The ecotoxicity of ionic liquids and traditional organic solvents on microalga *Selenastrum capricornutum*☆

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Received 30 September 2006; received in revised form 16 June 2007; accepted 1 July 2007
Available online 10 August 2007

Abstract

In this study, the effects of several ionic liquids and traditional organic solvents on the growth of the green microalga, *Selenastrum capricornutum*, were investigated. The toxicities were strongly related to the incubation time and chemical structures of the ionic liquids. The toxicities of tetrabutylphosphonium and tetrabutylammonium containing bromide anion were observed to decrease when the incubation time was raised from 48 to 96 h. Conversely, the toxicities of 1-butyl-3-methylimidazolium and 1-butyl-3-methylpyridinium containing bromide anion were found to increase with increasing incubation time. Of the ionic liquids tested, 1-butyl-1-methylpyrrolidinium bromide was found to be the least toxic, which is similar in toxicity level of dimethylformamide. In general, the toxicities of the ionic liquids were estimated to be two and four orders of magnitude greater than those of the organic solvents examined, although ionic liquids are being considered as green solvents.

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Keywords: Ionic liquids; Organic solvents; Toxicity; Ecotoxicity; *Selenastrum capricornutum*; Hormesis

1. Introduction

In recent years, room temperature ionic liquids have received attention as primary alternatives to the organic solvents traditionally used as conventional media in chemical processes (Holbrey and Seddon, 1999; Cull et al., 2000). Ionic liquids are non-volatile and non-flammable and possess high thermal, chemical and electrochemical stabilities, as well as favorable solvability behaviors (many organic, organometallic and inorganic compounds can be dissolved in ionic liquids). In addition, ionic liquids have a large electrochemical window and excellent solvent properties for a wide range of inorganic and organic materials (Fuller et al., 1997). With regard to the structure of ionic liquids, most are comprised of a bulky organic cation (Fig. 1) and various anions, which results in low melting point (less than 100 °C) salts. The characteristics of ionic liquids are known to be significantly altered by different cation and anion combinations. Due to their favorable properties, ionic liquids are suitable for use as media for catalysts, liquids crystals, and extraction, as well as in electrochemistry and separation (Sheldon, 2001; Welton, 1999; Seddon, 1997; Luo et al., 2006; Kubisa, 2004; Wilkes, 2004; Huddleston et al., 1998).

Because the ionic liquids have negligible vapor pressures, therefore, they can be considered as "green solvents". This property decreases the risk of exposure and loss of solvent
Several toxicity studies on the effects of ionic liquids toward organisms, including *Vibrio fischeri* (Docherty and Kulpa Jr, 2005), *Daphnia magna* (Bernot et al., 2005), animal cells (Stepnowski et al., 2004), algae (Latała et al., 2005), *Escherichia coli* (Lee et al., 2005), lactic acid-producing bacteria (Matsumoto et al., 2004a,b) and acetylcholinesterase (*Stock et al., 2004*) have recently been documented. Of these organisms, algae are now widely used as biological assay tools for environmental impact studies. The justification for using algae is related to their ecological role as primary producers in the transfer of energy to higher trophic levels; they are also simple to use and have potential economic benefits (Latała et al., 2005).

Therefore, this research has comparatively investigated the toxic effects of both ionic liquids and traditional organic solvents on the growth of the green microalga, *Selenastrum capricornutum*, using both the standard acute and chronic tests.

2. Materials and methods

2.1. Ionic liquids and other chemicals

1-butyl-3-methylimidazolium [BMIM], 1-butyl-3-methylpyridinium [BMPy] and 1-butyl-1-methylpyridinium [BMPyr] with bromide anion were supplied by C-TRI (Korea). Tetrabutylammonium bromide [TBA] [Br], tetrabutylphosphonium bromide [TBP] [Br] and 2-propanol (CAS No. 67-56-1, purity 99.5%) were purchased from Sigma-Aldrich and methanol (CAS No. 67-56-1, purity >99.5%) was purchased from Samchun Pure Chemical Co. (Korea). The CAS-numbers, molecular formulae and molecular weights of these toxicants are shown in Table 1.

2.2. Microalgal strain and cultivation

The green microalga used as the model algal strain in this study, *Selenastrum capricornutum* ATCC-22662, was obtained from the National Institute Environmental Research, Korea. The stock algae was cultivated in 250 ml Erlenmeyer flasks, containing 200 ml sterilized nitrate-enriched BBM medium prepared in triple distilled water, to avoid nitrogen limitation during the high-density culture (Yun and Park, 1997). The culture flask was agitated on a shaker at 170 rpm, and bubbled with air (1vvm), without sparger. Light was continuously supplied, with an average of 30 ± 5 μE m⁻² s⁻¹, using warm-white fluorescent tubes (Korea General Electric Co., Korea) located on top of the shaker. All the flasks were maintained in the shaker incubator at 25 ± 5 °C for 7 days.

