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Microalgal photosynthetic activity measurement system for rapid toxicity assessment

Chul-Woong Cho · Thi Phuong Thuy Pham · You-Chul Jeon · Jiho Min · Ho Young Jung · Dae Sung Lee · Yeoung-Sang Yun

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Abstract A systematic biosensor is constructed for the estimation of toxic compounds based on photosynthetic activity measurement in *Selenastrum capricornutum* cells. The photosynthetic response was evaluated as a function of light intensity, cell concentration and initial dissolved oxygen. The inhibitory effect of some toxicants (1-butyl-3-methylimidazolium tetrafluoroborate, methanol) on dissolved oxygen production was also determined. In all cases, a toxic response was detected (i.e. a dose-related inhibition of photosynthetic activity was observed). For the present system, a time of only 2 h was needed to predict EC50 values as compared to 96 h for a conventional algal assay based on algal growth rate. Thus, the developed biosensor was proved to be useful as a rapid and simple test method in environmental toxicity assessment.

C.-W. Cho · T. P. T. Pham · J. Min · Y.-S. Yun
Department of Bioprocess Engineering, Chonbuk National University, Chonbuk 561-756, Republic of Korea

Y.-C. Jeon
Central Research Institute of HYOSUNG Group, Kyeounggi 431-080, Republic of Korea

J. Min · Y.-S. Yun (✉)
Division of Environmental and Chemical Engineering and Research Institute of Industrial Technology, Chonbuk National University, Chonbuk 561-756, Republic of Korea
e-mail: ysyun@chonbuk.ac.kr

H. Y. Jung · D. S. Lee
Department of Environmental Engineering, Kyungpook National University, Sankyuk-dong, Buk-gu, Daegu 702-701, Republic of Korea

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Introduction

Algae are unicellular to multi-cellular plants that occur in fresh water, marine water, and terrestrial environment. Because they are considered to be primary producers in the aquatic environment, their ecological role is crucial in providing energy that sustains invertebrates and fish. The action of toxic substances on algae is therefore significant not only for the organisms themselves, but also for other links in the food chain. Recently, algal assay has emerged as an important method to evaluate the toxicity of chemicals present in water since a decrease in algal density may affect the aquatic ecosystem directly by reducing their biodiversity and primary products (Li et al. 2005). In addition, algal assays are relatively simple, quick, and inexpensive in comparison with bioassays using other organisms (Latała et al. 2005). Conventional response endpoints of laboratory toxicity tests undertaken with phytoplankton include final yield (biomass or cell densities) (Ma and Chen 2005; Lin et al. 2005), growth rate (Chen and Lin 2006; Wells and Coombe 2006), chlorophyll contents (Jay 1996; Li et al. 2005), phosphate uptake (Kaneko et al. 2004) and photosynthetic activity (Pardos et al. 1998). Most of the aforementioned endpoints result from chronic exposure of usually 3–4 d; whereas photosynthetic activity can be estimated in short duration, usually 2–4 h (Van der Heever and Grobbelaar 1996; Hall et al. 1996). Consequently, photosynthetic activity has been a common parameter monitored in laboratory toxicity bioassays with cultured algae.

Table 1 Previous experimental approaches using algal photosynthetic activity to detect the effects of toxicants

