# Electronic supplementary information (ESI)

# Fractionation of lignocellulosic biomass with the ionic liquid 1-butylimidazolium hydrogen sulfate

Pedro Verdía,[a] Agnieszka Brandt,[b] Jason P. Hallett,[b] Michael J. Ray[c] and Tom Welton*[b]

[a] Organic Chemistry Department, Faculty of Chemistry, University of Vigo, 36310 Vigo Spain
[b] Department of Chemistry, Imperial College London, London SW7 2AZ, UK
[c] Division of Biology, Department of Life Sciences, Imperial College London, London, SW7 2AZ, UK
* To whom correspondence should be addressed: t.welton@imperial.ac.uk

## 1. Ionic liquid synthesis details and characterisation:

### *1.1 $C_4Im:H_2SO_4$ = 1.00:0.50*

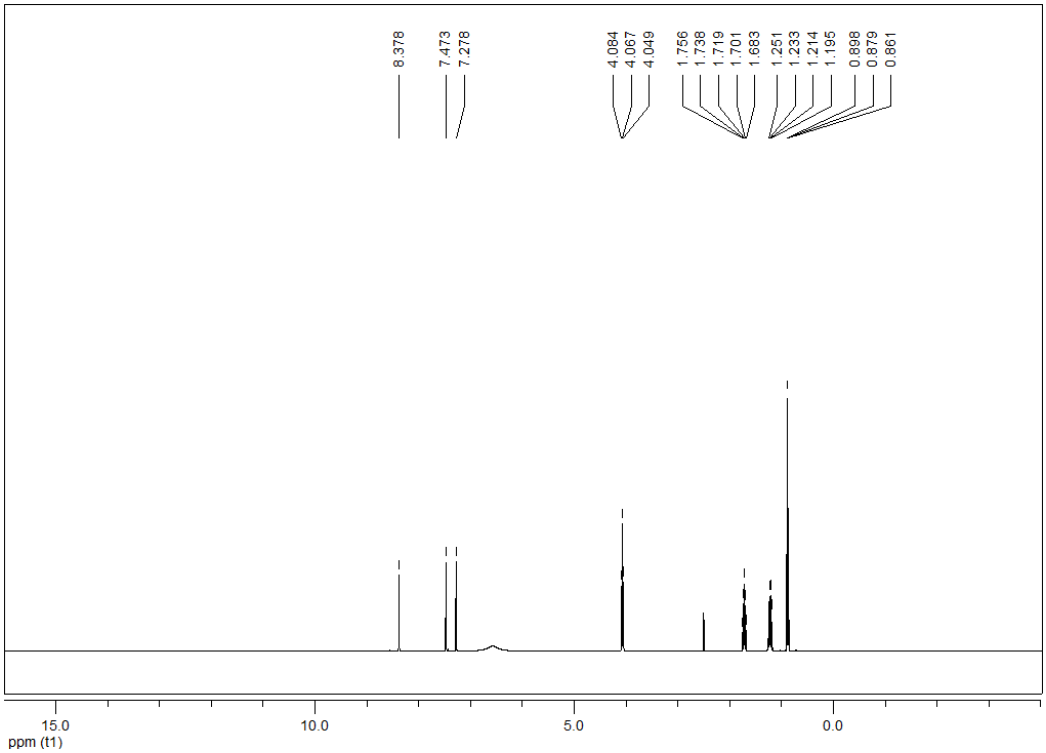
A solution of the $H_2SO_4$ (26.2 mL) in 75 ml water was added dropwise to a stirred solution of 1-butylimidazole (125 mL) in 75 ml water. The mixture was stirred at room temperature overnight, then treated with charcoal and filtered through neutral alumina and through a syringe with a 45 µm disc filter in order to remove alumina contamination carried over from the first filtration. Water was then removed by stirring and heating at 50 °C under reduced pressure for 48 hours. The ionic liquid was obtained as a viscous colourless liquid.

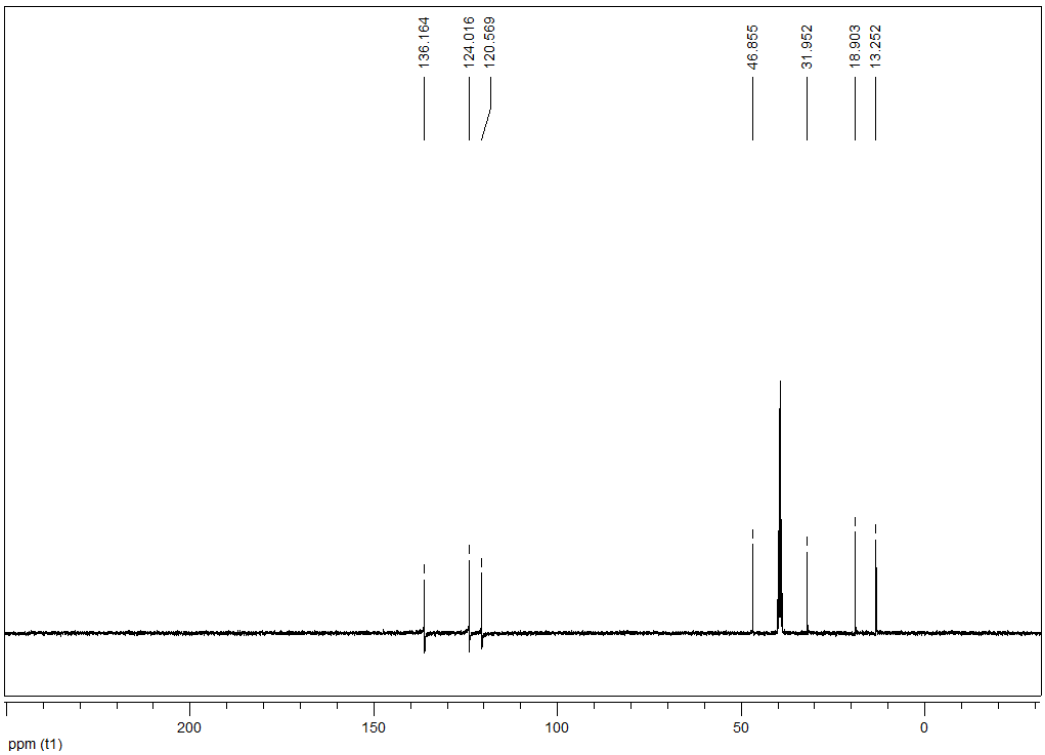
**$^1$H NMR (400 MHz, DMSO-$d_6$, δ):** 8.38 (s, 1 H, C(2)*H*), 7.47 (t, *J* = 1.3 Hz, 1 H, C(4)*H*), 7.28 (t, *J* = 1.3 Hz, 1 H, C(4)*H* ó C(5)*H*), 4.07 (t, *J* = 7.2 Hz, 2 H, NC$H_2$), 1.72 (td, 1H, $J_1$ = 7.4 Hz, $J_2$ = 14.8 Hz, 2 H, $NCH_2CH_2$), 1.22 (m, 2 H, $N(CH_2)_2CH_2$), 0.88 (t, *J* = 7.4 Hz, 3 H, $N(CH_2)_3CH_3$).

**$^{13}$C NMR (101 MHz, DMSO-$d_6$, δ):** 136.2 (*C*(2)H), 124.0 (*C*(5)H), 120.6 (*C*(4)H), 46.9 ($NCH_2$), 32.0 ($NCH_2CH_2$), 18.9 ($N(CH_2)_2CH_2$), 13.2 ($N(CH_2)_3CH_3$).

**m/z (LSIMS$^+$):** 41 ($(C_2H_3N)^+$, 12), 55 ($(C_3H_5N)^+$, 7), 57 ($(C_2H_5N_2)^+$, 7), 69 ($C_3H_5N_2$, 30), 82 ($C_4H_6N_2$, 8), 95 ($C_5H_7N_2$, 8), 125 ($(C_7H_{13}N_2)^+$, 100), 181 ($(C_7H_{13}N_2)^+$ + ($C_2H_4N_2$), 6).

**m/z (LSIMS$^-$):** 97 ($(HSO_4)^-$, 85), 177 ($(HSO_4)^-$ + $SO_3$, 10), 195 ($(HSO_4)^-$ + $H_2SO_4$, 100), 293 ($(HSO_4)^-$ + $(H_2SO_4)_2$, 10).




