Journal of Environmental Protection, 2011, 2, 298-303
doi:10.4236/jep.2011.23033 Published Online May 2011 (http://www.scirp.org/journal/jep)

Scientific Research

Toxicity and Antimicrobial Activities of Ionic Liquids with Halogen Anion

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Received January 2nd, 2011; revised February 18th, 2011; accepted March 24th, 2011.

ABSTRACT

To investigate the eco-toxicity of ionic liquids (*ILs*), *experiments on growth of three kinds of bacteria were carried out for six common ILs with halogen anion by a micro-calorimetric method at* 310 *K. The results indicate that the growth of all the bacteria was inhibited in the presence of ILs. In addition, all ILs at definite concentrations show some toxicity to Escherichia coli*, *Staphylococcus aureus and Bacillus subtilis. Anti-microbial activities of the ILs with halogen anion are strongly related to structures of the ILs. An increase in alkyl group chain length corresponds with an increase in toxicity*, *and the ILs with pyridinium cation exhibit stronger restraining effect than the same series ILs with imidazolium cation.*

Keywords**:** *Toxicity*, *Ionic Liquids*, *Inhibition*

1. Introduction

Ionic liquids (ILs) were applied in many fields such as biological and chemical reactions for they are non-flammable, sparsely volatile and thermally stable. For these properties, air pollution was prevented greatly. However, release of ILs into aquatic environments may lead to water pollution because of their high solubilities.

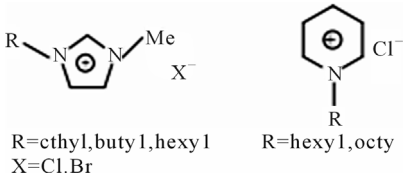
In recent years, some reports that risks of ionic liquids have been available in environment and organisms. Li Xiaoy *et al*. [1-5] studied the toxicity of ionic liquids to *Daphnia magna.* The results showed that the toxicity to *Daphnia magna* increased with the increasing of *n*-alkyl chain length. Zhang Feng *et al*. [6] carried out standard test methods for evaluating the toxicity of chemicals to three aquatic organisms. The results indicated that the inhibiting effects increased significantly with increasing concentrations of ILs. In addition, large-scale application, ILs released into the environments will be inevitable. These cause environmental and agricultural pollution. Some reports [7,8] on the germination rate of seeds and growth of seedlings have indicated different seeds have different reaction to ILs which shown eco-toxicity. Moreover, it is dangerous that poisoning organisms can be increased by increasing the food chain. The food chain of primary producers, primary consumers and predators cause acute and chronic toxicity or cancer. So studying the toxicity of ILs is important.

Microcalorimetry was confirmed to be valid as an alternative method in the study of metabolism of the cell. [9]. It is a useful tool for investigating the biological processes because it permits the continuous monitoring of the activity of a living process in situ without disturbing the system, and the heat evolved or adsorbed is strictly proportional to the rate of the biological processes [10,11]. With its abundant thermodynamic and kinetic information, micro-calorimetry has been widely applied in clinical analysis, pharmacology, ecology, biotechnology and agriculture [12,13].

In this paper, the effects of common ILs with Halogen anion (as shown in **Scheme 1**) on *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*) and *Bacillus subtilis* (*B. sutilis*) growth were investigated by micro-calorimetry, and the bacterial growth rate constants μ at different concentrations (c) of ILs aqueous solutions were calculated via power-time curves along with the generalized Logistic equation. The μ-c correlation equations were formulated. The results indicate antimicrobial activities are related to the structures of ILs, indicating a potential eco-toxicity of the ILs to the micro-organisms in the water.

2. Theory

The growth of bacteria is often limited by some external constraints, including substrate, product concentration, temperature, pH-values and so on. In the logarithmic growth phase, the number of bacteria and time are related according to [14,15]:



Scheme 1. Chemical structures of prepared Ils.

$$\frac{dN_t}{dt} = \mu N_t - \beta N_t^2 \quad (1)$$

where N_t represents the number of bacteria at time (t), μ is the growth rate constant, β is the deceleration rate constant, and t is the experimental time.