Organism	Test substances	Modulation	Ref.
Chlorella vulgaris	Potassium cyanide Methyl parathion diuron (DCMU), Paraquat	The detection of toxic agents in sunlight-exposured primary-source drinking waters based on fluorescence induction	Rodriguez et al. (2002)
Chlorella vulgaris	Diuron, Simazine, Atrazine, Glyphosate, Alachlor	An algal biosensor using a fluorescence-based optical fiber for determination of herbicides	Naessens et al. (2000)
Chlorella vulgaris	Cadmium, Lead	The detection of heavy metals using an immobilized algal biosensor alkaline phosphatase activity	Durrieu and Tran-Minh (2002)
Chlorella vulgaris	Atrazine, DCMU Toluene, Benzene	Development of a compact and disposable device for rapid toxicity testing on the basis of amperometric monitoring of oxygen generated photosynthetically by microalga *C. vulgaris* entrapped in an alginate gel or a polyion complex and immobilized directly on the surface of a transparent indium tin oxide electrode	Shitanda et al. (2005)
Selenastrum capricornutum	Zinc, Lead Cadmium, Benzene	Detection of the toxic effects of both organic and metallic toxicants using a closed-system algal toxicity test based on measuring dissolved oxygen production and growth rate	Lin et al. (2005)
Selenastrum capricornutum	Chlorophenols	Evaluation of the toxicity of chlorophenols using a closed-system algal toxicity test based on measuring dissolved oxygen production and growth rate	Chen and Lin (2006)
Selenastrum capricornutum	Cadmium, Zinc	Development of a short-term (4 h) test based on uptake and assimilation of radio-labeled carbon in *S. capricornutum*	Pardos et al. (1998)
Selenastrum capricornutum	Copper	Determination of the response of phytoplankton to Cu in natural, soft lake waters of different DOC content, and to test the modifying effect of ultraviolet radiation on response to Cu using the kinetics of in vivo chlorophyll *a* fluorescence	West et al. (2003)
Chlamydomonas reinhardtii	Copper, Nickel, Zinc, Lead	Determination if photosynthesis based on estimation of oxygen evolution and motility can be used as sensitive physiological parameters in toxicological studies of green unicellular alga	Danilov and Ekelund (2001)
Chlamydomonas reinhardtii	DCMU, FCCP	The report on a biosensor system in which pH changes due to the uptake or production of CO_2 by *Chlamydomonas* cells	Schubnell et al. (1999)
Scenedesmus obliquus	Atrazine, Metribuzin, Diuron derivatives, Diuron, Paraquat, Tetrabuthylazine	Measurement of the F684/F735 fluorescence ratio with a conventional fluorometer as an easy, rapid and sensitive assessment of the presence and toxicity of herbicides in a freshwater alga	Eullaffroy and Vernet (2003)
Plesiastrea versipora	Copper	Examination of the effects of copper on physiological interactions between symbiotic algae and their host, particularly with regard to two host signaling compounds that control algal carbon metabolism	Grant et al. (2003)
Spirogyra distenta	DCMU	Measurement of chlorophyll fluorescence from young and mature chloroplasts with a microscopic imaging system for monitoring the environmental degradation of aquatic ecosystems	Endo and Omasa (2004)
Spirulina subsalsa	Copper, Mercury, Atrazine, Carbaryl	The monitoring of the evolution of photosynthetic O_2 and the detection of alterations due to toxic effects caused by environmental pollutants	Campanella et al. (2000)
Phytoplankton, Bacteria	Cadmium	Evaluation of the effect of dredged sediment disposal using photosynthesis measured by the ^{14}C method and phosphate uptake by ^{32}P-PO_4 and heterotrophy by uptake of ^{14}C labeled glucose	Nalewajko (1995)
Phytoplankton, Periphyton, Epipsammon	Parquat, Simazine	Comparison of the sensitivities of three marine microalgal communities to two herbicides using photosynthesis (incorporation of ^{14}C) as a test parameter	Bonilla et al. (1998)

During the last decade microalgal photosynthesis has been shown to be an effective means to estimate the toxicity of toxicants (Table 1). The used methods detected mainly fluorescence induction, phosphate uptake, ^{14}C-assimilation, and oxygen evolution etc. Bioassays pertaining to photosynthesis, however, depend on light intensity and initial algal cell density. In this regard, light is the most important factor affecting microalgal photosynthetic activity (Aiba 1982; Becker 1994). Generally, most microalgal biosystems are limited by light, which is easily absorbed and scattered by the microalgal cells (Yun and Park 2001). It is therefore necessary to understand and quantify the light dependence of microalgal activity in order to specifically design an efficient biosensor based on photosynthetic activity for toxicity determination of pollutants.

