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Toxic effects of ionic liquid 1-octyl-3-methylimidazolium tetrafluoroborate on soil enzyme activity and soil microbial community diversity

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ABSTRACT

Ionic liquids (ILs) were considered as "green" solvents and have been used widely because of their excellent properties. But ILs are not as "green" as has been suggested, and the toxic effects of ILs on organisms have been shown in recent years. In the present study, the toxic effects of the IL 1-octyl-3-methylimidazolium tetrafluoroborate ([Omim]BF_4) on soil enzyme activity and soil microbial communities at three different concentrations (1.0, 5.0 and 10.0 mg/kg) and a control treatment over 40 days of incubation time (sampled on days 10, 20, 30 and 40) were examined under laboratory conditions. The concentrations of [Omim]BF_4 in soils were detected by high performance liquid chromatography (HPLC) and the results indicated that [Omim]BF_4 were maintained stable in the soil during the exposure period. However, the enzyme activity results showed that urease activity was stimulated on day 20 and then decreased after 30 days of incubation. The activity of β-glucosidase was stimulated after 20 days of incubation in both treatment groups. Moreover, both dehydrogenase and acid phosphatase were inhibited at a high level (10.0 mg/kg) only on day 20. The analysis of terminal restriction fragment length polymorphism (T-RFLP) revealed that the soil microbial community structures were altered by [Omim]BF_4 and that the soil microbial diversity and evenness of high levels (5.0 mg/kg and 10.0 mg/kg) treatments were decreased. Moreover, the dominant structure of the microbial communities was not changed by [Omim]BF_4. Furthermore, the abundance of the ammonia monooxygenase (amoA) genes of both ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) was examined using real time polymerase chain reaction (RT-PCR). The results revealed that the copy numbers of the amoA-gene were decreased by [Omim]BF_4 with the 5.0 and 10.0 mg/kg treatments. Based on the experiment, we concluded that high levels (5.0 and 10.0 mg/kg) of [Omim]BF_4 could have significantly toxic effects on soil enzyme activities and the diversity of the microbial communities.

1. Introduction

Designs of environmentally friendly solvents have attracted the attention of chemists in recent years. Ionic liquids (ILs), which were also called room temperature ILs, are a type of solvents that consist entirely of ions (Welton, 1999). ILs are called green solvents because of their unique physicochemical properties, such as nonflammability, synthetic flexibility, low vapor pressure and high polarity (Deetlefs and Seddon, 2010; Moosavi and Daneshvar, 2014). Therefore, ILs have been applied in many fields, including electrochemistry fields, separation processes and biosensors, and they constitute one of the focuses of study in chemistry (MacFarlane et al., 2010; Panda and Gardas, 2015; Patel and Lee, 2012; Wasserscheid and Keim, 2000).

However, because many ILs are used in many fields, they are released into and eventually accumulate in the environment (Bubalo et al., 2014). It is unlikely that ILs become air pollutants because of their low vapor pressure, but they are inevitably released into the water or the soil through accidental spills, effluents or irrigation (Liwarska-Bizukojc, 2011). As "green" solvents, ILs have already caused a great deal of controversy among chemists, and their toxicity has attracted comprehensive concern from scientists (Bubalo et al., 2014). In addition, the toxic effects of ILs on plants, aquatic organisms, and soil animals have been evaluated (Dong et al., 2013; Li et al., 2010; Liu et al., 2015a, 2015b). Moreover, because ILs possess some excellent

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properties, such as considerable solubility in water and resistance to degradation, their environmental risks will be increased with the large scale of the ILs usage (Liu et al., 2015a).

Imidazolium-based ILs is used most widely among the known ILs, as indicated by statistical data on the Web of Science. 1-octyl-3-methylimidazolium tetrafluoroborate ([Omim]BF_4) was studied widely in the chemical industry (Gutkowski et al., 2006), and its toxic effects on plants and zebrafish were reported by other researchers (Liu et al., 2015a, 2015b). However, the effects of [Omim]BF_4 on the diversity of the soil microbial community remain to be investigated. Guo et al. (2015a) reported that [Omim]Cl inhibited the functional diversity of soil microbial communities. As a major part of terrestrial ecosystems, soil performs important functions in sustainable agricultural production. Soil quality is considered as one of the integrative indicators of environmental quality and agricultural safety. Soil enzymes participate in many types of biological processes in the soil and always provide direct evaluations of the soil quality (Sharma et al., 2010). Moreover, a more abundant soil microbial community in addition to functional genes indicate a higher level of soil quality, resistance to disturbance and nutrient cycling (Epelde et al., 2010; Nielsen et al., 2002). The ammonia monooxygenase (amoA) genes of ammonia-oxidizing archaea (AOA) and ammonia-oxidizing bacteria (AOB) play an important role in N-cycling and nitrification, and the abundance of the amoA-gene reflects the functional health on the soil. Therefore, the present study on the toxic effects of [Omim]BF_4 on soil enzyme activities and the diversity of the soil microbial community seems extremely urgent, especially with the development of [Omim]BF_4 research and applications.

Terminal restriction fragment length polymorphism (T-RFLP) analysis is an effective and widely used method to detect the diversity of the soil microbial community structure, and real-time polymerase chain reaction (RT-PCR) has been widely used to evaluate the diversity of soil functional genes (Chen et al., 2011). Therefore, the aim of this study was to investigate the toxic effects of [Omim]BF_4 on soil enzyme activities, soil microbial community structure and the diversity of functional genes. This study will provide deep insights for the further study of the security of [Omim]BF_4 on the environment.

