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# Alkyl-Chain Length Effects of Imidazolium and Pyridinium Ionic Liquids on Photosynthetic Response of *Pseudokirchneriella subcapitata*

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**The toxicities of imidazolium- and pyridinium-based ionic liquids with various alkyl-chain lengths were investigated on the photosynthetic activity of the alga *Pseudokirchneriella subcapitata*. Our results show that an imidazolium compound with four carbons in the alkyl chain was the least toxic salt, and should be preferred over pyridinium entity.**

[**Key words:** ionic liquids, alkyl-chain length, toxicity, photosynthetic activity, *Pseudokirchneriella subcapitata*]

Being composed entirely of ions, ionic liquids (ILs) are technologically advanced solvents that can be designed to fit a specific application (1). Recently, ILs have been discussed widely in the context of green and/or sustainable chemistry, owing to their immeasurably low vapor pressure and nonflammablility. However, these green credentials are currently unproven in an aquatic environment. Up to now, the IL toxicity towards organisms such as the crustacean *Daphnia magna* (2–5), the snail *Physa acuta* (6) and the zebrafish *Danio rerio* (7) has been extensively studied. However, the effect of ILs on freshwater green algae has rarely been examined.

In this work, the toxicity of a variety of imidazolium- and pyridinium-derived ILs with different alkyl-chain lengths on the freshwater green alga *Pseudokirchneriella subcapitata* was investigated. The effect parameter was the photosynthetic response of algal cells as measured in terms of oxygen evolution rate. Toxicity test was conducted using a biosensor specifically designed for microalgal photosynthetic activity measurement.

The green unicellular freshwater alga *P. subcapitata* ATCC-22662 was kindly provided by the National Institute of Environmental Research (Korea). In our laboratory, this species was routinely cultivated in a 250-ml Erlenmeyer flask containing 200 ml of nitrate-enriched Bold's Basal medium by adding 58.8 mM $NaNO_3$ to avoid nitrogen limitation in high-density culture (8). The culture flask was continuously agitated on a rotary shaker at 170 rpm at 25±2°C and under continuous illumination of 30±5 $\mu Em^{-2} s^{-1}$.

The ILs employed in this study included 1-alkyl-3-methylimidazolium bromide and 1-alkyl-3-methylpyridinium bromide, comprising the alkyl moiety of *n*-propyl ($C_3$), *n*-butyl ($C_4$), *n*-hexyl ($C_6$) or *n*-octyl ($C_8$). The cations are abbreviated as $C_n$MIM and $C_n$MPy, where *n* represents the number of C atoms in the longer *n*-alkyl chain, M denotes the methyl group, and IM and Py denote the imidazolium and pyridinium, respectively. These compounds were obtained from C-TRI (Korea); their structural formulas are given in Fig. 1.

Algal acute toxicity assay based on photosynthetic activity measurement was carried out using a specifically designed system shown in the schematic diagram of Fig. 2. The most important part of this system was the reaction cell, which was a double-jacket cylinder made of Pyrex glass. During the experiments, the microalgal suspension and ILs were injected into the reaction vessel and a homogeneous mixture was formed by mixing with a small magnetic stirrer (0.5 cm in length). Light intensity was set at 1000±50 $\mu Em^{-2} s^{-1}$. The light source was a 150 W quartz halogen lamp (EKE; Ushio, Tokyo) set inside an optical fiber illuminator (A3200; Dolan-Jenner, Lawrence, MA, USA). A quantum sensor (LI-190A; Licor, Lincoln, NE, USA) connected to a light meter (LI-250; Licor) was positioned opposite to the illumination side so as to measure light transmittance. Since microalgal photosynthesis is temperature-sensitive, cooling water at 25±2°C from a water bath was continuously circulated through out double jacket of the reaction vessel. An oxygen

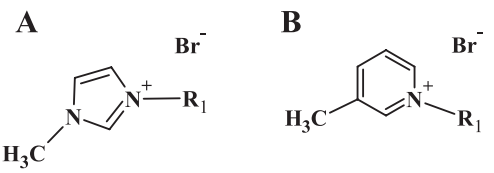


FIG. 1. Structural formulae of tested series of (A) dialkylimidazolium and (B) dialkylpyridinium compounds. $R_1$=*n*-propyl, *n*-butyl, *n*-hexyl, and *n*-octyl.

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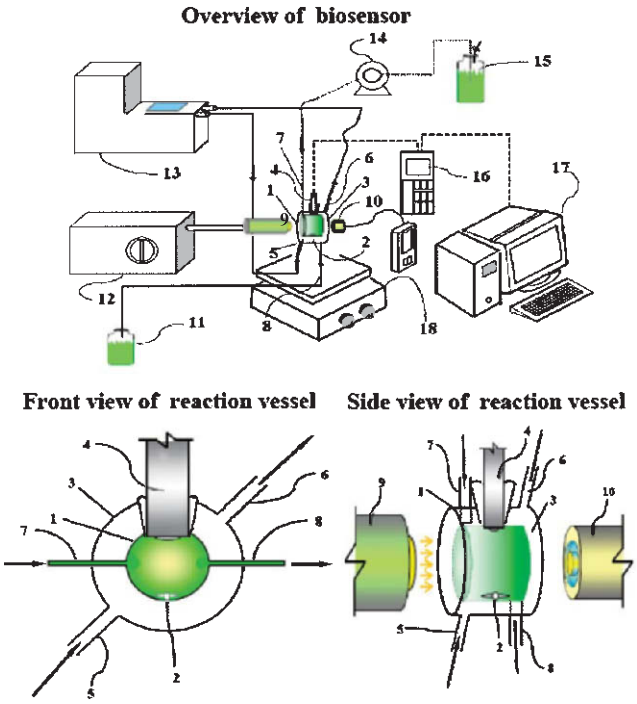


