Electronic Supplementary Information

Hydroxyl ammonium ionic liquids as media for biocatalytic oxidations

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Biodegradability test

Biodegradation is the natural process for the removal of organic substances from the environment. The determination of the biodegradability level of organic substances such as ILs is essential in order to estimate their environmental impact. Biodegradability assessment of ILs have been examined by measuring the Biochemical Oxygen Demand (BOD) [1],[2].

In this work, biodegradation tests were carried out according to a manometric method so as to determine the oxygen demand for the biochemical degradation of each organic substance after five days. VELP BOD manometric apparatus was used to measure the BOD of the IL inoculated samples. This method is based on the steady decrease of the pressure in a closed system as a result of oxygen consumption. The carbon dioxide which is produced is bounded by a strongly alkaline medium (KOH pellets above the solution) so as not to interfere with the final measurements. The nutrients prepared are:

- Ferric chloride hexahydrate: 0.25 g $FeCl_3 \cdot 6H_2O$ to a final volume of 1 L with distilled water.
- Calcium chloride anhydrous: 27.5 g $CaCl_2$ to a final volume of 1 L with distilled water.
- Magnesium sulfate heptahydrate: 22.5 g $MgSO_4 \cdot 7H_2O$ to a final volume of 1 L with distilled water.
- Phosphate salts solution (buffer): 8.5 g KH_2PO_4, 21.7 g K_2HPO4, 33.4 g $Na_2HPO_4 \cdot 7H_2O$ and 1.7 g NH_4Cl to a final volume of 1 L with distilled water.

This method consists of filling each BOD flask with specific amount of IL, 135 mL aqueous solution of nutrients and 15 mL microorganisms. The seed source of microorganisms was mixed liquor which was taken from a secondary sedimentation tank of urban waste water of Psyttaleia sewage treatment plant in Greece. A blank solution was also prepared, containing only nutrients and mixed liquor.

In general, two stages of degradation take place during the BOD test, carbonaceous and nitrogenous but in this work only the carbonaceous demand taken into account and the BOD values will be reported as CBOD (degradation of the organic carbon). Inhibition of nitrogenous bacteria was achieved by a thiourea solution (2 g thiourea to a final volume of 1 L with distilled water) which was also added to BOD samples (0.5 mL of the solution in each flask). The samples were kept at 20 ± 1°C in darkness in tightly closed bottles for an incubation period of 5 days.

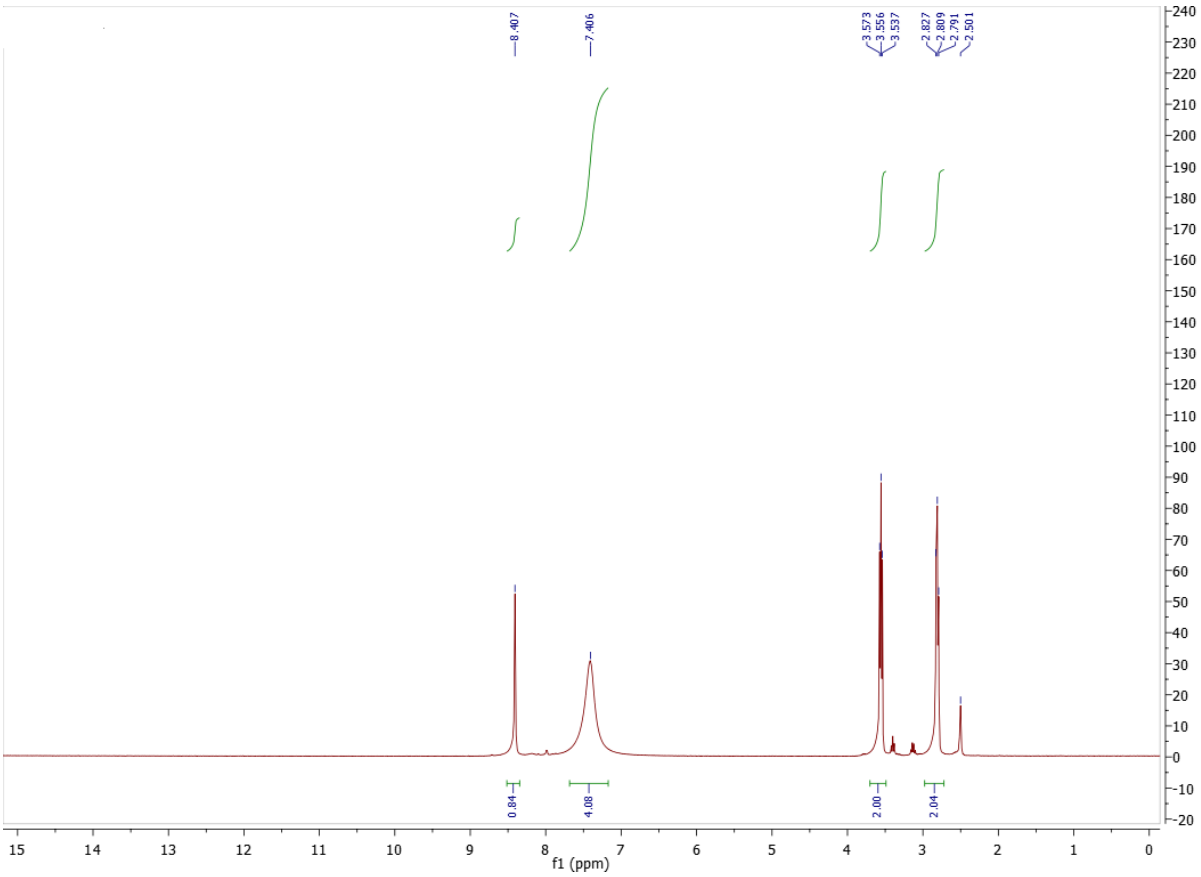


Fig. S1. ^{1}H NMR spectrum of *2-hydroxylethylammonium formate* (HEAF).