### *1.2 $C_4Im:H_2SO_4$ = 1.00:0.67*

A solution of 95-97% $H_2SO_4$ (26.6 mL) in 75 ml water was added dropwise to a stirred solution of 1-butylimidazole (100.0 mL) in 75 ml water. The mixture was stirred at room temperature overnight, then treated with charcoal and filtered through neutral alumina. Water was then removed by heating

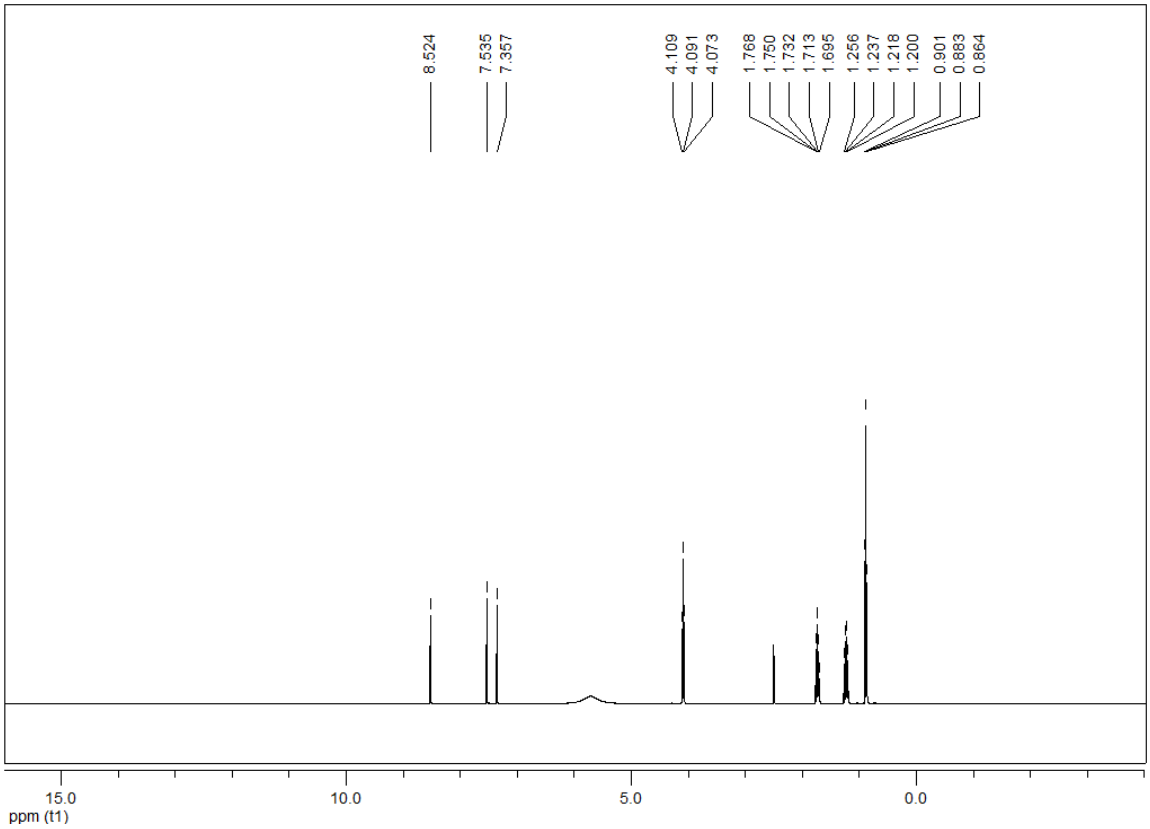
at 50 °C under reduced pressure for 5 hours to obtain a viscous colourless liquid (yield 141.9 g, 99.9 %).

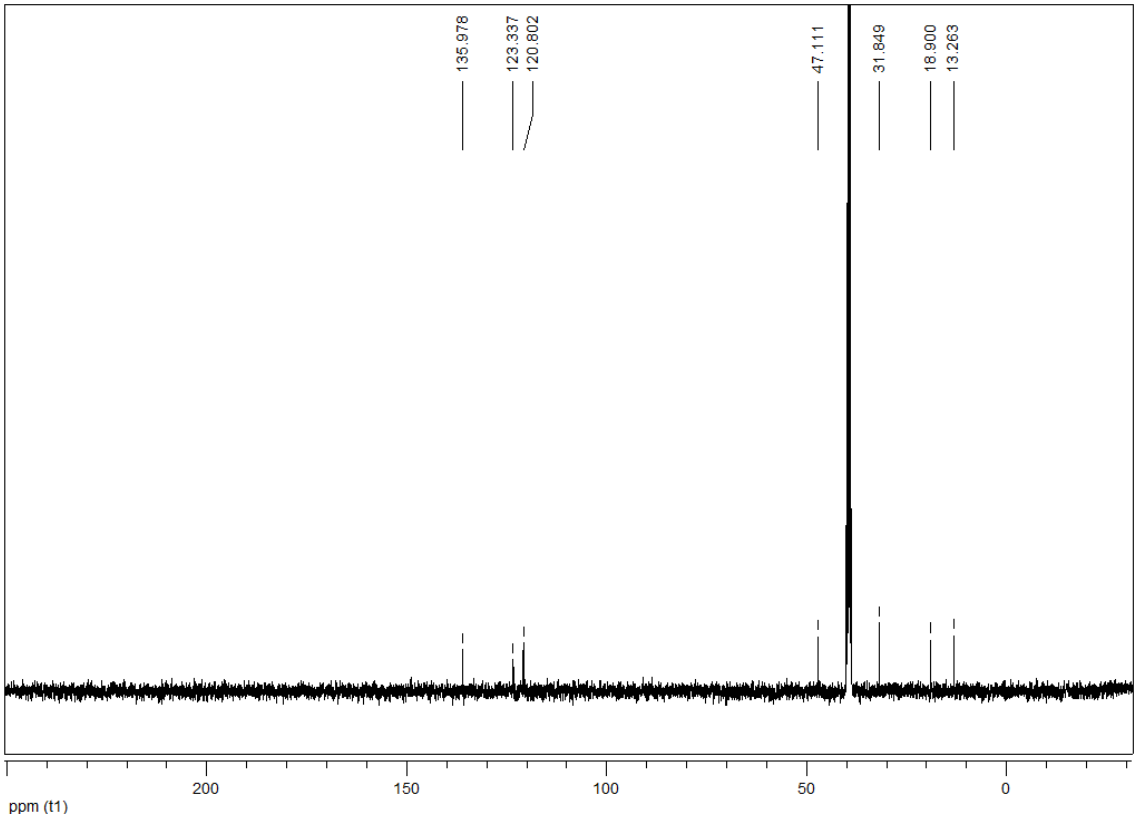
**$^{1}$H NMR (400 MHz, DMSO-$d_6$, δ):** 8.52 (s, 1H, C(2)*H*), 7.53 (t, 1H, *J* = 1.4 Hz, C(4)*H*), 7.36 (t, 1H, *J* = 1.4 Hz C(5)*H*), 4.09 (t, 2H, *J* = 7.2 Hz, NC$H_2$), 1.73 (td, 1H, $J_1$ = 7.4 Hz, $J_2$ = 14.8 Hz, 2 H, $NCH_2CH_2$), 1.23 (m, 2 H, $N(CH_2)_2CH_2$), 0.88 (t, *J* = 7.4 Hz, 3 H, $N(CH_2)_3CH_3$).

**$^{13}$C NMR (101 MHz, DMSO-$d_6$, δ):** 136.0(*C*(2)H), 123.3 (*C*(5)H), 120.8 (*C*(4)H), 47.1 ($NCH_2$), 31.8 ($NCH_2CH_2$), 18.9 ($N(CH_2)_2CH_2$), 13.3 ($N(CH_2)_3CH_3$).

**m/z (LSIMS$^+$):** 41 ($(C_2H_3N)^+$, 15), 55 ($(C_3H_5N)^+$, 7), 57 ($(C_2H_5N_2)^+$, 7), 69 ($C_3H_5N_2$, 43), 82 ($C_4H_6N_2$, 9), 95 ($C_5H_7N_2$ , 9), 125 ($(C_7H_{13}N_2)^+$, 100), 139 ($(C_4H_7N_2)^+$ + ($C_2H_4N_2$), 6), 181 ($(C_7H_{13}N_2)^+$ + ($C_2H_4N_2$), 8).

**m/z (LSIMS$^-$):** 97 ($(HSO_4)^-$, 100), 177 ($(HSO_4)^-$ + $SO_3$, 9), 195 ($(HSO_4)^-$ + $H_2SO_4$, 62), 217 ($(SO_4)^-$ + $H_2SO_3$ + $K^+$, 9), 293 ($(HSO_4)^-$ + $(H_2SO_4)_2$, 6).






