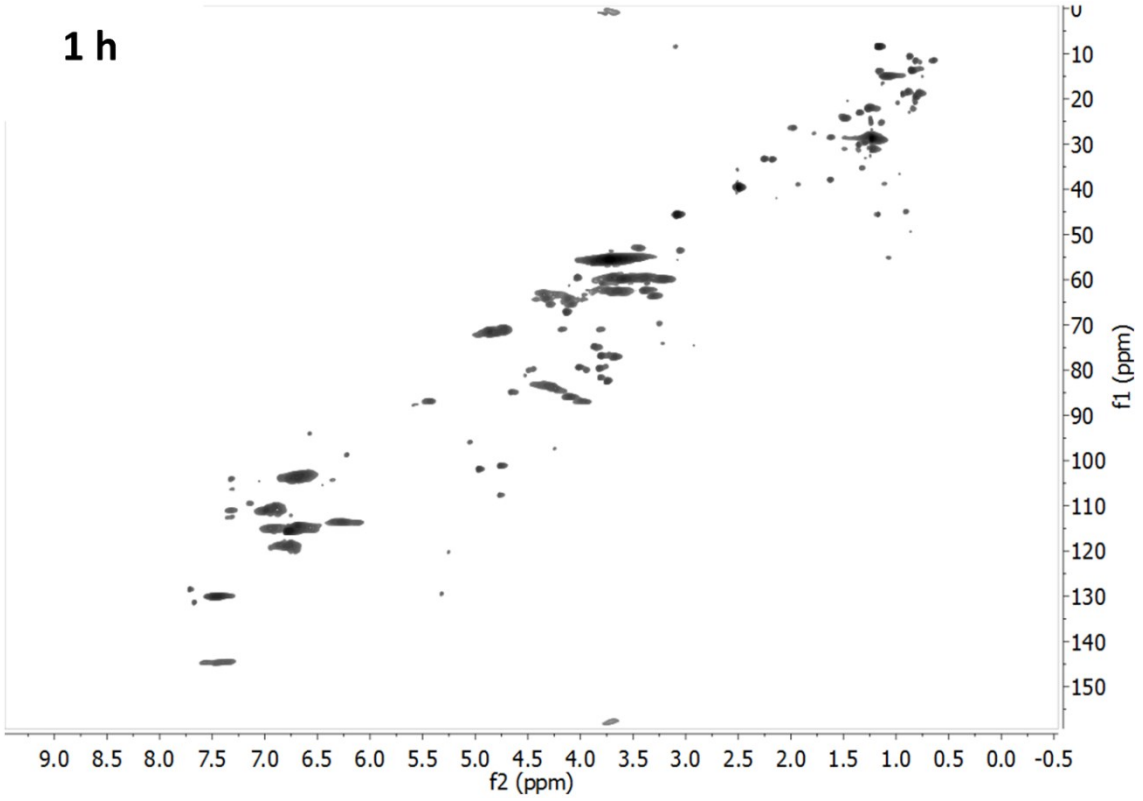

Figure S12: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution for 1 h.