By integrating Equation (1) with respect to time (t), the following equation was obtained:

$$N_t = K/(1+\alpha e^{-\mu t}) \quad (2)$$

$K\left(K=\mu/\beta\right)$ in Equation (2) represents the maximum bacterial number during the whole bacterial growth, and α is the final multiple of the initial bacterial number (being the integral constant).

Under the assumption that the heat production rate P_t is proportional to the bacterial number, and P_0 represents the heat production rate by one bacterium, then,

$$P_t = P_0 N_t, P_m = KP_0$$

Inserting it into Equation (2), the following equation was obtained:

$$P_t = P_m K/(1+\alpha e^{-\mu t}) \quad (3)$$

P_m in Equation (3) is the maximum heat production rate during the whole bacterial growth.

Using the experimental data P_t and t obtained from the power-time curves, the growth rate constant (μ) can be calculated by Equation (3).

3. Experimental and Material

3.1. Instrument

A thermometric eight channel Thermal Activity Monitor (3114/3236TAM Air, Sweden) in conjunction with the operating and analytical software was used in this experiment. With this instrument, reactions can be studied at any given temperature in the temperature range of 5˚C - 60˚C within ± 2 × 10^{-2}˚C uncertainty. The system is very sensitive, its detection limit is 1 × 10^{-5} W, and the baseline stability (over a period of 24 h) is 2 × 10^{-5} W. The measuring range contains between 60 mW and 600 mW. The maximum sample volume is 24 mL.

3.2. Materials

Standard strains of *E. coli*, *S. aureus* and *B. subtilis* were used as the test organism.

The beef culture medium was used, containing 1 g NaCl, 2 g peptone and 1 g beef extract in every 200 mL. The pH of medium was adjusted to 7.2 - 7.4 before autoclaving. The culture medium was sterilized in high pressure steam at 121℃ for 30 min before experiments.

Ionic liquids with halide anion used in this experiment were synthesized according to the published method [16-17]. Aqueous solutions of ILs at different concentrations were prepared using doubly distilled water.

3.3. Experimental Method

Ampoule mode was used in this experiment. The bacterial suspension with a volume of 8 mL was poured into each 24 mL glass ampoule in sterile conditions. After adding ILs aqueous solution at different concentrations into the ampoule, the ampoules were then sealed with caps and placed into channels. Power-time curves of continuous bacterial growth were recorded by computer. When the baseline was re-established and became stabilized, the process of bacterial growth was complete.

The micro-calorimeter was controlled at 310 K by thermostat in the whole process, which is the optimum growth temperature.

4. Results and Discussion

4.1. Power-Time Curves of Bacterial Growth

All ILs with halogen anion were tested for antimicrobial activities against *E. coli*, *S. aureus* and *B. subtilis*. The power-time curves of bacterial growth at 310 K in the absence and presence of ILs have been determined, and parts of the fit curves in logarithmic growth phase are plotted in **Figure 1**. It can be seen that the slopes of exponential growth phase at different IL concentrations are different. It can be concluded that the bacterial growth phase changes with adding the ILs aqueous solution into the culture medium.

4.2. Bacterial Growth Rate Constants

The data of P_t and t were obtained from **Figure 1**. According to Equation (3), the bacterial growth rate constants (μ) were calculated, and shown in **Tables 1-3**. The growth rate constants (μ) of the *E. coli*, *S. aureus* and *B. subtilis* gradually decrease with the increase of the IL concentration (c). This is mainly attributed to the inhibitory effect of the halide ILs to some cells in the bacteria suspension. The survivors remain metabolizing continuously at a lower level of heat production rate, depending on the concentration of IL in the solution.

4.3. Growth Rate Constants VS Concentrations and Structure of ILs

The growth rate constants decrease with the increase of IL concentrations. The results indicate that the ILs with

Table 1. Growth rate constants μ/min^{-1} of *E. Coli* at different concentrations of ILs at 310 K.

