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Influence of Ionic Liquid 1- butyl-3-methylimidazolium Chloride on the Soil Micro-Ecological System

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Abstract: In order to evaluate the influence of ionic liquid 1- butyl-3-methylimidazolium chloride ([Bmim]Cl) on the soil micro-ecological system, the toxicity of [Bmim]Cl to soil microorganisms and its impact on soil physico-chemical properties were investigated. Three soil samples, which were taken from the rape land, nursery land and the broad bean land respectively, were used for this study. The toxicity test results show that the [Bmim]Cl inhibited the growth of soil microorganisms including bacteria and actinomycetes. This inhibition became stronger with the [Bmim]Cl concentration increasing. The EC50 of soil bacteria was close to that of the *Vibrio fischeri,* and the EC50 of soil actinomycetes was near to that of the *Pseudokirchneriella subcapitata*. The soil physico-chemical properties test results indicate that the organic mass and the soluble salts in soil increased with the increase of the [Bmim]Cl concentration. The [Bmim]Cl also caused the pH change in the soil micro-ecological system. It suggests that the ionic liquid [Bmim]Cl would influence the soil micro-ecological system by inhibiting the growth of soil microorganisms and altering the soil physico-chemical properties when it contaminated the soil system.

Keywords: Ionic liquid, [Bmim]Cl, soil micro-ecological system, microorganism, physico-chemical property.

1. INTRODUCTION

Ionic liquids (ILs) are a group of new organic salts that exist as liquids at a relatively low temperature (<100 ℃). They are composed entirely of ions, typically large organic cations and small inorganic anions [1, 2]. Interests in ILs have steadily grown in recent years because they have many attractive properties, such as chemical and thermal stability, non-flammability and immeasurably low vapor pressure, which provide the possibility for clean manufacturing in chemical-related industry [3]. Researches on the applications of ILs have become one of the most active areas in green chemistry, the ILs have been widely used in organic synthesis, separation, biotransformation, new material preparation and renewable resource utilization [4-8]. Although most of these applications are still in a laboratory scale, some of them have been coming into pilot or commercial stage since the BASF first successfully used the IL in an industrial scale in 2003 [9, 10]. Compared using the traditional volatile organic solvents, the industrial application of ILs can help to reduce air pollution because of their immeasurably low vapor pressure, but it is still possible to release them into environment by accidental spills or effluents, which might cause water or soil pollution [11-13]. Therefore, much attention should be paid on their influence on the environment and ecology when they are used in an industrial scale. The impact of ILs on the environment and ecology is closely related to their bioaccumulation, toxicity and degradability. Based on the reported data on their bioaccumulation, toxicity and degradability in recent years, the ILs might cause water or soil pollution as the commonly used chemicals [11-13]. However, as we know, there are no reports on the influence of ILs on their contaminated soil micro-ecological system. This work is to deal with how the ILs will affect their contaminated soil micro-ecological system. In this work, the most commonly used IL, 1- butyl-3-methylimidazolium chloride ([Bmim]Cl) was selected as the model IL. Three soil samples, which were taken from the rape land, nursery land and the broad bean land respectively, were used as the model soil micro-ecological system. The toxicity of [Bmim]Cl to soil microorganisms and its impact on soil physico-chemical properties were investigated to evaluate its influence on the soil micro-ecological system.

2. MATERIALS AND METHODS

All experiments were carried out three times. The data reported are expressed as the mean values ± standard deviation.

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2.1. Materials and Chemicals

Soil samples were taken from different sites (rape land, nursery land and the broad bean land) in Wuhan, Hubei province, China. All soil samples were collected in the beginning of April in 2010. The soil samples from the rape land, nursery land and the broad bean land were chosen to evaluate the influence of [Bmim]Cl on the soil micro-ecological system with different biological and physico-chemical characteristics.

Sampling was carried out at 5 randomly chosen points from each site. Samples were collected at a depth of 1~15 cm, after removing the top layer. All samples from one site were mixed, then were air-dried, homogenized by sieving to less than 2 mm to separate roots and large objects, and stored in a polyethylene bag at room temperature for later use.

Each soil sample from one site was divided eight portions, one portion was used as the control, and the remaining seven portions were added the suitable amount of [Bmim]Cl to their required [Bmim]Cl concentration respectively. In this study, their [Bmim]Cl concentration were set at 10000 ppm, 1000 ppm,100 ppm, 10 ppm, 1 ppm, 0.1 ppm and 0.01 ppm respectively. These soil samples with different [Bmim]Cl concentrations were stored in polyethylene bags for 15 days at room temperature, and then they were used to determine their biological and physico-chemical properties.

The [Bmim]Cl used in this study was obtained from Henan Lihua Pharmaceutical Co. Ltd., China. All other chemicals employed in this study were of reagent grade and purchased from Wuhan Chemicals & Reageng Corp., China.