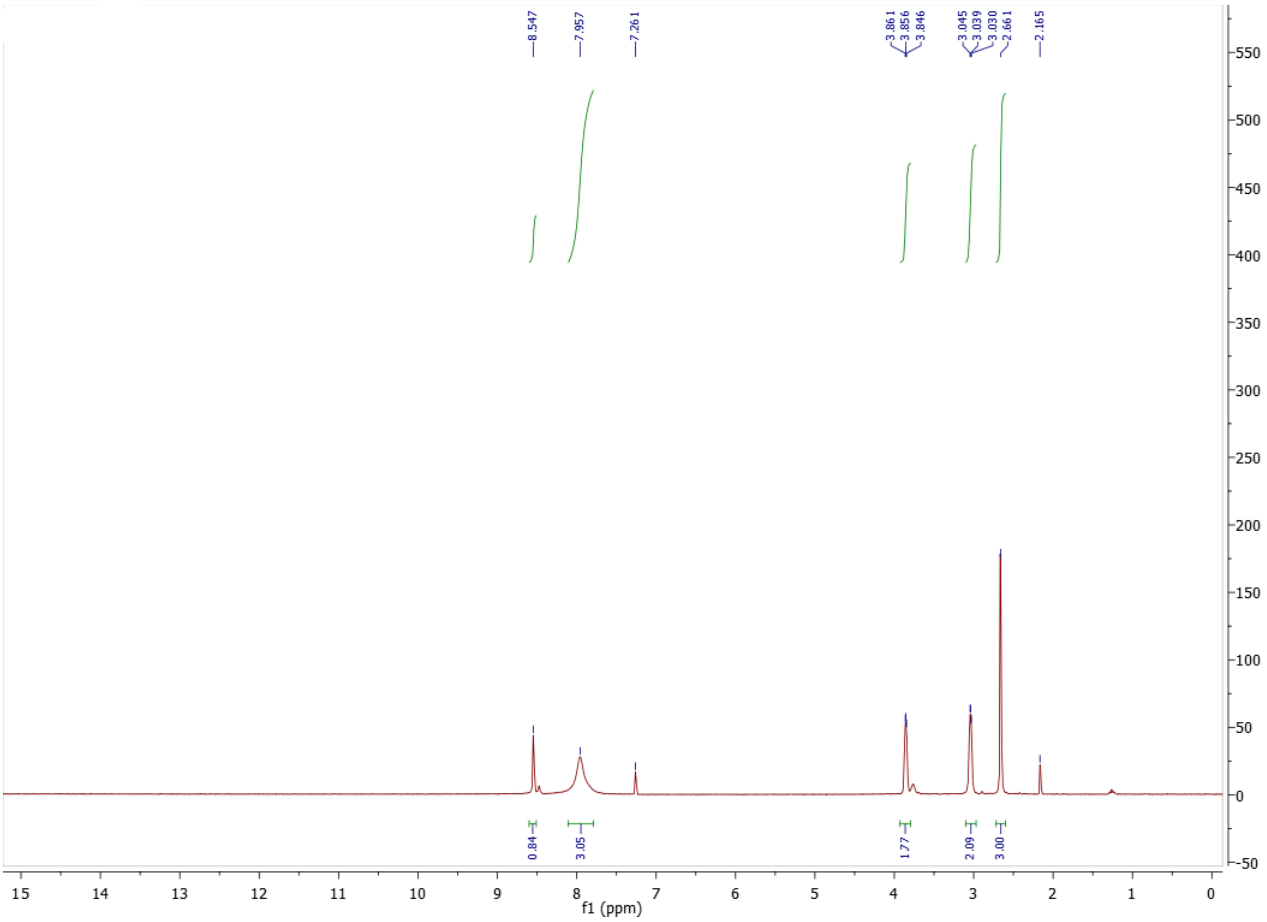


Fig. S2. ^{1}H NMR spectrum of *2-hydroxy-N-methylethanaminium formate* (HMEAF).

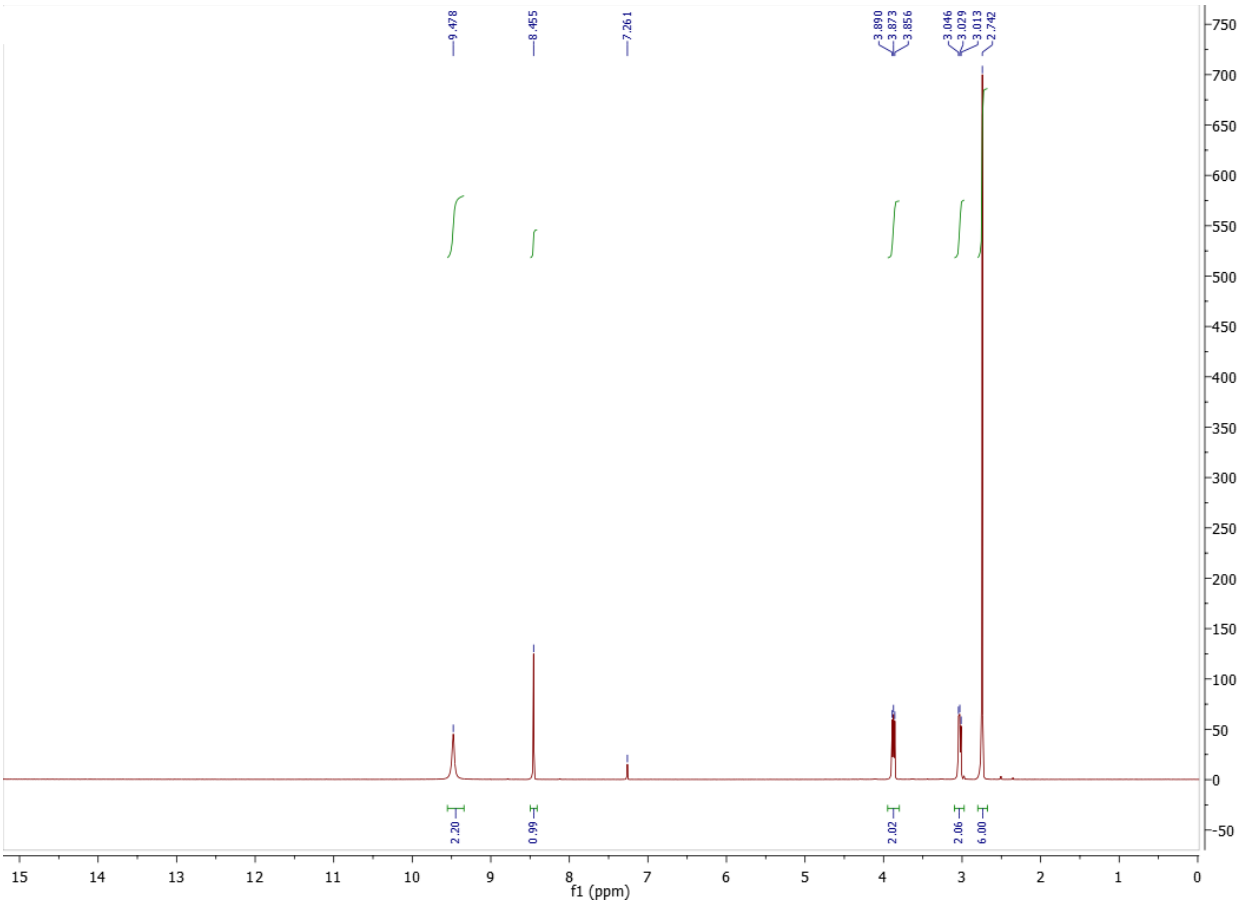


Fig. S3. ^{1}H NMR spectrum of *2-hydroxy-N,N-dimethylethanaminium formate* (HDMEAF).

