A pH-adjusted method to measure urease activity in soils spiked with 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF_4]

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Abstract

Ionic liquids (ILs) commercialization has exponentially increased in recent years because of their very different industrial utilities. The ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF_4] is widely used in different applications. However, the number of studies about the toxic effects of [BMIM][BF_4] on the environment is very limited, especially for soils. This is striking since terrestrial ecosystems would be the first to be reached by this compound as a result of any possible accidental spill. Soil biochemical properties, and particularly the enzymatic activities, are very sensitive parameters to detect any perturbation in soil functioning. Among all the enzymes, the urease is one of the most widely used because it is highly sensitive to the presence of different exogenous agents. In this study, we carried out laboratory experiments with two soils of contrasting pH to adjust the parameters and experimental conditions of the method to determine urease activity in soils spiked with [BMIM][BF_4].

Keywords: 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF_4], urease activity, soil contamination, soil enzymes.

1. Introduction

ILs have attracted special attention, because of both the infinity of cation-anion combinations (Plechcova y Seddon, 2008) and their negligible volatility (Pérez de los Ríos y Hernández, 2014), property this latter due to which they have been considered as “green fluids”. However, the lack of studies about their toxic effects on the environment does not allow assigning them this appellation.

The 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF_4] is a widely used ionic liquid in different applications. However, the number of studies about the toxic effects of this IL on the environment and, especially, on soils is very limited. This is striking since terrestrial ecosystems would be the first to be reached by this compound as a result of any possible accidental spill. Soil is a non-renewable resource, at least within a human lifespan (Fitzpatrick, 1980); therefore preserving soil quality is a priority in environmental conservation (Cihacek et al., 1996). Among all the soil properties, soil biochemical properties, and particularly the enzymatic activities, are highly sensitive parameters to detect any perturbation in soil functioning. The urease, one enzyme of the nitrogen cycle that catalyses the hydrolysis of urea, is one of the most widely used in the evaluation of changes in soils, because it is highly sensitive to the presence of different exogenous agents.

The method of urease activity determination is based on the measurement of ammonia released from soil in a buffered medium (pH 7.1-8.0) with phosphate buffer, using urea as a substrate (Nannipieri et al., 1980). However, depending on soil characteristics and changes induced by the presence of exogenous agents, modifications in the analytical method could be needed to determine the activity of this enzyme.

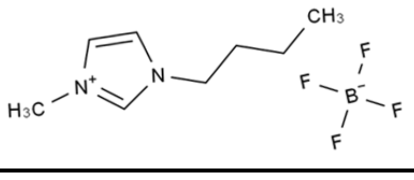
The aim of this study was to adjust the parameters and experimental conditions of the method to determine urease activity in soils of contrasting characteristics and spiked, under controlled laboratory conditions, with various doses of [BMIM][BF_4].

2. Material and methods

2.1. Chemical

1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM][BF_4]) was purchased from IOLITEC (Heilbronn, Germany) with the highest available purity (99%). The [BMIM][BF_4] standard solutions were prepared by dilution of the stock solution in distilled water. Table 1 shows the empirical formula (E.F.), chemical abstract service (CAS) registry number, molecular weight (M.W.) and chemical structure of [BMIM][BF_4].

Table 1. Physicochemical data and structure of [BMIM][BF_4].

Compound	E.F.	CAS number	M.W.	Structure
[BMIM][BF_4]	$C_8H_{15}BF_4N_2$	174501-65-6	226	

2.2. Soils

Two soils of contrasting pH, an agricultural soil (crop soil) with acid pH (pH_{H2O}=5.31) and a forest soil (forest soil) with neutral-to-alkaline pH (pH_{H2O}=7.74) were selected for the study. The surface horizon (0–10 cm) of both soils was sampled and the samples were transported in isothermal bags to the laboratory and maintained at 4 °C until their analysis. An aliquot of each sample was air-dried (20 °C) and used for analysis of general soil properties. Table 2 shows the main general properties of the soils analyzed.

Table 2. Main general properties of forest and crop soils.

Soil	pH H_2O	pH KCl	Total C (%)	Total N (%)	C/N	Texture
Forest soil	7.74 ± 0.01	7.18 ± 0.01	5.23 ± 0.10	0.28 ± 0.01	18	Sandy loam
Crop soil	5.31 ± 0.00	4.17 ± 0.01	2.26 ± 0.04	0.19 ± 0.00	12	Loamy sand

2.3. Urease activity method

Reaction mixtures for urea hydrolysis consisted of 1 g of soil, 4 ml of 0.2 M phosphate buffer (obtained by mixing different volumes of NaH_2PO_4 0.2 M and Na_2HPO_4 0.2 M) and 1 ml of 1.067 M urea. After 1.5 hours incubating at 37 °C, the NH_4^+ released was determined with an ammonia-selective gas electrode (METROHM Ltd., Herisau, Switzerland). The enzymatic activity is expressed in µmol NH_3 g^{-1} h^{-1}. Ordinarily, the pH of the phosphate buffer used to determine urease activity in acidic soils is 8.0, while for soils with pH above 7.0 a phosphate buffer of pH 7.0 is used.