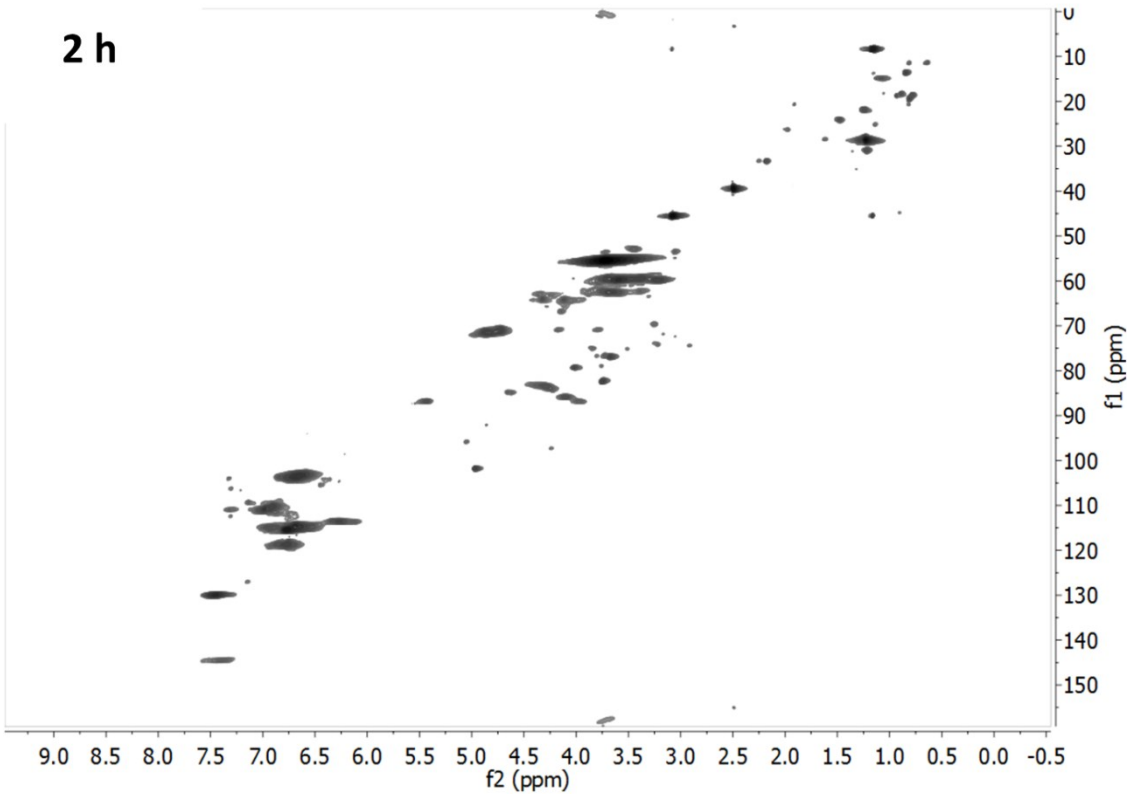

Figure S13: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution for 2 h.

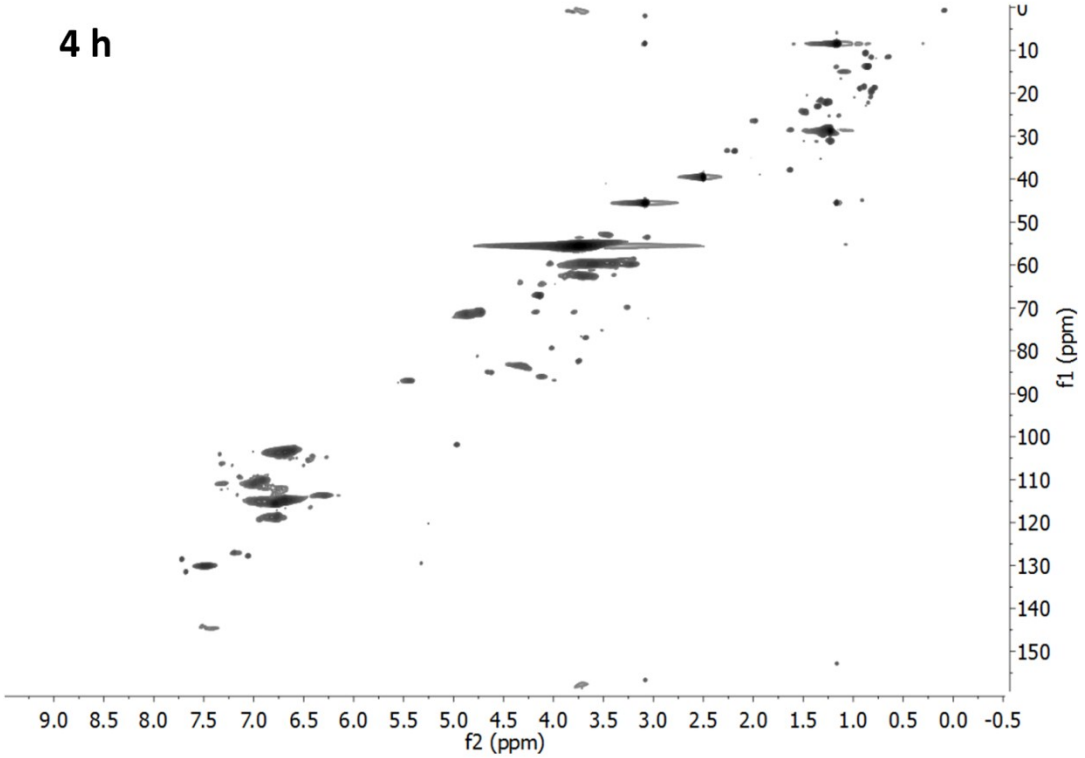

Figure S14: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution for 4 h.