2.2. Analytical Methods

2.2.1. Determination of Biological Properties

2.2.1.1. Determination of the Number of Living Microorganisms

The number of living microorganisms (bacteria or actinomycetes) in soil samples was estimated by viable count on serial spread plates [14]. A series of 10–fold dilution for the sample was prepared starting with 9 ml of sterilized phosphate buffered saline added to 1.0 g soil sample. The flask was then closed and the contents were stirred for 30 min, and 1.0 ml suspension of sample was added to 9.0 ml of sterilized phosphate buffered saline. The dilutions were repeated to 7 continuous dilutions. Finally, 200 µl from each serial dilutions of the sample suspension was spread over an agar plate with beef extract peptone medium for bacteria and an agar plate with Gause's No.1 synthetic medium for actinomycetes. All plates were in triplicates and each soil sample was diluted for three independent measurements. Finally, the plates were incubated two or three days at 28℃ until colonies appeared (2 days for bacteria and 3 days for actinomycetes), and colony forming units (CFU) were count which varied from 30 to 300.

2.2.1.2. Calculation of the EC50 of Microorganisms

The concentration of added [Bmim]Cl which inhibits 50% of the growth of microorganisms (bacteria and actinomycetes) comparing with the control culture was defined as EC50, and it was calculated by a regression method [15].

2.2.2. Determination of Physico-Chemical Properties

The organic matter in each soil sample was determined using a $K_2Cr_2O_7$-H_2SO_4 method as described in Chinese National Standard GB9834--88. The soluble salts in each soil sample was measured following the procedures described in Chinese Agricultural Standard NY/T1121.16-2006. The pH value in each soil sample was determined according to the method described in ISO10390-2005.

3. RESULTS AND DISCUSSION

3.1. Toxicity of [Bmim]Cl to the Soil Microorganisms

The soil microorganisms are an important element in soil micro-ecological system and play a vital role in soil micro-ecological system. The toxicity of [Bmim]Cl to the soil microorganisms is an important part of its influence on the soil micro-ecological system. Table **1** listed the number of microorganisms in different soil samples at different [Bmim]Cl concentrations. As indicated in Table **1**, the number of microorganisms including bacteria and actinomycetes decreased with the increase of [Bmim]Cl concentration for all soil samples. It demonstrated that the [Bmim]Cl could inhibit the growth of such soil microorganisms as bacteria and actinomycetes. Table **2** listed the EC50 of microorganisms including bacteria and actinomycetes of different soil samples. As shown in Table **2**, the EC50 values were at relatively lower concentrations for both microorganisms. It indicated that the inhibition of the [Bmim]Cl to the growth of both microorganisms was not just simply due to the pH shift because the [Bmim]Cl concentration changed, but it came from its toxicity to these microorganisms. For all soil samples, the EC50 of bacteria was greater than that of actinomycetes. It indicated that actinomycetes in soil was more sensitive to [Bmim]Cl than bacteria, that is, the [Bmim]Cl had a stronger inhibition on the growth of actinomycetes. For different soil samples, the EC50 of microorganisms including bacteria and actinomycetes existed difference and it showed that the [Bmim]Cl had a different extent influence on the growth of the soil microorganisms which come from the soil micro-ecological system with different biological and physico-chemical characteristics. Compared with the reported EC50 of pure cultures [16-19], the EC50 of soil bacteria was close to that of the *Vibrio fischeri*, and the EC50 of soil actinomycetes was near to that of the *Pseudokirchneriella subcapitata*. It seems that the toxicity of ILs to soil microorganisms is close to that of some well-investigated organisms, such as *Vibrio fischeri* and *Pseudokirchneriella subcapitata*. Whether the ILs toxicity data to these well-investigated organisms can be used to predict the toxicity of ILs to soil microorganisms, further research work are needed because the soil microbial community are far more complicated than the pure cultures. Anyway, the [Bmim]Cl would influence the soil micro-ecological system by inhibiting the growth of soil microorganisms including bacteria and actinomycetes. The well-established IL toxicity data of some pure cultures could provide useful information in the prediction of its toxicity to soil microorganisms.

Table 1. The Number of Microorganisms in Different Soil Samples at Different [Bmim]Cl Concentrations

	Soil type	[Bmim]Cl concentration (ppm)							
		10000	1000	100	10	1	0.1	0.01	control
Bacteria number (10^7CFU.g^{-1})	Rape land	1.30±0.08	3.20±0.17	7.65±0.54	11.06±1.03	11.51±0.87	11.22±0.79	11.75±0.86	11.80±0.92
	Broad bean land	1.20±0.23	1.25±0.35	3.85±0.045	4.35±0.46	4.85±0.63	5.05±0.73	5.29±0.39	5.45±0.47
	Nursery land	0.13±0.01	0.95±0.02	2.45±0.39	4.50±0.81	5.45±0.43	5.37±0.54	5.53±0.61	5.50±0.79
Actinomycetes number (10^6CFU.g^{-1})	Rape land	0.30±0.02	0.65±0.06	1.80±0.02	3.00±0.31	3.35±0.54	3.69±0.43	3.87±0.49	4.05±0.75
	Broad bean land	1.00±0.13	2.5±0.83	3.00±0.38	5.00±0.58	9.00±0.87	9.45±0.48	10.80±0.70	11.51±0.82
	Nursery land	0.10±0.02	0.15±0.02	0.50±0.07	0.85±0.13	1.33±0.19	1.42±0.21	1.73±0.19	1.95±0.16