The main purpose of this research was to design systematically a rapid and simple biosensor assessment system which can be applied to estimate the toxic effects of chemicals to microalgal photosynthetic activity. Moreover, the paper studied the influence of various incident light intensities and different initial algal cell densities on volumetric and specific oxygen evolution rate during photosynthesis. In toxicity assays, 1-butyl-3-methylimidazolium tetrafluoroborate and methanol were selected as tested compounds. Methanol is one of the most commonly used organic solvents in industrial processes. Although other work in the literature has focused on the toxicity of this solvent toward microalga, according to European Centre for Ecotoxicology and Toxicology of Chemicals (1996), the experimental protocols applied in these studies are unsuitable for assessing the effects of volatile compounds including methanol. With respect to 1-butyl-3-methylimidazolium tetrafluoroborate, this is a non-volatile solvent. When discharged into the aqueous environment, it might cause serious effect to aquatic microorganism, especially to the algae, primary producers of organic matter required by animals in freshwater food chains. However, until now, the toxicity of this chemical on microalgal photosynthetic activity has not been investigated.

Materials and methods

Microalgal strain and cultivation

The freshwater microalga, *Selenastrum capricornutum* ATCC-22662, used in the experiments as test organism, was supplied by National Institute Environmental Research (Korea). *S. capricornutum* was cultured in nitrate-enriched BBM medium by adding 58.8 mM $NaNO_3$ so as to avoid nitrogen limitation in the high-density culture (Yun and Park 1997).

Preparation of microalgal suspensions for short-term testing

The green algal cells in the late exponential phase were centrifuged at 3,000 × *g* for 5 min at room temperature, followed by washing with the fresh medium. After repeating centrifugation and washing three times, the suspension was diluted in series to prepare different concentration (0.048, 0.095 and 0.182 g cell/l). Dry cell weight was measured by drying 5 ml of the suspension at 70°C in a drying oven for 24 h after being filtered through a pre-dried and pre-weighed 0.45 μm cellulose nitrate membrane filter (Whatman, Ann Arbor, MI). In order to easily measure the dry weight, an equation, $D_w = 0.1329 \times OD_{438}$, g cell/l, was determined according to the close correlation between dry cell weight and optical density (Stain 1973).

Chemicals

The toxicants tested included an ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM] [BF_4]) supplied by C-TRI, Korea and an organic solvent (methanol) which was purchased from SAMCHUN Pure Chemical Company, Korea.

Design of the microalgal photosynthetic activity measurement system

A schematic diagram of the toxicity estimation system used in this research is shown in Fig. 1. The system consists of a water bath for constant temperature maintenance, a peristaltic pump, an illuminator (A3200, Dolan-Jenner, USA), a quantum sensor (LI-190A, Licor, USA), a light meter (LI-250, Licor, USA), a dissolved oxygen meter and a personal computer for data acquisition using Hach software. As shown in Fig. 1, the reaction vessel was a double-jacket cylinder made of Pyrex glass. Magnetic stirring with a small bar (0.5 cm in length) put inside the vessel made microalgal suspensions homogeneous. The working volume of the reaction cell was 3.58 ml and the light path length was 1.8 cm. Since microalgal photosynthesis is known to be temperature sensitive, cooling water at 25 ± 2°C was continuously circulated through the double-jacket. The oxygen probe (Ingold) was placed in the circular top of the reaction cell and used for measuring the concentration of dissolved oxygen generated by algal photosynthesis. The light beam was supplied from a duck-neck-like optical fiber connection to one straight side of the reaction cell. The convex lens was equipped in the head of optical fiber connection. By adjusting the position of the lens, it was possible to make the light beam parallel to the

