# **ELECTRONIC SUPPLEMENTARY INFORMATION**

# How to produce easily biodiesel using a green biocatalytic approach in sponge-like ionic liquids

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**Table 1.** Phase behaviour of IL/triolein/methanol (left column) and IL/methyl oleate (rigth column) mixtures at 60°C. For IL/triolein/methanol mixtures, the following ratios, A, 9.1/16.2/74.7; B, 18.5/14.6/66.9; C, 31.0/12.3/56.7; D, 47.6/9.4/43.0; E, 63.8/6.5/29.7; F, 73.7/4.2/22.1 (w/w/w). For IL/methyl oleate mixtures, the following ratios were assayed: a, 10/90; b: 20/80; c, 33/67; d, 50/50; e, 66/34; f, 75/25. See Experimental section for further details.

IL	IL/triolein/methanol mixtures	Methyl oleate/IL mixtures
[C4tma][NTf2]		
[C <sub>12</sub> tma][NTf <sub>2</sub> ]		
[C <sub>14</sub> tma][NTf <sub>2</sub> ]		
[C <sub>16</sub> tma][NTf <sub>2</sub> ]		
[C <sub>18</sub> tma][NTf <sub>2</sub> ]	Sease	

#### Determination of enzymatic activity.

For each IL (*i.e.*  $[C_{12}tma][NTf_2]$ ,  $\Box$ ;  $[C_{14}tma][NTf_2]$ ,  $\blacktriangle$ ;  $[C_{16}tma][NTf_2]$ ,  $\blacksquare$ ; or  $[C_{18}tma][NTf_2]$ ,  $\bigcirc$ , see next Figure), triolein (1 mmol) was added into a screw-capped vial (3.0 mL total capacity), containing 885 mg of IL and methanol (6 mmol). The resulting final mixtures at a 1/6 (mol/mol) triolein/methanol ratio gave a 47.7/43.0/9.3 (w/w/w) IL/triolein/methanol ratio. For each case, the mixture was previously incubated in an orbital shaker (IKA KS4000i, Germany) for 30 min at 1,000 rpm and at 60°C to melt the IL. The reaction was started by adding 150 mg Novozym 435, and the reaction system was incubated at 60°C for 1 h. To obtain the time-course profiles depicted in the next Figure, 20 µL aliquots were taken at 0, 15, 30, 45 and 60 min, and suspended in 480 µL dodecane/isopropanol (95:5, v/v) solutions, and the resulting biphasic mixtures were strongly shaken for 3 min, and then centrifuged at 15,000 rpm for 10 min to extract biodiesel. Finally, 350 µL of dodecane/isopropanol extracts (upper phase) were added to 150 µL of 100 mM ethyl decanoate and 100 mM tributyrin (internal standards) solution in dodecane/isopropanol (95:5, v/v), and the final solution was analyzed by CG. For each IL, initial activity (see Table 2) was determined by using the methyl oleate content in the reaction medium at 0, 15 and 30 min of reaction time, because of its linear profile. One unit of activity was defined as the amount of enzyme that produces 1 µmol of methyl oleate per min.



### NMR analysis

#### Cooling/centrifugation protocol for biodiesel extraction from sponge-like ILs

Samples of each upper liquid phase obtained from methyl oleate /IL mixtures by using the iterative cooling  $[C_{12}tma][[NTf_2]],$ centrifugation protocol were prepared as follows: for  $[C_{14}tma][[NTf_2]],$  $[C_{16}tma][[NTf_2], [C_{12}tma]][[NTf_2]]$  cases,  $40 \mu L$  aliquots were dissolved in 0.5 mL acetone- $\delta_6$  containing trifluoroacetic acid (TFA, 10 µL) as internal standard. For the [C<sub>18</sub>tma][NTf<sub>2</sub>] case, a 80 µL aliquot of the upper phase were dissolved in 340  $\mu$ L acetone- $\delta_6$  containing TFA (80  $\mu$ L) as internal standard. Samples were analyzed by 188 MHz <sup>19</sup>F NMR on a Bruker AC 200E spectrometer, the residual IL being quantified with respect to standard IL solutions (*i.e.* [C<sub>12</sub>tma][NTf<sub>2</sub>], [C<sub>14</sub>tma][NTf<sub>2</sub>], [C<sub>16</sub>tma][NTf<sub>2</sub>] or [C<sub>18</sub>tma][NTf<sub>2</sub>], respectively) in acetone- $\delta_6$  containing TFA (( $\delta$ = 76.79 ppm).





