Chemosphere 210 (2018) 762–768

Contents lists available at ScienceDirect

Chemosphere

journal homepage: www.elsevier.com/locate/chemosphere

Evaluating toxicity of 1-octyl-3-methylimidazolium hexafluorophosphate to microorganisms in soil

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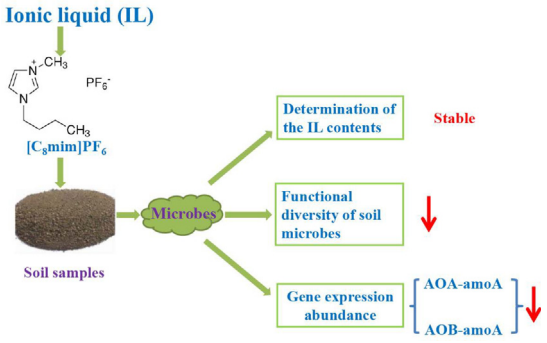
HIGHLIGHTS

- The levels of amoA genes was used to study the toxicity of [Omim]PF_6 in brown soil.
- Functional diversity of soil microbes was used to study the toxicity of [Omim]PF_6.
- The concentrations of investigated IL in soil relatively maintained stable.
- The investigated IL has potential risks if they were released to soil by accident.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 19 May 2018
Received in revised form
16 July 2018
Accepted 18 July 2018
Available online 19 July 2018

Handling Editor: A. Gies

Keywords:
High-performance liquid chromatography
Quantitative real-time polymerase chain reaction
Biolog
Ammonia-oxidizing archaea
Ammonia-oxidizing bacteria

ABSTRACT

Ionic liquids (ILs) were widely applied because of their excellent properties. The present investigation studied the toxicity of the IL 1-octyl-3-methylimidazolium hexafluorophosphate ([Omim]PF_6) to the soil microbial population and community diversity with dose (1.0, 2.0, 4.0, 6.0, and 8.0 mg kg^{-1}) and exposure time (7, 10, and 13 d). The results show the IL was stable during the entire experimental period. The Biolog-ECO plate results indicated that the average well color development (AWCD) in the 6.0 and 8.0 mg kg^{-1} treatments was lower than these in the other treatments. The diversity indices of the Biolog analysis were significantly reduced. The abundance of the ammonia-oxidizing archaea (AOA-) and the ammonia-oxidizing bacteria (AOB-) ammonia monooxygenase (amoA) genes was measured by the real-time polymerase chain reaction (RT-PCR). In the treatments of 4.0, 6.0 and 8.0 mg kg^{-1}, the abundance of amoA genes of the AOA- and AOB- were inhibited by IL [Omim]PF_6.

1. Introduction

Ionic liquids (ILs), considered green solvents, entirely consist of ions (Olivier, 1999). Because of excellent physicochemical properties, they are extensively used and studied (Deng et al., 2015; Dołżonek et al., 2017; Liu et al., 2013; Sintra et al., 2017). Ionic

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https://doi.org/10.1016/j.chemosphere.2018.07.105
0045-6535/

liquids have recently been an intense research area in chemistry (Ning et al., 2012; Ueno and Watanabe, 2011).

As an industrial product, ILs can be released into the environment and accumulated in the water or soil (Pham et al., 2010). Ionic liquids have been considerably utilized in industrial and commercial fields because of their thermostability and good solubility (Marsh et al., 2004). However, knowledge about the ecotoxicity of ILs is limited. In fact, chemists have been concerned about different aspects of ionic liquids and their toxicity has also drawn attention (Liu et al., 2014a, b; Zhang et al., 2018c, 2017a; 2017b, 2017c; Zhou et al., 2018). Furthermore, the risks of ILs to the ecosystem will increase with their heavy usage in industry and research because of their degradation resistance and high environmental stability.

Imidazolium-based ILs were the most extensively used for applications and scientific studies (Dommert et al., 2012). Guo et al. (2015), Sun et al. (2017) and Zhang et al. (2018b) have evaluated the toxicity of the ILs 1-octyl-3-methylimidazolium chlorine ($[C_8mim]Cl$), -tetrafluoroborate ($[C_8mim]BF_4$), -nitrate ($[C_8mim]NO_3$) to soil microorganisms, respectively. However, the IL $[Omim]PF_6$, which has the same alkyl chain and different anion with the aforementioned ILs, has been widely applied widely in industry (Han and Row, 2010). Though, its toxicity to earthworms (Liu et al., 2016), plants (Liu et al., 2014b) and zebrafish (Du et al., 2012) has been studied, but studies of the toxicity of ILs to the soil microorganism community are rare, especially those related to functional diversity. Thus, we evaluated the effects of $[Omim]PF_6$ on the soil microbial community functional diversity in the present study.