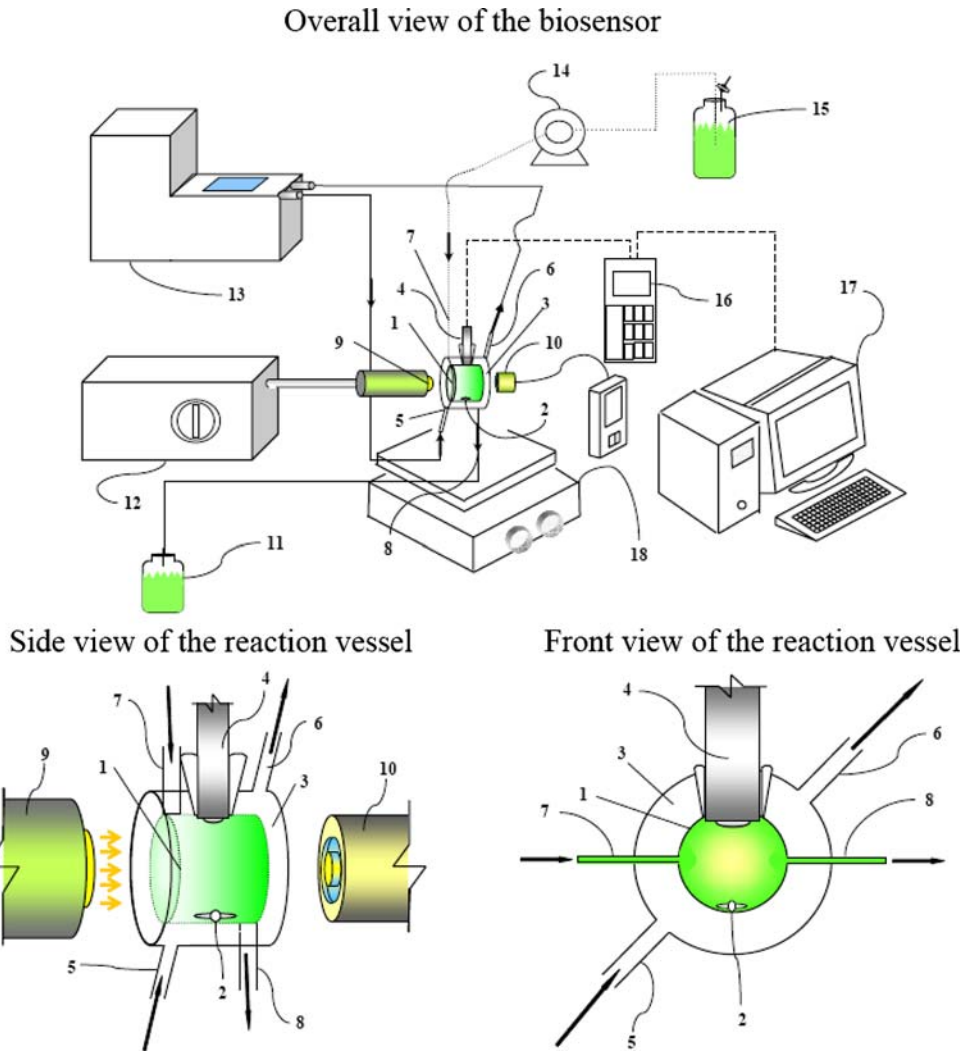

Fig. 1 Schematic diagram of the photosynthetic activity measurement system. 1, reaction cell; 2, magnetic bar; 3, cooling water jacket; 4, dissolved oxygen electrode; 5, inlet of cooling water; 6, outlet of cooling water; 7, inlet of sample; 8, outlet of sample; 9, convex lens; 10, quantum sensor; 11,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

axial direction without dispersion. It was confirmed that the oxygen probe, inlet/outlet gates, and stirring bar had negligible effects on light penetration. The quantum sensor connected with the light meter was positioned opposite to the illumination side in order to measure the transmitted light. The light source was supplied by a 150 W quartz halogen lamp equipped inside the optical fiber illuminator. The incident photon flux density was controlled by the scale of illuminator aperture. The light absorption by Pyrex glass, cooling water, and pure water was negligible compared to absorption by microalgal cells.

For the toxicity tests, mixture of previously prepared algal broth and toxicant was subsequently filled in the reaction vessel after being exposed for 10 min and stripped by a gas mixture at a rate of 75 ± 10 ml/min to reduce the initial dissolved oxygen level to 2–3 mg/l. The gas mixture used in this experiment contained 99% N_2 and 1% CO_2 so as to supply an extra carbon source for algal photosynthesis. The oxygen produced by algal organisms was recorded every minute during 10 min period by a computer directly connected to the system. It took almost 2 h to carry out the whole experiment in order to obtain complete concentration-response curves. A similar procedure was adapted for control samples in which deionized water instead of toxicants was used. In each experiment, the volumetric oxygen evolution rate was measured from the slope of linearity between dissolved oxygen (DO) and time. The specific oxygen evolution rate was calculated by dividing the volumetric oxygen evolution rate with the cell concentration (Jeon et al. 2005).

