녹조류 *Selenastrum capricornutum*에 관한 이온성액체의 독성

조철웅[1], 팜티풍튀[1], 윤영상[1,2,*]
[1]전북대학교 생물공정공학과, [2]전북대학교 환경화학공학부
(ysyun@chonbuk.ac.kr[*])

Toxicity of Ionic Liquids on Green Microalga *Selenastrum capricornutum*

Chul-Woong Cho[1], Thi Phuong Thuy Pham[1] and Yeoung-Sang Yun[1, 2 *]
[1]*Department of Bioprocess Engineering,* [2]*Department of Chemical Engineering*
Chonbuk National University
(ysyun@chonbuk.ac.kr[*])

1. Introduction

Ionic liquids (ILs) are organic salts that have melting points around room temperature. They have recently received tremendous attention due to their low volatility and subsequent potential to replace volatile organic compounds [1, 2]. The key advantage of ILs is the fact that the cationic (Fig.1) as well as the anionic components can be varied and thus the whole solvent properties can be tailored to match a particular application. Thereby, they are called “designer solvents”. However, the product designs for this promising group of compounds should take account not only of technological needs but also of ecotoxicological hazards. Therefore, this study aimed to evaluate the toxicity of conventional ILs regarding the alkyl-chain length, cationic and anionic effects.

In this paper, we reported our results of the investigation of antimicrobial activities of ILs.

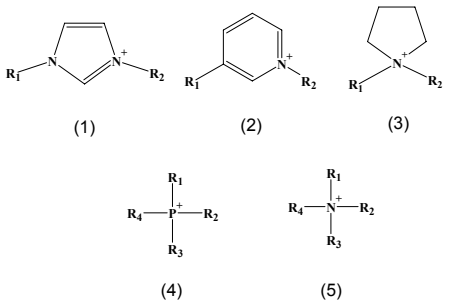

Fig.1. Five important cationic classes of ILs: (1) imidazolium-, (2) pyridinium-, (3) pyrrolidinium-, (4) phosphonium-, (5) ammonium-class.

2. Materials and Methods

2.1. Ionic Liquids and Other Chemicals

1-propyl-3-methylimidazolium [PMIM], 1-butyl-3-methylimidazolium [BMIM] (Br, BF_4, and PF_6), 1-hexyl-3-methylimidazolium [HMIM], 1-octyl-3-methylimidazolium [OMIM], 1-butyl-3-methylpyridinium [BMPy] and 1-butyl-1-methylpyrrolidinium [BMPyrr] Br were supplied by C-TRI, Korea. Tetrabutylammonium [TBA], tetrabutylphosphonium [TBP] Br, and BMIM (Cl, $C_8H_{17}OSO_3$, and CF_3SO_3) were purchased from Sigma-Aldrich.

2.2. Test methods

The ILs toxicity tests were referred to methods recommended in the EPA guidelines [3] and the OECD guidelines [4]. Experiments were conducted in a 250ml Erlenmeyer flask containing 55ml of sterilized culture nitrate-enriched BBM medium made from triple distilled water so as to avoid nitrogen limitation in the high-density culture [5]. Test flasks were inoculated with 5ml of 7 days cultured algal samples. The appropriate volume of known concentration of ILs was added to each flask from a single stock solution. The flasks were randomly placed on a shaking incubator operating at 170 rotations per minute, and were kept at $25 \pm 5\,^{o}C$ with a 24 h light from warm-white fluorescent tubes which provided an approximate uniform illumination of $30 \pm 5\ \mu E\ m^{-2}\ s^{-1}$. At the each determined date of the exposure period, optical density of algal biomass was estimated at 438 nm using spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan). The dry cell weigh, corresponding to optical density, was determined through linear relation (dry cell weight = 0.1329 * optical density). All experiments were done in duplicates; whereas control experiments were triplicated. Data treatment was carried out with the Sigma Plot (Sigma-plot 8.02).