2.4. Analysis of soils

The two soils were analysed for pH in water and in 1 M KCl, total carbon and nitrogen content and texture following the methods described in Guitián and Carballas (1976). The pH in water and KCl in all the spiked samples was measured following the same method used for the soils without addition of [BMIM][BF_4].

To measure the pH of the mixtures of the soils spiked with the different doses of [BMIM][BF4] and the buffer solutions at diverse pHs, soil:buffer mixtures at the same proportion used for the analysis of urease were prepared and the pH measured before and after 1.5 h of incubation at 37 °C.

2.5. Experiment set-up

Six different solutions of [BMIM][BF4] were prepared by dilution of this compound in water, to give final concentrations of 1, 10, 25, 50, 75 and 100%. Thereafter, the soils were spiked with 0.1 ml of each of the solutions per gram of soil and the soil-[BMIM][BF_4] mixture was maintained at 20 °C for one day, before the start of the diverse measurements. The soil soil-[BMIM][BF_4] mixture were analysed for: pH in water and in 1 M KCl, pH of the soil-[BMIM][BF_4]

mixture with phosphate buffer at different pHs and urease activity at the selected buffer pHs. A soil sample without addition of [BMIM][BF_4], but to which identical quantity of water than for the soil samples with spiked [BMIM][BF_4] was added, was maintained as a control.

3. Results and Discussion

[BMIM][BF_4] has a clear and strong acidifying effect on the pH of both soils. The acidifying effect on forest calcareous soil was more intense with the 25 and 50 % doses, while on the cropped acidic soil acidification was more intense with increasing [BMIM][BF4] doses (Table 3).

Table 3. Variations in pH_{H2O} and pH_{KCl} values of soils spiked with different [BMIM][BF_4] doses.

[BMIM][BF_4] dose (%)	Forest Soil		Crop Soil	
	pH H_2O	pH KCl	pH H_2O	pH KCl
0	7.84	7.20	5.17	4.10
1	7.63	7.14	5.21	4.13
10	6.80	6.56	5.04	4.10
25	6.45	6.31	4.86	4.04
50	6.40	6.37	4.68	3.93
75	6.72	6.67	4.53	3.88
100	7.21	7.08	4.50	3.91

The urease activity determination is based on the measurement of ammonia released from soil at optimum pH for the activity (pH 7.1 to 8.0) in buffered medium, and therefore the pH of the phosphate buffer had to be selected for each soil and [BMIM][BF_4] dose.

Table 4. pH values before (b.i.) and after incubation (a.i.) of forest soil with different phosphate buffer solutions.

[BMIM][BF_4] dose (%)	phosphate buffer pH 7.0		phosphate buffer pH 7.5		phosphate buffer pH 8.0	
	b.i.	a.i.	b.i.	a.i.	b.i.	a.i.
0	6.92	6.93	7.31	7.27	7.65	7.56
1	6.92	6.90	7.30	7.27	7.63	7.54
10	6.84	6.79	7.20	7.17	7.55	7.41
25	6.74	6.73	7.17	7.12	7.44	7.30
50	6.79	6.72	7.14	7.07	7.45	7.32
75	6.76	6.71	7.13	7.08	7.44	7.35
100	6.77	6.73	7.13	7.09	7.47	7.39

The pHs of the forest soil spiked with the different doses of [BMIM][BF_4] were very close to the optimum pH value for urease activity (Table 3), and thus the pHs of the buffer solutions tested were 7.0, 7.5 and 8.0. However, the pHs of spiked crop soil were considerably lower and, in consequence, the pHs of the buffer solutions tested (7.0, 7.6, 8.0, 8.7 and 8.9) were higher than in the forest soil (Tables 4 and 5).

Tables 4 and 5 show the pH values with the tested phosphate buffer solutions of forest and crop soils, respectively, spiked with the different solutions of [BMIM][BF4] before and after incubation. The pH values of the soil-phosphate buffer reaction mixtures in forest soil spiked with the different doses of [BMIM][BF_4] were into the optimal range for urease activity measurement after the addition of the pH 7.5 phosphate buffer, since the pH after the incubation ranged from 7.07 (50%) to 7.27 (0 and 1%), with a mean value of 7.15±0.09.