### *1.3 $C_4$Im:H2SO4 = 1.00:0.80*

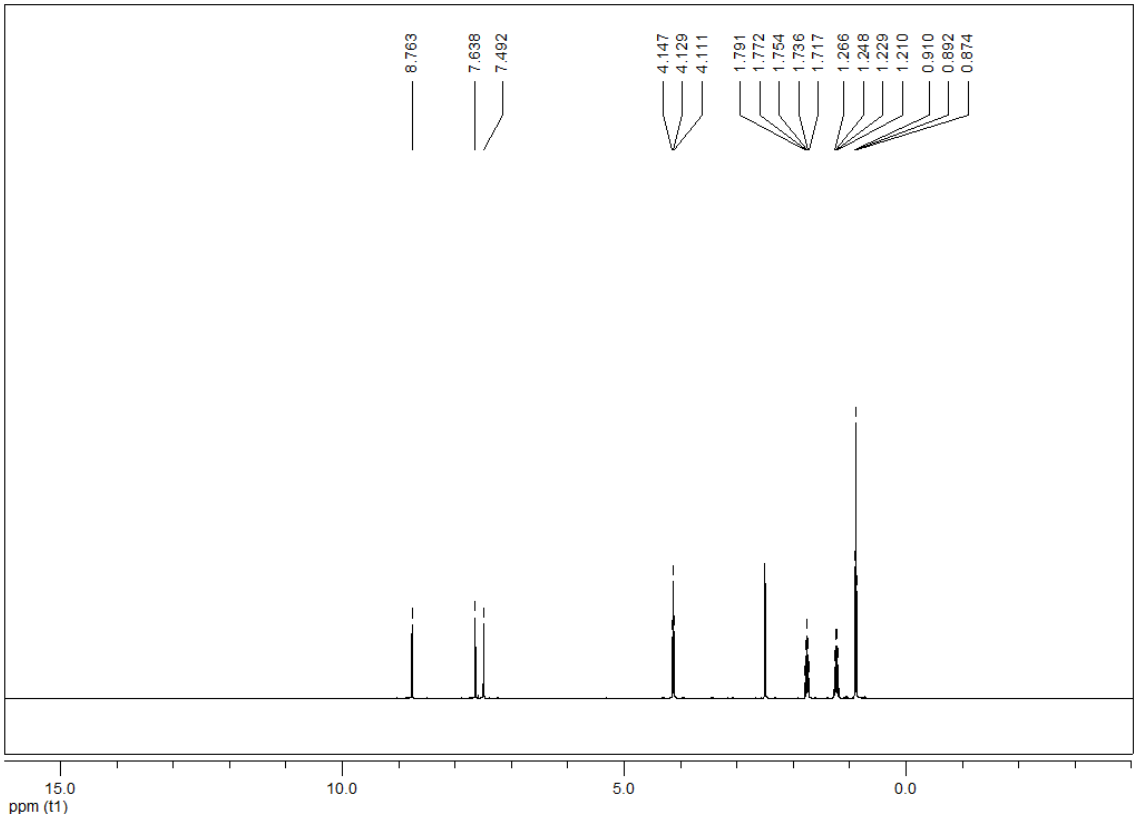
A solution of $H_2SO_4$ (30.1 mL) in 75 mL water was added dropwise to a stirred solution of 1-butylimidazole (90.0 mL) in 75 mL water. The mixture was stirred at room temperature overnight, then treated with charcoal and filtered through neutral alumina and through a syringe with 45 µm disc filter in a second filtration step in order to remove alumina contamination carried over from the first filtration. Water was then removed by heating at 50 °C under reduced pressure for 48 hours. The product was obtained as a viscous colourless liquid (133.8 g, 98.3 % with a water content of 1.6 %).

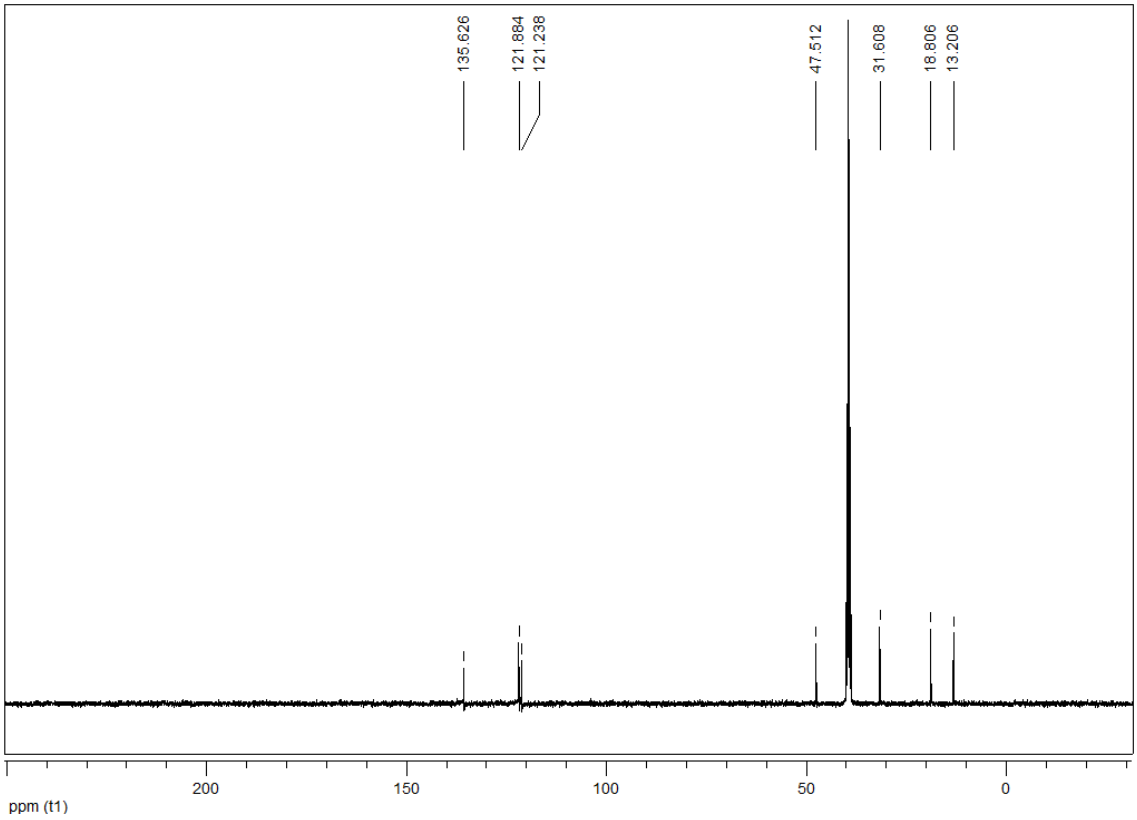
**$^1$H NMR (400 MHz, DMSO-$d_6$, δ):** 8.76 (s, 1 H, C(2)*H*), 7.64 (t, *J* = 1.5 Hz, 1 H, C(4)*H*), 7.49 (t, *J* = 1.4 Hz, 1 H, C(5)*H*), 4.13 (t, *J* = 7.2 Hz, 2 H, NC$H_2$), 1.75 (m, 2 H, NCH$_2$C$H_2$), 1.24 (m, 2 H, N(CH$_2$)$_2$C$H_2$), 0.89 (t, *J* = 7.4 Hz, 3 H, N(CH$_2$)$_3$C$H_3$).

**$^{13}$C NMR (101 MHz, DMSO-$d_6$, δ):** 135.6 (*C*(2)H), 121.9 (*C*(5)H), 121.2 (*C*(4)H), 47.5 (NCH$_2$), 31.6 (NCH$_2$*C*H$_2$), 18.8 (N(CH$_2$)$_2$*C*H$_2$), 13.2 (N(CH$_2$)$_3$*C*H$_3$).

**m/z (LSIMS$^+$):** 41 ((C$_2$H$_3$N)$^+$, 11), 69 (C$_3$H$_5$N$_2$, 33), 82 (C$_4$H$_6$N$_2$, 6), 125 ((C$_7$H$_{13}$N$_2$)$^+$, 100).

**m/z (LSIMS$^-$):** 97 ((HSO$_4$)$^-$, 100), 177 ((HSO$_4$)$^-$ + (SO$_3$), 9), 195 ((HSO$_4$)$^-$ + H$_2$SO$_4$, 98), 219 ((HSO$_4$)$^-$ + H$_2$SO$_4$ + Na$^+$, 12), 279 ((HSO$_4$)$_2^-$ + Rb$^+$, 10), 293 ((HSO$_4$)$^-$ + (H$_2$SO$_4$)$_2$, 11).



