Electronic Supplementary Information

Quantitative Glucose Release from Softwood after Pretreatment with Low-cost Ionic Liquids

Florence J. V. Gschwend, Clementine L. Chambon, Marius Biedka, Agnieszka Brandt, Paul S. Fennell and Jason P. Hallett*

[a] Department of Chemical Engineering, Imperial College London, London, SW7 2AZ, UK

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 the hydrogen sulfate ionic liquids (1-hydrogen-3-butylimidazolium hydrogen sulfate [HBim][HSO_4], triethylammonium hydrogen sulfate [TEA][HSO_4] and *N,N*-dimethylbutylammonium hydrogen sulfate [DMBA][HSO_4]) followed by mixing until a homogenous solution was obtained. The water content was confirmed by Karl-Fischer titration in triplicate.

The required amount ±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 1.05 and 1.15 g of chopped pine was added for biomass to solvent ratios of 1:10 to 1:2 g/g. Between 0.55 and 0.60 g of chopped pine was added to 10±0.05 g of ionic liquid/water master-mix was used for the biomass to solvent ratio of 1:20 g/g. The vials were 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 left to cool at room temperature.

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

300 mg (on ODW basis) of air-dry biomass or pulp was weighed out into a 100 mL 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 again every 15 min for one hour, they were then diluted with 84 mL distilled water sealed. The samples were autoclaved (Sanyo Labo Autoclave ML5 3020 U) for one hour at 121°C and left to cool. The samples were then filtered through filtering ceramic crucibles of a known weight. The filtrate was stored in two plastic tubes and the remaining residue washed with distilled water. The crucibles were placed into a convection oven (VWR Venti-Line 115) at 105°C for 24±2 hours. They were placed in a desiccator for 15 min and the weight recorded. The crucibles were then placed into a muffle oven (Nabertherm + controller P 330) and ashed to constant weight at 575°C. The crucible weight after ashing was recorded. The content of acid insoluble lignin (AIL) was determined according to equation 1.

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

where $Weight_{crucibles\ plus\ AIR}$ is the weight of the oven-dried crucibles plus the acid insoluble residue, $Weight_{crucibles\ plus\ ash}$ is the weight of the crucibles after ashing to constant temperature at 575°C.

The supernatant was used for the determination of acid soluble lignin content (ASL) by UV analysis at 240 nm (equation 2) using aPerkin Elmer Lambda 650 UV/Vis spectrometer.

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

A is the absorbance at 240 nm, l is the path length 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.

Calcium carbonate was added to the second liquid fraction until pH 5 was reached. The liquid was passed through a 0.2 µm PTFE syringe filter and subsequently submitted to HPLC analysis (Shimadzu, Aminex HPX-97P from Bio-Rad, 300 x 7.8 mm, purified water as mobile phase at 0.6 ml/min, column temperature 85°C, de-ashing columns were used as pre-filters) 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 prepared 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:

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

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

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 the C6 sugars glucose, galactose and mannose and 0.88 for the C5 sugars xylose and arabinose) and ODW is the oven-dried weight of the sample in mg.

Saccharification Assay

100±10 mg (ODW basis) of air-dried was placed into a Sterilin tube and the weight recorded. Three enzyme only blanks were run with 100 µL of purified water instead of biomass in order to correct for sugar residues present in the enzyme solutions. 9.9 mL solution made from 5 mL 100mM 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 and 50 µL of Novozymes experimental enzyme mixture NS-22201 was added, the tubes closed and placed into an Stuart Orbital Incubator (S1500) at 50°C and 250 rpm.

For wet samples, moisture contents were determined again directly prior to saccharification. 100±10 mg (on and ODW basis) of air-dried or wet biomass were placed into a Sterilin tube and the weight recorded. Three blanks were run with 100 µL of purified water instead of biomass in order to correct for sugar residues present in the enzyme solutions. The water contained in the biomass sample was (calculated using its moisture content and the total sample mass) subtracted from 1.5 mL. The difference was added as water using a pipette. 8.4 mL solution consisting of 5 mL 1M sodium citrate buffer at pH 4.8, 40 µL tetracyline antibiotic solution (10 mg/mL in 70% ethanol), 30 µL cycloheximide antibiotic solution (10 mg/mL in purified water), 3.38-3.41 mL purified water and 20-50 µL of Novozymes experimental enzyme mixture NS-22201 (kindly provided directly by Novozymes) were added, the tubes closed and placed into an Stuart Orbital Incubator (S1500) for 7 days at 50°C and 250 rpm.

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.

Additional Spectra and Numerical Values

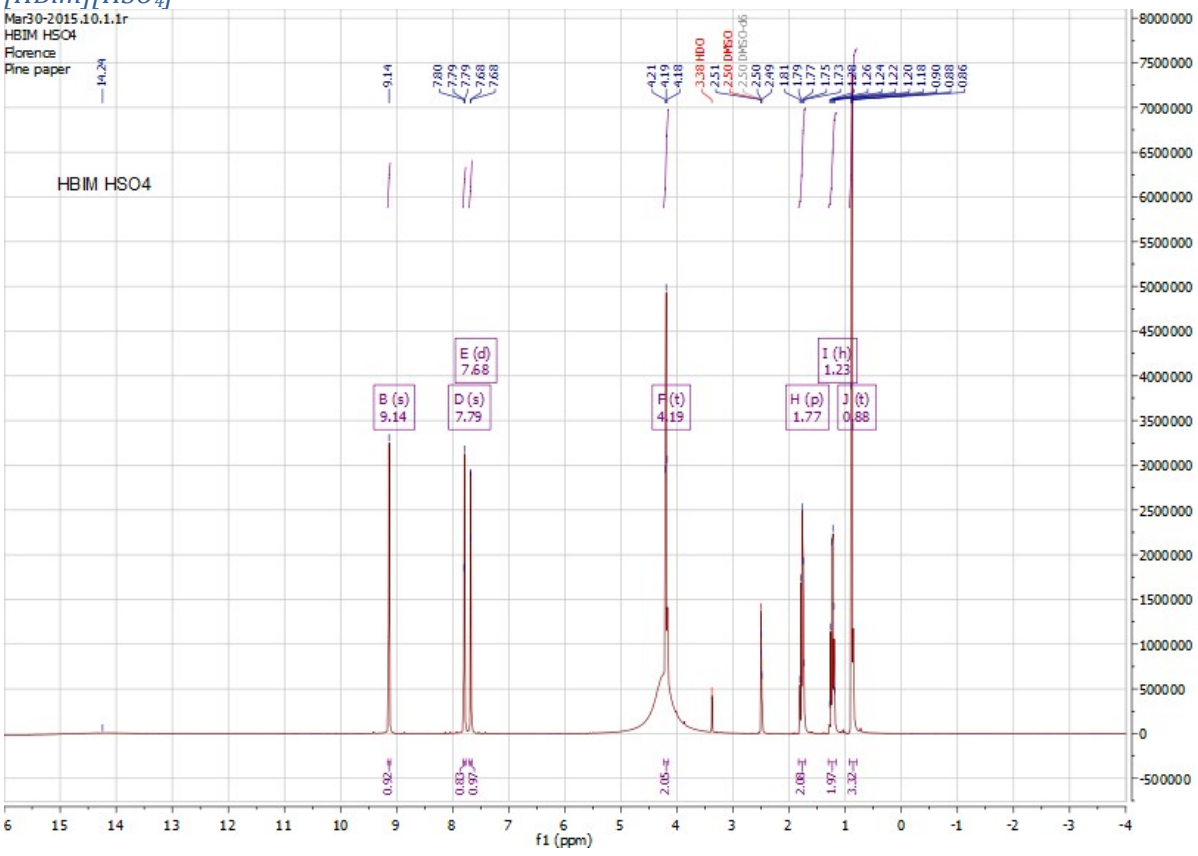
Table S1 Composition of pine pulp as determined by compositional analysis, as well as lignin yield, after pretreatment with [HBim][HSO_4] with a biomass to solvent ratio of 1:10 g/g and a final water content of 20wt%.