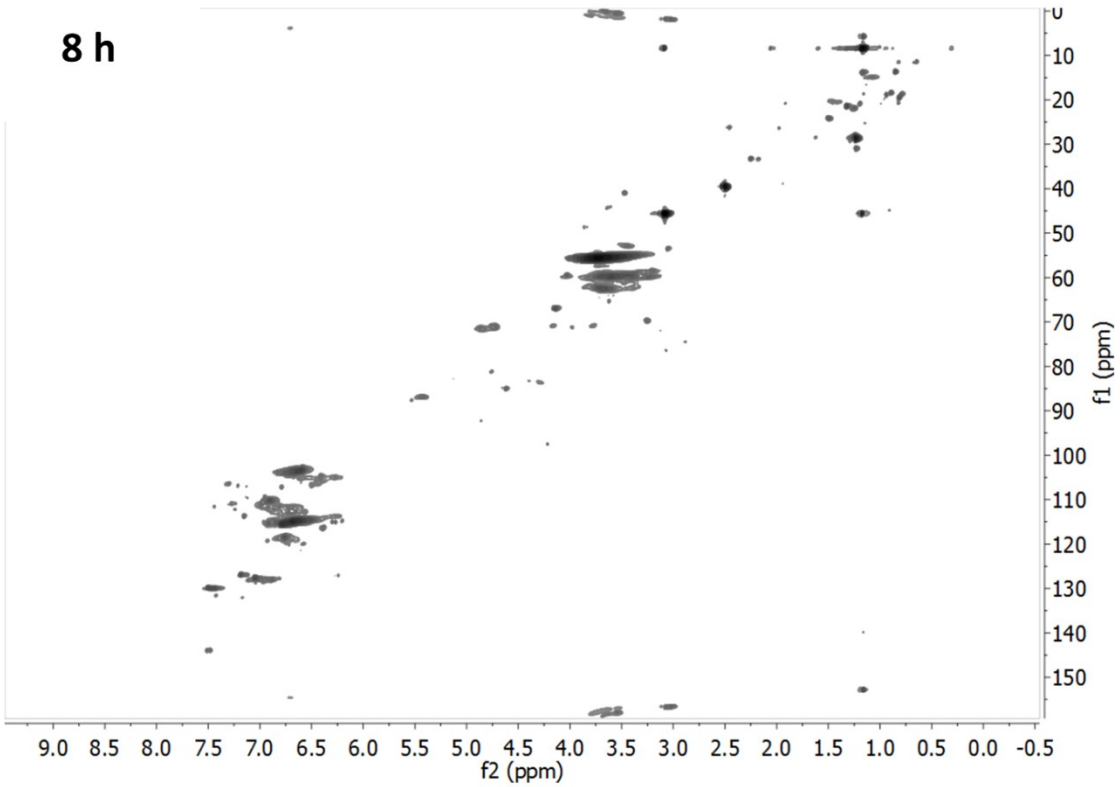

Figure S15: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution for 8 h.

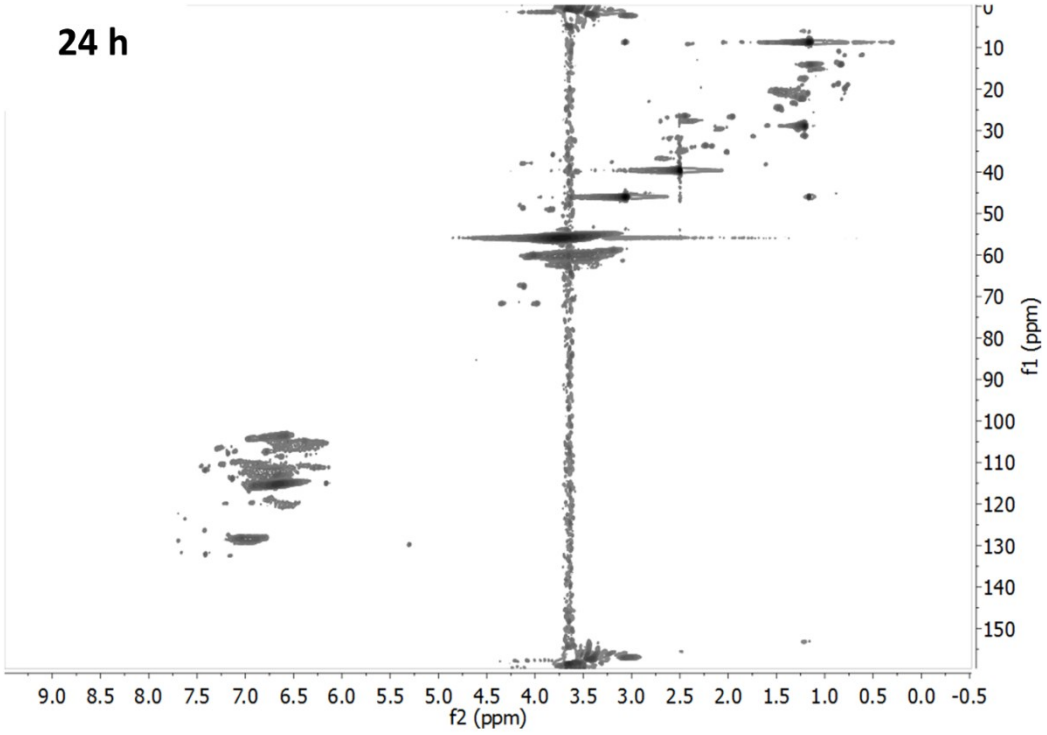

Figure S16: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution for 24 h.