Photosynthesis- irradiance model and parameter estimation

In order to quantify the photosynthetic activity, a general photosynthesis-irradiance model (Yun and Park 2003) can be applied.

$$A_X = \frac{A_m I_O}{K + I_O} - R_X \quad (1)$$

where A_X is the specific photosynthetic activity (g O_2/g cell min), and A_m, K and R_X are maximum specific activity (g O_2/g cell min), half constant ($\mu Em^{-2}\ s^{-1}$) and specific respiration rate (g O_2/g cell min), respectively. The symbol I_O represents the incident light intensity ($\mu Em^{-2}\ s^{-1}$). The respiration rate (R_X) was determined by measuring the specific oxygen consumption rate of algal suspensions in the dark. The maximum photosynthetic activity (A_m) and the half constant (K) were estimated using the Marquardt–Levenberg nonlinear regression algorithm (Marquardt 1963).

Cell growth effect test

The conventional cell growth effect test was done according to methods recommended in the U.S. EPA (1996) and the OECD guidelines (2002). In this test, the organisms were exposed to various concentrations of toxicants for 96 h and growth of cultures was measured at 438 nm wavelength using a UV-spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan). The percentage inhibition of the cell growth I_a was determined from the equation:

$$I_a(\%) = \frac{A_c - A_t}{A_c} \times 100 \quad (2)$$

where A_c is the mean value of area under the curve on the control group and A_t is the mean value for area under the curve in the treatment group.

Effect data modeling

A feasible concentration-response curve was fitted to the multinomial data with the nonlinear least-squares method using the logistic model for the relationship of cell viability and inhibition to the decadic logarithm of the examined concentrations ($\log_{10}$ of the actual concentration), which can be written as follows:

$$P = \frac{1}{1 + (c/c^a)^b} \quad (3)$$

where c is the concentration of the substance to which the cells are exposed, P is the physiological response, normalized with positive and negative controls to the interval from 0 (c = 0) to 100 (negative control). c^a represents the $\log_{10}$EC50, and b is the slope of the function on a logit-log-scale. Calculations were carried out using the Sigma-plot 8.02. In some cases, algal growth increased up to a particular concentration and reduced thereafter. Therefore, the concentration-response curves were fitted with the linear-logistic model proposed by Brain and Cousens (1989) and reparameterized as indicated by van Ewijk and Hoekstra (1993) for the case of a subtoxic stimulus.

$$P = \frac{1 + fx}{1 + (2 \times fx_o + 1)(x/x_o)^{b'}} \quad (4)$$

where b' is a parameter without intuitive interpretation and f is the parameter describing hormesis. If $f > 0$, then the curve shows an increase for low concentrations.

Results and discussion

Effect of light intensity

In general, the algal photosynthetic activity is enhanced as the light intensity increases up to the point where photosynthetic apparatus becomes saturated at higher photon flux densities. In order to estimate the effect of light intensity, algal photosynthetic activity was measured at various light incidents in the presence and absence of toxicant ([BMIM] [BF_4]).

As can be gleaned from Figs. 2 and 3, more oxygen was generated when light intensity increased from 0 to 1,200 $\mu Em^{-2}\ s^{-1}$. However, in condition without light, dissolved oxygen concentration was slightly decreased due to algal respiration. In comparison between the oxygen production by algae in the presence and absence of toxicant, it was observed that the inhibition of algal photosynthetic activity was significantly different at different light intensities. The inhibition percentages of respiration and algal photosynthesis by [BMIM] [BF_4] were 55, 6.5, 31.7 and 25% at light intensities of 0, 100, 500 and 1,200 $\mu Em^{-2}\ s^{-1}$, respectively. This variability in the toxicity of [BMIM] [BF_4] demonstrates that the results of an algal photosynthesis inhibition assay can differ considerably under different light conditions. This of course makes the comparability more difficult and should be avoided by providing stringent rationales for a light regime during the test. Keeping this point in view, the specific oxygen evolution rate was calculated and plotted against the light intensities in order to obtain an optimum illumination power for microalgal photosynthesis. Fig. 4 reveals that the generated oxygen initially increased with increase in light intensity and reached a plateau at higher light intensities. As light intensities ranged between 1,000 and 1,200 $\mu Em^{-2}\ s^{-1}$, specific oxygen evolution rates were observed to be almost constant. Therefore, the light intensity between 1,000 $\mu Em^{-2}\ s^{-1}$ and 1,200 $\mu Em^{-2}\ s^{-1}$