T	t	Glucan	Hemicelluloses[a]	Lignin[b]	Ash	Extractives	Mass loss[c]	Lignin precip.[d]
Untreated pine		43.4	19.1	32.2	0.8	4.46	0.0	N/A
120°C	1 h	40.3	9.3	22.2	0.0	N/A	28.2	3.9
120°C	4 h	38.6	4.6	14.9	0.1	N/A	41.8	9.6
120°C	8 h	37.1	2.9	12.5	0.0	N/A	47.5	13.8
150°C	30 min	40.3	8.3	15.8	0.0	N/A	35.6	8.2
150°C	1 h	39.2	4.0	6.5	0.0	N/A	50.3	16.9
150°C	2 h	34.7	3.0	6.4	0.0	N/A	55.9	24.4
150°C	4 h	28.1	0.5	11.0	0.0	N/A	60.4	23.1
150°C	8 h	17.0	0.5	20.8	0.0	N/A	61.7	17.4
150°C	16 h	4.9	0.5	28.8	0.0	N/A	65.8	11.5
170°C	15 min	38.8	6.2	20.2	0.0	N/A	27.9	6.9
170°C	30 min	37.5	1.6	7.3	0.0	N/A	53.6	18.3
170°C	45 min	28.2	0.0	6.5	0.1	N/A	65.1	27.5
170°C	1 h	24.2	0.3	9.1	0.0	N/A	66.4	31.6
170°C	4 h	0.1	0.4	27.0	0.0	N/A	72.5	18.1

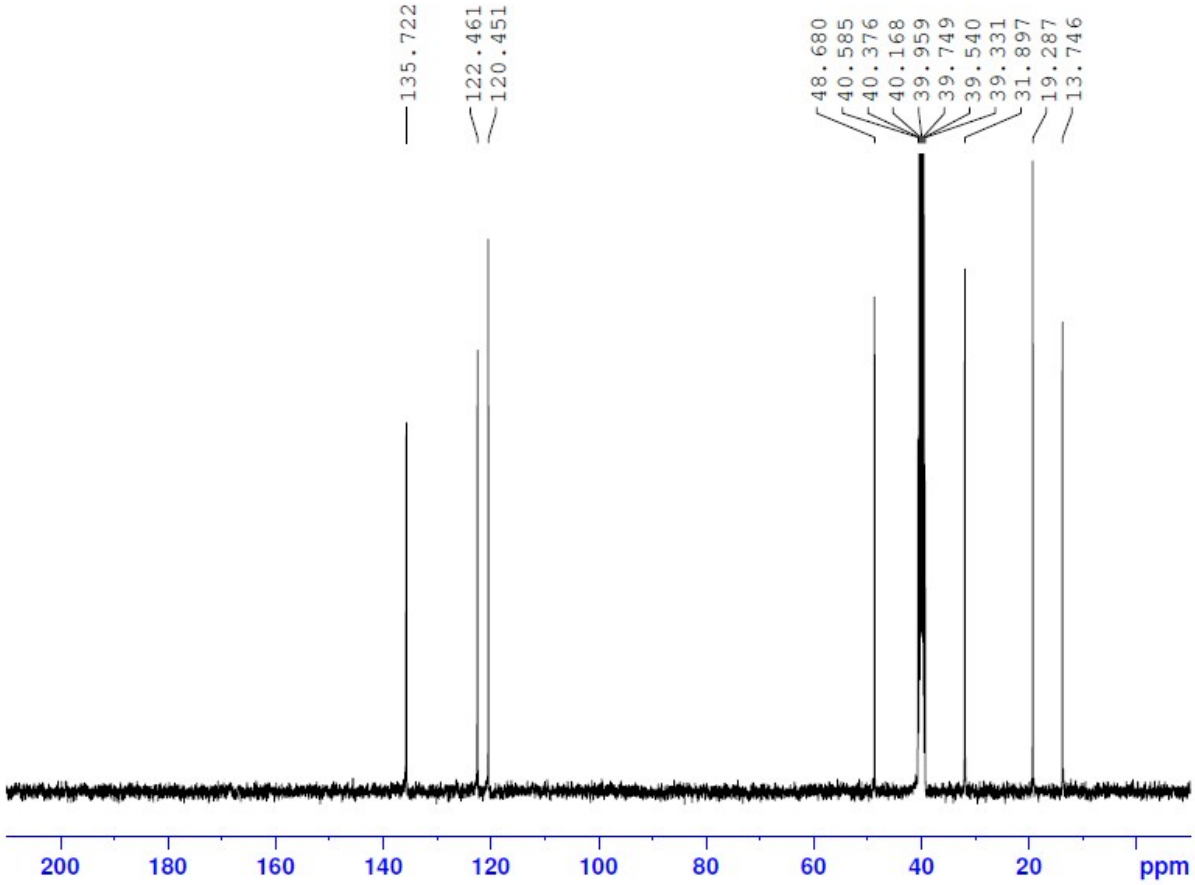
[a]Sum of mannose, xylose, arabinose and galactose; [b]Sum of acid soluble and acid insoluble lignin; [c]Mass dissolved in IL during pretreatment. [d]Lignin precipitate as percentage of initial biomass weight.

Proton and Carbon NMR spectra of ILs:

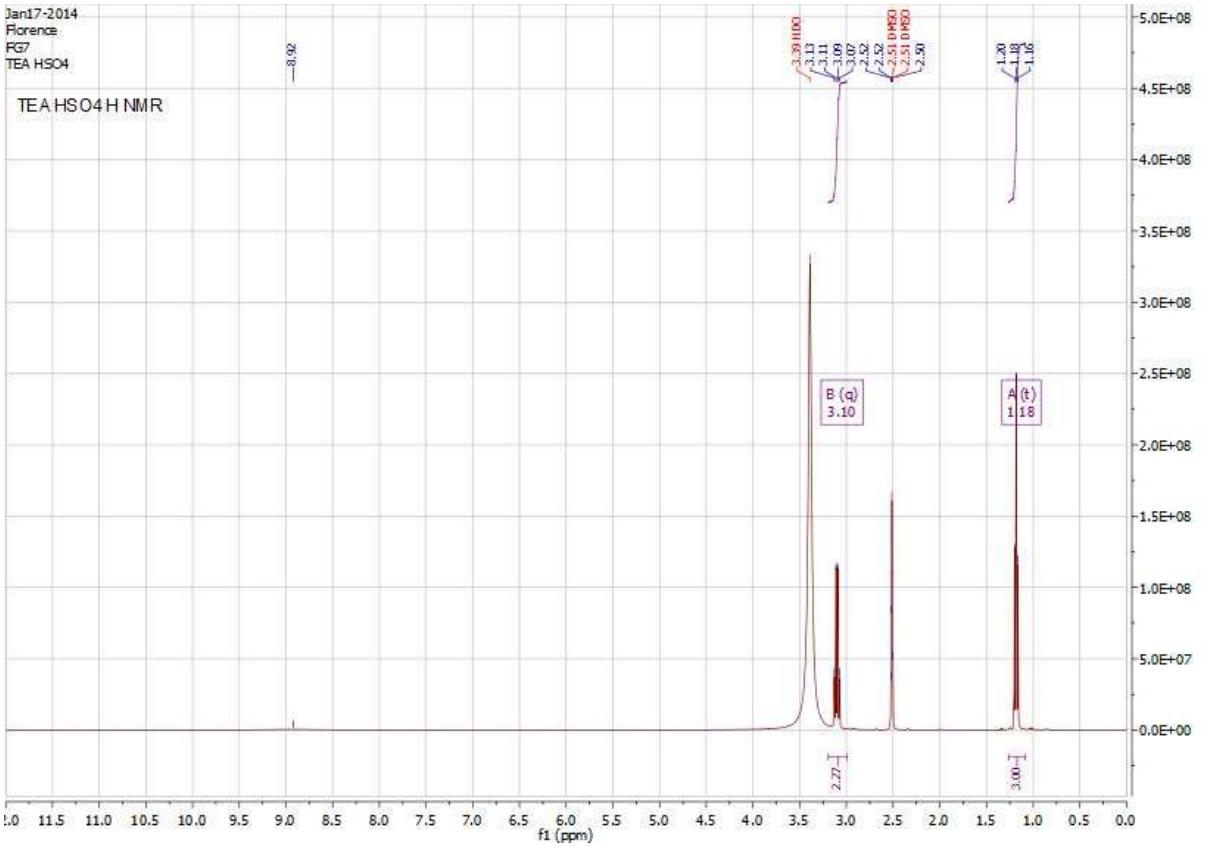
[HBim][HSO$_4$]