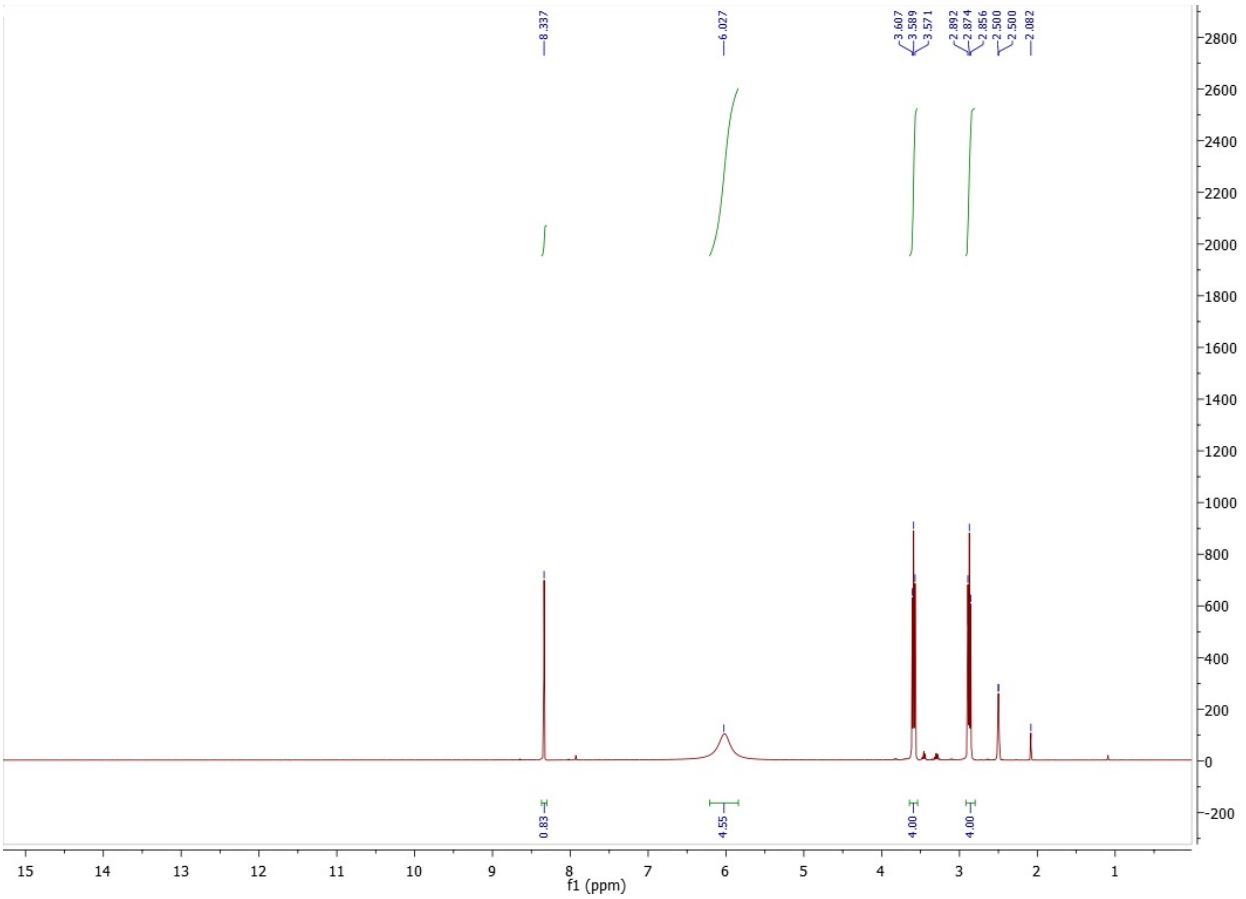


Fig. S4. ^{1}H NMR spectrum of *bis(2-hydroxyethyl)ammonium formate* (BHEAF).

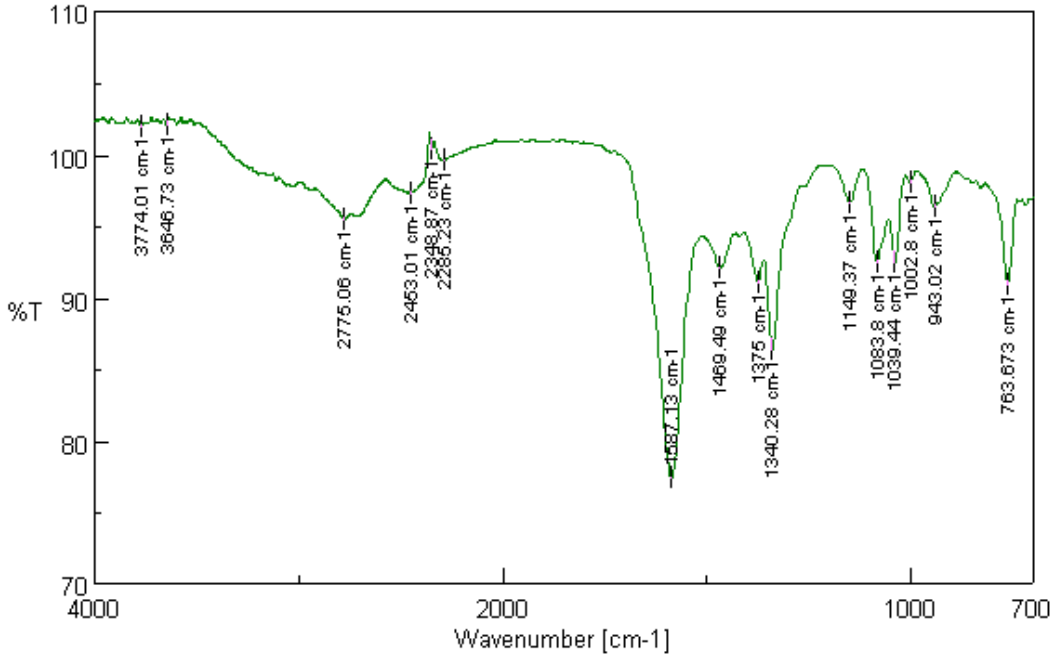


Fig. S5. ATR spectrum of 2-*hydroxylethylammonium formate* (HEAF).