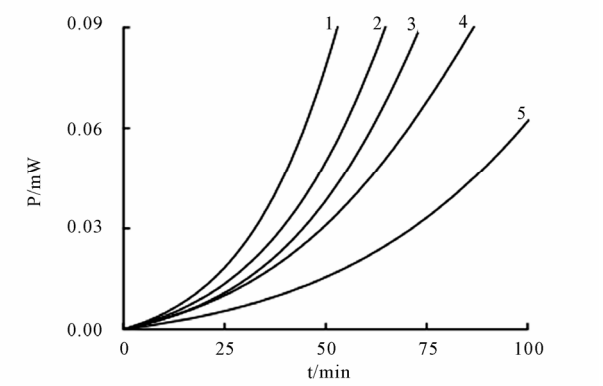
ILs							
EMIMCl	c(mmol/L)	4.985	7.431	16.924	25.123	30.001	39.617
	μ (min^{-1})	0.06029	0.05459	0.045	0.04277	0.03509	0.02689
BMIMCl	c(mmol/L)	1.44	9.132	11.133	13.784	18.084	
	μ (min^{-1})	0.06456	0.05515	0.04788	0.04211	0.03701	
HMIMCl	c(mmol/L)	0.537	0.892	1.422	1.773	2.122	2.643
	μ (min^{-1})	0.05583	0.04861	0.04152	0.03558	0.02063	0.01481
HPyCl	c(mmol/L)	0	0.646	1.288	1.928	3.197	
	μ (min^{-1})	0.09126	0.0768	0.04322	0.03798	0.01876	
OPyCl	c(mmol/L)	0	0.235	0.352	0.584	0.699	
	μ (min^{-1})	0.09126	0.07036	0.04481	0.0341	0.02268	
BMIMBr	c(mmol/L)	0	3.491	6.947	13.757	20.435	26.985
	μ (min^{-1})	0.09126	0.08043	0.07743	0.06148	0.0582	0.03734

Table 2. Growth rate constants μ/min^{-1} of *S. aureus* at different concentrations of ILs at 310 K.

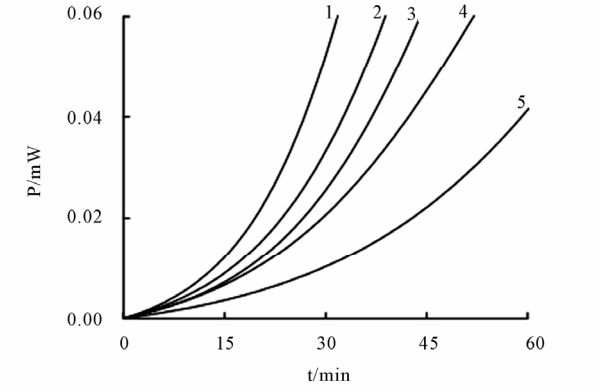
ILs							
EMIMCl	c(mmol/L)	0	1.508	2.508	3.998	4.985	5.967	8.885
	μ (min^{-1})	0.10048	0.07313	0.06869	0.05495	0.04731	0.03729	0.0259
BMIMCl	c(mmol/L)	0	1.44	2.155	2.866	3.574	4.278	
	μ (min^{-1})	0.10048	0.07859	0.05113	0.04211	0.04367	0.03254	
HMIMCl	c(mmol/L)	0	0.178	0.529	0.701	0.87	1.038	
	μ (min^{-1})	0.10048	0.09172	0.07211	0.06802	0.03621	0.02643	
HPyCl	c(mmol/L)	0	0.0646	0.129	0.256	0.32	0.477	
	μ (min^{-1})	0.10048	0.09299	0.0827	0.0677	0.05613	0.03075	
OPyCl	c(mmol/L)	0	0.0235	0.0352	0.0468	0.0584		
	μ (min^{-1})	0.10048	0.05	0.03573	0.03095	0.0271		
BMIMBr	c(mmol/L)	0	4.358	6.947	8.662	12.913		
	μ (min^{-1})	0.10048	0.09307	0.07162	0.06862	0.05074		

Table 3. Growth rate constants μ/min^{-1} of *B. subtilis* at different concentrations of ILs at 310 K.