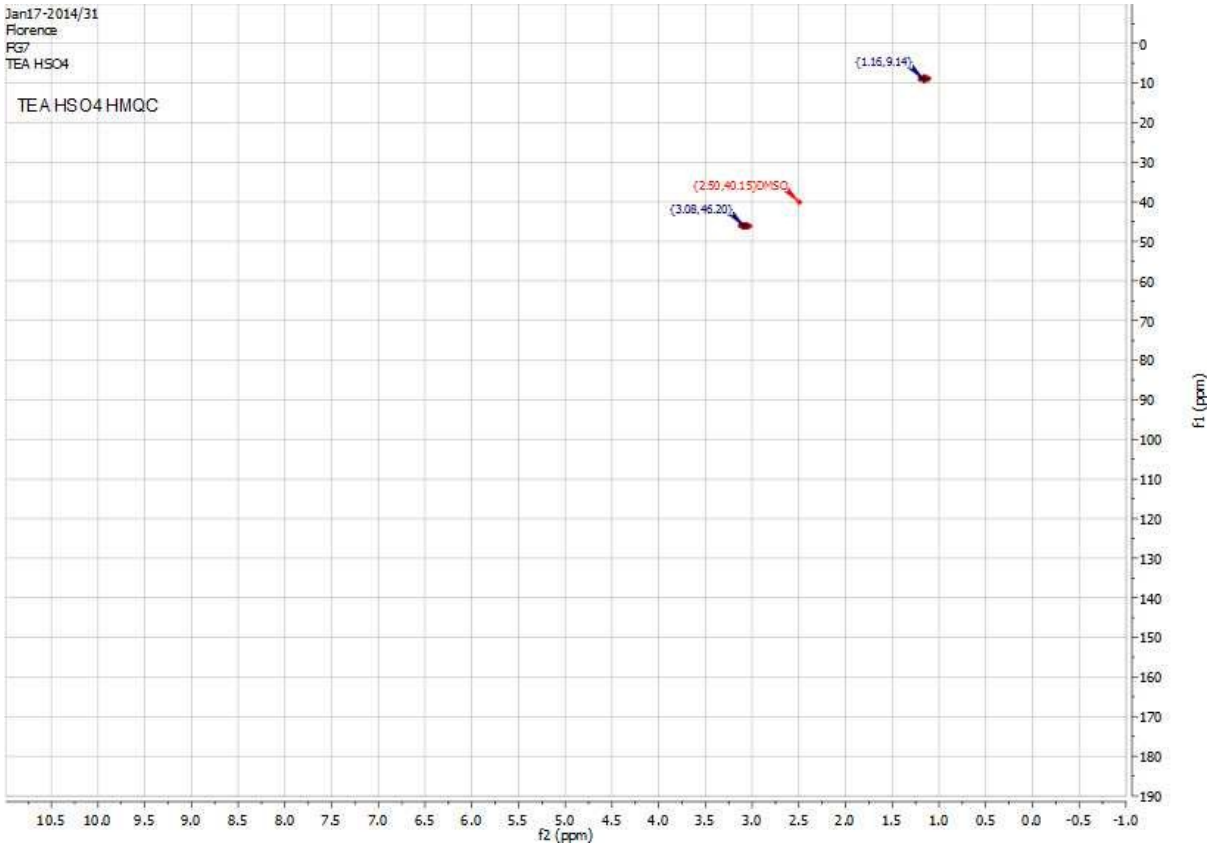


[HBim][HSO$_4$]

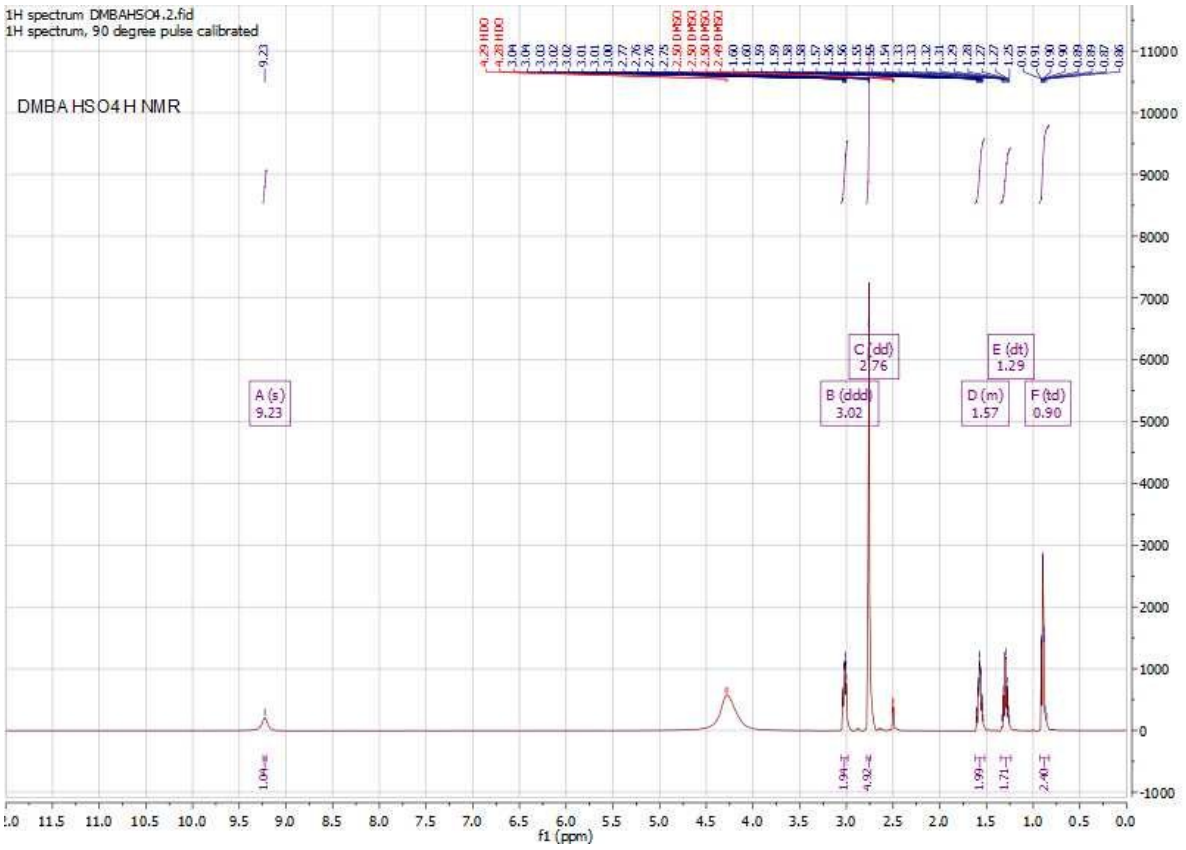

135.722
122.461
120.451
48.680
40.585
40.376
40.168
39.959
39.749
39.540
39.331
31.897
19.287
13.746
200
180
160
140
120
100
80
60
40
20
ppm

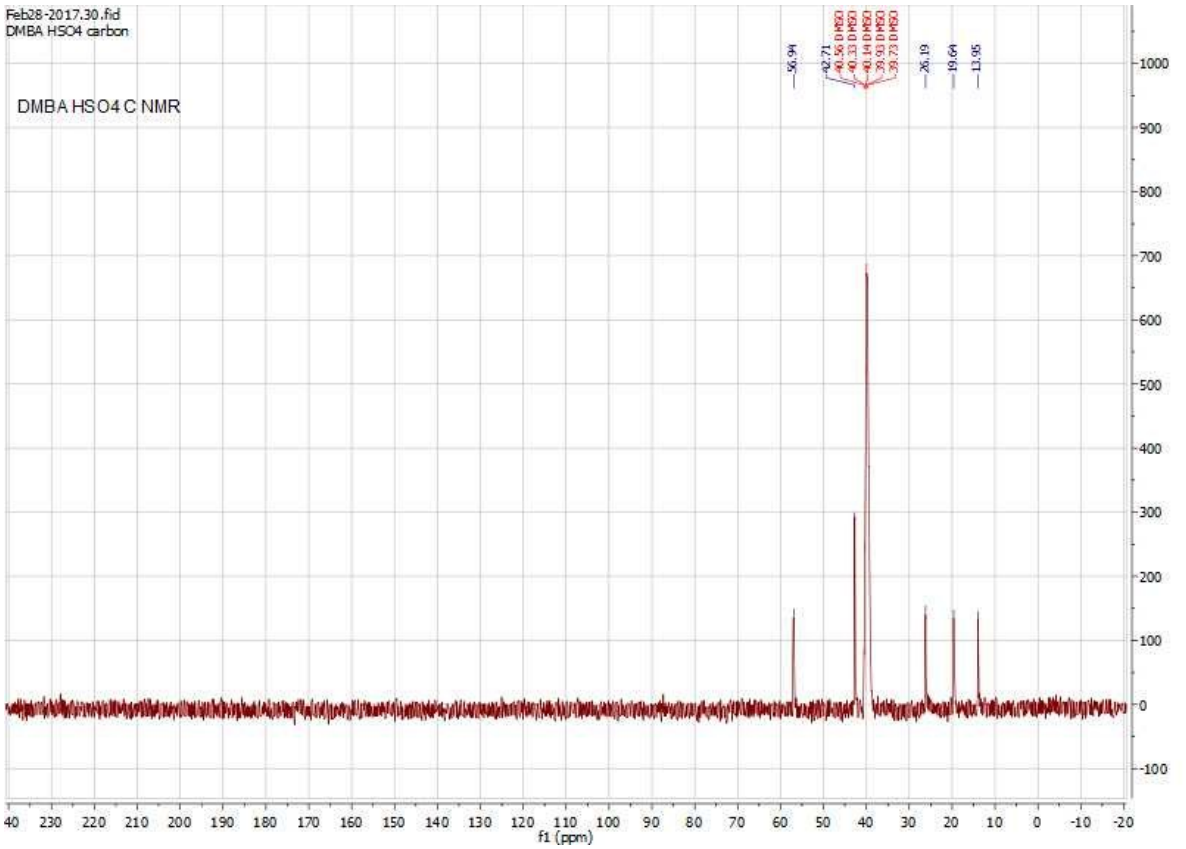
[TEA][HSO$_4$]