FIG. 2. Schematic diagram of photosynthetic activity measurement system and reaction cell. 1, Reaction cell; 2, magnetic bar; 3, cooling water jacket; 4, dissolved oxygen electrode; 5, inlet of cooling water; 6, outlet of cooling water; 7, inlet of sample; 8, outlet of sample; 9, convex lens; 10, quantum sensor; 11, wastewater; 12, quartz halogen illuminator; 13, water bath; 14, peristaltic pump; 15, sample reservoir; 16, dissolved oxygen meter; 17, computer and 18, magnetic stirrer.

probe (Ingold) was placed in the circular top of the reaction cell and used for measuring the concentration of the dissolved oxygen generated by algal photosynthesis.

Prior to the test, a cell suspension was prepared by centrifuging algal cells in the late exponential phase and resuspending them in a fresh medium to yield a concentration of approximately 0.19 g/*l*. A mixture of the previously prepared algal broth and the toxicant was subsequently used to fill in the reaction vessel after being exposed for 10 min and stripped by a gas mixture to reduce the initial dissolved oxygen level to 2 to 3 mg/*l*. The oxygen produced by the algal organisms was recorded every minute during a 10-min period by a computer directly connected to the system. Complete concentration-response curves were obtained by conducting the experiment for almost 2 h.

In each experiment, the percentage inhibition of the algal photosynthetic activity was calculated using the recorded oxygen evolution data. The $EC_{50}$ values (concentration which leads to a 50% reduction in photosynthetic activity of the exposed organisms relative to that of the control) were obtained by fitting dose-response curves to the multinomial data by the nonlinear least-squares method using the logistic model of the relationship of photosynthetic activity and inhibition to the decadic logarithm of examined concentrations. Calculations were carried out using the Sigma Plot software (Sigma-plot 8.02).

The acute toxicity test conducted in this study involved the use of a photosynthetic activity measurement system. This system provided a rapid means of determining the acute toxic effects of aqueous compounds by measuring decreases in the concentration of volumetric oxygen generated from the green alga *P. subcapitata*. A decrease in generated oxygen concentration served as an indirect measurement of the toxicity of the tested compound. The ecotoxicological test data from the experiments are shown in Table 1. There was a strong correlation of toxicity and the alkyl side-chain length for both alkylmethylimidazolium and alkylmethylpyridinium with a monotonic decrease in $EC_{50}$ value for the algal photosynthetic activity with increasing chain length of ILs. This relationship can be quantified using a linear regression on the logarithm of the $EC_{50}$ value versus the number of carbon atoms at $R_1$ ($nR_1$). For pyridinium, the equation is:

$$\log_{10}EC_{50}\,(\mu M) = -0.58(nR_1) + 6.45 \qquad (1)$$

where $R^2 \approx 0.989$. The corresponding equation for imidazolium moiety is

$$\log_{10}EC_{50}\,(\mu M) = -0.21(nR_1) + 5.33 \qquad (2)$$

where $R^2 \approx 0.84$, except $C_3$MIM, since its $EC_{50}$ value could not be determined. Decisively lower effective concentrations were obtained for the ILs with an octyl chain in comparison to those with propyl, butyl or hexyl chains. Additionally, it was observed that pyridinium salts were more toxic than imidazolium compounds, whereas the ILs with pyridinium as the cationic core structure inhibited the enzyme acetylcholinesterase stronger than the imidazolium analogue (9).

Figure 3 shows the photosynthetic activity inhibition of *P. subcapitata* exposed to 5 different concentrations (from 10 µM to 0.1 M) of imidazolium- and pyridinium-derived ILs. The figure indicates that the tested ILs inhibited the microalgal photosynthetic activity at relative low concentrations. In the cases of $C_4$MIM, $C_3$MPy, $C_4$MPy and $C_8$MPy with a Br anion, the 10 µM concentration had no inhibitory potency during the experiment, which enabled the photosynthetic activity of *P. subcapitata* to recover sufficiently so as to attain a final oxygen evolution rate close to that of the control sample or even higher. The aforementioned hormetic

TABLE 1. Ranges of observed $EC_{50}$ values estimated by short-term inhibition of photosynthesis of *P. subcapitata* exposed to imidazolium- and pyridinium-based ionic liquids and organic solvents

| Imidazolium-based IL | $EC_{50}$ (mM) | Pyridinium-based IL | $EC_{50}$ (mM) | Organic solvent | $EC_{50}$ (mM) |
|---|---|---|---|---|---|
| $C_3$MIM Br | >1000 | $C_3$MPy Br | 53.70 (28.84–100) | Methanol | 2570 (1995–3311) |
| $C_4$MIM Br | 23.99 (7.94–72.44) | $C_4$MPy Br | 10.72 (2.57–44.67) | Dimethyl-formamide | 2089 (1175–3715) |
| $C_6$MIM Br | 19.05 (6.31–57.54) | $C_6$MPy Br | 1.48 (0.26–8.51) | 2-Propanol | 589 (417–832) |
| $C_8$MIM Br | 3.47 (0.79–15.14) | $C_8$MPy Br | 0.055 (0.015–0.19) | | |

The values in parentheses with $EC_{50}$ indicate 95% confidential interval.