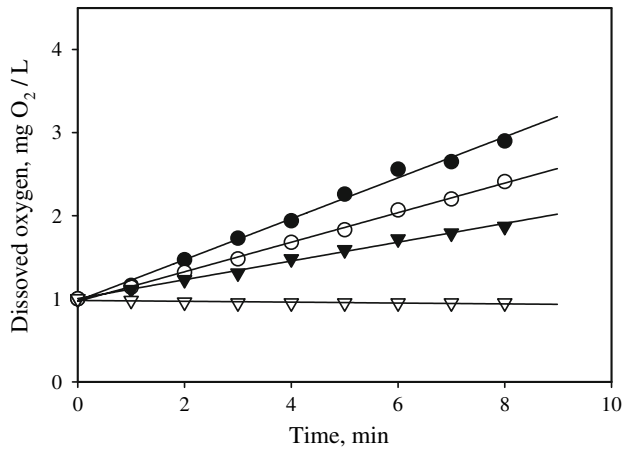

Fig. 2 Oxygen production by alga in different light intensities in the presence of toxicant. Volumetric oxygen evolution rates were evaluated using data in a linear range. The algal concentration was 0.095 g cell/l and the concentration of stimulated toxicant ([BMIM] [BF_4]) was 101.5 μM in all cases. The intensities of stimulated daylight were (▽) 0 $\mu Em^{-2}\ s^{-1}$, (▼) 100 $\mu Em^{-2}\ s^{-1}$, (○) 500 $\mu Em^{-2}\ s^{-1}$ and (●) 1,200 $\mu Em^{-2}\ s^{-1}$. Estimated volumetric activities were (▽) −0.0043 ± 0.0014, (▼) 0.1152 ± 0.0027, (○) 0.1778 ± 0.0027 and (●) 0.2498 ± 0.0040 mg O_2/l min

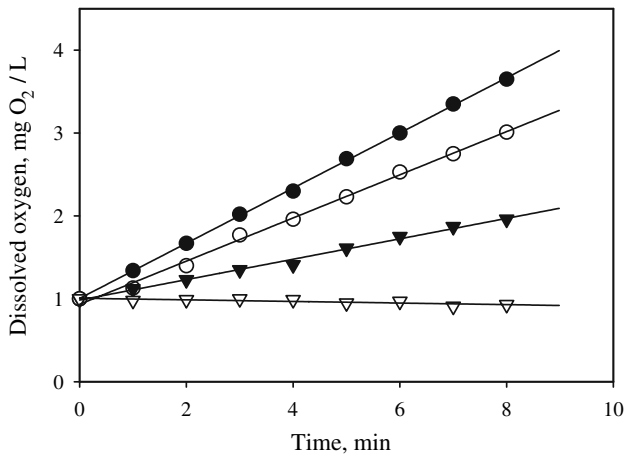

Fig. 3 Oxygen production by alga in different light intensities in the absence of toxicant. Volumetric oxygen evolution rates were evaluated using data in a linear range. The algal concentration was 0.095 g cell/l in all cases. The intensities of stimulated daylight were (▽) 0 $\mu Em^{-2}\ s^{-1}$, (▼) 100 $\mu Em^{-2}\ s^{-1}$, (○) 500 $\mu Em^{-2}\ s^{-1}$ and (●) 1,200 $\mu Em^{-2}\ s^{-1}$. Estimated volumetric activities were (▽) −0.0097 ± 0.0030, (▼) 0.1232 ± 0.0039, (○) 0.2603 ± 0.0075, (●) 0.3327 ± 0.0025 mg O_2/l min

was considered to be suitable for estimating the photosynthesis of *S. capricornutum* in the present system.