### *1.4 $C_4Im:H_2SO_4$ = 1.00:0.99*

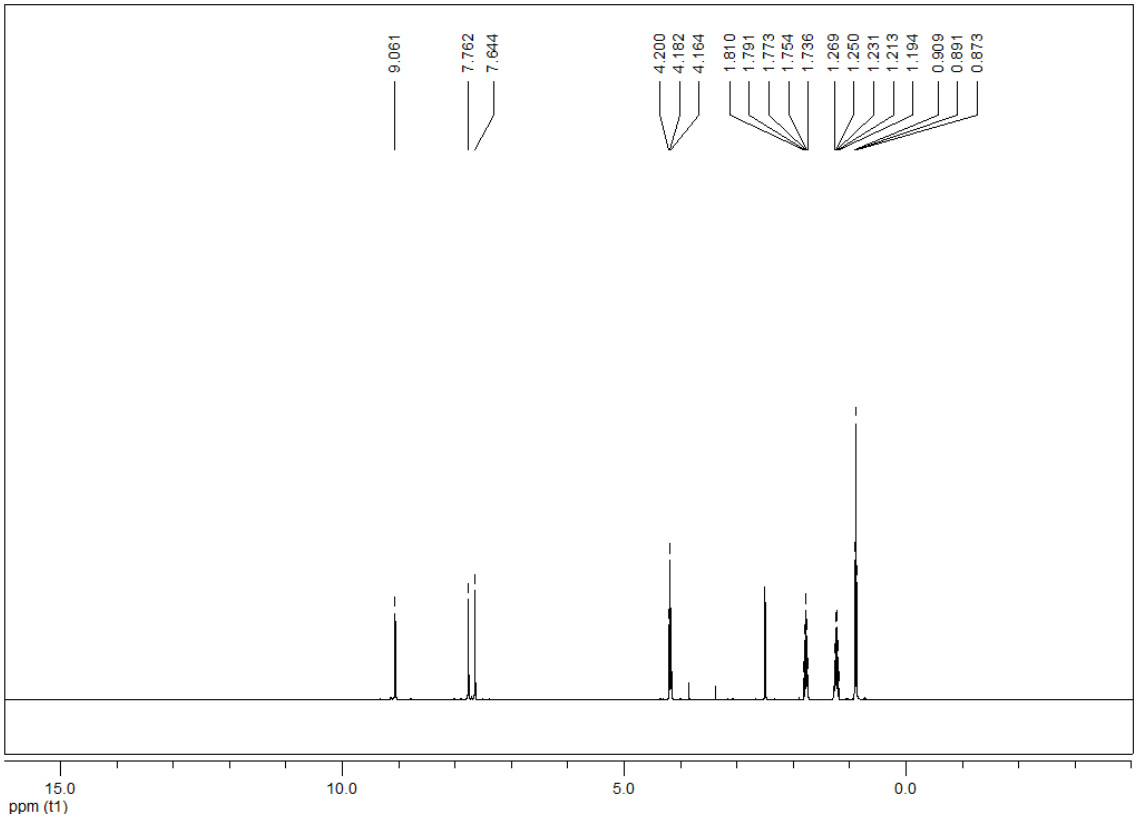
A solution of $H_2SO_4$ (41.4 mL) in 75 ml water was added drop wise to a stirred solution of 1-butylimidazole (100.0 mL) in 75 mL water. The mixture was stirred at room temperature overnight, then treated with charcoal, filtered through neutral alumina and through a syringe with 45 µm disc filter in a second filtration step in order to remove alumina contamination carried over from the first filtration. Water was then removed by heating at 50 °C under reduced pressure for 48 hours. The product was obtained as a viscous colourless liquid (yield 162.1 g, 98.2% with a water content of 0.87 %).

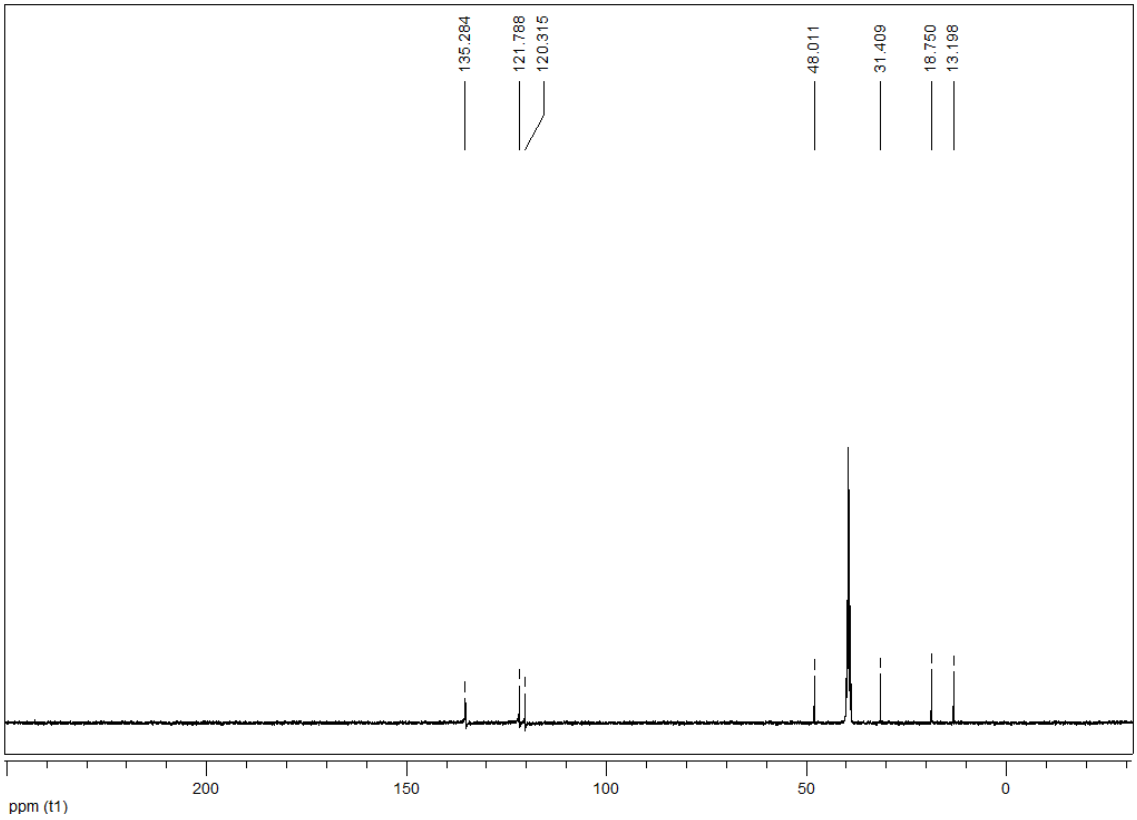
**$^1$H NMR (400 MHz, DMSO-$d_6$, δ):** 9.06 (s, 1 H, C(2)*H*), 7.76 (s, 1 H, C(4)*H* ó), 7.64(t, *J* = 1.4 Hz 1 H, C(5)*H*), 4.18 (t, *J* = 7.3 Hz, 2 H, NC$H_2$), 1.77 (m, 2 H, $NCH_2CH_2$), 1.24 (m, 2 H, $N(CH_2)_2CH_2$), 0.89 (t, *J* = 7.4 Hz, 3 H, $N(CH_2)_3CH_3$).

**$^{13}$C NMR (101 MHz, DMSO-$d_6$, δ):** 135.3 (*C*(2)H), 121.8 (*C*(5)H), 120.3 (*C*(4)H), 48.0 ($NCH_2$), 31.4 ($NCH_2CH_2$), 18.8 ($N(CH_2)_2CH_2$), 13.2 ($N(CH_2)_3CH_3$).

**m/z (LSIMS$^+$):** 41 ($(C_2H_3N)^+$, 9), 55 ($(C_3H_5N)^+$, 4), 57 ($(C_2H_5N_2)^+$, 4), 69 ($C_3H_5N_2$, 24), 82 ($C_4H_6N_2$, 6), 95 ($C_3H_5N_2$, 5), 125 ($(C_7H_{13}N_2)^+$, 100), 139 $((C_4H_7N_2)^+ + (C_2H_4N_2)$, 2), 181 $((C_7H_{13}N_2)^+ + (C_2H_4N_2)$, 2), 249 $((C_7H_{13}N_2)^+ + ((C_7H_{12}N_2)$, 5), 347 $((C_7H_{13}N_2)_2^+ (HSO_4)^-$, 4).

**m/z (LSIMS$^-$):** 97 ($(HSO_4)^-$, 77), 177 $((HSO_4)^- + SO_3$, 9), 195 $((HSO_4)^- + (H_2SO_4)$, 100), 219 $((HSO_4)^- + H_2SO_4 + Na^+$, 6), 293 $((HSO_4)^- + (H_2SO_4)_2$, 15).