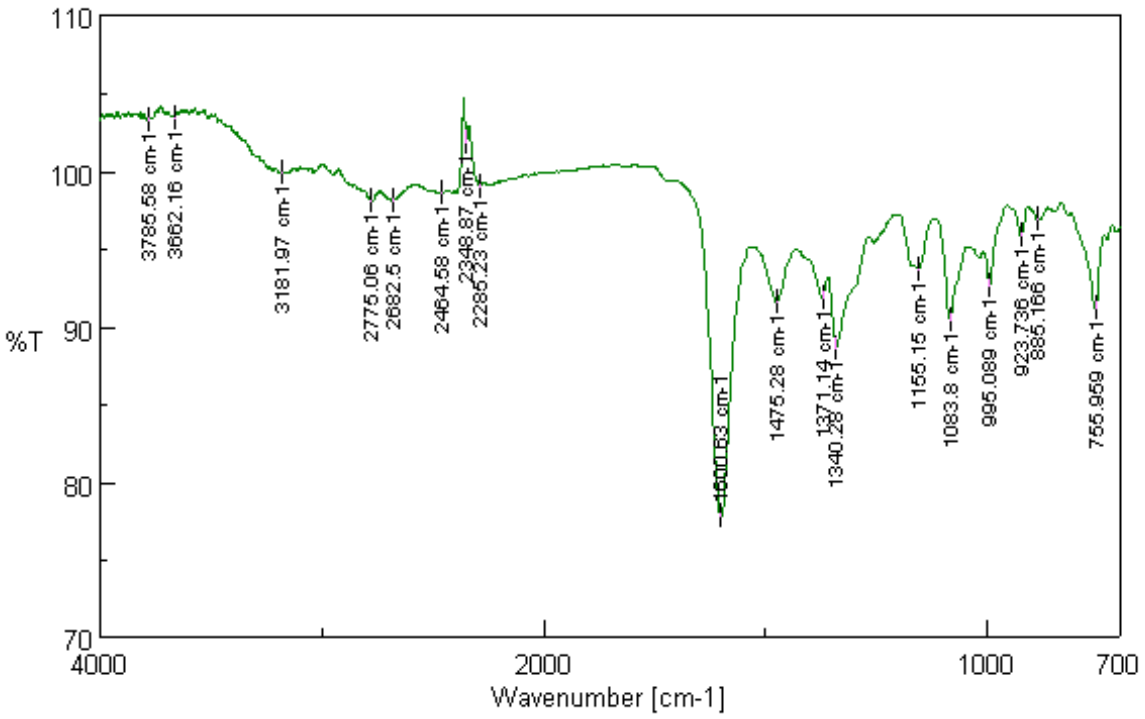


Fig. S6. ATR spectrum of *2-hydroxy-N,N-dimethylethanaminium formate* (HDMEAF).

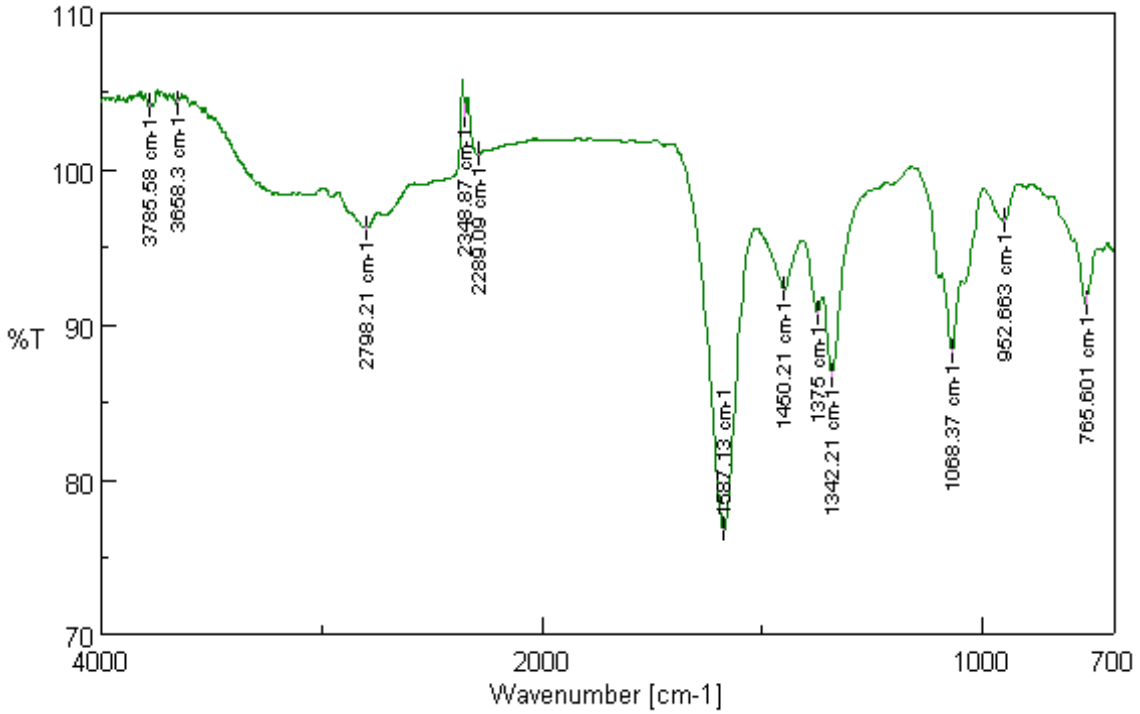


Fig. S7. ATR spectrum of *bis(2-hydroxyethyl)ammonium formate (BHEAF)*

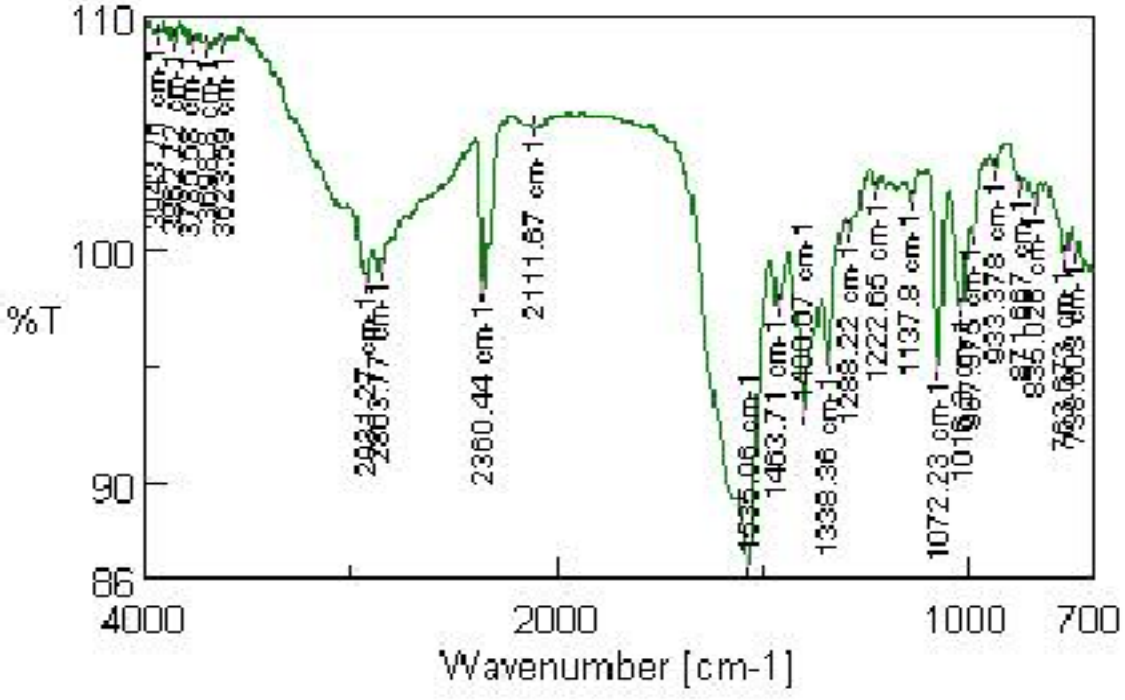


Fig. S8. ATR spectrum of 2-hydroxylethylammonium formate (HEAF):

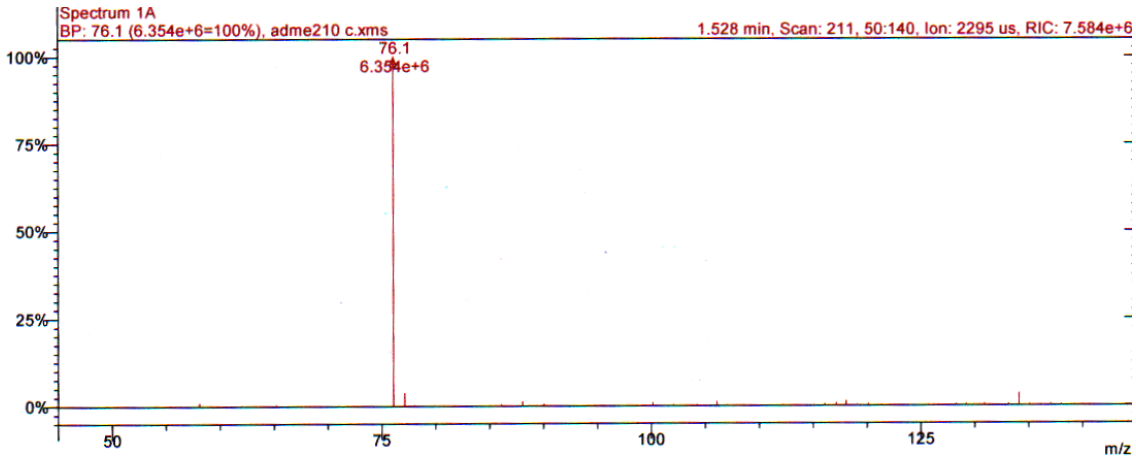


Fig. S9. MS spectrum of *2-hydroxy-N-methylethanaminium formate* (HMEAF).

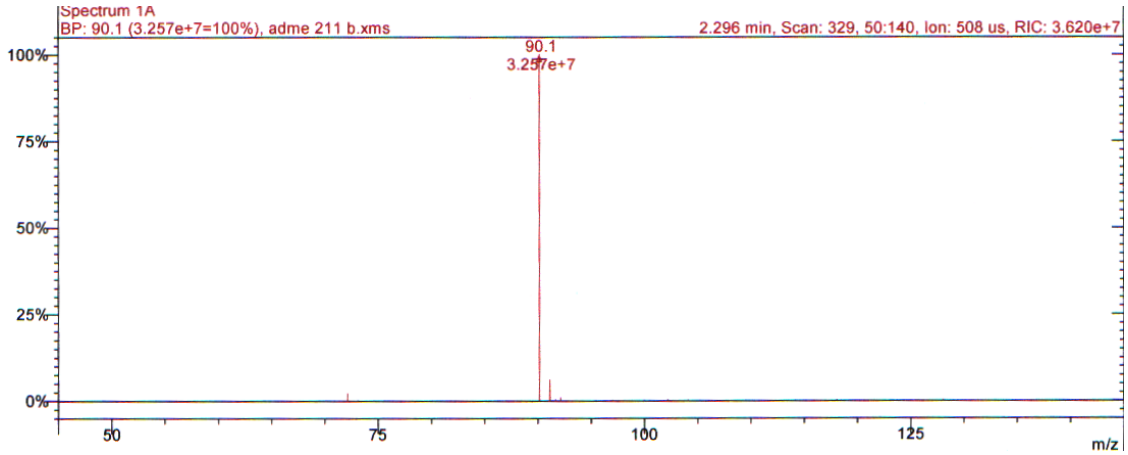


Fig. S10. MS spectrum of *2-hydroxy-N,N-dimethylethanaminium formate* (HDMEAF).

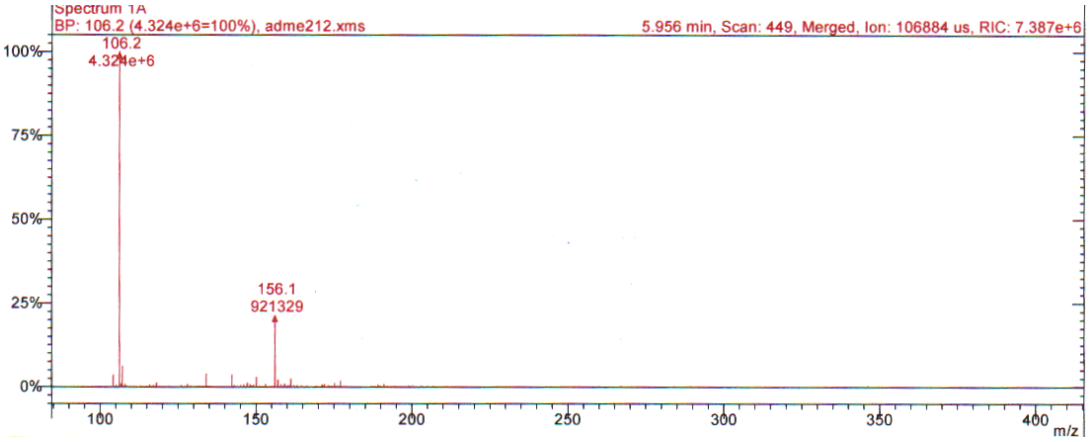


Fig. S11. MS spectrum of *bis(2-hydroxyethyl)ammonium formate* (BHEAF).

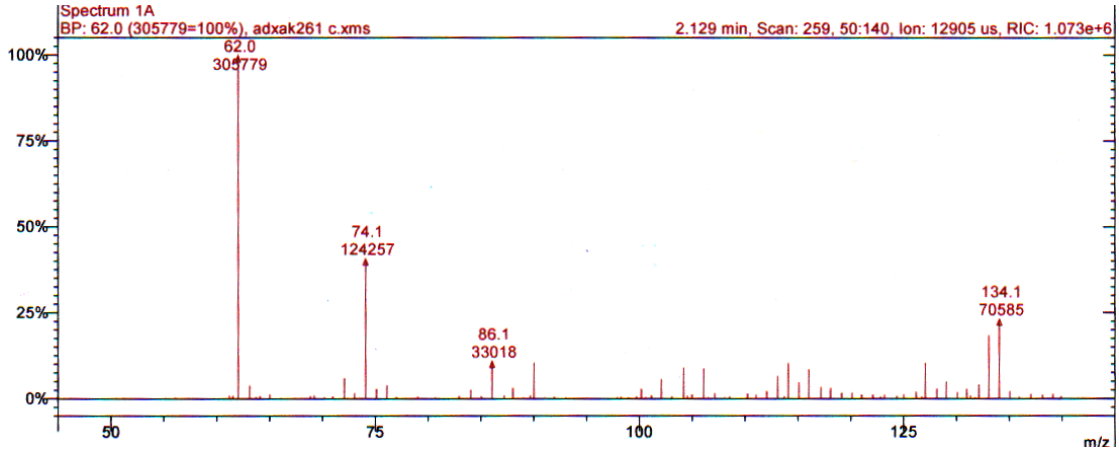


Fig. S12. MS spectrum of *2-hydroxylethylammonium formate* (HEAF).

The UV–Vis spectroscopic measurements were performed on a double-beam UV-vis spectrophotometer (UV-1601 Shimadzu, Tokyo, Japan) in a standard 1 cm path length quartz cuvette.

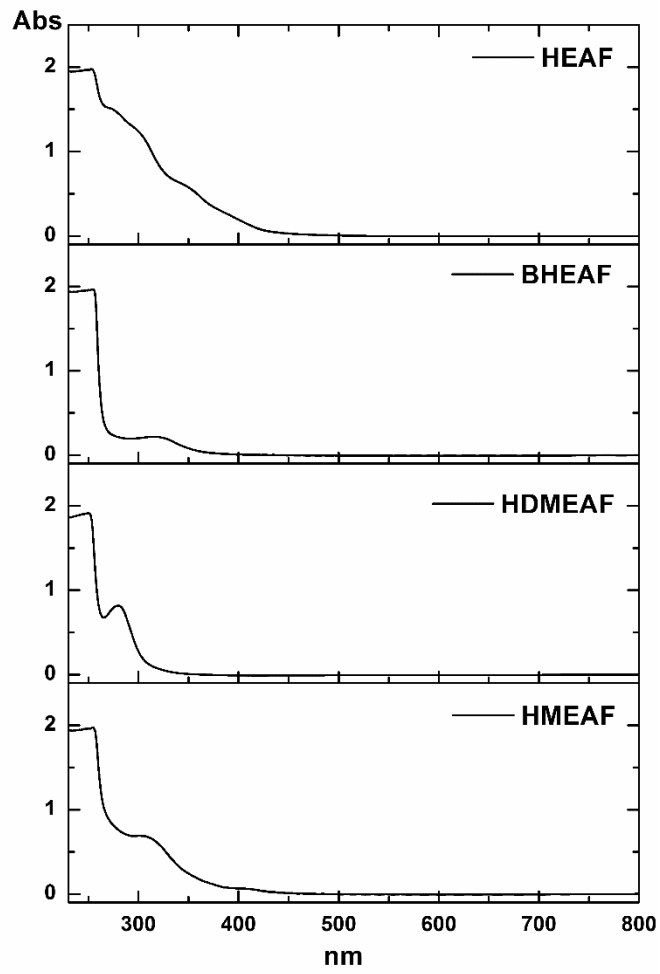
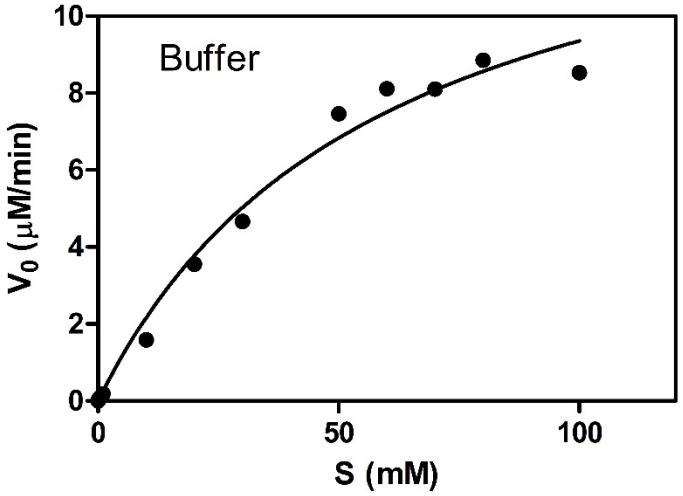
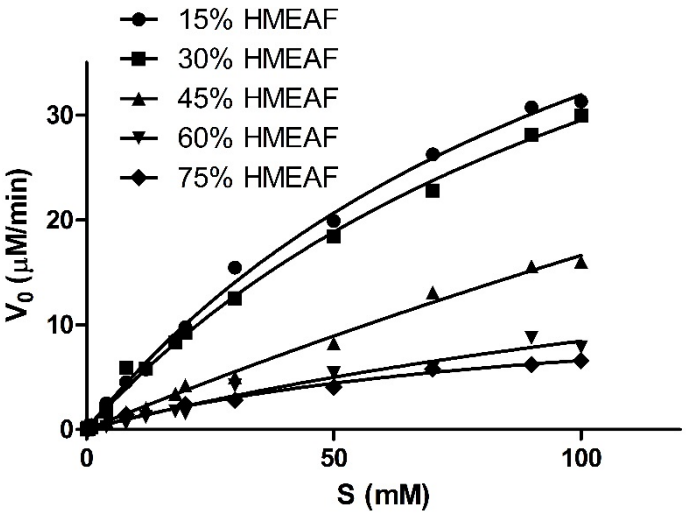
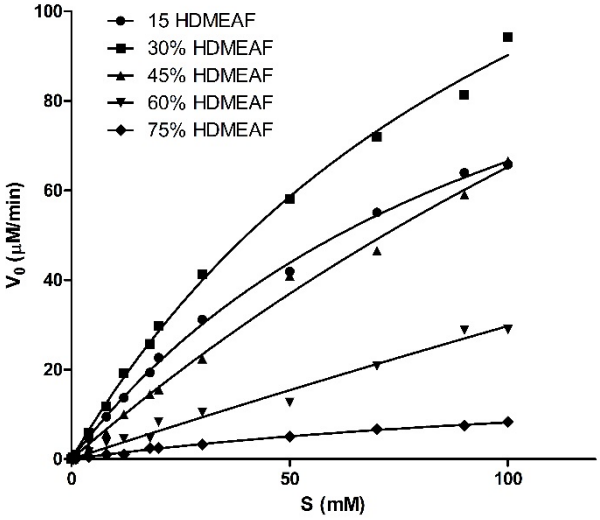
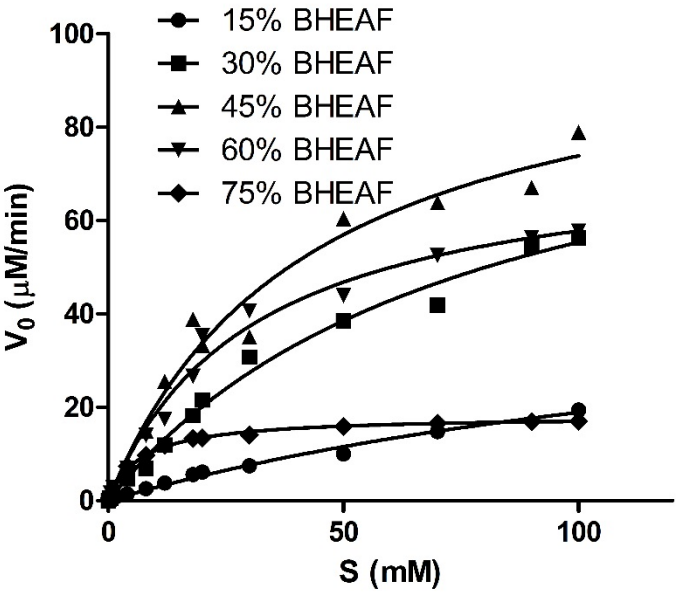