2. Materials and methods

2.1. Soil and chemicals

Soil (without any prior application of ILs) was collected at a depth of 0–15 cm from the test field of Shandong Agricultural University (Taian, China). The soil samples were incubated at 60% maximum water-holding capacity (WHC) for a week at 25 °C (Hinojosa et al., 2004) after being passed through a 2-mm sieve. Physical and chemical properties of the soil samples are as follows: pH 6.93, organic matter 13.91 g/kg, available N 93.41 mg/kg, available P 35.24 mg/kg, available K 73.12 mg/kg, and the maximal water holding capacity 18.51%.

The IL [Omim]BF_4 (99% purity, CAS No. 244193-52-0) was obtained from Chengjie Chemical Co. Ltd. (Shanghai, China). All other chemicals and reagents used in the following experiments were analytical grade and purchased from Sangon Biological Engineering Technology and Service Co. Ltd (Shanghai, China), Takara Biotechnology Co. Ltd (Dalian, China).

2.2. Soil treatment with IL

Three treatments, i.e., 1.0, 5.0 and 10.0 mg [Omim]BF_4 per kilogram soil and a control treatment with equal deionized water were performed in laboratory experiments. [Omim]BF_4 aqueous solutions were made with concentrations of 0.1, 0.5, 1.0 mg mL^{-1} and were then thoroughly mixed with 0.5 mL solutions of 50 g soil, and the control treatment was mixed with 0.5 mL equal deionized water of 50 g soil. The soils were placed in a 125 mL brown glass bottle as a microcosm system and plugged with a cotton pad. Each of the treatments for all four of the sampling times had three replications. Every treatment was incubated in the dark for 40 days at 25 °C, and the soil moisture content level was held at 60% of the maximum water holding capacity by adding sterile distilled water and by weighing. Samples from every treatment were collected after 10, 20, 30 and 40 days of incubation for soil enzyme assessment and DNA extraction for further PCR analysis.

2.3. Determination of the [Omim]BF_4 concentrations

[Omim]BF_4 in the soil samples of different treatment groups was extracted by extraction solutions (90% methanol and 10% saturated ammonium chloride solution) and EDTA-Na_2 (the final concentration in the extraction solutions was 1g/L) according to the method described by Nichthauser et al. (2009). Mixed 2 g of each soil sample was mixed with 5 mL of extraction solution and then vortexed for 30 s, sonicated for 50 min, and then shaken for 1 h at 160 rpm. After that, the samples were centrifuged at 4000 rpm for 10 min, and the supernatants were passed through the 0.22 μL syringe filter.

The concentrations of [Omim]BF_4 in the supernatant were measured by a high performance liquid chromatography (Agilent 1260, USA) using the method described previous (Zhou et al., 2015). The mixture of acetonitrile (30%, v/v) with 25 mmol/L of phosphate buffer (KH_2PO_4/H_3PO_4) in 0.5% triethylamine (pH 3.0) was used as the mobile phase in the present study. A C-18 column (Eclipse XDB-C_{18}, 4.6×250 mm, 5 μm) was used to separate the samples, the column temperature was maintained at 30 °C, the flow rate was 0.8 mL/min, the injection volume was 10 μL, and the samples were detected using an UV/VIS variable wavelength detector (Agilent, VWD G1314A, USA) at 212 nm.

2.4. Soil enzyme assays

Four soil enzymes were selected to measure in response to all treatments in this study, i.e., urease, dehydrogenase, acid phosphatase and β-glucosidase, and activities of these enzymes were determined using a spectrophotometry methods as described previously.

The urease activity was expressed as mg NH_4^+-N produced by per kilogram of dry soil 24 h^{-1}. Soil (5 g) and 1.0 mL methylbenzene were mixed, followed by incubation for 15 min at room temperature in a 50 mL volumetric flask. Then, 5 mL of 10% urea and 10 mL citrate buffer were added at 37 °C for 24 h of incubation followed by adding 38 °C distilled water to a 50 mL total. Subsequently, 1 mL of filter liquor was dispensed into a new 50 mL volumetric flask, and water was added to 10 mL. Then, 4 mL of sodium phenate and 3 mL of sodium hypochlorite were added, and followed with sealed and shaken the flask. Finally, the mixed solution was measured at 578 nm after 20 min of incubation using an ultraviolet-visible spectrophotometer (Shimadzu, UV-2600, Japan) (May and Douglas, 1976).

The dehydrogenase activity was expressed as micrograms of triphenyl formazan (TPF) per gram of dry soil every 24 h. The enzyme activity was measured as described previously (Rossel et al., 1997). In the experiment, 5.0 g of soil samples with 5 mL of 2,3,5-triphenyl tetrazolium chloride (TTC) solution (0.5% by weight) were incubated at 30 °C for 24 h. Then, 40 mL of methanol was added into each test tube, and the suspension was mixed for approximately 1 h in an oscillator. During the procedure, the soluble TTC was reduced to the red compound of TPF. Subsequently, the mixed suspension was filtered through filter paper, and the concentration of the red TPF was measured at 485 nm using the ultraviolet-visible spectrophotometer (Shimadzu, UV-2600, Japan).

The acid phosphatase activity was tested as described by Dick et al. (1996). In the experiment 1 g of soil with 0.2 mL of methylbenzene, 4 mL of modified universal buffer (pH 6.5) and 1 mL of 0.05 M 4-nitrophenyl phosphate disodium salt were incubated at 37 °C for 1 h. Then 1 mL of 0.5 M $CaCl_2$ and 4 mL of 0.5 M NaOH were added to stop