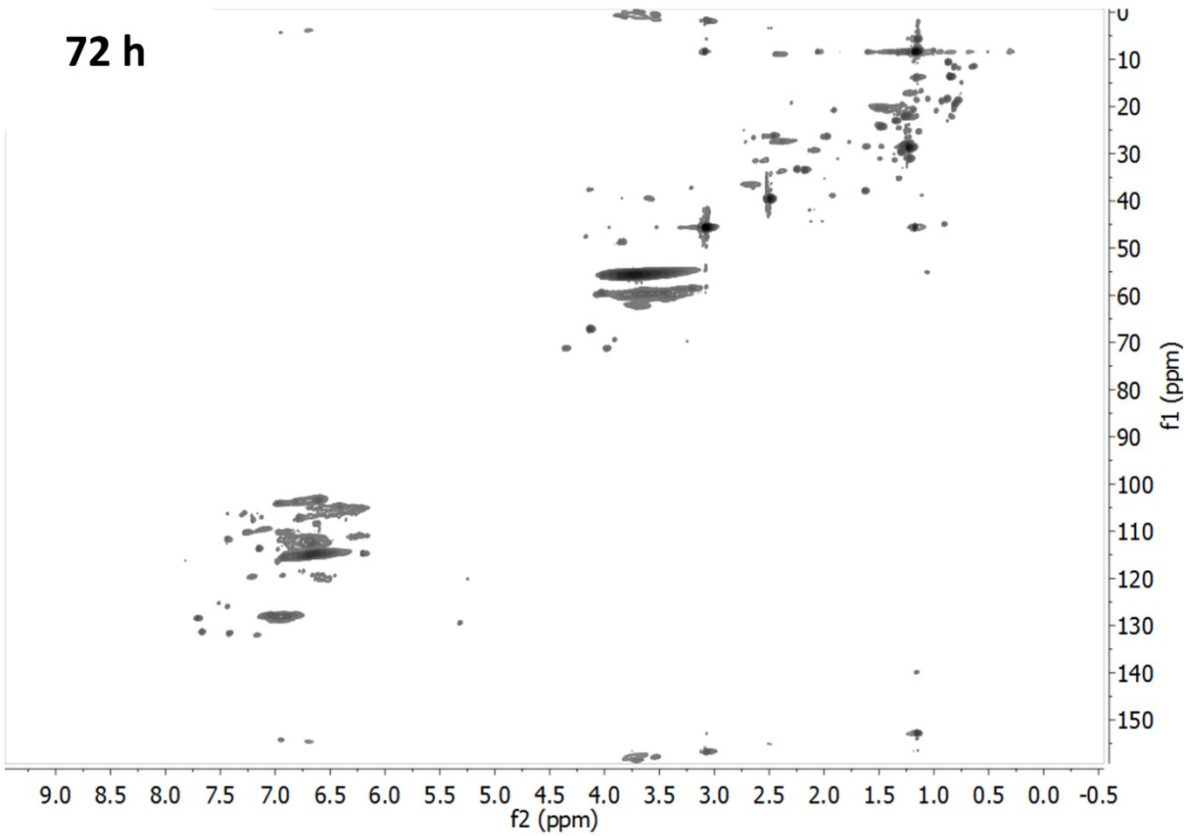

Figure S17: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution for 72 h.

Table S8: Volume integrals for side chain and aromatic peaks (time course study)

Peak	f2/ppm (1H)	f1/ppm (13C)	1h	2h	4h	8h	24 h	72 h	30 min	1h acid	2h acid	4h acid
beta-O-4	4.8	71.1	1.2	1.2	1.0	0.8	0.4	0.4	1.0	0.8	0.6	0.3
beta-beta	4.6	84.8	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1	0.1
beta-5	5.4	86.8	0.2	0.2	0.2	0.3	0.1	0.1	0.2	0.2	0.2	0.1
S	6.7	103.6	1.5	1.5	1.6	1.7	1.3	1.4	1.6	1.8	1.6	1.0
S cond.	6.4	105.4	0.3	0.4	0.5	1.0	1.5	1.8	0.3	0.8	1.1	1.0
G2	6.9	110.9	1.0	1.0	1.0	1.0	1.0	1.1	1.0	1.1	1.1	1.0
G2 cond.	6.7	112.1	0.3	0.4	0.6	1.1	1.9	2.2	0.4	0.8	1.3	1.3
G5	6.8	114.7	2.8	3.1	3.6	5.0	7.0	7.4	3.4	4.5	5.8	5.3
G6	6.8	119.0	0.7	0.7	0.7	0.7	0.5	0.6	0.7	0.6	0.6	0.5
H	6.9	127.9	0.2	0.2	0.4	1.1	1.9	2.4	0.2	0.6	1.4	1.4
PCA	7.5	129.9	0.8	0.7	0.6	0.4	0.1	0.1	0.9	0.8	0.4	0.1