<sup>19</sup>*F* NMR spectrum of the upper phase from  $[C_{12}tma][NTf_2]$  (with the washing-by-water step)



<sup>19</sup>*F* NMR spectrum of [ $C_{14}$ tma][NTf<sub>2</sub>] standard ( $\delta$ =-79.9 ppm)



<sup>19</sup>F NMR spectrum of the upper phase from  $[C_{14}tma][NTf_2]$  (without the washing-by-water step)



<sup>19</sup>F NMR spectrum of the upper phase from  $[C_{14}tma][NTf_2]$  (with the washing-by-water step)



<sup>19</sup>*F* NMR spectrum of  $[C_{16}tma][NTf_2]$  standard ( $\delta$ =-79.9 ppm)



<sup>19</sup>F NMR spectrum of the upper phase from  $[C_{16}tma][NTf_2]$  (without the washing-by-water step)



<sup>19</sup>F NMR spectrum of the upper phase from  $[C_{16}tma][NTf_2]$  (with the washing-by-water step)



<sup>19</sup>F NMR spectrum of [ $C_{18}$ tma][NTf<sub>2</sub>] standard ( $\delta$ =-79.9 ppm)



<sup>19</sup>F NMR spectrum of the upper phase from  $[C_{18}tma][NTf_2]$  (without the washing-by-water step)



<sup>19</sup>F NMR spectrum of the upper phase from  $[C_{18}tma][NTf_2]$  (with the washing-by-water step)



### Extraction of glycerol and methanol from model reaction mixture in [C<sub>18</sub>tma][NTf<sub>2</sub>] IL.

Samples (80  $\mu$ L) of each three phases were dissolved in 340  $\mu$ L acetone- $\delta_6$  containing TFA (80  $\mu$ L, IS), then 300MHz <sup>13</sup>C NMR spectra were recorded in a Bruker AC 300E or Bruker AC 600E. For the case of middle D<sub>2</sub>O phase, samples (80  $\mu$ L) were also dissolved in 340  $\mu$ L D<sub>2</sub>O containing TFA (80  $\mu$ L, IS). Standards solutions of glycerol and methanol in either acetone- $\delta_6$  or D<sub>2</sub>O, containing TFA ( $\delta$ = -117.83, -115.92, -114.01, -112.10 ppm) were used for quantification.



<sup>13</sup>C NMR spectrum of glycerol ( $\delta$ = -71.53, -61.95 ppm) and methanol ( $\delta$ = -48.29 ppm) standards in D<sub>2</sub>O

<sup>13</sup>C NMR spectrum of the middle D<sub>2</sub>O phase





 $^{13}C$  NMR spectrum of glycerol ( $\delta$ = -75.95, 65.69 ppm) and methanol ( $\delta$ = -48.52 ppm) standards in acetone- $\delta_6$ 

 $^{13}C$  NMR spectrum of the upper phase (extracted methyl oleate) in acetone- $\delta_6$ 



 $^{13}C$  NMR spectrum of [ $C_{18}$ tma][NTf<sub>2</sub>] bottom phase solid (IL) in acetone- $\delta_6$ 



## Sponge-like behviour of [C<sub>16</sub>tma][NTf<sub>2</sub>] and [C<sub>18</sub>tma][NTf<sub>2</sub>]

Determination of the IL content in the solid bottom phase after 5 soaking/wringing out cycles

Samples (40  $\mu$ L) of bottom phase ([C<sub>16</sub>tma][NTf<sub>2</sub>] and [C<sub>18</sub>tma][NTf<sub>2</sub>]) were dissolved in 500  $\mu$ L acetone- $\delta_6$  containing TFA (10  $\mu$ L, IS), then analyzed by 188 MHz <sup>19</sup>F NMR in a Bruker AC 200E spectrometer, the residual IL being quantified with respect to standard IL solutions (*i.e.* [C<sub>16</sub>tma][NTf<sub>2</sub>] or [C<sub>18</sub>tma][NTf<sub>2</sub>], respectively) in acetone- $\delta_6$  containing TFA ( $\delta$ = -76.79 ppm).





 $^{19}F$  NMR spectrum of [ $C_{18}$ tma][NTf<sub>2</sub>] bottom phase in acetone- $\delta_6$ 