### *1.5 $C_4Im:H_2SO_4$ = 1.00:1.01*

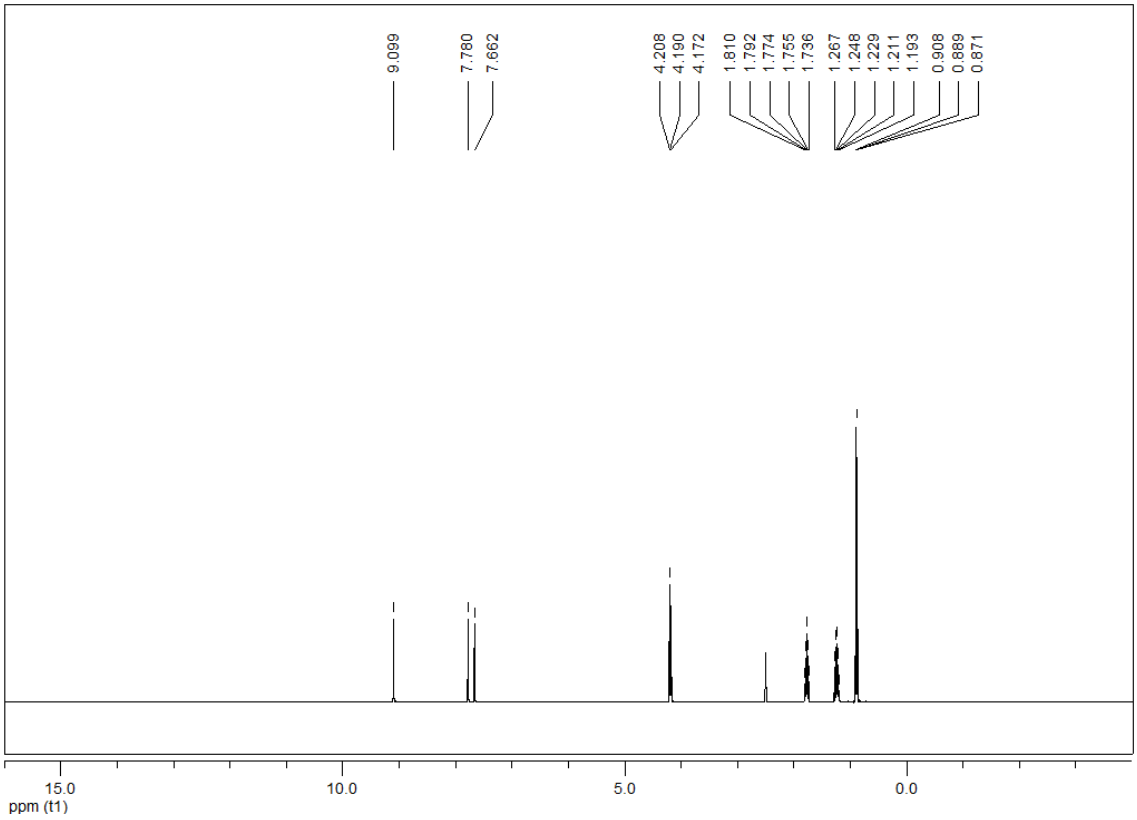
A solution of the $H_2SO_4$ (42.3 mL) in 75 ml water was added drop wise to a stirred solution of 1-butylimidazole (100.0 mL) in 75 ml water. The mixture was stirred at room temperature overnight, then treated with charcoal, filtered through neutral alumina and through a syringe with 45 µm disc filter in a second filtration step in order to remove any alumina contamination carried over from the first filtration. Water was then removed by heating at 50 °C under reduced pressure for 48 hours. A viscous colourless liquid (yield 160.3 g, 96.3 %, with a water content of 0.33 %) was obtained.

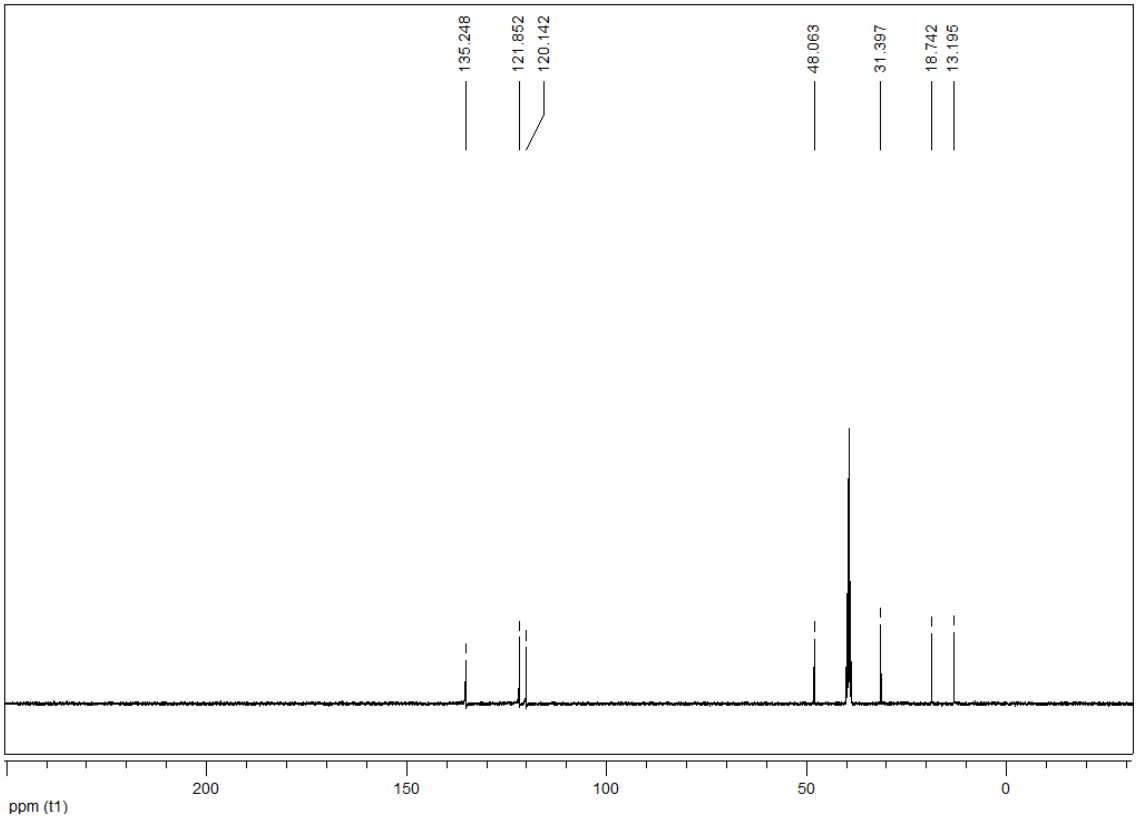
**$^1$H NMR (400 MHz, DMSO-$d_6$, δ):** 9.10 (s, 1 H, C(2)*H*), 7.78 (t, *J* = 1.6 Hz, 1 H, C(4)*H*), 7.66 (t, *J* = 1.6 Hz, 1 H, C(5)*H*), 4.19 (t, *J* = 7.2 Hz, 2 H, NC$H_2$), 1.78 (qd, $J_1$ = 7.5 Hz, $J_2$ = 13.0 Hz, 2 H, $NCH_2CH_2$), 1.24 (m, 2 H, $N(CH_2)_2CH_2$), 0.89 (t, *J* = 7.4 Hz, 3 H, $N(CH_2)_3CH_3$).

**$^{13}$C NMR (101 MHz, DMSO-$d_6$, δ):** 135.2 (*C*(2)H), 121.8 (*C*(5)H), 120.1 (*C*(4)H), 48.1 ($NCH_2$), 31.4 ($NCH_2CH_2$), 18.7 ($N(CH_2)_2CH_2$), 13.2 ($N(CH_2)_3CH_3$).

**m/z (LSIMS$^+$):** 41 ($(C_2H_3N)^+$, 9), 69 ($C_3H_5N_2$, 22), 125 ($(C_7H_{13}N_2)^+$, 100).

**m/z (LSIMS$^-$):** 97 ($(HSO_4)^-$, 73), 177 ($(HSO_4)^-$ + $SO_3$, 9), 195 ($(HSO_4)^-$ + $H_2SO_4$, 100), 219 ($(HSO_4)^-$ + $H_2SO_4$ + $Na^+$, 7), 293 ($(HSO_4)^-$ + $(H_2SO_4)_2$, 19).