High-performance liquid chromatography (HPLC) can accurately detect the $[Omim]PF_6$ content in different treatments (Zhou et al., 2015). As Preston-Mafham et al. (2002) stated, biolog-ECO plates are a widely used and effective method to evaluate soil microbial functional diversity because they measure the utilization patterns of diverse carbon sources. Additionally, the AOA- and AOB-amoA genes have been determined to indicate a healthy soil environment and soil microbial activity (Avrahami et al., 2003). The copy number and level of functional genes in the soil were measured by the real-time polymerase chain reaction (RT-PCR) (Chen et al., 2011; Rutgers et al., 2016; Sala et al., 2008). The present study evaluated IL effects on soil microorganisms and provide a basic theoretical platform for future studies of its ecotoxicity, the environmental safety, and the degradation of ILs in the environment.

2. Materials and methods

2.1. Chemicals and soil

The IL $[Omim]PF_6$ (99% purity, CAS No. 304680-36-2) was bought from the Chengjie Chemical Co. Ltd. (Shanghai, China). Other chemicals, of analytical grade, were provided by the Solarbio Science &Technology Company (Beijing, China).

Soil was taken from the corn fields in a research farm of Shandong Agricultural University from the depth of 0–15 cm. The large soil particles were separated from the soil by using a 2-mm mesh sieve (Hinojosa et al., 2004). The soil was incubated at 60% of the water-holding capacity (WHC) at 25 °C for 7 d. Table 1 shows the physicochemical properties of the soil.

2.2. Application of $[Omim]PF_6$

In each treatment, 0.5 mL of different concentrations of an aqueous solution of $[Omim]PF_6$ (0, 0.06, 0.12, 0.24, 0.36 and 0.48 g L^{-1}) were added to 30 g soil. Next, 125 mL brown glass bottles were used to hold the soil with cotton pads. Three replications of each treatment were incubated in the dark at 25 °C for up to 13 days. Deionized water was weighed to adjust the soil moisture to 60% of the WHC. The soil from each bottle was mixed for the concentration analysis, the Biolog-ECO plates test, and DNA extraction for PCR analyses on days 7, 10, and 13.

Table 1
Soil physical and chemical properties.

Soil type	Brunisolic soil
pH	6.93
Soil water ratio (%)	18.5
TOC (g kg^{-1})	13.9
Available N (g kg^{-1})	93.4
Available P (g kg^{-1})	35.2
Available K (mg kg^{-1})	73.1
Clay (%)	14.6
Sand (%)	59.3
Silt (%)	26.1

TOC: Total Organic Carbon, N: nitrogen, P: phosphorus, K: potassium. The methods used for measuring available N and available P were according to Han et al. (2015). The methods used for measuring available K were according to Yang et al. (2016).

2.3. Determination of the $[Omim]PF_6$ contents

Following the study of Guo et al. (2016), the $[Omim]PF_6$ was extracted from the soil using extraction solutions of 90% methanol, 10% saturated NH_4Cl and EDTA-Na_2 (1 g L^{-1}). The sample of 4.0 g soil from each treatment was extracted with extraction solutions. Then, the soil mixtures were vortexed for 30 s, sonicated for 50 min and shaken for 1 h. The soil solutions were subsequently centrifuged by the centrifuge at 2057 g for 10 min (Centrifuge 5804R, Eppendorf China Limited, China).

Following the study of Zhou et al. (2017), the $[Omim]PF_6$ content in the supernatant was determined by High-performance liquid chromatography (Agilent 1260, Agilent Technologies Inc., USA). The mobile phase was phosphate buffer (KH_2PO_4/H_3PO_4, 25 mM) and ultrapure water at a ratio of 70:30.

2.4. Biolog-ECO plates assay

The effects of $[Omim]PF_6$ on the soil community level of physiological profiles (CLPP) were evaluated by Biolog-ECO plates as described previously (Rutgers et al., 2016). Incubated soil (10 g) was added to 90 mL 0.85% (*m/v*) NaCl sterile physiological solution in a 250 mL flask and then shaken for 1 h. After allow the solution to settle for 10 min, 1 mL resulting solution was serially diluted in 9 mL of sterile 0.85% NaCl (*m/v*) until a 10^{-3} dilution was produced. Next, the supernatant (150 μL) was used to inoculate each well in a Biolog plate. The plates were incubated in the dark at 25 °C for 7 days, then the optical density (OD) was measured using a plate reader (Multiskan MK3 ELISA) at 590 nm every 24 h to indicate microbial growth.

The average well color development (AWCD) was used to reflect the intensity of the microbial activity according to Biolog data analysis described by Garland (1996) using the following formula:

$$AWCD = \frac{1}{31}\sum_{i=1}^{31}(C - R) \quad (1)$$

where C: OD value of a well, i and R: OD value of the control.

The richness, advantage of the dominant species and evenness of the soil microbial community were evaluated by the Shannon, Simpson and Mclntosh indices. According to the method of Zhao et al. (2010), the three diversity indices were calculated by using the OD-values at 72 h exposure using the following formulae: