

Effects of three ionic liquids on microbial activity of an organic soil. Microcalorimetric study.

T. Sixto^{1,2,3}, C. Ucha^{1,2,3}, C. Trasar-Cepeda¹, O. Reyes², J. Salgado³.

¹Departamento de Bioquímica del Suelo. IIAG-CSIC. Santiago de Compostela. Spain.

²Departamento de Fisiología Funcional. Universidade de Santiago de Compostela. Spain.

³NaFoMAT Group. Departamento de Física Aplicada. Universidade de Santiago de Compostela. Spain.

e-mail: j.salgado.carballo@usc.es

Abstract

Ionic liquids are considered as a potential green replacement for traditional volatile organic solvents, but their impact on the environment is not sufficiently studied.

The effect of the addition of aqueous solutions of different amounts (from 0% to 75%) of three ionic liquids, two based imidazolium, dimethylimidazolium dimethylphosphate ([MMIM][DMP] and 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF₄]), and choline dihydrogen phosphate ([Chol][DHP]) on the microbial activity of an acidic, organic soil was determined in this work. For this propose, an isothermal microcalorimeter, TAM III of TA Instruments was used. This apparatus allows estimating the variation of the metabolic activity trough the variation of the heat released after the addition of the ILs to the soil.

The results showed that the most toxic IL is [BMIM][BF₄] which presented a large stress effect and subsequent death on soil microorganisms for the highest concentration of the IL. On the contrary, [Chol][DHP] showed stimulation of the microbial activity for all the concentrations.

Key words: Ionic liquids, toxicity, isothermal calorimetry, soil microbial activity.

1. Introduction

Despite the increasing number of papers reporting studies aiming to determine the toxic effects of different ionic liquids (ILs), the knowledge of their toxicity is still an open question [1].

ILs present low volatility making them an attractive alternative to volatile organic solvents. ILs are unlikely air contaminants, however, ILs could contaminate environmental recipients like soils and groundwater [2].

The toxic effects of ILs vary considerably across their type, test conditions and morphology of the model organisms [3]. Thus a systematic study to know their

effects with regard to structure moieties of ILs would be of practical use as a guideline before the widespread use of these novel solvents.

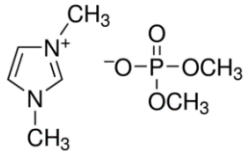
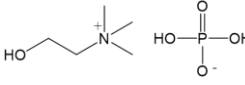
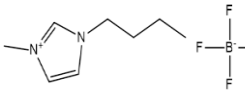
In this work, a descriptive view about the effect of three ionic liquids, two based imidazolium, 1,3-dimethylimidazolium dimethylphosphate ([MMIM][DMP] and 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF₄]), and choline dihydrogen phosphate ([Chol][DHP]) on the microbial activity of an acidic, organic soil using an isothermal microcalorimeter TAM III of TA Instruments [4] is reported. Calorimetry is an important tool that allow monitoring the soil microbial activity during long periods of time without disturbing the system, giving qualitative and quantitative indicators that can inform about the soil status [5].

2. Materials and methods

2.1. Chemicals

The main characteristic and identifications of the selected ionic liquids are indicated in Table 1. All these ILs were purchased to Iolitec and were used without further purifications.

TABLE 1. Main characteristics of the ionic liquids: CAS identification number, structure, molecular mass and purity

Ionic liquid	Short Name [CAS Number Id.]	Structure	Mm (g·mol ⁻¹)	Purity
1,3-dimethylimidazolium dimethylphosphate	[MMIM][DMP] [654058-04-5]		222.18	> 0.99
Choline Dihydrogenphosphate	[Chol][DHP] [83846-92-8]		201.16	> 0.99
1-butyl-3-methylimidazolium tetrafluoroborate	[BMIM][BF ₄] [174501-65-6]		226.02	>0.98

2.2 Experimental

2.2.1 Soil

A soil, rich in organic matter, acidic and located in Negreira - A Coruña (NW Spain) was selected for this work and its main characteristics (pH, contents of total C and N, sand, lime, clay, AlO₃ and FeO₃) are presented in Table 2. The effect of the addition (0.1 ml g⁻¹ soil) of six different concentrations of aqueous solutions (75, 50, 25, 10, 1

and 0% (control) in weight in distilled water) of the ILs on microbial activity of these soils was studied by calorimetry.

Table 2. Main characteristics of the selected soil.

pH H ₂ O	4.39±0.02	%sand	68
pH KCl	3.69±0.00	%lime	21
%C _t	10.99±0.12	%clay	11
%N _t	0.58±0.02	texture	Sandy loam
C/N	19	%Al ₂ O ₃	1.20±0.02
		%Fe ₂ O ₃	0.95±0.07

2.2.2 Isothermal Microcalorimetry

Experiments were performed using an isothermal microcalorimeter TAM III- TA Instruments. This apparatus is used to evaluate the short-term effect of the addition of different amounts of ILs under closed and static experimental environment. Measurements were carried out at 25 °C in hermetically sealed 5 ml stainless steel ampoules closed with a Teflon coated septum and an steel cap. Soil samples of 1 g size at water-holding capacity, treated with 0.2 ml of a glucose solution in water with a concentration of 6.25 g l⁻¹ to activate the metabolism of soil microorganisms, were used as control [6]. The heat released by the microorganisms was recorded until the total consumption of glucose [7]. Three replicates were performed for each case.

3. Results

The power-time curves of the selected soil treated with all the doses of the three ILs [BMIM][BF₄], [MMIM][DMP] and [Chol][DHP] are presented in the Figure 1. This technology has also been used by other authors [8] for detecting the toxic effects of cadmium on several bacteria, obtaining similar shapes and behaviour than for the ILs in the present study. The curves are characterized by three different phases: growth, stationary and decline phases.

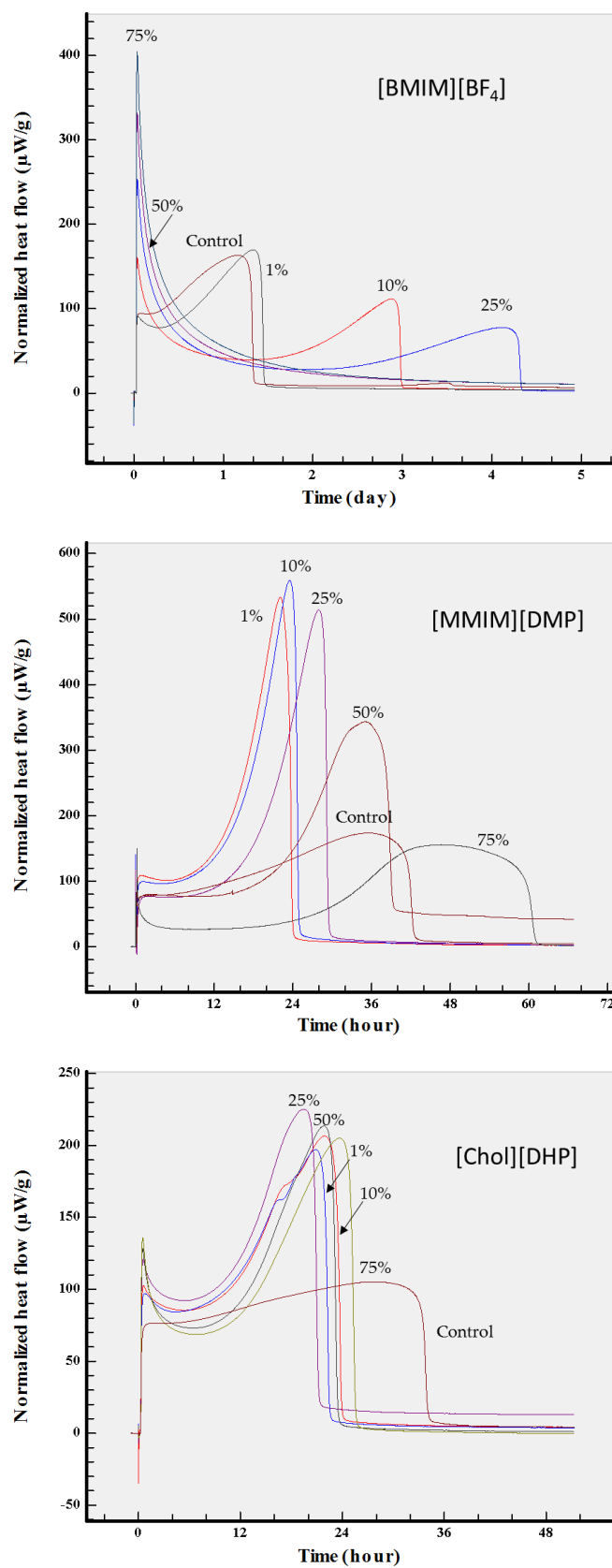


Figure 1. Power –time curves of Negreira soil treated with all the doses of the three ILs.

The comparison of power-time curves of the soil treated with the intermediate (25%) and highest (75%) doses of the three ILs is presented in Figure 2.

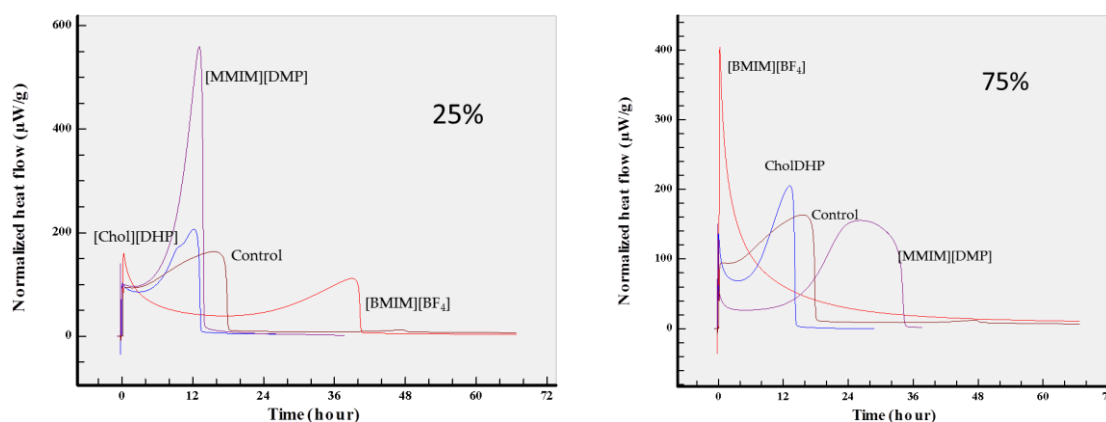


Figure 2. Power-time curves of Negreira soil treated with the intermediate (25%) and the highest (75%) concentrations of the three ILs.

Table 3 collects the mean values of heat released by soil microorganisms during the glucose consumption and the time of the maximum of power time curves for the different doses of the ILs [BMIM][BF₄], [MMIM][DMP] and [Chol][DHP] added to Negreira soil.

Table 3. Mean values of heat released by soil microorganism and time of the maximum of the peak in power-time curves of the Negreira soil treated with the different doses of the selected ILs

Sample	ΔH (kJ g ⁻¹)	t_{max} (min)
Control	14.3	2000
1% [BMIM][BF ₄]	14.3	2100
10% [BMIM][BF ₄]	16.0	130/4100
25% [BMIM][BF ₄]	20.4	70/7900
50% [BMIM][BF ₄]	22.4	70/10500
75% [BMIM][BF ₄]	12.9	360
1% [MMIM][DMP]	14.7	1300
10% [MMIM][DMP]	15.3	1400
25% [MMIM][DMP]	15.5	1700
50% [MMIM][DMP]	14.8	2000
75% [MMIM][DMP]	14.1	2850
1% [Chol][DHP]	12.8	1250
10% [Chol][DHP]	13.9	1300
25% [Chol][DHP]	13.2	1160
50% [Chol][DHP]	13.4	1300
75% [Chol][DHP]	14.0	1900

Expanded uncertainties $U(\Delta H) < 4\%$ and $U(t) < 10\%$ (0.95 level of confidence, $k=2$)

The results indicate that the effect of the three ILs on this soil is very different. Firstly, [BMIM][BF₄] provokes a delay in the growth phase for intermediate concentrations and the o highest dose yields an intense and short peak compatible with a remarkable stress, with the corresponding increase in the heat released, and subsequent dead of the microorganism [4]. The ILs [MMIM][DMP] and [Chol][DHP] do not lead to total inhibition of the microbial growth, and even a stimulation of microbial activity is observed for all the concentrations for both ILs (Figure 1).