2.3. Toxicity tests

The ionic liquids toxicity tests were conducted using the methods recommended by the EPA (EPA, 1996) and via the OECD guidelines (OECD, 2002). Experiments were performed in 250 ml Erlenmeyer flasks, containing 55 ml of sterilized culture medium, inoculated with 5 ml samples of 7-day cultured algae. Solutions of ionic liquids and organic solvents were subsequently added to the test flasks. The ionic liquids and organic solvents tested were completely miscible with water, forming a homogeneous phase. Concentrations of these toxicants were adjusted in the range between 0.1 M and 1.26 M to obtain complete concentration-response curves. The flasks then were placed on a shaker incubator at 170 rpm and 25 °C, with 24 h light supplied via warm-white fluorescent tubes, with an average illumination of 30 ± 5 μE m⁻² s⁻¹. At each determined exposure date, the optical density of the algal biomass was estimated at 438 nm using a spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan). The dry cell weight, corresponding to the optical density, due to evaporation; thereby, reducing air pollution. Even though ionic liquids potentially have high technical and commercial uses, little data exist with regard to their toxicological properties. Based on their assumed properties, i.e. their high solubilities and stabilities, the release of ionic liquids into aquatic environments may lead to water pollution, with subsequent risk to aquatic organisms. Ionic liquids are also poorly decomposed by microorganisms (Garcia et al., 2005; Gathergood et al., 2004, 2006). The adsorption of ionic liquids onto a range of bacterial surfaces has also been found to be minimal, implying that their transport through subsurface groundwater would be unimpeded (Gorman-Lewis and Fein, 2004). Thus, it can be hypothesized that ionic liquids may pose environmental risks to aquatic ecosystems and their accurate data on toxicities are likely to be of foremost importance.
was determined from the linear relationship; dry cell weight (g/l) = 0.1329 x optical density. All experiments were performed in duplicate, but the control experiments were conducted in triplicate.

2.4. Data treatment

The protocol for the growth inhibition bioassay was based on standard procedures in the OECD guidelines (OECD, 2002). The average specific growth rate, \( \mu \), for a specific period is calculated as the logarithmic increase in the biomass:

\[
\frac{\ln C_f - \ln C_i}{t_f - t_i} = \mu \text{d}^{-1},
\]

(1)

where \( \mu_{i-f} \) is the average specific growth rate from initial moment time, \( i \), to the final moment time, \( f \), and \( C_i \) and \( C_f \) are the initial and final biomass concentrations (mg/l), respectively; and \( t_i \) and \( t_f \) the initial and final moment times (d), respectively.

The percent inhibition of the growth rate (% \( I \)) for each treatment replicates can be calculated from

\[
\%I = \frac{\mu_C - \mu_T}{\mu_C} \times 100.
\]

(2)

where \( \mu_C \) and \( \mu_T \) are the specific growth rates of the control and treatment (d\(^{-1}\)), respectively.

The concentration-response curves, where feasible, were fitted to the multinomial data using the non-linear least-squares method and logistic model, to obtain the relationships of the cell viability and inhibition to the concentration of the substance to which the cells are exposed, \( x \). The inhibition % was calculated as:

\[
P = \frac{1}{1 + (x/x_0)^b}.
\]

(3)

where \( x \) is the concentration of the substance to which the cells are exposed, \( P \) the physiological response, normalized to the positive and negative controls, with intervals from 1 (c = 0) to 0 (negative control); \( x_0 \) represents the EC\(_{50}\) and \( b \) the slope of the function on a logit-log-scale. Calculations were carried out with the Sigma Plot software (Sigma-plot 8.02).

The algal growth was found to be increased with increasing concentration, but then decreased with additional increases in concentration. Therefore, the concentration–response curves were fitted using the linear-logistic model proposed by Brain and Cousens (1989), which has been reparameterized in the work of van Ewijk and Hoekstra (1993) for the case of a subtoxic stimulus

\[
P = \frac{1 + f x}{1 + (2 \times f x_0 + 1)(x/x_0)^b}\]

(4)

where \( b' \) is a parameter without intuitive interpretation and \( f \) the parameter describing hormesis. If \( f > 0 \), the curve shows an increase at lower concentrations. The confidence intervals of the fitted EC\(_{50}\) values could be derived, as they are model parameters in both the logistic and reparameterized linear-logistic models.