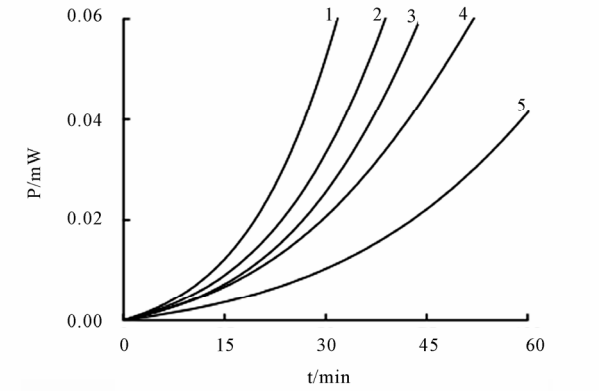
ILs							
EMIMCl	c(mmol/L)	0	2.508	4.985	9.848	14.594	
	μ (min^{-1})	0.11248	0.09984	0.0799	0.06637	0.0472	
BMIMCl	c(mmol/L)	0	1.798	3.574	5.327	7.06	8.771
	μ (min^{-1})	0.11248	0.10884	0.09289	0.07786	0.06444	0.04914
HMIMCl	c(mmol/L)	0	0.716	2.844	5.286	7.005	8.703
	μ (min^{-1})	0.11248	0.09693	0.0766	0.04609	0.03397	0.01186
HPyCl	c(mmol/L)	0	0.646	1.288	1.928	2.564	3.197
	μ (min^{-1})	0.11248	0.10393	0.0887	0.07373	0.06781	0.05921
OPyCl	c(mmol/L)	0	0.59	1.177	1.693	2.342	
	μ (min^{-1})	0.11248	0.09185	0.08533	0.0607	0.03813	
BMIMBr	c(mmol/L)	0	1.625	3.229	4.814	6.379	9.454
	μ (min^{-1})	0.112 48	0.091 81	0.079 63	0.0712	0.064 19	0.039 72



(a)The power-time curves of *E.coli* at different concentrations of EMIMCI (1)7.41 mmol/L; (2)16.924 mmol/L; (3)25.123 mmol/L; (4)30.001 mmol/L; (5)39.617 mmol/L



(b)The power-time curves of *S.aureus* at different concentrations of HMIMCI (1)0.178 mmol/L; (2)0.529 mmol/L; (3)0.701 mmol/L; (4)0.87 mmol/L; (5)1.038 mmol/L



(c)The power-time curves of *B.subtilis* at different concentrations of HPyCl (1)0 mmol/L; (2)0.646 mmol/L; (3)1.288 mmol/L;(4)1.928 mmol/L; (5)2.564 mmol/L

Figure 1. The power-time curves of *E. coli*, *S. aureus* and *B. subtilis* at different ionic liquid concentrations in logarithmic growth phase at 310 K.

halogen anion show significant inhibition to *E. coli*, *S. aureus* and *B. subtilis* growth. Moreover, their activities are greatly affected by the alkyl chain length of the cation ring, and variety of the cation.

For example, the growth rate constants of *E. coli* in the presence of ILs follow the order: [BMIM]Br > [EMIM]Cl > [BMIM]Cl > [HMIM]Cl. With the same anion Cl^-, the longer the alkyl chain length of the imidazolium ring is, the lower the bacterial growth rate constant is. And with the same cation $[BMIM]^+$, the growth rate constant in the presence of [BMIM]Br is higher than [BMIM]Cl. The growth rate constants of *B. subtilis* in the presence of ILs follow the order: [HMIM]Cl > [HPy]Cl >[OPy]Cl. With the same anion, the toxicity of pyridinium ring is stronger than that of the imidazolium ring. The inhibitory activity of [OPy]Cl is the highest among all ILs.

All μ-c relationship can be well represented by equations, as listed in **Table 4**. The correlations between μ and c of *E. coli*, *S. aureus* were formulated by quadratic equations, and the correlations between $\mu$and c of *B. subtilis* were formulated by linear equations. The results suggest all ILs may have the same inhibition mechanism on the *E. coli*, *S. aureus* and *B. subtilis* growth.

