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Effects of Three Ionic Liquids on Microbial Activity of an Organic Soil. Microcalorimetric Study †

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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][BF_4]) 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 through the variation of the heat released after the addition of the ionic liquids (ILs) to the soil. The results showed that the most toxic IL is [BMIM][BF_4] 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.

Keywords: 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, but could contaminate environmental recipients like soils and groundwater [2].

The toxic effects of ILs vary considerably across their type, test conditions and the 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.

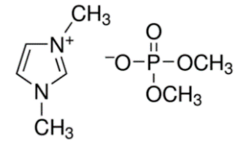
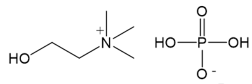
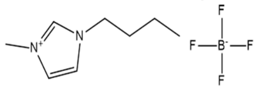
In this work, a descriptive view about the effect of three ionic liquids, two based imidazolium, 1,3-dimethylimidazolium dimethylphosphate ([MMIM][DMP] and 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) and choline dihydrogen phosphate ([Chol][DHP]) on the microbial activity of an acidic, organic soil using an isothermal microcalorimeter TAM III of TA Instruments [4], is reported. Calorimetry is an important tool that allows the monitoring of 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 (ILs) 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 (ILs): CAS identification number, structure, molecular mass and purity.

Ionic Liquid	Short Name [CAS Number Id.]	Structure	Mm ($g \cdot mol^{-1}$)	Purity
1,3-dimethylimidazolium dimethylphosphate	[MMIM][DMP] [654058-04-5]		222.18	>0.99
Choline dihydrogen phosphate	[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 it main characteristics (pH, contents of total C and N, sand, lime, clay, AlO_3 and FeO_3) 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.

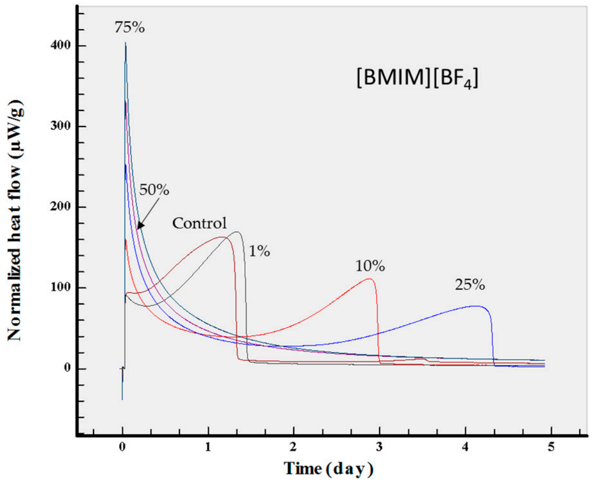
pH H_2O	4.39 ± 10.02	%sand	68
pH KCl	3.69 ± 0.00	%lime	21
$\%C_t$	10.99 ± 0.12	%clay	11
$\%N_t$	0.58 ± 0.02	texture	Sandy loam
C/N	19	$\%Al_2O_3$	1.20 ± 0.02
		$\%Fe_2O_3$	0.95 ± 0.07

2.2.2. Isothermal Microcalorimetry

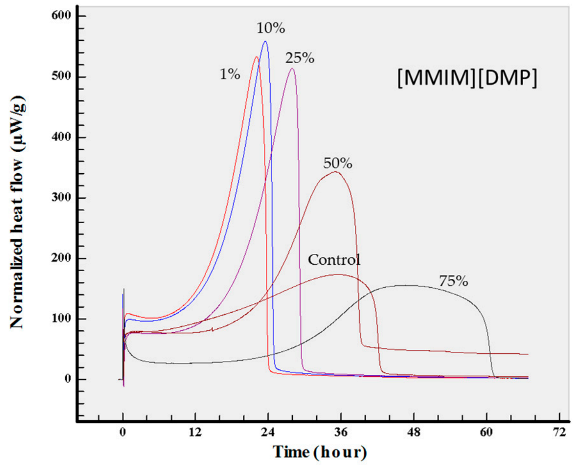
Experiments were performed using an isothermal microcalorimeter TAM III-TA Instruments. This apparatus is used to evaluate the short-term effect of the addition of different amounts of ILs under a closed and static experimental environment. Measurements were carried out at 25 °C in hermetically sealed 5 ml stainless steel ampules closed with a Teflon™ coated septum and a 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 the 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