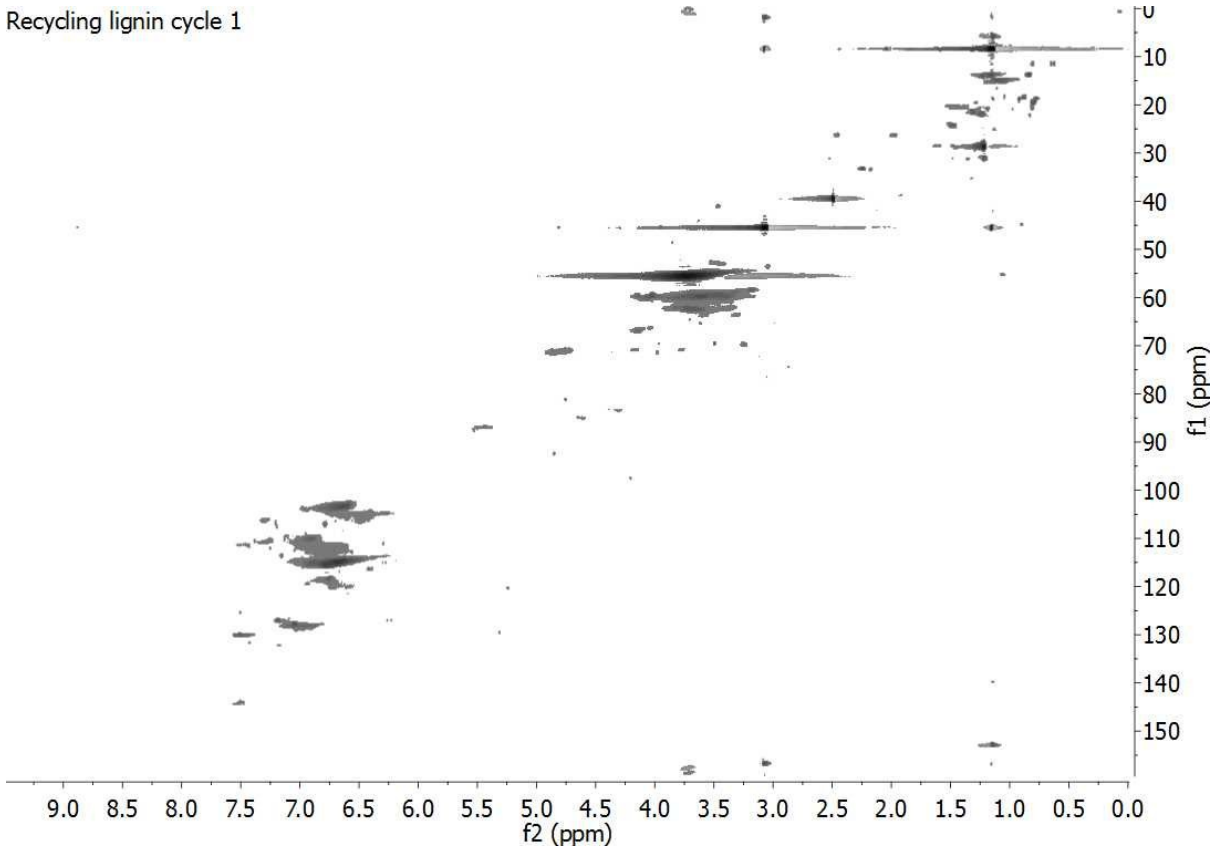

Figure S18: HSQC-NMR spectrum of lignin isolated using [TEA][HSO_4] with 20% water solution.