Jan17-2014
Florence
FG7
TEA HSO4
TEA HSO4 H NMR
8.92
3.39 HDO
3.13
3.11
3.09
3.07
2.52
2.52
2.51 DMSO
2.51 DMSO
2.50
1.20
1.18
1.16
B (q)
3.10
A (t)
1.18
2.27
3.00
f1 (ppm)


Jan17-2014/31
Florence
FG7
TEA HSO4
TEA HSO4 HMQC
(1.16,9.14)
(2.50,40.15)DMSO
(3.08,46.20)
f2 (ppm)
f1 (ppm)

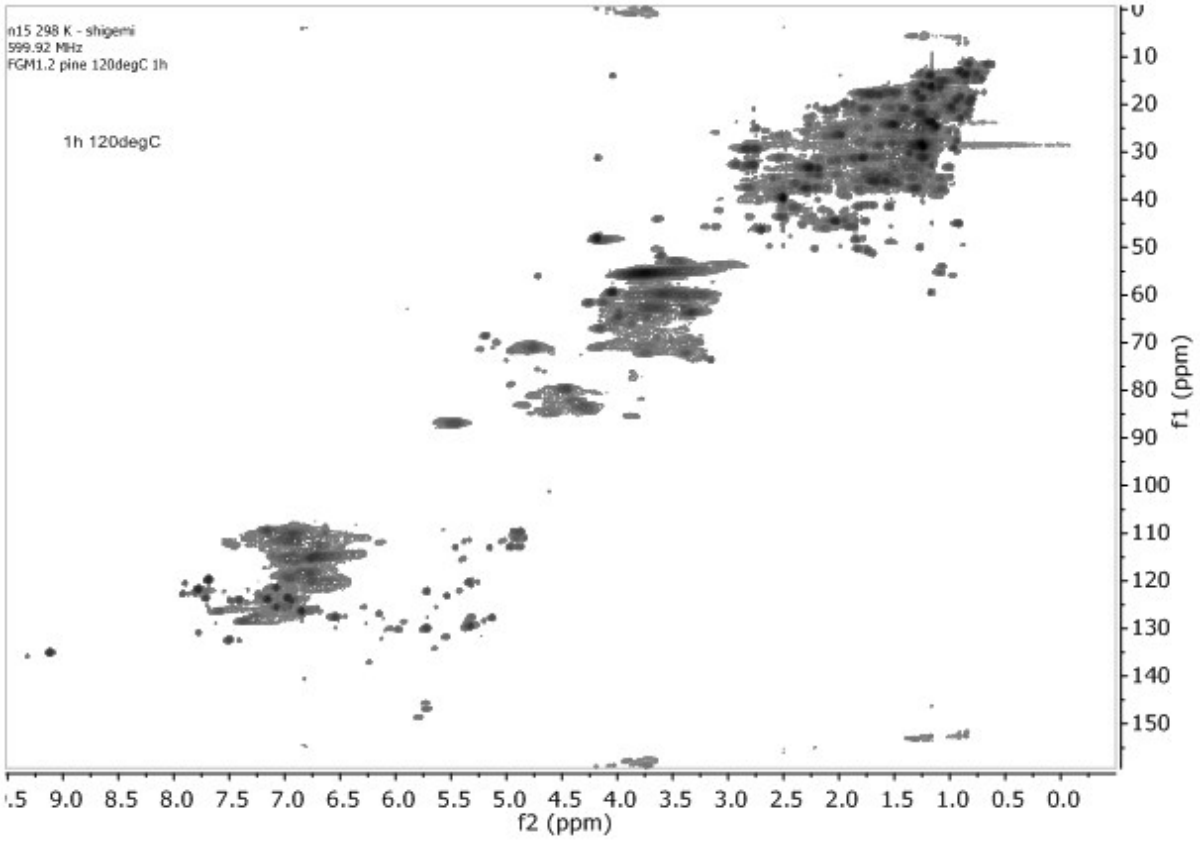
[DMBA][HSO$_4$]

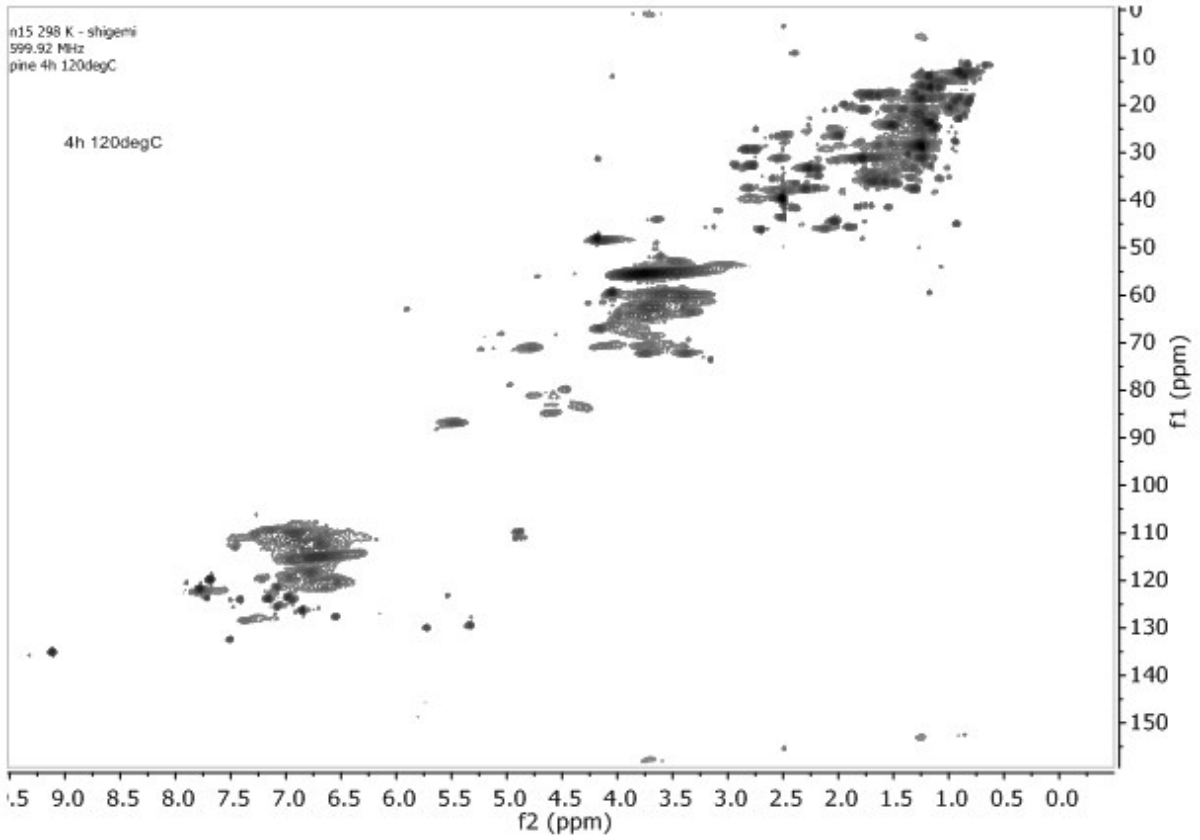

1H spectrum DMBAHSO4.2.fid
1H spectrum, 90 degree pulse calibrated
DMBA HSO4 H NMR
A (s) 9.23
B (ddd) 3.02
C (dd) 2.76
D (m) 1.57
E (dt) 1.29
F (td) 0.90
f1 (ppm)