Table 5 pHs values before (b.i.) and after incubation (a.i.) of crop soil with different phosphate buffer solutions.

[BMIM][BF_4] dose (%)	**phosphate buffer pH 7.0**		**phosphate buffer pH 7.6**		**phosphate buffer pH 8.0**		**phosphate buffer pH 8.7**		**phosphate buffer pH 8.9**	
	b.i.	**a.i.**	**b.i.**	**a.i.**	**b.i.**	**a.i.**	**b.i.**	**a.i.**	**b.i.**	**a.i.**
0	6.95	6.75	7.32	7.16	7.65	7.56	7.83	7.66	-	-
1	6.93	6.70	7.20	7.12	7.60	7.51	7.81	7.64	-	-
10	-	-	7.06	6.99	7.40	7.20	7.59	7.41	-	-
25	-	-	-	-	-	-	7.44	7.24	7.64	7.36
50	-	-	-	-	-	-	7.39	7.17	7.57	7.29
75	-	-	-	-	-	-	7.40	7.20	7.62	7.31
100	-	-	-	-	-	-	7.39	7.19	7.55	7.28

As regards the crop soil, the phosphate buffer solution with higher pH that can be used is the unmixed solution of Na_2HPO_4 0.2 M (pH 8.9). After the addition of this solution, the pH values of the reaction mixture for the most acidic samples (soil spiked with the doses 25, 50, 75 and 100%) were within the optimal pH range (Table 5). Regarding the soil samples with 0, 1 and 10% doses, the reaction mixtures with the pH values more similar to those of the 25, 50, 75 and 100% doses were selected (phosphate buffer pH 8.0 for 0 and 1% and phosphate buffer pH 8.7 for 10%). Thus, the urease activity in cropped soil spiked with different doses of [BMIM][BF_4] was determined at similar pH values and into the optimal pH range (mean value 7.39±0.11).

Figure 1 shows the urease activity obtained for the forest and crop soils with different doses of [BMIM][BF_4] at the selected phosphate buffer solutions.

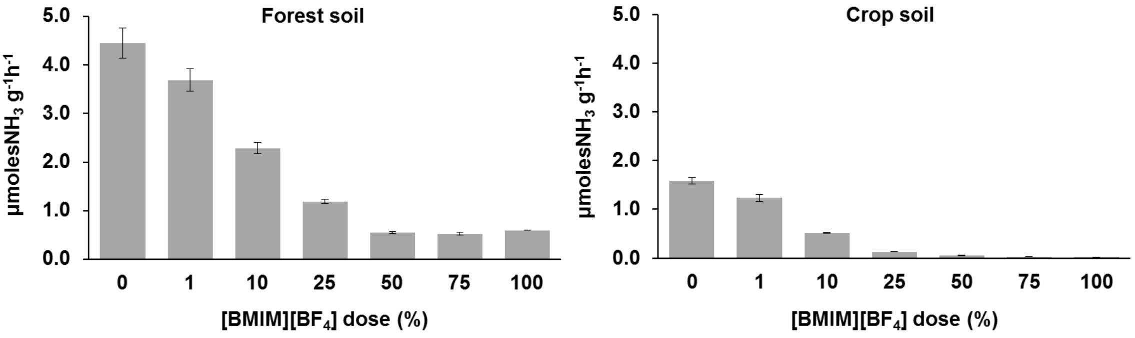


Figure 1. Urease activity in forest and crop soils spiked with different doses of [BMIM][BF_4].

The negative effect of [BMIM][BF_4] on urease activity was clear, even at the lowest dose used (1%). The urease activity was strongly affected in both the forest and the crop soil, with a significant decrease in the activity as the dose of [BMIM][BF_4] increased. The dose of 10% of [BMIM][BF_4] reduced urease activity about 50% in both soils. In the case of the soil with neutral-alkaline pH (forest soil), the activity in the soil with the 50% dose was a 80% lower than in control soil and there are scarce differences in the effect of [BMIM][BF_4] with doses higher than 50%. The urease activity in the soil with acidic pH (crop soil) at the dose of 25% was strongly reduced and the activity was practically zero at doses above 50%.

4. Conclusions

The method for determination of urease in soils is adequate for its use in soils spiked with [BMIM][BF_4], but the pH of the phosphate buffer has to be selected so as to assure that the pH of the soil-buffer reaction mixtures is well above 7.

The addition of [BMIM][BF_4] to the soils caused a strong reduction of the urease activity and there is a direct relation between the concentration of [BMIM][BF_4] added to the soils and the reduction of the activity.

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