### *1.6 $C_4Im:H_2SO_4$ = 1.00:1.50*

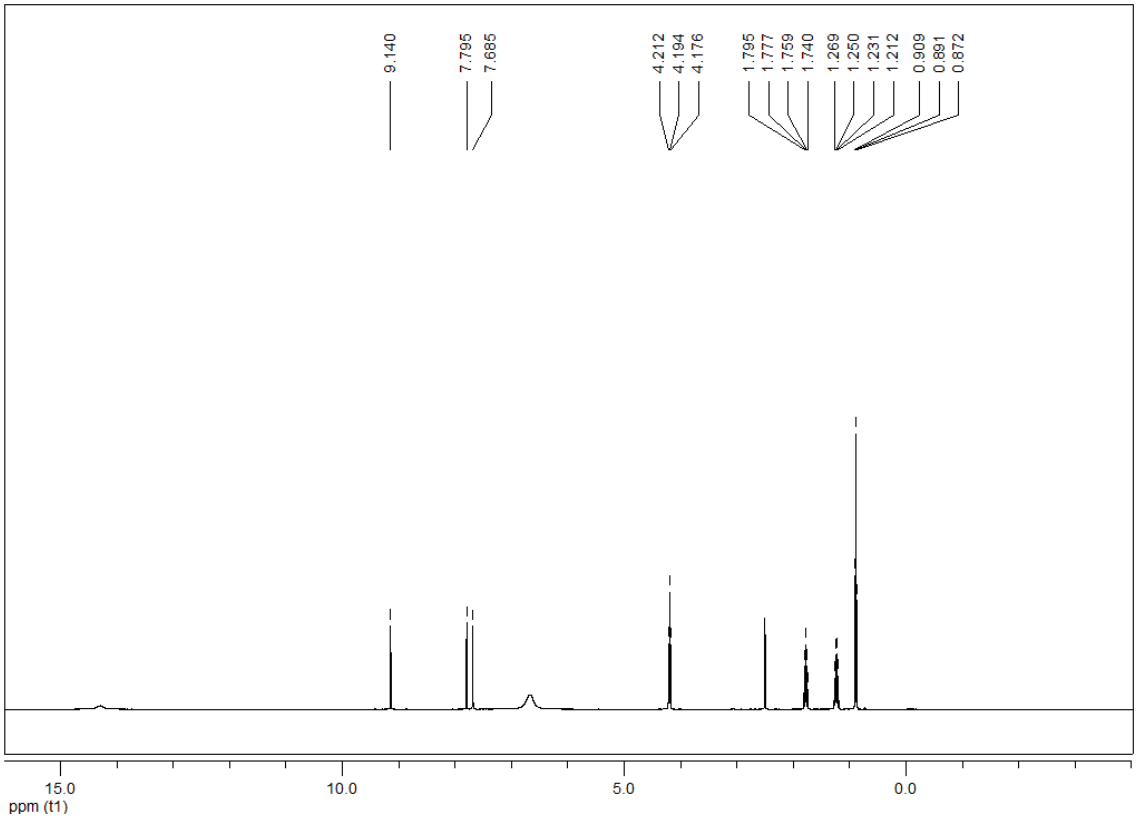
A solution of the $H_2SO_4$ (56.5 mL) in 50 ml water was added drop wise to a stirred solution of 1-butylimidazole (90.0 mL) in 75 ml water. The mixture was stirred at room temperature overnight, treated with charcoal and filtered through neutral alumina. Water was then removed by stirring at 60 $^oC$ under reduced pressure overnight until a water content of 0.89% was achieved.

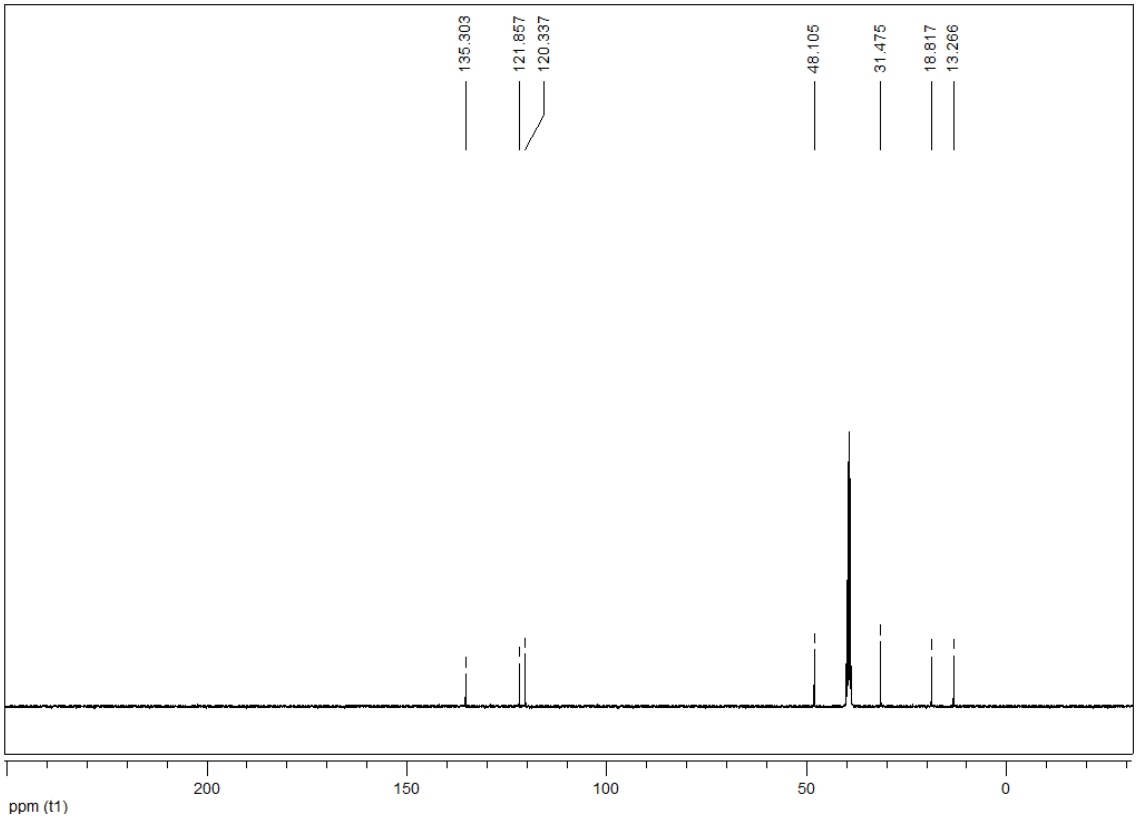
**$^1$H NMR (400 MHz, DMSO-$d_6$, δ):** 9.14 (s, 1 H, C(2)*H*), 7.79 (t, *J* = 1.6 Hz, 1 H, C(4)*H*), 7.69 (t, *J* = 1.5 Hz, 1 H, C(5)*H*), 4.19 (t, *J* = 7.2 Hz, 2 H, NC$H_2$), 1.78 (m, 2 H, $NCH_2CH_2$), 1.24 (m, 2 H, $N(CH_2)_2CH_2$), 0.89 (t, *J* = 7.4 Hz, 3 H, $N(CH_2)_3CH_3$).

**$^{13}$C NMR (101 MHz, DMSO-$d_6$, δ):** 135.3 (*C*(2)H), 121.9 (*C*(5)H), 120.3 (*C*(4)H), 48.1 ($NCH_2$), 31.5 ($NCH_2CH_2$), 18.8 ($N(CH_2)_2CH_2$), 13.3 ($N(CH_2)_3CH_3$).

**m/z (LSIMS$^+$):** 41 (($C_2H_3N$)$^+$, 21), 55 (($C_3H_5N$)$^+$, 7), 57 (($C_2H_5N_2$)$^+$, 8), 69 ($C_3H_5N_2$, 8), 82 ($C_4H_6N_2$, 13), 95 (($C_5H_7N_2$), 10), 125 (($C_7H_{13}N_2$)$^+$, 100), 249 (($C_7H_{13}N_2$)$^+$ + ($C_7H_{12}N_2$), 4), 14), 347 (($C_7H_{13}N_2$)$_2^+$ ($HSO_4$)$^-$,11).

**m/z (LSIMS$^-$):** 97 (($HSO_4$)$^-$, 72), 177 (($HSO_4$)$^-$ + $SO_3$, 9), 195 (($HSO_4$)$^-$ + $H_2SO_4$, 100), 293 (($HSO_4$)$^-$ + ($H_2SO_4$)$_2$, 15).





## 2. Biomass pretreatment, compositional analysis and enzymatic saccharification results:

**Table 1** Pulp recovered after pretreatment of 1 g (ODW) *Miscanthus* (including mass fraction not accounted for in carbohydrate or lignin fractions) and its moisture content.

| Pretreatment solvent | Time (h) | Air-dried weight (g) | Moisture (%) | Recovery (%) | Mass loss (%) |
|---|---|---|---|---|---|
| $H_2O$ | 24 | 0.8635 | 4.41 | 85.18 | 14.82 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 4 | 1.1168 | 9.48 | 101.06 | -1.06 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 24 | 0.7832 | 7.23 | 72.66 | 27.34 |
| $C_4Im:H_2SO_4$ = 1.00:0.50 | 24 | 1.2173 | 16.51 | 101.62 | -1.62 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 4 | 0.8945 | 6.92 | 85.92 | 14.08 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 24 | 0.6335 | 6.70 | 61.00 | 39.00 |
| $C_4Im:H_2SO_4$ = 1.00:0.67 | 24 | 0.8905 | 6.79 | 85.66 | 14.34 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 4 | 0.7770 | 6.89 | 72.35 | 27.65 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 24 | 0.6780 | 7.19 | 62.87 | 37.13 |
| $C_4Im:H_2SO_4$ = 1.00:0.80 | 24 | 0.8865 | 7.32 | 82.14 | 17.86 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 4 | 0.6540 | 7.60 | 60.43 | 39.57 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 24 | 0.5975 | 7.58 | 55.19 | 44.81 |
| $C_4Im:H_2SO_4$ = 1.00:0.99 | 24 | 0.6280 | 6.93 | 58.45 | 41.55 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 2 | 0.5627 | 4.56 | 53.70 | 46.30 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 4 | 0.5202 | 4.61 | 49.62 | 50.38 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 24 | 0.4294 | 4.67 | 40.93 | 59.07 |
| $C_4Im:H_2SO_4$ = 1.00:1.01 | 24 | 0.4965 | 3.94 | 47.69 | 52.31 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 0.25 | 0.9837 | 11.86 | 86.70 | 13.30 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 4 | 0.7170 | 1.25 | 73.28 | 26.72 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 24 | 0.5785 | 2.95 | 57.95 | 42.05 |
| $C_4Im:H_2SO_4$ = 1.00:1.50 | 24 | 1.3190 | 309 | 131.99 | -31.99 |