3. Results and Discussion

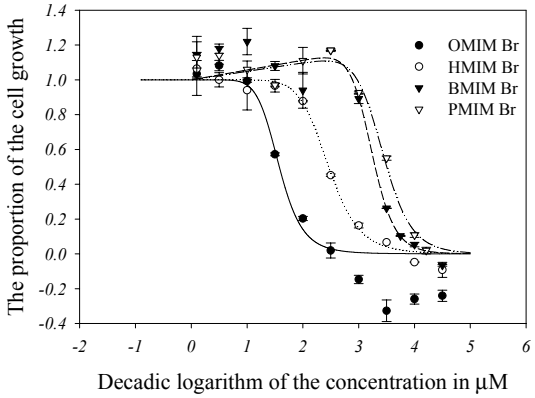

Fig. 1. Influence of alkyl chain on toxicity of ILs to alga, *S. capricornutum*

3.1. Alkyl-chain length effect

All imidazolium-based ILs inhibited the algal growth with EC_{50} values varying between 38 and 1047μM. A correlation between alkyl-chain length of ILs and inhibitory potency was observable. A longer chain length resulted in a stronger inhibition of the algal growth (Fig 2.). This is due to the fact that ILs with longer alkyl-chain possess more lipophilic properties [6].

The above mentioned relationship can be quantified by a linear regression on the logarithms of the EC_{50} values versus the number of carbon atoms at R_2 (nR_2). The equation is $\log_{10}(EC_{50}) = -0.316(nR_2) \pm 4.2$ with $R^2 \approx 0.965$.

3.2. Anion effect

Table 1. Imidazolium-based ILs with various anions and their $\log_{10}EC_{50}$ on algal growth after 96h.

Compounds	Molecular formula	$Log_{10}EC_{50}$/μM	EC_{50}/μM
[BMIM] CF_3SO_3	$C_9H_{15}F_3N_2O_3S$	2.79±0.29	616
[BMIM] PF_6	$C_8H_{15}F_6N_2P$	2.64±0.28	436
[BMIM] BF_4	$C_8H_{15}BF_4N_2$	2.1±0.042	126
[BMIM] Cl	$C_8H_{15}ClN_2$	3.03±0.77	1071
[BMIM] $C_8H_{17}OSO_3$	$C_{16}H_{32}N_2O_4S$	3.33±0.07	2137
[BMIM] Br	$C_8H_{15}BrN_2$	3.02±0.19	1047

The influence of the anions of ILs on the inhibition of algal growth was estimated using BMIM as a countercation. The effect concentrations for all the ILs investigated are listed in Table 1. The obtained EC_{50} values ranged from 126 μM to 2137 μM. The BMIM $C_8H_{17}OSO_3$ was the least toxic among tested ILs. On the other hand, BMIM BF_4 is the most toxic. This might be due to the chemical hydrolysis leading to fluoride formation, thus enhancing toxicity through the decomposition products. Additionally, it is known that the fluoride ion is a potent inhibitor of the Na^+-K^+-ATPase which is located at the cell surface and thus may interfere with processes essential in cell self-maintenance [7].

3.3. Cation effect

Table 2. $Log_{10}EC_{50}$ values for cationic effect of ILs with Br as counteranion.

Chemicals	$Log_{10}EC_{50}$/μM		
	48 hours	72 hours	96 hours
[BMIM] Br	3.46±0.062	3.36±0.073	3.02±0.19
[BMPy] Br	3.93±0.08	3.70±0.06	3.69±0.12
[BMPyrr] Br	3.67±0.28	3.97±0.2	4.09±0.22
[TBA] Br	2.97±0.13	3.68±0.133	3.35±0.25
[TBP] Br	1.90±0.23	2.19±0.16	2.35±0.11

In order to evaluate the cationic toxicity of ILs on algal growth rate, we selected various chemicals, such as imidazolium, pyridinium, pyrrolidinium, ammonium, and phosphonium with bromide as counteranion. It was observed that the toxicity of TBP and TBA decreased when incubating time was

raised from 48 to 96 h (Table 2). In contrary, toxicity of BMIM and BMPy was found to increase according to incubating time. Among tested ILs, BMPyrr Br was found to be least toxic and TBP Br is the most toxic. Generally, as being shown by the results estimated for 96 hours, the ILs structure with four substituents was more toxic than cyclo compounds.

4. Conclusion

The observed results revealed that the EC_{50} values decreased in regard with the increasing alkyl-chain length of ILs. Also, we found pyrrolidinium as cation and $C_8H_{17}OSO_3$ as anion were the least toxic among tested ILs. On the other side, cationic phosphonium salt and anionic BF_4 were the most toxic. We might think that toxicity mechanism of ILs is algal membrane disruption because ILs are similar to pharmaceuticals which attack lipid structure [8]. Further research should be done for better understanding the correlation between the structure and toxic effects of ILs.

5. References

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