3. Results

The microalgal growth rates in the presence of ionic liquids and traditional organic solvents were determined on the basis of the algal growth rate after toxicant addition, with the relative activity being defined as the ratio of the cell concentration in the sample compared to that in the control. The estimated EC\(_{50}\) values for all ionic liquids are listed in Table 2, which ranged from 0.079 to 12.3 mM. However, the parameters (\( f, b \) and \( b' \)) in Table 2 seem to provide no additional biological insight, but are included for the sake of completeness of the fitted dose–response curves.

From our results, the growth of *S. capricornutum* was significantly affected, even at low [BMIM] [Br] concentrations (Fig. 2). The EC\(_{50}\) values for [BMIM] [Br] obtained after 48 and 72 h of incubation ranged from 2.50 to 3.33 mM and from 1.94 to 2.71 mM, respectively. The effect of incubation time on the EC\(_{50}\) values obtained at 48 and 72 h was unclear, but a trend of increasing toxicity was observed after 96 h incubation. The algal growth rates were also found to be affected in the presence of both [BMPy] [Br] and [BMPyr] [Br]. The toxicity of [BMPyr] [Br] showed a decreasing trend, particularly when increasing the incubation time from 48 to 72 h, but this stabilized after 96 h of incubation (Fig. 3). Conversely, the incubation time had no influence on the toxicity of [BMPyr] [Br] (Fig. 4).

3.1. Effect of ionic liquids on algal growth rate

The EC\(_{50}\) values of [TBA] [Br] increased when increasing the incubation time from 48 to 72 h (Fig. 5). However, further increases in the incubation time resulted in reduction of the EC\(_{50}\) values. The presence of [TBP] [Br] strongly affected the algal growth rate, with an EC\(_{50}\) value after 48 h of incubation in the order of 0.047–0.134 mM (Fig. 6). A decrease in the toxicity was observed when increasing the incubation time from 48 to 96 h. However, [TBP] [Br] was found to be more toxic than [TBA] [Br]. To summarize, [TBP] [Br] was the most toxic and [BMPyr] [Br] the least toxic of the ionic liquids tested, with differences of between 10- and 100-fold. Therefore, the cationic structure of [BMPyr] [Br] was the most environmentally friendly type.
Table 2

<table>
<thead>
<tr>
<th></th>
<th>Log_{10}EC_{50}/\mu M</th>
<th>f</th>
<th>b</th>
<th>Log_{10}EC_{50}/\mu M</th>
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<th>Log_{10}EC_{50}/\mu M</th>
<th>f</th>
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<tbody>
<tr>
<td>[BMIM] [Br]</td>
<td>3.46 ± 0.062</td>
<td>20.1</td>
<td>1.4</td>
<td>3.36 ± 0.073</td>
<td>18.2</td>
<td>2.4</td>
<td>3.02 ± 0.19</td>
<td>0.0556</td>
<td>16.1</td>
</tr>
<tr>
<td>[BMPy] [Br]</td>
<td>3.46 ± 0.062</td>
<td>18.2</td>
<td>1.7</td>
<td>3.70 ± 0.06</td>
<td>12.8</td>
<td>2.7</td>
<td>3.69 ± 0.12</td>
<td>0.0788</td>
<td>10.4</td>
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<tr>
<td>[BMPyr] [Br]</td>
<td>3.67 ± 0.28</td>
<td>0.0487</td>
<td>17.5</td>
<td>3.97 ± 0.2</td>
<td>0.0727</td>
<td>11.5</td>
<td>4.09 ± 0.22</td>
<td>0.0788</td>
<td>10.4</td>
</tr>
<tr>
<td>[TBA] [Br]</td>
<td>2.97 ± 0.13</td>
<td>7.88</td>
<td>6.8</td>
<td>3.68 ± 0.133</td>
<td>0.0594</td>
<td>8.26</td>
<td>3.35 ± 0.25</td>
<td>8.78</td>
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<tr>
<td>[TBP] [Br]</td>
<td>1.90 ± 0.23</td>
<td>7.62</td>
<td>4.7</td>
<td>2.19 ± 0.16</td>
<td>6.79</td>
<td>2.58</td>
<td>5.85 ± 0.08</td>
<td>28.3</td>
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<tr>
<td>Methanol</td>
<td></td>
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<tr>
<td>Dimethylformamide</td>
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<td>2-Propanol</td>
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</table>

f is the parameter introduced to describe hormesis, b' a parameter with no intuitive meaning, and b the slope parameter of the logistic regression (see Eqs. (3) and (4)).
The toxicities of traditional organic solvents (methanol, dimethylformamide, and 2-propanol) toward *S. capricornutum* were tested for comparison with those of ionic liquids. The data showed that of tested solvents, dimethylformamide was the most toxic with an EC50 value ranging between 17.4 and 31.6 mM. It was also worth noting that the toxic effect of dimethylformamide was similar to that of [BMPyrr][Br], the least toxic ionic liquid in this study. In general, the toxicities of commonly used organic solvents were estimated to be two and four orders of magnitude less than those of ionic liquids.