Fig. S13. UV-vis spectra (230-800 nm) of all ILs used in this study.









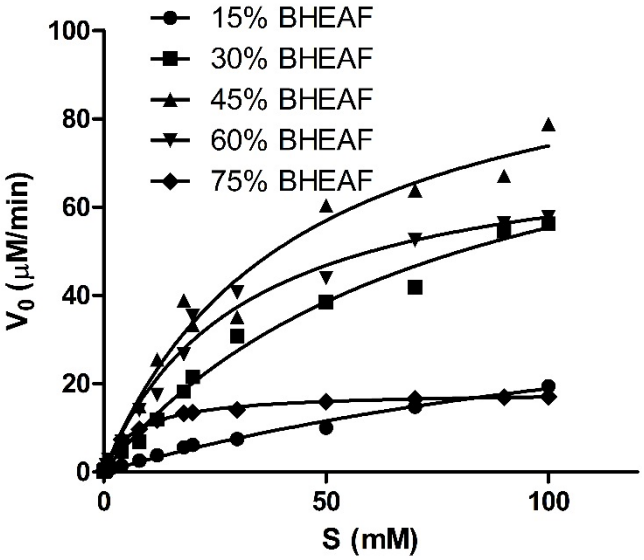


Fig. S14. Michaelis Mented plots for cyt c catalyzed oxidation of guaiacol with H_2O_2 in buffer and in various Ils-based media.

Table S1. Apparent kinetic parameters K_m^{app} (μM) and V_{max}^{app} (μM min^{-1}) of guaiacol oxidation with H_2O_2 by cyt c in the presence of various amounts of ILs (0-75% v/v).

% of IL in reaction medium (v/v)	HEAF		HMEAF		HDMEAF		BHEAF	
	K_m^{app}	V_{max}^{app}	K_m^{app}	V_{max}^{app}	K_m^{app}	V_{max}^{app}	K_m^{app}	V_{max}^{app}
0	58.6 ±1.1	18.8 ±2.5	58.6 ±1.1	18.8 ±2.5	58.6 ±1.1	18.8 ±2.5	58.6 ±1.1	18.8 ±2.5
15	93.3 ±2.2	70.96 ±2.9	120.3 ±5.3	70.3 ±4.2	107.2 ±5.4	137.9 ±5.9	173.2 ±10.9	51.6 ±3.5
30	110 ±1.2	188.7 ±8.5	63.3 ±3.1	43.5 ±3.6	117.2 ±6.8	196.0 ±9.8	77.5 ±5.6	98.2 ±6.8
45	111.1 ±1.4	397.4 ±10.8	118.2 ±6.8	32.1 ±3.9	88.7 ±4.3	108.7 ±7.3	42.2 ±3.6	105.1 ±12.1
60	45.2 ±2.5	231.8 ±12.8	113.5 ±7.2	16.8 ±2.8	281.2 ±8.6	107.8 ±6.4	30.8 ±6.8	75.6 ±9.8
75	34.3 ±1.6	164.4 ±19.3	99.3 ±7.1	13.1 ±1.2	143.2 ±5.9	20.2 ±3.6	7.6 ±4.3	18.4 ±2.3

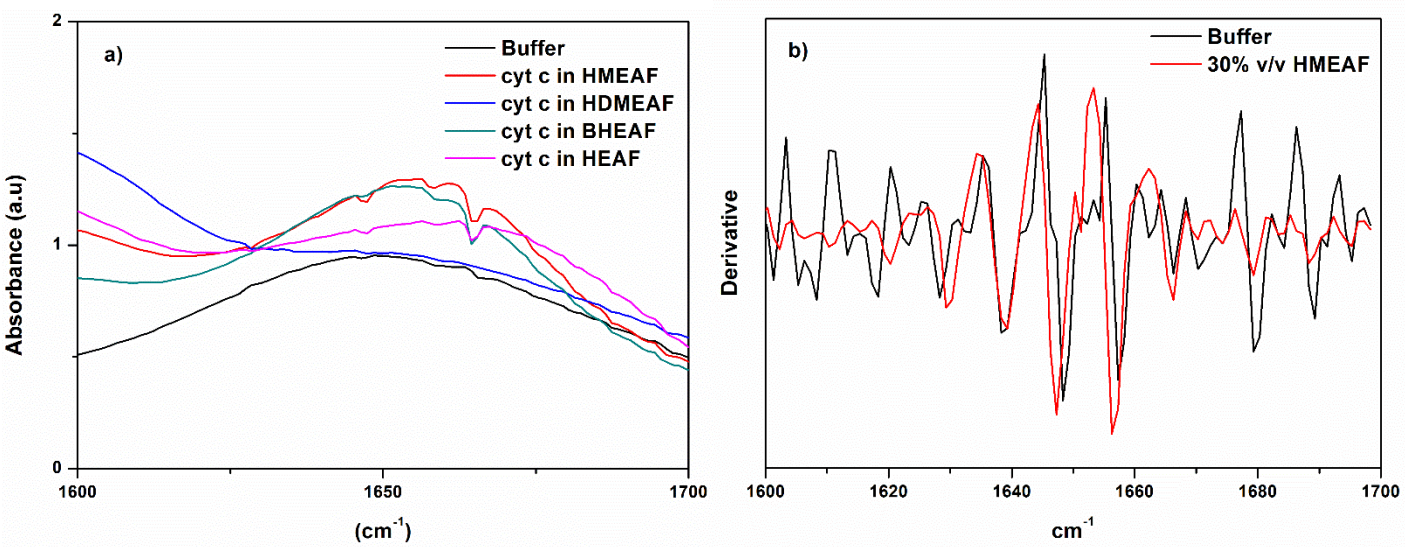


Fig. S15. a) Comparison of ATR spectra of the Amide I region of cyt c in 50 mM phosphate buffer pH 7.0 and 30 % v/v of all ILs studied, b)Comparison of the second derivative spectra in the Amide I region of cyt c in 50mM phosphate buffer pH 7.0 and 30% v/v HMEAF.