**Table 2** Yield of precipitate recovered from pretreatment liquor (relative to initial biomass weight).

| Pretreatment solvent | Time (h) | Yield (%) |
|---|---|---|
| $H_2O$ | 24 | 1.03 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 4 | 4.84 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 24 | 8.65 |
| $C_4Im:H_2SO_4$ = 1.00:0.50 | 24 | 1.97 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 4 | 3.20 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 24 | 12.64 |
| $C_4Im:H_2SO_4$ = 1.00:0.67 | 24 | 5.11 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 4 | 7.30 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 24 | 13.93 |
| $C_4Im:H_2SO_4$ = 1.00:0.80 | 24 | 8.25 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 4 | 11.40 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 24 | 17.64 |
| $C_4Im:H_2SO_4$ = 1.00:0.99 | 24 | 19.29 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 2 | 16.60 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 4 | 18.73 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 24 | 24.34 |
| $C_4Im:H_2SO_4$ = 1.00:1.01 | 24 | 21.58 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 0.25 | 0.69 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 4 | 9.86 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 24 | 1.55 |
| $C_4Im:H_2SO_4$ = 1.00:1.50 | 24 | 6.66 |

**Table 3** Glucose yields during enzymatic saccharification (relative to theoretical maximum).

| Pretreatment solvent | Time (h) | Glucose yield 24 h (%) | Glucose yield 48 h (%) | Glucose yield 72 h (%) | Glucose yield 96 h (%) |
|---|---|---|---|---|---|
| Enzyme blank | - | 0.00 | 0.00 | 0.00 | 0.00 |
| Untreated *Miscanthus giganteus* | - | 4.07 | 4.41 | 5.04 | 5.42 |
| $H_2O$ | 24 | 4.54 | 5.43 | 5.69 | 5.97 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 4 | 5.45 | 5.98 | 5.97 | 6.15 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 24 | 25.65 | 30.09 | 33.54 | 34.64 |
| $C_4Im:H_2SO_4$ = 1.00:0.50 | 24 | 13.62 | 15.75 | 18.62 | 18.80 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 4 | 6.85 | 8.97 | 9.76 | 10.18 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 24 | 47.03 | 66.06 | 70.32 | 75.82 |
| $C_4Im:H_2SO_4$ = 1.00:0.67 | 24 | 20.59 | 25.65 | 26.95 | 33.50 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 4 | 23.18 | 29.84 | 31.80 | 35.99 |

| | | | | | |
|---|---|---|---|---|---|
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 24 | 59.43 | 70.84 | 79.47 | 88.58 |
| $C_4Im:H_2SO_4$ = 1.00:0.80 | 24 | 25.57 | 29.89 | 30.99 | 32.23 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 4 | 55.66 | 63.31 | 64.80 | 71.46 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 24 | 72.64 | 81.81 | 86.57 | 89.71 |
| $C_4Im:H_2SO_4$ = 1.00:0.99 | 24 | 11.01 | 14.15 | 15.36 | 18.03 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 2 | 51.33 | 58.08 | 61.55 | 64.58 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 4 | 65.87 | 72.94 | 75.34 | 79.76 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 24 | 74.37 | 75.09 | 79.49 | 83.92 |
| $C_4Im:H_2SO_4$ = 1.00:1.01 | 24 | 9.76 | 10.70 | 10.70 | 11.50 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 0.25 | 8.68 | 10.54 | 10.82 | 10.98 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 4 | 21.70 | 20.14 | 19.48 | 20.50 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 24 | 0.36 | 0.00 | 0.00 | 0.00 |
| $C_4Im:H_2SO_4$ = 1.00:1.50 | 24 | 0.00 | 0.00 | 0.00 | 0.00 |

**Table 4** Xylose + mannose + galactose yields during enzymatic saccharification (relative to theoretical maximum)..

| **Pretreatment solvent** | **Time (h)** | **Xylose + Mannose + Galactose yield 24 h (%)** | **Xylose + Mannose + Galactose yield 48 h (%)** | **Xylose + Mannose + Galactose yield 72 h (%)** | **Xylose + Mannose + Galactose yield 96 h (%)** |
|---|---|---|---|---|---|
| Enzyme blank | - | 0.0 | 0.00 | 0.00 | 0.00 |
| Untreated *Miscanthus giganteus* | - | 0.0 | 0.00 | 0.00 | 0.00 |
| $H_2O$ | 24 | 8.31 | 7.08 | 7.42 | 7.76 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 4 | 6.74 | 7.42 | 7.39 | 7.74 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 24 | 23.64 | 25.77 | 27.42 | 28.13 |
| $C_4Im:H_2SO_4$ = 1.00:0.50 | 24 | 17.66 | 19.14 | 21.32 | 22.07 |

| | | | | | |
|---|---|---|---|---|---|
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 4 | 12.66 | 10.90 | 13.23 | 13.60 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 24 | 31.57 | 35.05 | 37.46 | 38.94 |
| $C_4Im:H_2SO_4$ = 1.00:0.67 | 24 | 27.11 | 25.59 | 27.85 | 32.13 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 4 | 22.25 | 25.27 | 26.00 | 28.98 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 24 | 23.98 | 28.36 | 30.62 | 34.29 |
| $C_4Im:H_2SO_4$ = 1.00:0.80 | 24 | 19.41 | 21.05 | 21.80 | 22.62 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 4 | 22.51 | 24.87 | 24.47 | 28.68 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 24 | 8.89 | 9.64 | 10.07 | 10.46 |
| $C_4Im:H_2SO_4$ = 1.00:0.99 | 24 | 8.77 | 9.60 | 9.52 | 10.66 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 2 | 23.43 | 25.22 | 26.29 | 27.52 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 4 | 19.05 | 20.53 | 20.95 | 22.44 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 24 | 4.76 | 4.89 | 5.14 | 5.66 |
| $C_4Im:H_2SO_4$ = 1.00:1.01 | 24 | 3.89 | 4.01 | 3.90 | 4.20 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 0.25 | 12.18 | 13.67 | 13.07 | 13.72 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 4 | 0.00 | 0.00 | 0.00 | 0.00 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 24 | 0.00 | 0.00 | 0.00 | 0.00 |
| $C_4Im:H_2SO_4$ = 1.00:1.50 | 24 | 0.00 | 0.00 | 0.00 | 0.00 |

**Table 5** Solid content in air-dried pulps and moisture content (oven-dried basis) for pre-treated pulps.

| Pretreatment solvent | Time (h) | % Total solids | % Moisture |
|---|---|---|---|
| $H_2O$ | 24 | 95.81 | 4.41 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 4 | 91.35 | 9.48 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 24 | 93.26 | 7.23 |
| $C_4Im:H_2SO_4$ = 1.00:0.50 | 24 | 85.83 | 16.51 |

| | | | |
|---|---|---|---|
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 4 | 93.52 | 6.92 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 24 | 93.72 | 6.70 |
| $C_4Im:H_2SO_4$ = 1.00:0.67 | 24 | 93.64 | 6.79 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 4 | 93.55 | 6.89 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 24 | 93.29 | 7.19 |
| $C_4Im:H_2SO_4$ = 1.00:0.80 | 24 | 93.18 | 7.32 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 4 | 92.94 | 7.60 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 24 | 92.96 | 7.58 |
| $C_4Im:H_2SO_4$ = 1.00:0.99 | 24 | 93.52 | 6.93 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 2 | 95.64 | 4.56 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 4 | 95.59 | 4.61 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 24 | 95.53 | 4.67 |
| $C_4Im:H_2SO_4$ = 1.00:1.01 | 24 | 96.21 | 3.94 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 0.25 | 89.40 | 11.86 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 4 | 98.78 | 1.25 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 24 | 97.13 | 2.95 |
| $C_4Im:H_2SO_4$ = 1.00:1.50 | 24 | 97.02 | 3.09 |
| Untreated *Miscanthus giganteus* | - | 93.60 | 6.83 |

**Table 6** Biomass recovered after extraction.

| Sample | Initial air-dried mass (g) | Air-dried mass after extraction (g) |
|---|---|---|
| 1 | 3.000 | 2.830 |
| 2 | 3.000 | 2.835 |

**Table 7** Total solids and moisture content of untreated *Miscanthus giganteus* recovered after extraction.

| Sample | $W_{foil}$ | $W_{foil + sample}$ | $W_{foil + dry\ sample}$ | % Total solids | % Moisture |
|---|---|---|---|---|---|
| 1 | 0.397 | 0.816 | 0.780 | 91.4 | 9.4 |
| 2 | 0.439 | 0.819 | 0.786 | 91.3 | 9.5 |