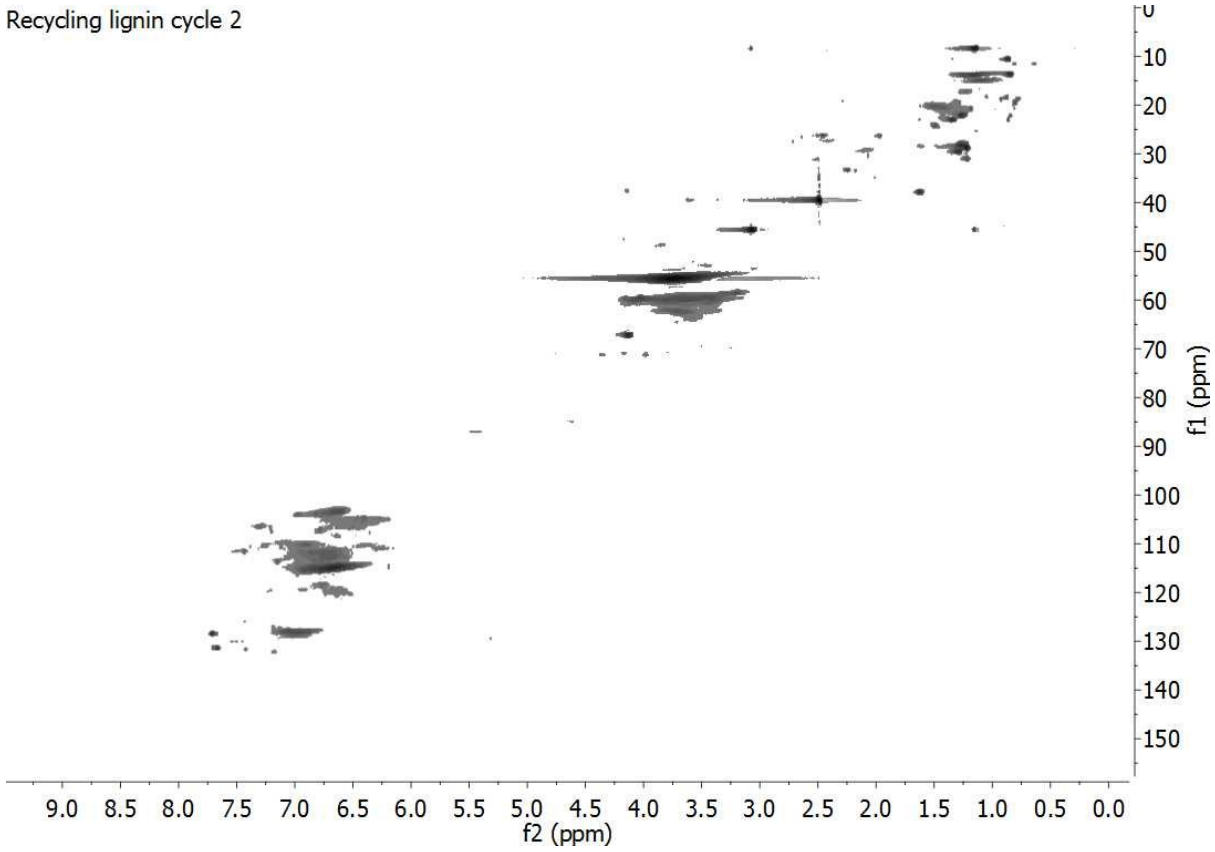

Figure S19: HSQC-NMR spectrum of lignin isolated using recycled [TEA][HSO_4] with 20% water solution.