4. Discussion

In order to explore the influence of the cationic compartment within ionic liquids, compounds with different cationic structures, but with Br as the same anion, were selected, such as [BMIM][Br], [BMPy][Br], [BMPyrr][Br], [TBA][Br] and [TBP][Br]. According to the data obtained, hormetic effects (i.e., stimulatory effects occurring in response to low levels of exposure to agents that are harmful at high levels of exposure) were observed to be related to incubation time as well as the cationic component. Considering [BMIM][Br], a high hormetic effect was found after incubation time of 96 h. Also, a slight hormetic effect was seen in the raw data after 48 and 72 h of incubation at below inhibitory concentrations (Fig. 2). A similar phenomenon was observed on IPC-81 leukemia and HeLa cells by Ranke et al. (2004) and Stepnowski et al. (2004), respectively. However, no hormetic effect was seen for [TBP][Br] and for [BMPy][Br] during the test period. In case of [BMPyrr][Br] and [TBA][Br], this kind of subtoxic stimulus was identical regardless of incubation time.

The toxicity of chemicals in the environment is closely related to the lipophilic coefficient (log *P*; the ratio of the concentration of a chemical in octanol and in water at equilibrium and at a specified temperature) used in many environmental studies. The log *P* values of the organic solvents were −0.77, 0.05 and −1.01 for methanol, 2-propanol, and dimethylformamide, respectively (Iredale et al., 1991; Hansen and Meyer, 1990). The octanol–water coefficients of the ionic liquids were difficult to obtain, with the exception of those for [BMIM][Br] (log *P* = −2.48) and [BMPy][Br] (log *P* = −2.4) (Ropel et al., 2005). From these limited data, the octanol–water coefficients of ionic liquids were lower than those of the reference organic solvents. Therefore, the octanol–water coefficients may not be a guideline in comparing the toxicities of ionic liquids with those of organic solvents.

Compared to traditional organic solvents, the ionic liquids were found to be more toxic. This can be explained by the loss of volatile organic solvents during the experiment period. Additionally, large headspace in the test vessels may cause a significant portion of the volatile compound to partition from the aqueous phase into the headspace until equilibrium is reached. In contrast to conventional solvents, the exposure to pure ionic liquids might differ substantially due to their negligible vapor pressures (Ranke et al., 2004). On injection of high concentration of toxicant into the reaction vessel, the optical density of the alga was observed to decrease, implying that the algal membrane was more quickly destroyed at higher toxicant concentrations. According to this phenomenon, the suggested toxicity mechanism of ionic liquids may be one of algal membrane disruption, with the action of ionic liquids being similar to that of pharmaceuticals, i.e., attack of the lipid structure (Escher et al., 2005).

5. Conclusions

Recently, ionic liquids have attracted a lot of interest as alternatives to organic solvents in several chemical processes, but the available toxicological and/or ecotoxicological data pertaining to their use are limited. Therefore, toxicities of typical ionic liquids were investigated toward an alga, *S. capricornutum*, since they are primary producers, either directly or indirectly, of the organic matter required by animals in fresh water food chains. The toxic effects were determined based on the OECD and EPA methods.

The toxicity of ionic liquids is dependent on the cation structure and incubation time. The toxicities of cyclic compounds, such as [BMIM] and [BMPy] containing [Br] as the anion, generally increase with increasing incubation time. However, in the case of quaternary salts, such as [TBA][Br] and [TBP][Br], the reverse was observed. The degree of toxicity is also related to the cation.

To compare the toxic effects of ionic liquids and organic solvents, the microalga was cultivated in the presence of various water miscible solvents; dimethylformamide, 2-propanol and methanol. The ionic liquids were generally found to be two-to-four orders of magnitude more toxic than the organic solvents. It is likely that the differences in
the toxicities of ionic liquids and organic solvents depend on the experimental organisms employed; therefore, toxicology studies, using various organisms, will be required. Any ecotoxicity and toxicity evaluations of ionic liquids should also take into account their overall bioavailability, which will be affected by their negligible vapor pressures of ionic liquids compared to conventional solvents.

References


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