**Table 8** Extractives content of untreated *Miscanthus giganteus.*

| Sample | $Weight_{extractives}$ (g) | $ODW_{sample}$ (g) | Extractives (%) |
|---|---|---|---|
| 1 | 0.170 | 2.742 | 6.2 |
| 2 | 0.165 | 2.740 | 6.0 |

**Table 9** Composition of recovered pulps and untreated *Miscanthus giganteus.*

| Pretreatment solvent | time (h) | Glu | Xyl | Gal | Ara | Man | ASL | AIL | Ash | Extracts |
|---|---|---|---|---|---|---|---|---|---|---|
| $H_2O$ | 24 | 43.0 | 3.8 | 0.0 | 1.1 | 0.0 | 2.9 | 19.5 | 0.4 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 4 | 45.5 | 16.9 | 0.0 | 0.4 | 0.1 | 4.6 | 17.9 | 0.6 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 24 | 66.7 | 18.4 | 0.0 | 1.2 | 0.5 | 4.2 | 12.9 | 0.4 | - |
| $C_4Im:H_2SO_4$ = 1.00:0.50 | 24 | 44.8 | 14.9 | 0.0 | 0.9 | 0.4 | 4.3 | 13.2 | 1.2 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 4 | 44.2 | 14.8 | 0.0 | 1.1 | 2.7 | 5.3 | 14.6 | 0.7 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 24 | 66.6 | 18.5 | 0.0 | 0.3 | 2.6 | 3.4 | 8.5 | 0.9 | - |
| $C_4Im:H_2SO_4$ = 1.00:0.67 | 24 | 46.8 | 21.5 | 0.0 | 2.0 | 1.7 | 4.5 | 16.9 | 0.9 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 4 | 61.6 | 15.5 | 0.0 | 1.1 | 1.4 | 3.2 | 9.1 | 0.9 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 24 | 72.3 | 12.1 | 0.0 | 1.0 | 1.3 | 2.3 | 3.6 | 0.9 | - |
| $C_4Im:H_2SO_4$ = 1.00:0.80 | 24 | 54.3 | 11.8 | 0.0 | 1.4 | 0.9 | 3.6 | 8.4 | 0.7 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 4 | 73.2 | 19.2 | 0.0 | 0.2 | 0.1 | 2.3 | 4.3 | 0.2 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 24 | 75.6 | 7.5 | 0.0 | 1.2 | 0.1 | 0.8 | 1.8 | 0.4 | - |
| $C_4Im:H_2SO_4$ = 1.00:0.99 | 24 | 50.8 | 6.2 | 0.0 | 0.8 | 0.0 | 2.5 | 6.8 | 0.4 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 2 | 74.2 | 14.7 | 0.0 | 1.6 | 0.0 | 2.6 | 6.0 | 2.5 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 4 | 78.2 | 11.2 | 0.0 | 1.8 | 0.0 | 2.5 | 3.7 | 2.5 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 24 | 86.1 | 3.8 | 0.0 | 0.0 | 0.0 | 1.4 | 4.1 | 2.6 | - |
| $C_4Im:H_2SO_4$ = 1.00:1.01 | 24 | 72.4 | 4.9 | 0.0 | 0.0 | 0.0 | 1.6 | 4.5 | 1.8 | - |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 0.25 | 46.9 | 17.6 | 2.2 | 1,366 | 0.0 | 3.4 | 18.3 | 1.8 | - |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 4 | 43.1 | 0.3 | 1.6 | 0.0 | 0.0 | 0.4 | 34.4 | 1.4 | - |
| Untreated *Miscanthus giganteus* | - | 43.2 | 19.4 | 2.5 | 3.2 | 1.2 | 3.5 | 20.4 | 0.9 | 5.6 |

**Table 10** Glucan digestibility after enzymatic saccharification.

| Pretreatment solvent | Pretreatment time (h) | Glucose yield 24 h (%) | Glucose yield 48 h (%) | Glucose yield 72 h (%) | Glucose yield 96 h (%) |
|---|---|---|---|---|---|
| Untreated *Miscanthus giganteus* | - | 3.9 | 4.2 | 4.8 | 5.2 |
| $H_2O$ | 24 | 5.4 | 6.4 | 6.7 | 7.0 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 4 | 5.1 | 5.6 | 5.6 | 5.8 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 24 | 22.9 | 26.8 | 29.9 | 30.9 |
| $C_4Im:H_2SO_4$ = 1.00:0.50 | 24 | 13.0 | 15.0 | 17.8 | 17.9 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 4 | 7.4 | 9.7 | 10.5 | 11.0 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 24 | 49.7 | 69.9 | 74.6 | 80.6 |
| $C_4Im:H_2SO_4$ = 1.00:0.67 | 24 | 20.9 | 26.2 | 27.5 | 34.2 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 4 | 22.4 | 28.8 | 30.8 | 34.7 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 24 | 57.2 | 68.1 | 76.4 | 85.2 |
| $C_4Im:H_2SO_4$ = 1.00:0.80 | 24 | 24.9 | 29.1 | 30.2 | 31.4 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 4 | 54.6 | 62.1 | 63.6 | 70.0 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 24 | 76.9 | 86.1 | 91.0 | 94,.5 |
| $C_4Im:H_2SO_4$ = 1.00:0.99 | 24 | 16.5 | 21.3 | 23.1 | 27.2 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 2 | 55.7 | 63.1 | 66.8 | 70.1 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 4 | 73.4 | 81.3 | 84.0 | 88.9 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 24 | 91.3 | 92.2 | 97.6 | 97.6 |
| $C_4Im:H_2SO_4$ = 1.00:1.01 | 24 | 12.2 | 13.4 | 13.4 | 14.4 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 0.25 | 9.2 | 11.2 | 11.5 | 11.7 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 4 | 28.6 | 27.0 | 25.8 | 27.0 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 24 | 0.0 | 0.0 | 0.0 | 0.0 |
| $C_4Im:H_2SO_4$ = 1.00:1.50 | 24 | 0.0 | 0.0 | 0.0 | 0.0 |

**Table 11** Xylose + mannose + galactose digestibility after enzymatic saccharification.

| Pretreatment solvent | Pretreatment time (h) | Xylose + Mannose + Galactose yield 24 h (%) | Xylose + Mannose + Galactose yield 48 h (%) | Xylose + Mannose + Galactose yield 72 h (%) | Xylose + Mannose + Galactose yield 96 h (%) |
|---|---|---|---|---|---|
| Untreated *Miscanthus giganteus* | - | 0.0 | 0.0 | 0.0 | 0.0 |
| $H_2O$ preteated | 24 | 59.2 | 50.5 | 52.9 | 55.3 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 4 | 9.1 | 10.0 | 10.0 | 10.5 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.50/20% | 24 | 40.0 | 43.7 | 46.4 | 47.6 |
| $C_4Im:H_2SO_4$ = 1.00:0.50 | 24 | 26.2 | 28.4 | 31.6 | 32.7 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 4 | 18.7 | 16.1 | 19.6 | 20.2 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.67/20% | 24 | 56.0 | 62.4 | 66.7 | 69.5 |
| $C_4Im:H_2SO_4$ = 1.00:0.67 | 24 | 30.0 | 28.4 | 30.9 | 35.6 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 4 | 42.3 | 48.1 | 49.4 | 55.2 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.80/20% | 24 | 65.4 | 77.2 | 84.1 | 94.2 |
| $C_4Im:H_2SO_4$ = 1.00:0.80 | 24 | 43.2 | 46.8 | 48.5 | 50.3 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 4 | 45.0 | 49.8 | 49.0 | 56.9 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:0.99/20% | 24 | 48.9 | 53.2 | 55.6 | 57.7 |
| $C_4Im:H_2SO_4$ = 1.00:0.99 | 24 | 61.6 | 67.7 | 67.3 | 75.7 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 2 | 68.8 | 74.1 | 77.2 | 80.9 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 4 | 79.8 | 86.0 | 87.7 | 94.0 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.01/20% | 24 | 71.8 | 73.3 | 77.4 | 85.2 |
| $C_4Im:H_2SO_4$ = 1.00:1.01 | 24 | 38.9 | 40.1 | 39.0 | 41.9 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 0.25 | 16.47 | 18.49 | 17.68 | 18.56 |
| $C_4Im:H_2SO_4/H_2O$ = 1.00:1.50/20% | 4 | 0.0 | 0.0 | 0.0 | 0.0 |
| $C_4Im:H_2SO_4/H_2O$ = | 24 | 0 | 0 | 0.0 | 0.0 |

| | | | | | |
|---|---|---|---|---|---|
| 1.00:1.50/20% | | | | | |
| $C_4Im:H_2SO_4$ = 1.00:1.50 | 24 | 0.0 | 0.0 | 0.0 | 0.0 |