4. Conclusions

Microcalorimetric technique is used to determine the effects of three ILs, [BMIM][BF₄], [MMIM][DMP] and [Chol][DHP], on soil microbial activity, observing his viability for their harmful effect characterization. Results showed that the most harmful IL is [BMIM][BF₄].

Acknowledgements

Authors acknowledge V. Piñeiro (RIAIDT-USC) the technical support. This work was supported by Spanish MINECO and FEDER Program through projects CGL2015-66857-C2-1-R and AGL2017-86075-C2-2-R (GESFIRE), as well as by Xunta de Galicia through the ED431C 2016/001 and IN607A 2017/6 projects and Galician Network of Ionic Liquids (ReGaLIs) ED431D 2017/06

References

- [1] B. Peric, J. Sierra, E. Martí, R. Cruañas, M.A. Garau, J. Arning, U. Bottin-Weber, S. Stolte, (Eco)toxicity and biodegradability of selected protic and aprotic ionic liquids, *J. Hazard. Mater.* 261 (2013) 99–105. doi.org/10.1016/j.jhazmat.2013.06.070.
- [2] M. Amde, J.F. Liu, L. Pang, Environmental Application, Fate, Effects, and Concerns of Ionic Liquids: A Review, *Environ. Sci. Technol.* 49 (2015) 12611–12627. doi:10.1021/acs.est.5b03123.
- [3] M. Cvjetko Bubalo, K. Radošević, I. Radojčić Redovniković, J. Halambek, V. Gaurina Srček, A brief overview of the potential environmental hazards of ionic liquids, *Ecotoxicol. Environ. Saf.* 99 (2014) 1–12. doi:10.1016/j.ecoenv.2013.10.019.
- [4] J. Salgado, J.J. Parajó, T. Teijeira, O. Cruz, J. Proupín, M. Villanueva, J.A. Rodríguez-Añón, P. V. Verdes, O. Reyes, New insight into the environmental impact of two imidazolium ionic liquids. Effects on seed germination and soil microbial activity, *Chemosphere.* 185 (2017) 665–672. doi:10.1016/j.chemosphere.2017.07.065.
- [5] N. Barros, J. Salgado, S. Feijóo, Calorimetry and soil, *Thermochim. Acta.* 458 (2007) 11–17. doi:10.1016/j.tca.2007.01.010.

- [6] L. Núñez Regueira, J.A. Rodríguez-Añón, J. Proupín-Castiñeiras, M. Villanueva-López, O. Núñez-Fernández, Study of the influences of different forest species on the microbial activity in soils, *J. Therm. Anal. Calorim.* 84 (2006) 7–13. doi:10.1007/s10973-005-7200-z.
- [7] L. Wadsö, F. Gómez Galindo, Isothermal calorimetry for biological applications in food science and technology, *Food Control* 20 (2009), 956-961. doi:10.1016/j.foodcont.2008.11.008.
- [8] H.Y. Chen, J. Yao, Y. Zhou, H.L. Chen, F. Wang, N. Gai, R.S. Zhuang, B. Ceccanti, T. Maskow, G. Zaray, Investigation of the toxic effect of cadmium on *Candida humicola* and *Bacillus subtilis* using a microcalorimetric method, *J. Hazard. Mater.* 159 (2008) 465–470. doi:10.1016/j.jhazmat.2008.02.040.



© 2018 by the authors. Submitted for possible open access publication under the terms and conditions of the Creative Commons Attribution (CC BY) license (<http://creativecommons.org/licenses/by/4.0/>).