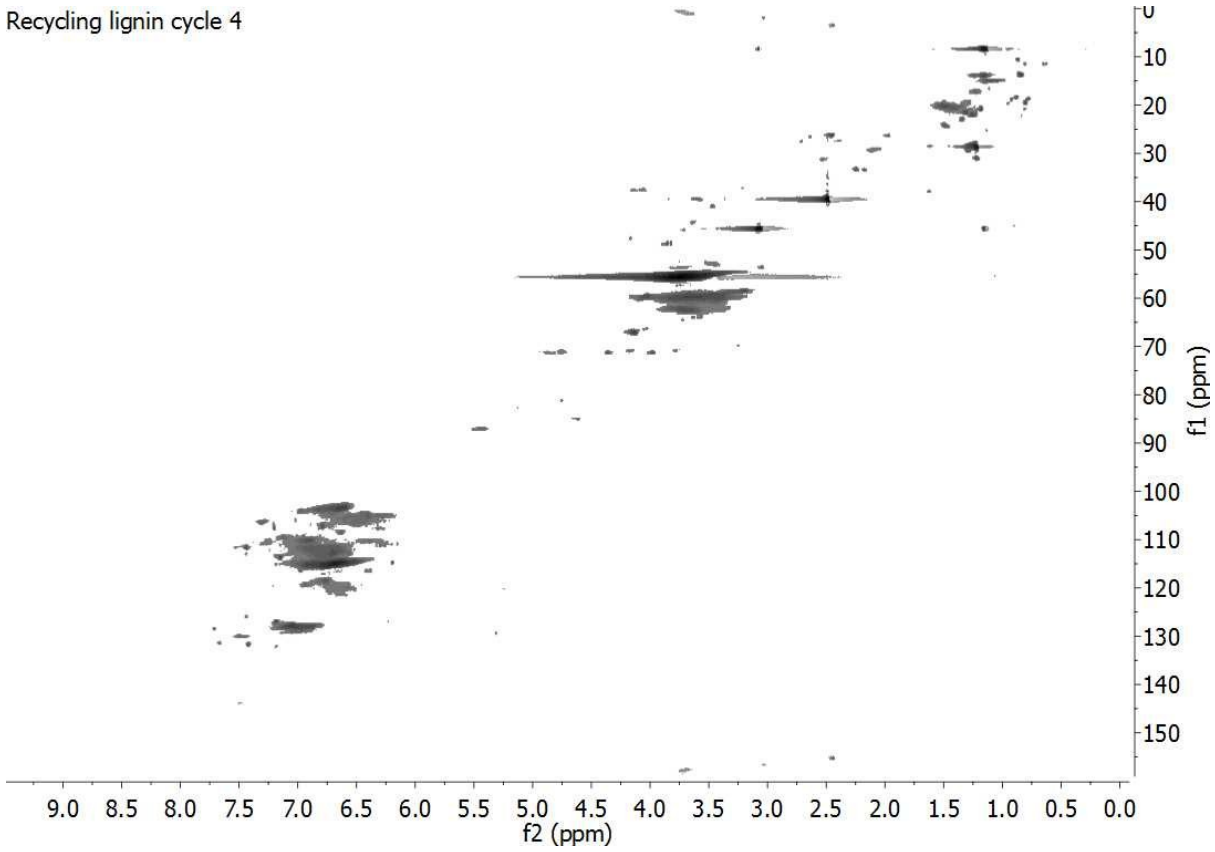

Figure S20: HSQC-NMR spectrum of lignin isolated using three times recycled [TEA][HSO_4] with 20% water solution.

Table S8: Mass Balance of Hemicelluloses, numerical data expressed as weight percentage of initial content in biomass.

Time (h)	In pulp	Dissolved Xyl	Dissolved Ara	Dissolved furfural	Unaccounted
0	100.00	0.00	0.00	0.00	0.00
1	62.52	29.68	3.59	1.33	2.88
2	49.67	37.84	4.28	5.66	2.55
4	30.31	40.38	5.12	12.49	11.71
8	22.27	30.57	4.26	20.54	22.36
12	15.89	24.90	4.02	29.11	26.08
16	12.55	13.05	2.66	27.82	43.91
24	0.00	10.18	1.78	31.42	56.62

Table S9: Mass Balance of Glucan, numerical data expressed as weight percentage of initial content in biomass.

Time (h)	In pulp	Dissolved Glu	Dissolved HMF	Unaccounted
0	100	0.00	0.00	0.00
1	89.46	0.58	0.00	9.96
2	94.33	1.15	0.03	4.48
4	96.11	1.97	0.30	1.61
8	89.33	2.01	0.43	8.23
12	90.36	2.33	0.56	6.76
16	85.47	1.55	0.58	12.40
24	84.56	2.14	1.00	12.30

Table S10: Mass Balance of Lignin, numerical data expressed as weight percentage of initial content in biomass.

Time (h)	In pulp	Precipitated lignin	Unaccounted
0	100.00	0.00	0.00
1	46.04	27.25	26.71
2	28.30	40.16	31.54
4	14.60	58.60	26.80
8	12.05	75.54	12.41
12	14.64	79.24	6.12
16	12.30	82.08	5.61
24	28.61	72.85	-1.47

X-ray photoelectron spectroscopy (XPS) analysis of acid-insoluble ash

Method: Analysis of the ash in pulp Ash samples were obtained as part of the compositional analysis. In brief, air-dried pulp was swollen and hydrolysed in concentrated and dilute sulfuric acid. The lignin and ash were separated from the solubilised carbohydrates by filtration, dried and the organics removed at 575°C in a muffle furnace for 48 h.

XPS was carried out using a Thermo K-alpha spectrometer utilising Al Kα radiation (1486.6 eV) and a quartz crystal monochromator set in a 250 mm Rowland circle. The X-ray spot was focussed at the sample to a size of 400 μm. The base pressure was 10^{-9}bar, and the analyser was a double focusing 180° hemisphere with mean radius 125 mm which was run in constant analyser energy mode. The pass energy was set to 200 eV for survey scans and 20 eV for high resolution regions. The detector was a 128 channel position sensitive detector. The energy scale of the instrument was regularly calibrated using a three point (Cu, Ag, Au) scale. Drops of the samples were placed directly onto a stainless steel plate. These were placed in a loadlock and the pressure reduced to 10^{-7} mbar by pumping down overnight. There was no significant outgassing or boiling of the samples during this process. After attaining the required pressure, samples were transferred to the analysis chamber. Charge compensation was achieved using a dual beam flood gun which applies both electrons and low energy Ar^{+} ions to the sample.