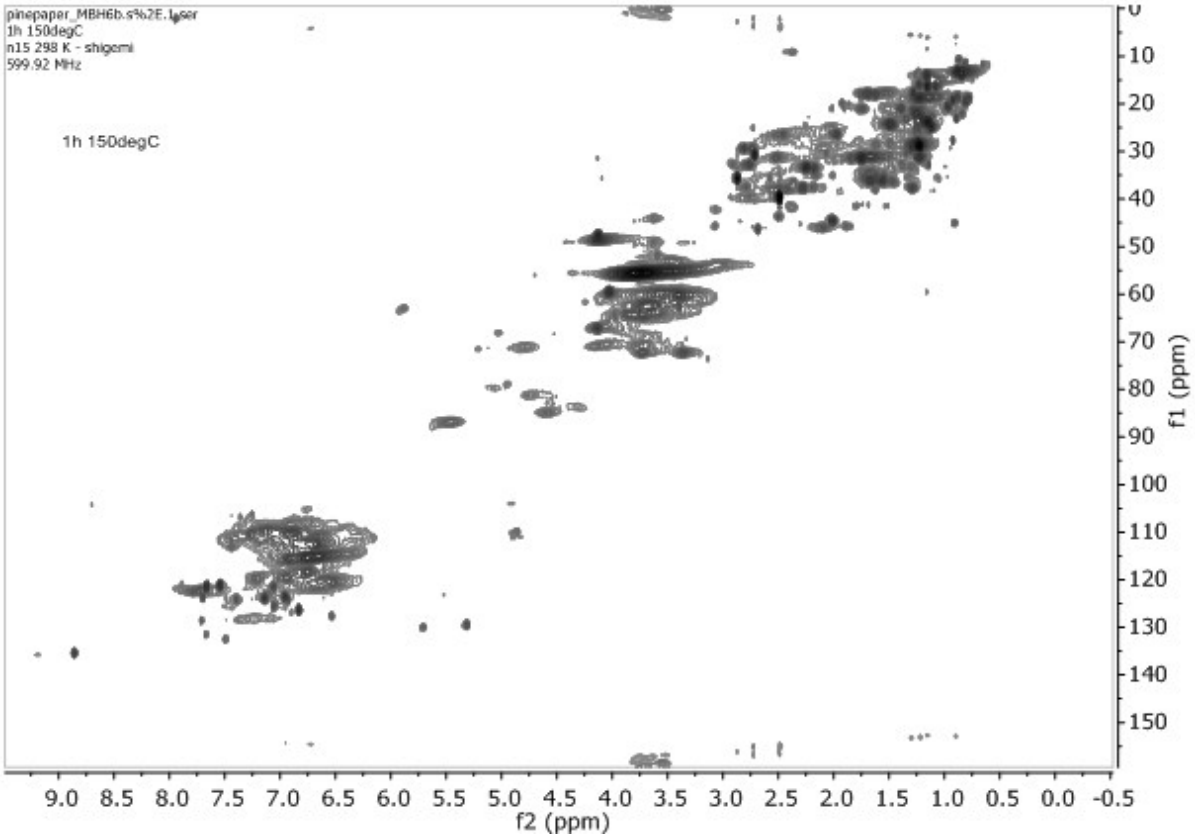

Feb28-2017.30.fid
DMBA HSO4 carbon
DMBA HSO4 C NMR
f1 (ppm)

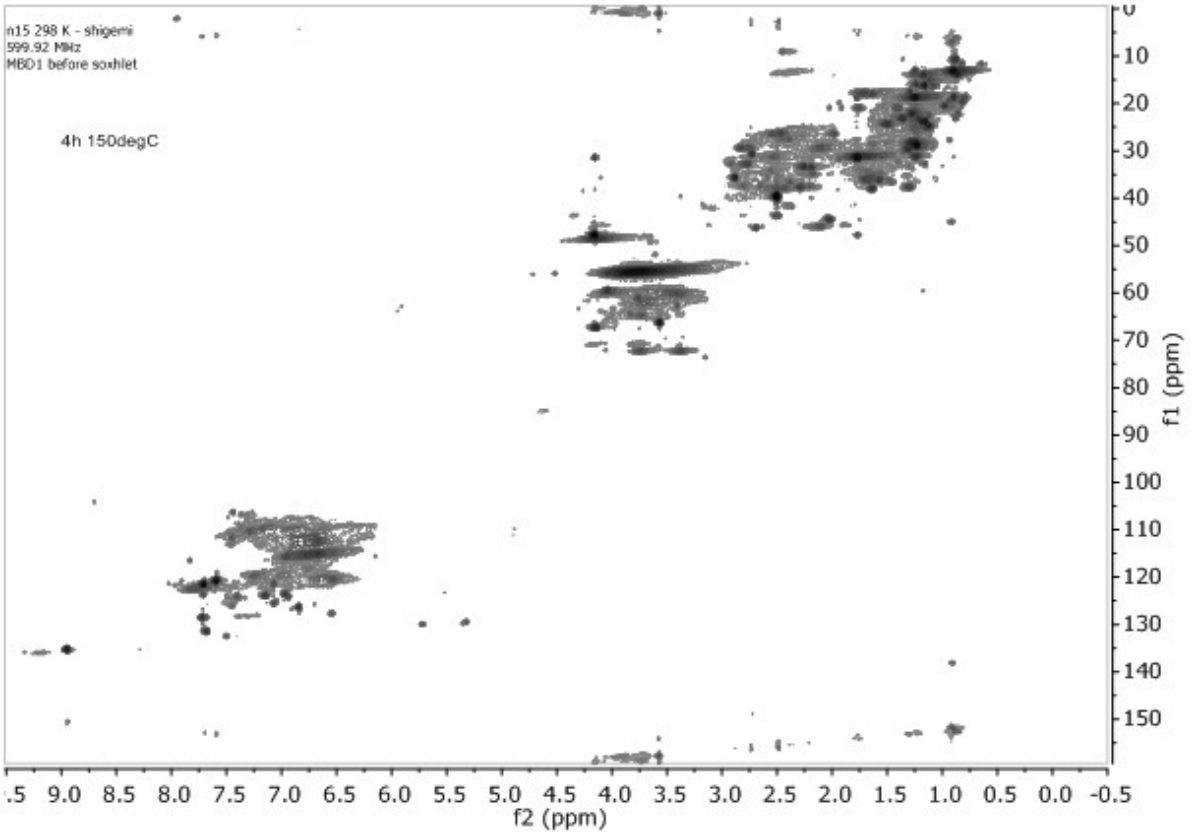
HSQC-NMR raw spectra:

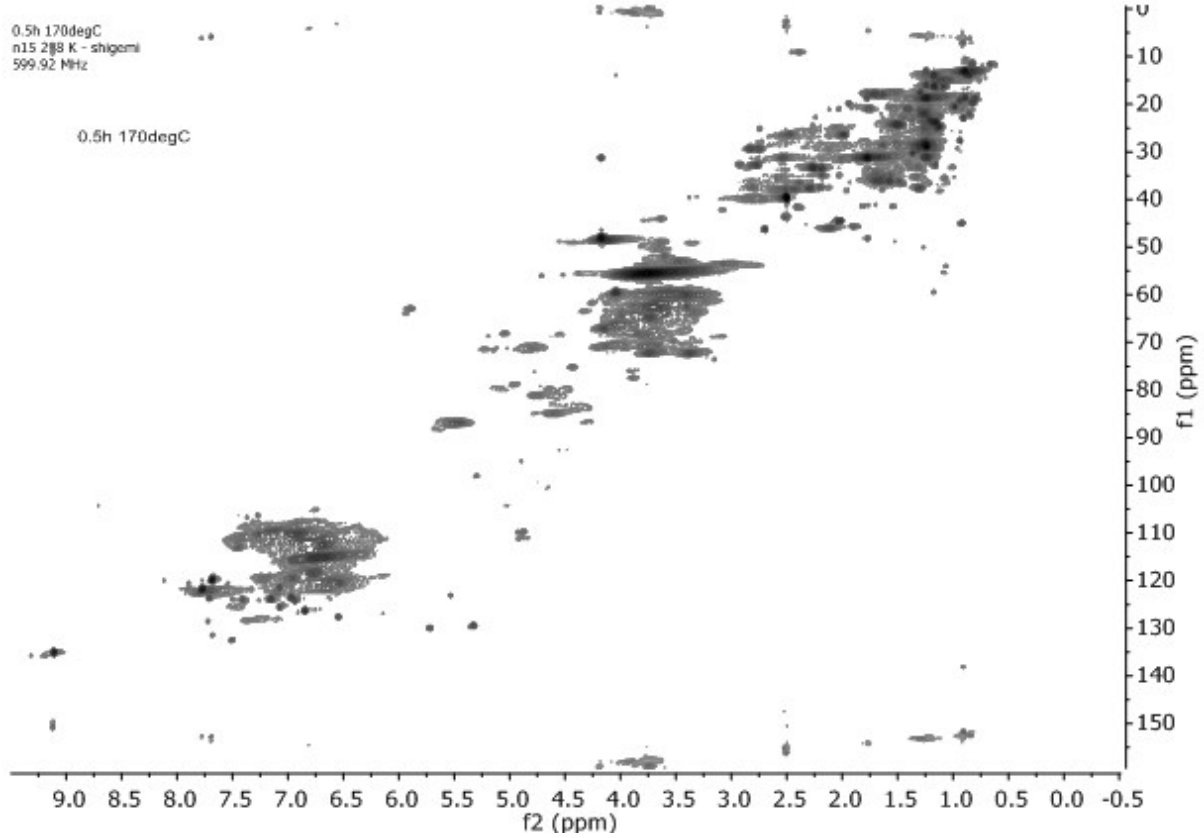
[HC_4im][HSO_4], 10% biomass loading

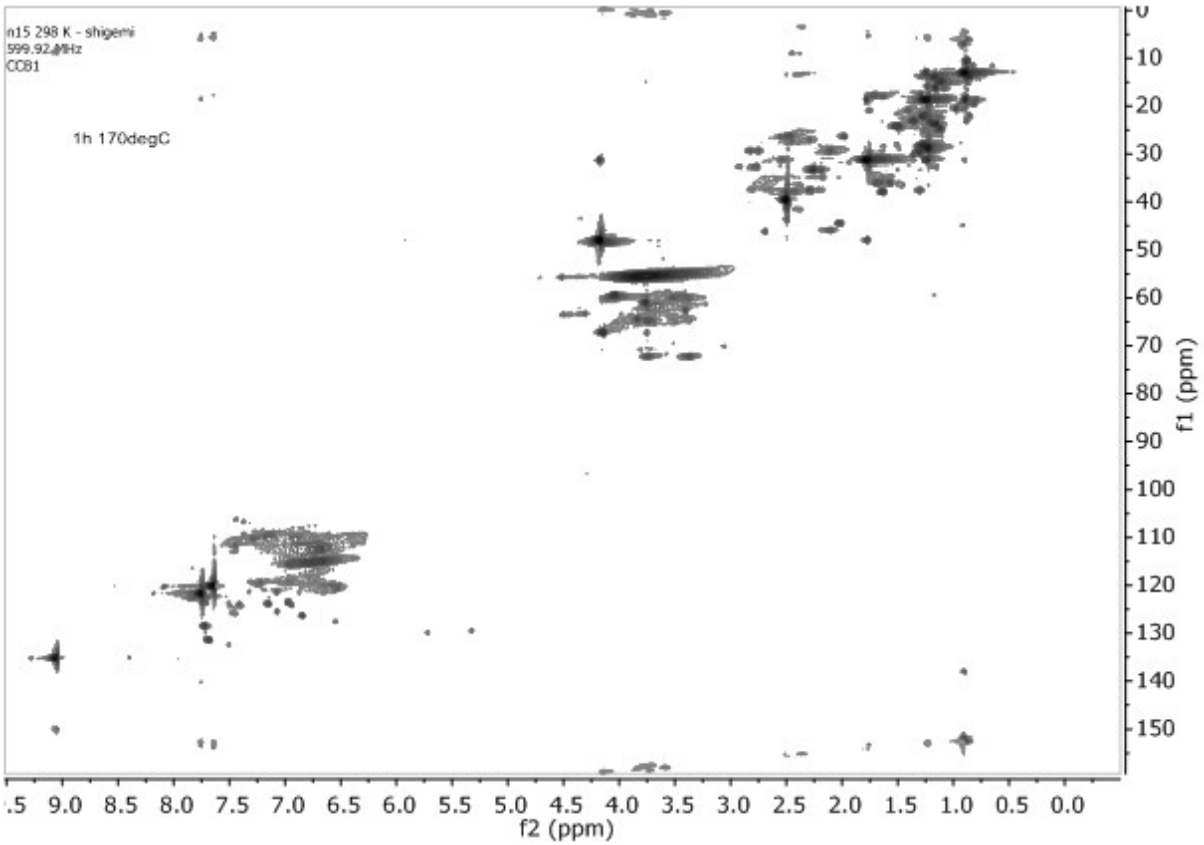




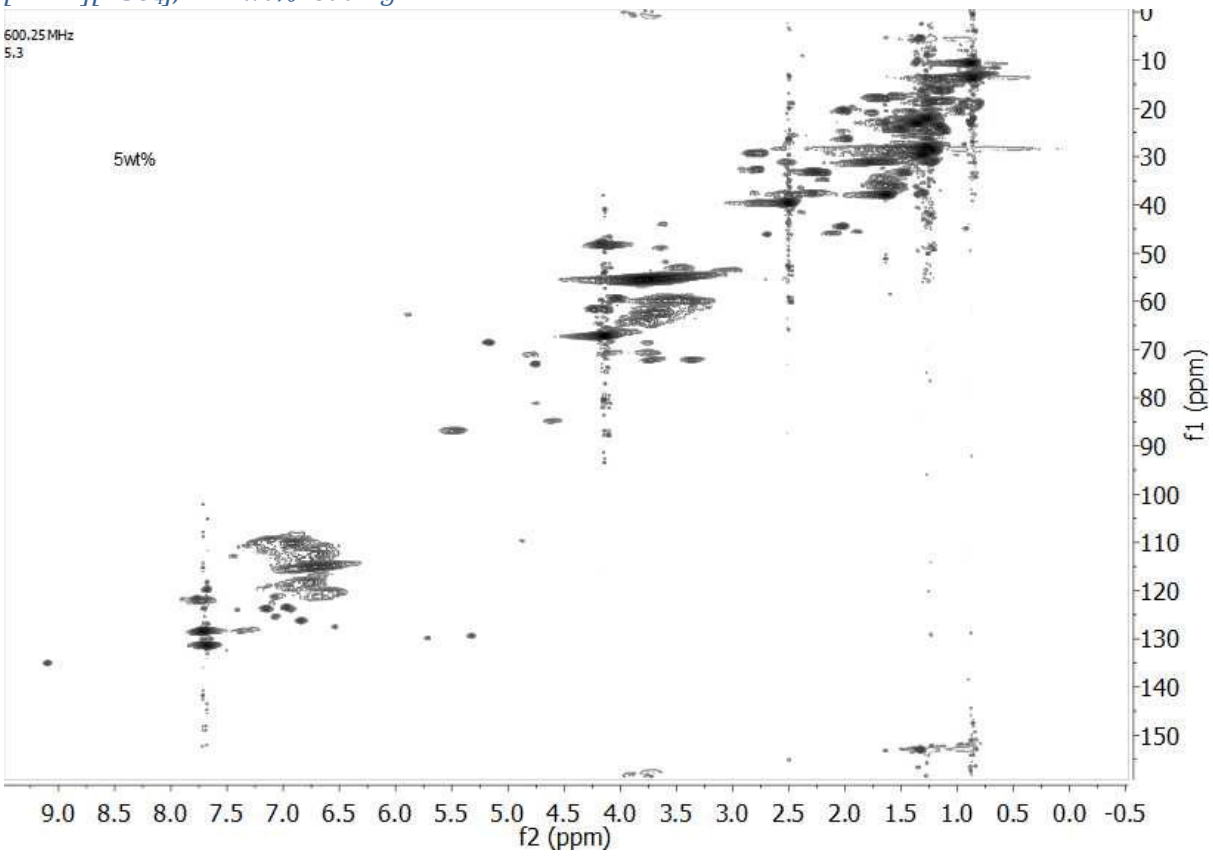

pinepaper_MBH6b.s%2E.ser
1h 150degC
n15 298 K - shigemi
599.92 MHz
1h 150degC
f1 (ppm)
f2 (ppm)

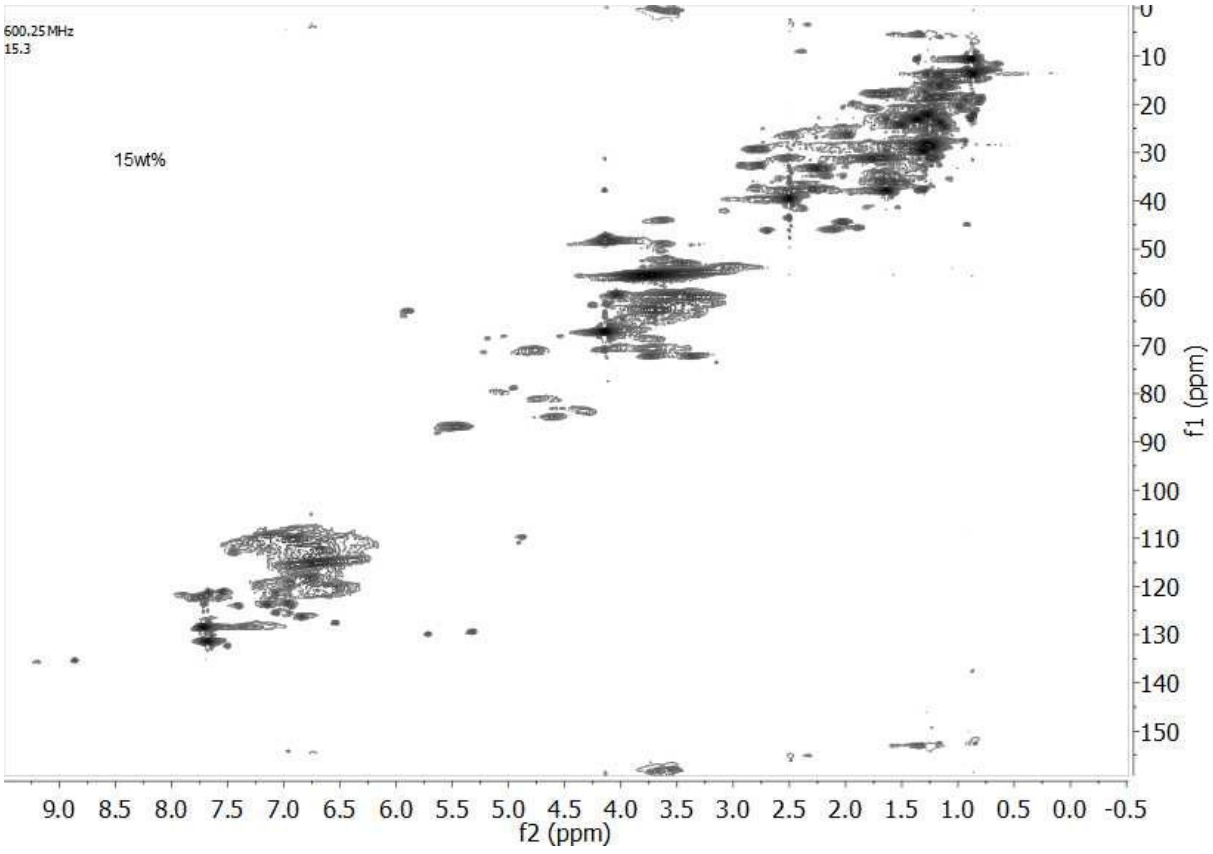

n15 298 K - shigemi
599.92 MHz
MBD1 before soxhlet
4h 150degC
f1 (ppm)
f2 (ppm)