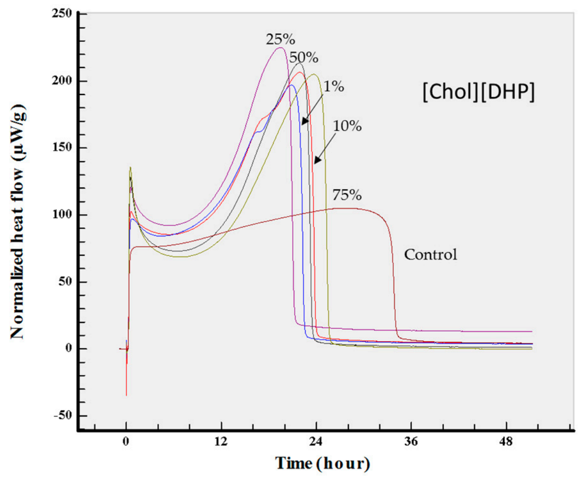
The power-time curves of the selected soil treated with all the doses of the three ILs [BMIM][BF_4], [MMIM][DMP] and [Chol][DHP] are presented in the Figure 1. This technology has also been used by other authors [8] for detecting the toxic effects of cadmium on several bacteria, obtaining similar shapes and behavior than for the ILs in the present study. The curves are characterized by three different phases: Growth, stationary and decline phases.



(**a**)



(**b**)



(**c**)

Figure 1. Power–time curves of Negreira soil treated with all the doses of the three different ionic liquids (ILs), (**a**) 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF4], (**b**) 1,3-dimethylimidazolium dimethylphosphate [MMIM][DMP], and (**c**) Choline dihydrogen phosphate [Chol][DHP].

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.

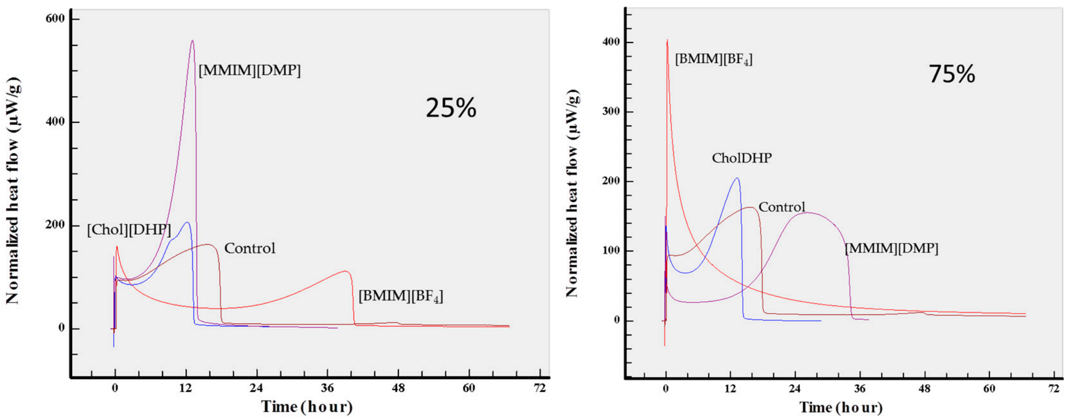


Figure 2. Power-time curves of Negreira soil treated with the intermediate (25%) and the highest (75%) concentrations of the three ILs.

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][BF_4], [MMIM][DMP] and [Chol][DHP] added to the 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(ΔH) < 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][BF_4] provokes a delay in the growth phase for intermediate concentrations and the highest dose yields an intense and short peak compatible with a remarkable stress, with the corresponding increase in the heat released, and subsequent death of the microorganism [4]. The ILs [MMIM][DMP] and [Chol][DHP] do not lead to any total inhibition of the microbial growth, and even a stimulation of microbial activity is observed with respect to all the concentrations for both ILs (Figure 1).

4. Conclusions

Microcalorimetric technique is used to determine the effects of three ILs, [BMIM][BF_4], [MMIM][DMP] and [Chol][DHP], on soil microbial activity, observing its viability for harmful effect characterization. Results showed that the most harmful IL is [BMIM][BF_4].

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