Table 2. The EC50 of Microorganisms of Different Soil Samples

Microorganism type	Land type	EC50 (ppm)
Bacteria	Rape land	453.93±20.70
	Broad bean land	489.42±14.47
	Nursery land	868.29±34.31
Actinomycetes	Rape land	81.23±4.27
	Broad bean land	83.13±2.96
	Nursery land	7.50±1.34

3.2. Influence of [Bmim]Cl on the Soil Physico-Chemical Properties

The impact of [Bmim]Cl on the soil physico-chemical properties is another important part of its on the soil micro-ecological system besides its toxicity to soil microorganisms. Table **3** listed some important physico-chemical properties of different soil samples at different [Bmim]Cl concentrations. As indicated in Table **3**, the organic mass and the soluble salts in soil samples increased with the increase of the [Bmim]Cl concentration. This is because the [Bmim]Cl itself was an organic compound and it had strong solubility to salts in the soil samples. The [Bmim]Cl also caused the pH change in the soil micro-ecological system. When the [Bmim]Cl concentration was greater than 100 ppm, the soil

Table 3. The Physico-Chemical Properties of Different Soil Samples at Different [Bmim]Cl Concentrations

	Soil type	[Bmim]Cl concentration (ppm)							
		10000	1000	100	10	1	0.1	0.01	control
Organic matter (mg.g^{-1})	Rape land	31.78±1.59	25.30±1.42	22.11±1.15	20.69±1.43	19.29±0.97	17.85±1.02	15.20±1.07	15.06±1.35
	Broad bean land	22.11±2.13	18.01±1.59	15.47±1.67	13.96±2.49	13.05±1.71	12.69±1.63	11.96±1.56	10.97±1.55
	Nursery land	75.40±1.77	69.14±2.37	67.45±1.97	65.70±1.29	62.24±2.11	60.59±2.23	58.70±1.43	58.23±2.12
Soluble salts (mg.g^{-1})	Rape land	1.57±0.08	1.23±0.06	1.19±0.06	0.97±0.05	0.85±0.05	0.76±0.04	0.73±0.04	0.74±0.04
	Broad bean land	1.8±0.07	1.44±0.09	1.32±0.07	1.17±0.05	1.05±0.04	0.92±0.05	0.79±0.03	0.74±0.03
	Nursery land	2.84±0.09	1.65±0.08	1.46±0.07	1.13±0.05	0.91±0.05	0.78±0.03	0.67±0.04	0.62±0.03
pH	Rape land	5.82±0.02	6.51±0.02	6.93±0.03	6.88±0.05	6.85±0.03	6.89±0.06	6.91±0.02	6.96±0.03
	Broad bean land	6.29±0.04	6.62±0.03	6.81±0.03	6.90±0.04	6.88±0.04	6.96±0.05	6.93±0.05	7.04±0.05
	Nursery land	6.08±0.04	6.44±0.05	7.05±0.05	7.03±0.03	7.07±0.05	7.03±0.04	7.07±0.05	7.02±0.05

pH decreased with the [Bmim]Cl concentration increasing. When the [Bmim]Cl concentration was less than 100 ppm, the soil pH was almost the same as the control. This is the result of interaction between the [Bmim]Cl and the soil microorganisms. At lower [Bmim]Cl concentration, although the [Bmim]Cl itself cause the pH to decrease because of its acidity, the soil microorganisms had the ability to adjust the soil pH and the soil pH could remain almost unchanged. However, at higher [Bmim]Cl concentration, the [Bmim]Cl cause the pH to decrease and, at the same time, the soil microorganisms lost the ability to adjust the soil pH because of its toxicity, the final result was that the soil pH decreased.

CONCLUSIONS

This work investigated the toxicity of [Bmim]Cl to soil microorganisms and its impact on soil physico-chemical properties to evaluate its influence on the soil micro-ecological system. The main conclusions are as follows:

1) The [Bmim]Cl inhibited the growth of soil microorganisms including bacteria and actinomycetes. This inhibition became stronger with the [Bmim]Cl concentration increasing. Based on the EC50 of the soil samples, the actinomycetes were more sensitive to the [Bmim]Cl than the bacteria.

2) The organic mass and the soluble salts in soil increased with the increase of the [Bmim]Cl concentration. The [Bmim]Cl also caused the pH change in the soil micro-ecological system.

3) The [Bmim]Cl would influence the soil micro-ecological system by inhibiting the growth of soil microorganisms and altering the soil physico-chemical properties when it contaminated the soil system.

CONFLICT OF INTEREST

None declared.

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