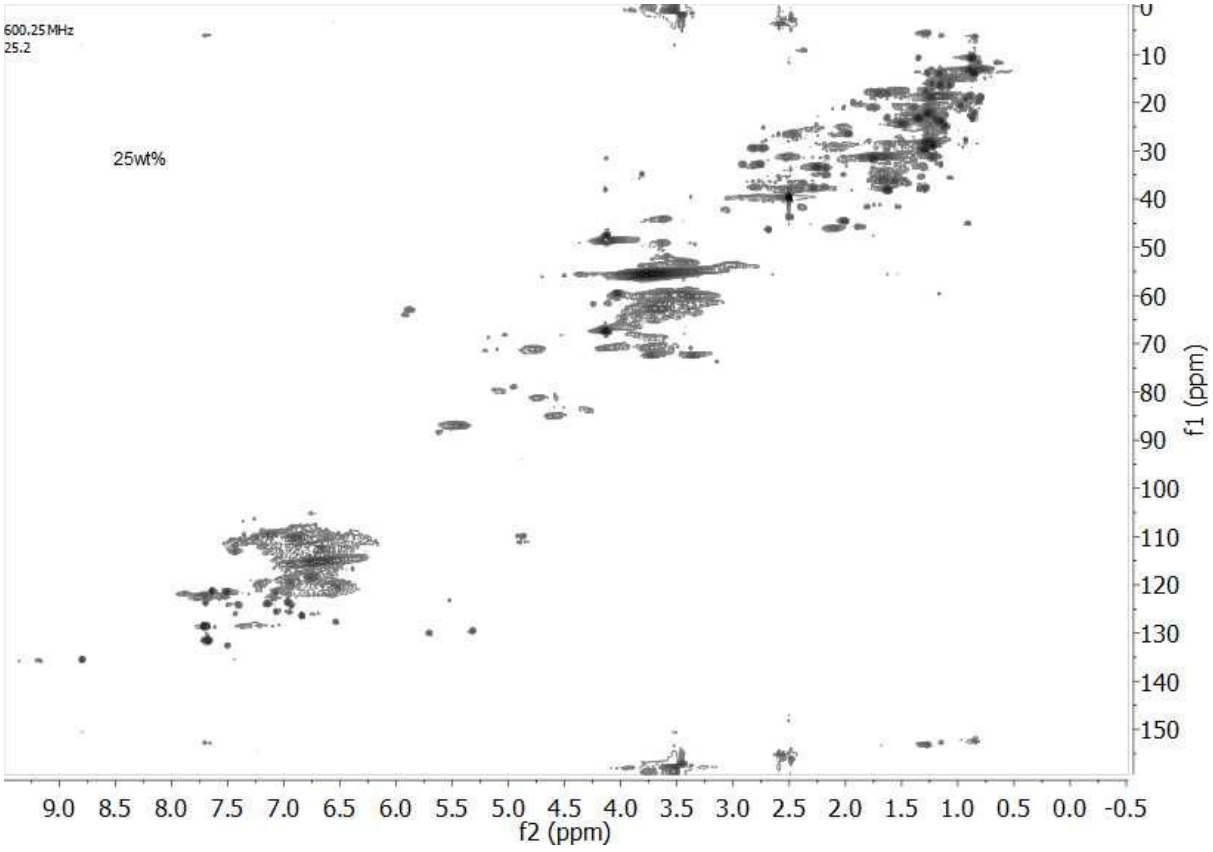

0.5h 170degC
n15 298 K - shigemi
599.92 MHz
0.5h 170degC
f1 (ppm)
f2 (ppm)

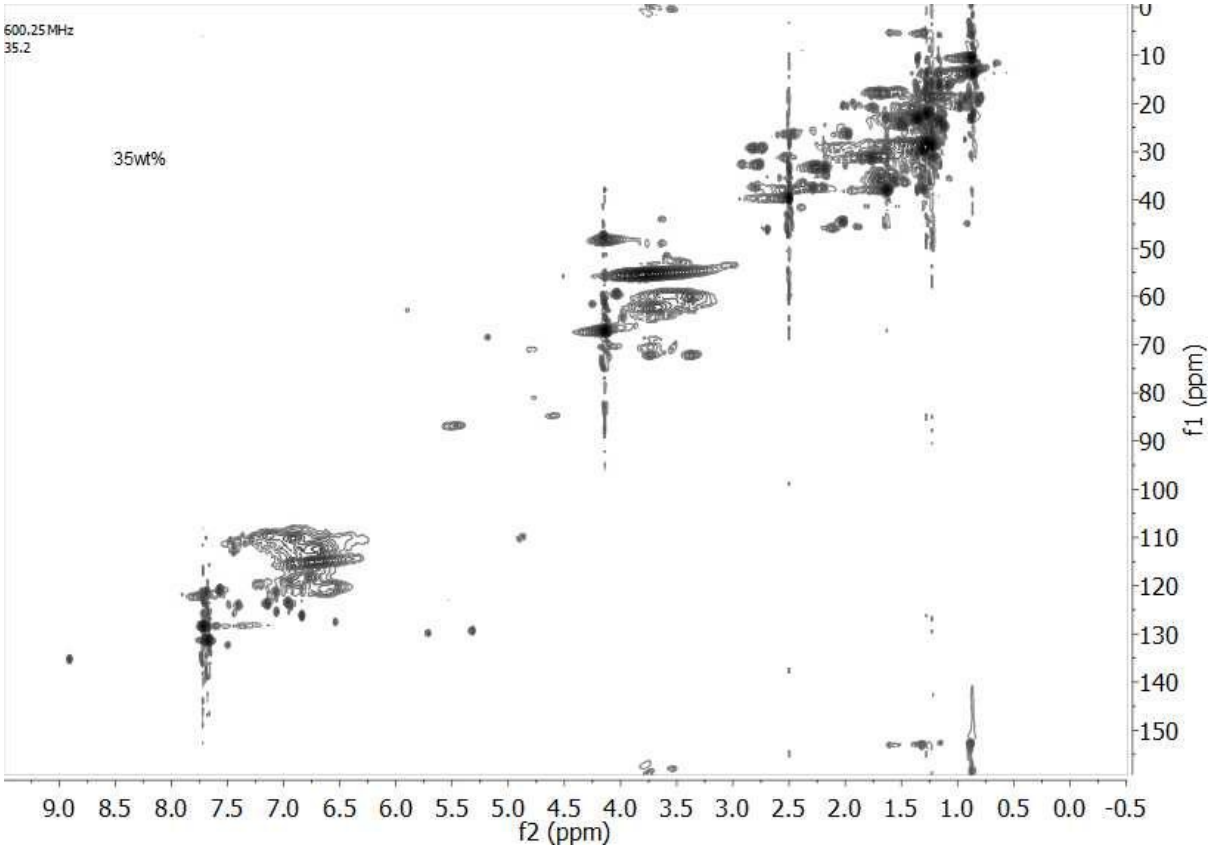

n15 298 K - shigemi
599.92 MHz
CO81
1h 170degC
f1 (ppm)
f2 (ppm)