4.4. Inhibition Mechanism

E. coli, *S. aureus* and *B. subtilis* are significantly inhibited by the IL-treatments. The toxic effect of ILs may be related to a common cellular structure or process. It is assumed that the toxicity mechanism of ILs is through interaction with the cell wall and membrane, leading to a membrane disruption [18]. ILs consisting of cation-anion pairs is similar to the structure of surfactants, pesticides and antibiotics that attack lipid structure, and induce polar narcosis due to their interfacial properties, and may cause membrane-bound protein disruption [19]. Moreover, disruption to cell membrane is related to the alkyl chain length of the cation ring and variety of the cation of ILs.

4.5. Conclusions

The effects of six halide ionic liquids on the bacterial growth were studied by microcalorimetry. The ionic liquids studied show inhibition activities on the metabolism of *E. coli*, *S. aureus* and *B. subtilis*, and may follow the same inhibition mechanism on the bacteria growth. The antimicrobial activities of ILs are associated with the alkyl side chain length of cation and the variety of the cation**.** With the same anion, the longer the alkyl side chain length of cation is, the stronger the antibacterial activitie of the IL is. The toxicity of pyridinium ring is stronger than that of the imidazolium ring. The microcalorimetry can be effectively used to study the microbial growth and toxic properties of ILs. The antimicrobial effects of ILs should be considered in their potential

Table 4. μ-c correlation equations.

bacteria	Ionic liquids	$\mu - c$ equations	
E.coli	[EMIM]Cl	$\mu = 1.74\times10^{-6}c^2 - 9.76\times10^{-4}c + 0.0633$	$R^2 = 0.9738$
	[BMIM]Cl	$\mu = -1.22\times10\,c\ - 1.50\times10\,c + 0.0672$	$R^2 = 0.9708$
	[HMIM]Cl	$\mu = 5.70\times10^{-4}c^2 - 0.0179c + 0.0650$	$R^2 = 0.9974$
	[BMIM]Br	$\mu = -5.16\times10^{-6}c^2 - 1.69\times10^{-3}c + 0.0890$	$R^2 = 0.9662$
	[HPy]Cl	$\mu = 5.30\times10^{-3}c^2 - 0.0403c + 0.0936$	$R^2 = 0.9679$
	[OPy]Cl	$\mu = 4.70\times10^{-2}c^2 - 0.132c + 0.0924$	$R^2 = 0.9684$
S. aureus	[EMIM]Cl	$\mu = 6.30\times10^{-4}c^2 - 0.0137c + 0.0979$	$R^2 = 0.9873$
	[BMIM]Cl	$\mu = 2.24\times10^{-3}c^2 - 0.0258c + 0.102$	$R^2 = 0.9519$
	[HMIM]Cl	$\mu = -4.78\times10^{-2}c^2 - 0.0226c + 0.0993$	$R^2 = 0.9702$
	[BMIM]Br	$\mu = -6.52\times10\,c\ - 3.22\times10\,c + 0.102$	$R = 0.9575$
	[HPy]Cl	$\mu = -5.93\times10^{-2}c^2 - 0.118c + 0.100$	$R^2 = 0.9986$
	[OPy]Cl	$\mu = 24.9c^2 - 2.69c + 0.100$	$R^2 = 0.9983$
B. subtilis	[EMIM]Cl	$\mu = -2.99\times10^{-5}c + 0.109$	$R^2 = 0.973$
	[BMIM]Cl	$\mu = -7.57\times10^{-3}c + 0.118$	$R^2 = 0.981$
	[HMIM]Cl	$\mu = -1.11\times10^{-2}c + 0.108$	$R^2 = 0.9929$
	[BMIM]Br	$\mu = -7.20\times10^{-3}c + 0.107$	$R^2 = 0.9769$
	[HPy]Cl	$\mu = -1.73\times10^{-2}c + 0.112$	$R^2 = 0.9673$
	[OPy]Cl	$\mu = -3.06\times10^{-2}c + 0.114$	$R^2 = 0.9716$

industrial applications and overall risk assessment.

5. Acknowledgements

Shandong Province Natural Scientific Foundation of China (ZR2009BM035), and Shandong Province Science Research Reward Foundation for Excellent Young and Middle-aged Scientists (2008BS02021).

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