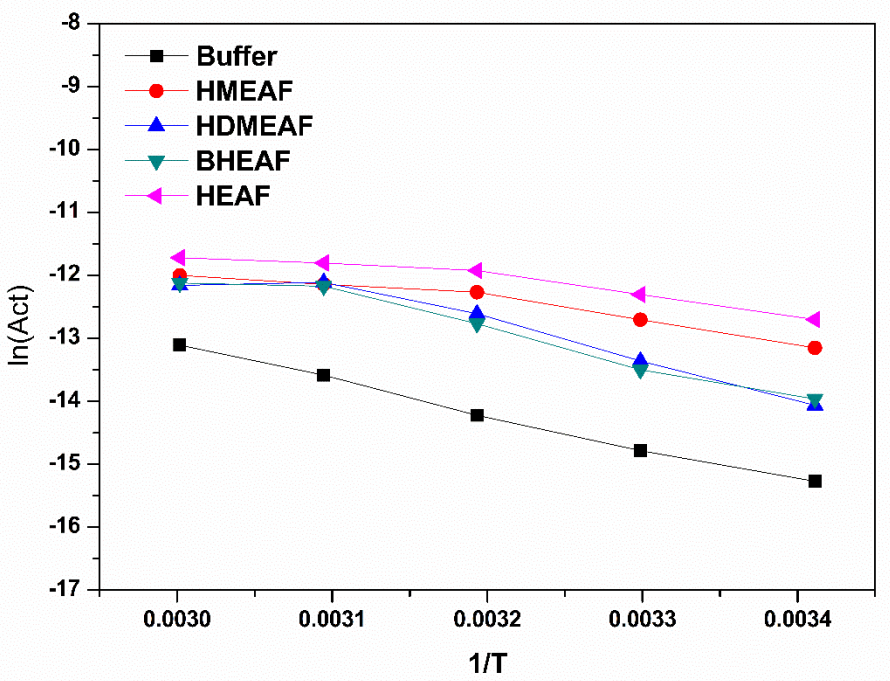


Fig. S16. Arrhenious plots of cyt c activity in buffer and all ILs studied.

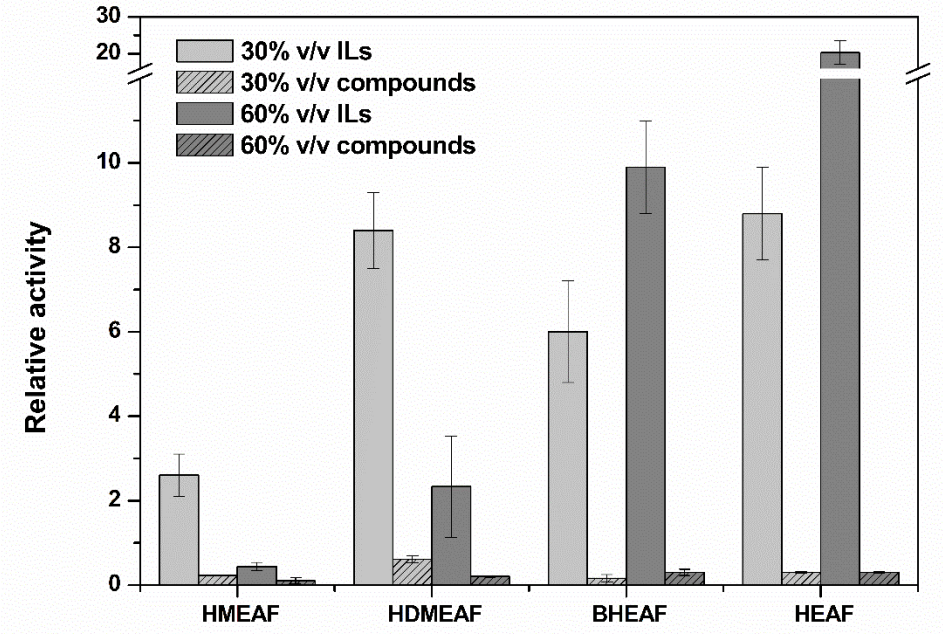


Fig. S17. Relative peroxidase activity of cyt c for the oxidation of guaiacol in the presence of various amounts of hydroxyl ammonium-based ILs and its equimolar amounts of individual components. As 1.0 is indicated the peroxidase activity of cyt c in 50 mM phosphate buffer pH 7.0. Initial reaction rate in buffer: 10 μM/min.

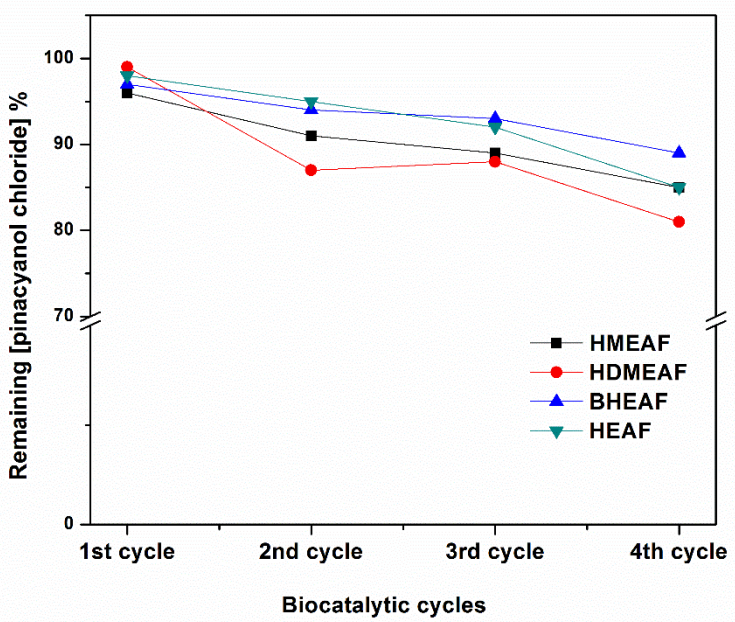


Figure S18. Recycle of ILs used as media in the decolorization of pinacyanol chloride catalyzed by immobilized cyt c.

References

[1] A. Tzani, A. Douka, A. Papadopoulos, E.A. Pavlatou, E. Voutsas, A. Detsi, ACS Sustainable Chem. Eng. 1 (2013) 1180–1185.
[2] A. Romero, A. Santos, J. Tojo, A. Rodríguez, J. Hazard. Mater. 151 (2008) 268–273.