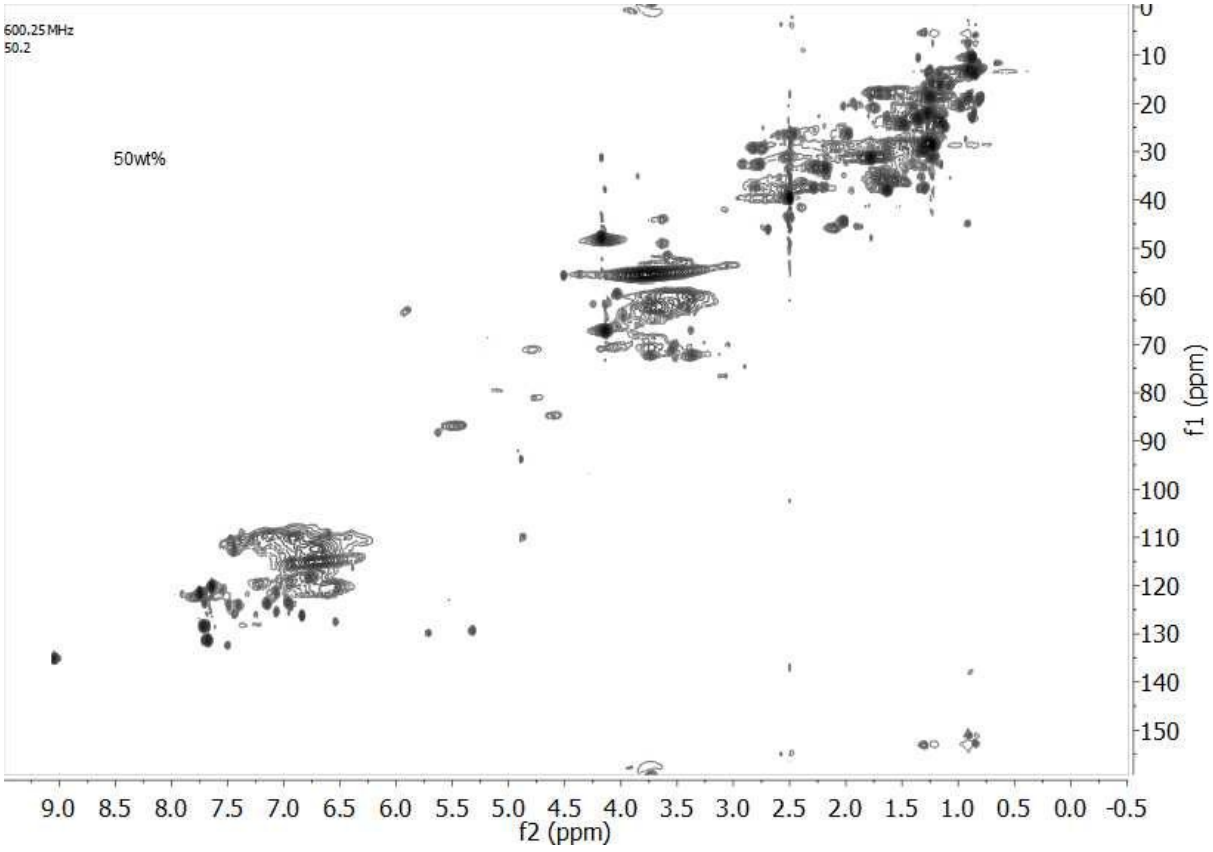
[HBim][HSO_4], 5-50wt% loading



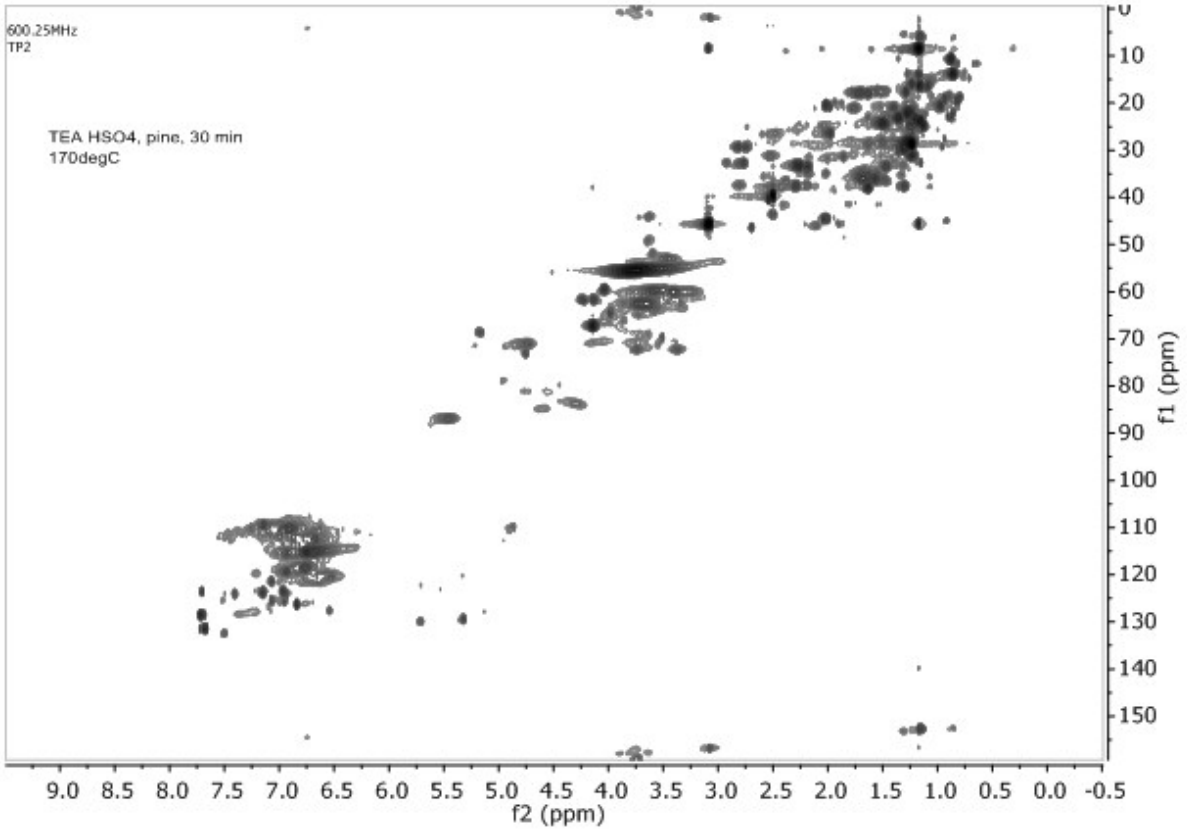


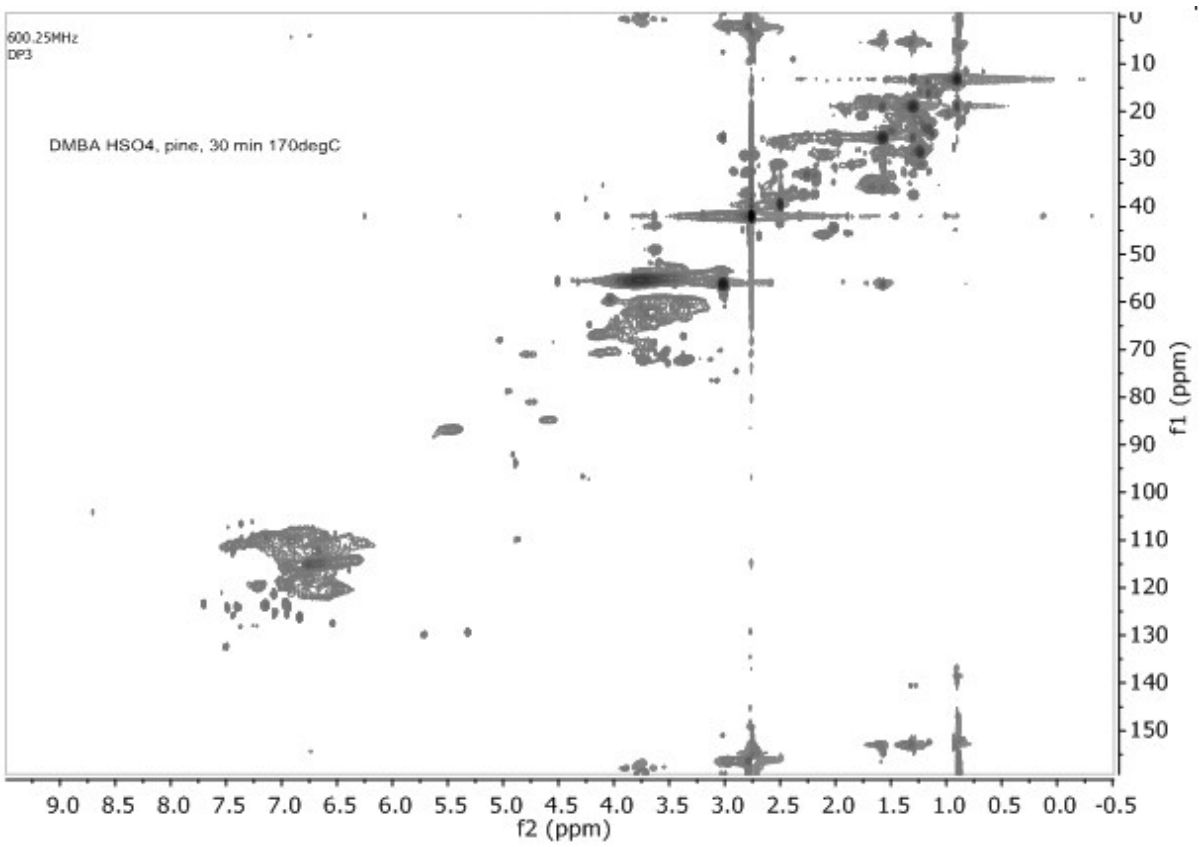

600.25 MHz
25.2
25wt%
f1 (ppm)
f2 (ppm)


600.25 MHz
35.2
35wt%
f1 (ppm)
f2 (ppm)



IL comparison




600.25MHz
DP3
DMBA HSO4, pine, 30 min 170degC
f1 (ppm)
f2 (ppm)