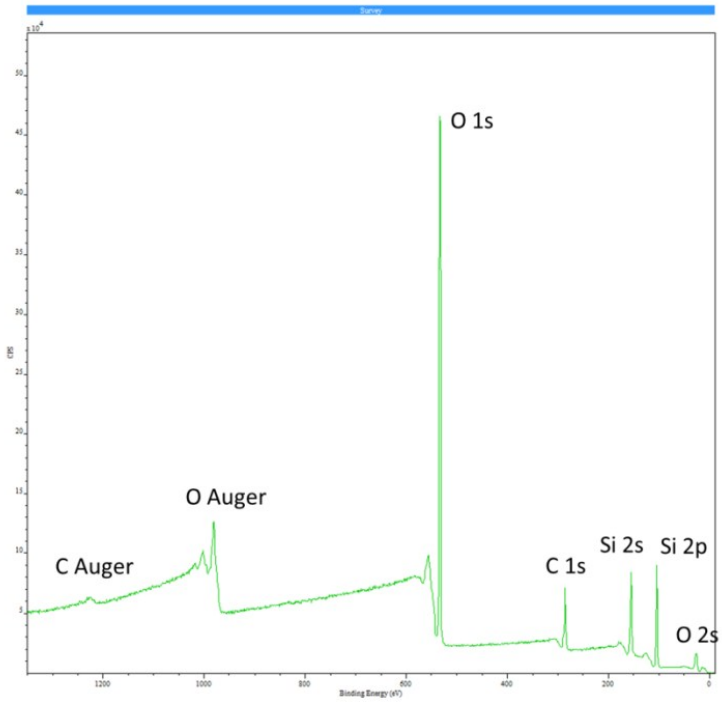

Figure S21: XPS spectrum of acid insoluble ash

The ash retained after compositional analysis was a white powder. We analysed the ash with X-ray photoelectron spectroscopy (XPS). It shows that the sample was composed of three elements: O, C and Si. The sample composition O:C:Si was ~3:1:1. As the sample was a solid, it is expected that the carbon signal is at least partly due to adventitious carbon contamination present at the outer surface. Therefore, the residue after compositional analysis was most likely SiO_2. The Miscanthus feedstock we used contains silicate as many grasses do, although in lower quantities. In addition, XRD measurements showed that the silica was amorphous. This was observed for all recycling ash samples.

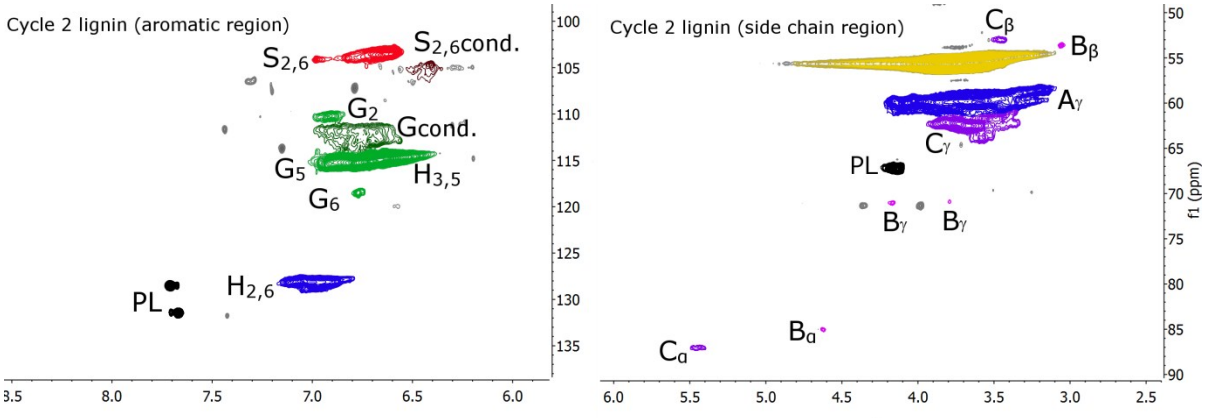

Figure S22: Annotated HSQC NMR spectrum of cycle 2 lignin isolated from the recycled [TEA][HSO_4] solution.

Economic Modelling

Preliminary economic models were made using the assumptions presented in Table 4. Flowsheet modelling was performed using Aspen Plus V8.0. Equipment sizing and cost estimates were made using Peters et al, *Plant Design and Economics for Chemical Engineers*, McGraw Hill, New York, 5th edition, 1988. All costs were converted to 2014 USD using the Chemical Engineering Plant Cost Index. Economic models assumed a 10-year fixed plant lifetime for depreciation, 330 days operation per year, 20% tax rate and a 13% interest rate.

Table S11: Economic estimates (Process considerations)

Assumptions	
Cost of biomass	$90/tonne
Price of cellulose pulp (biofuel grade)	$240/tonne
Price of lignin (heating value)	$130/tonne
Price of furfural	$1000/tonne
Price of acetic acid	$600/tonne
Solvent cost	$1.24/kg
IL recycling rate	0.5%
Capital cost for pretreatment	$118.5 mio
Plant size	770,000 tonnes per annum
Biomass loading	0.1:1, 0.3:1
Pulp yield	51.3%
Lignin yield	25%
Furfural yield	15%
Acetic acid yield	5%
Biomass not valorized	3.7%

Cellulose pulp	$123
Lignin	$49
Furfural	$150
acetic acid	$30
REVENUE	$353
Solvent	$16
Biomass	$90
Water	$6
Capital	$20
Energy	$49
COST	$181
NET	**$171**
GROSS MARGIN	49%