Effect of cell concentration

As far as the effect of cell concentration was concerned, algal photosynthesis was examined at different concentrations of algal culture with light intensity fixed at 1,000 $\mu Em^{-2}\ s^{-1}$. The results implied that algal cell

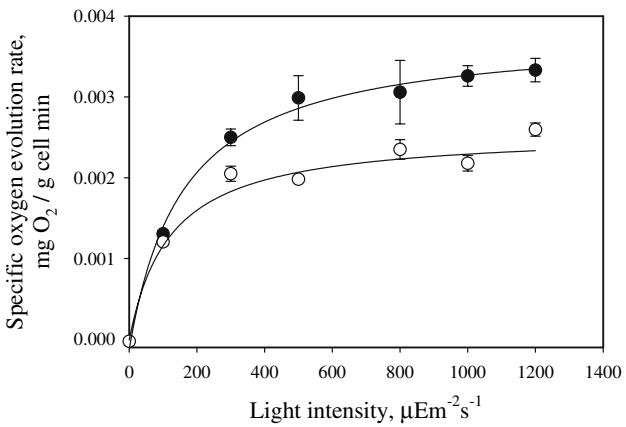

Fig. 4 Specific oxygen evolution rate as a function of incident photon flux density in the presence (○) and absence (●) of toxicant. Data points and error bars were average values and standard deviation of two or three replicated experimental results. Solid lines represent the calculated results from the photosynthesis-irradiance model (Eq. 1). The algal concentration was 0.095 g cell/l in all cases and the concentration of toxicant ([BMIM] [BF_4]) applied was 101.5 μM

concentration notably affected the oxygen evolution rate with the increasing of oxygen generated directly proportional to algal dry cell weight (Fig. 5). In the presence of [BMIM] [BF_4], the oxygen evolution rate was inhibited and the levels of inhibitory effect were different at different algal cell densities. To be precise, the inhibition percentages of photosynthetic activity by [BMIM] [BF_4] were 46, 34 and 34% at cell concentrations of 0.048 g cell/l, 0.095 g cell/l and 0.182 g cell/l, respectively. From these observations, it was clear that at low cell concentrations, the inhibition percentage was comparatively higher. This may be due to the relationship between toxicity and photoinhibition (Göksan et al. 2003), which is more likely to occur at low concentration because the light intensity to which microalgae are actually exposed is not reduced by mutual shading (Evers 1991; Contreras-Flores et al. 2003; Richmond 2000). Although experiments were not conducted at higher cell concentrations beyond 0.182 g cell/l, it was supposed that mutual shading might be occurred at the extreme cell density (Grobbelaar and Soeder 1985). Thus, 0.095 g cell/l of algal culture was optimized through the following experiments.

Effect of initial dissolved oxygen concentration

In order to investigate the influence of initial dissolved oxygen concentration on algal photosynthesis, experiments were conducted at different initial DO levels ranged from 0.78 to 6.68 mg O_2/l. These initial concentrations were selected randomly by controlling the supplied amount of gas mixture containing N_2 and CO_2. However, these gases were not applied in case of the highest DO concentration of

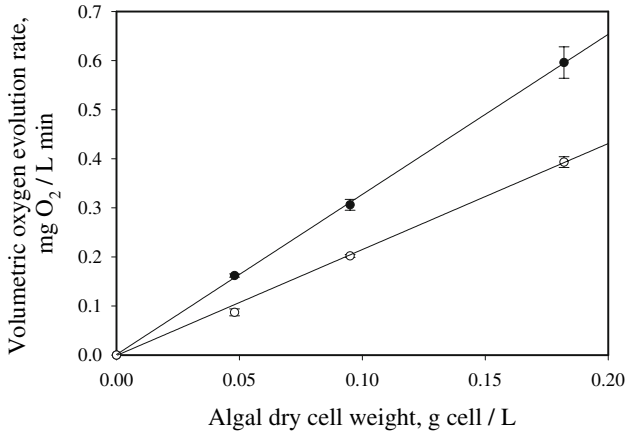

Fig. 5 Oxygen production by alga in different cell concentrations in the presence (○) and absence (●) of toxicant. Volumetric oxygen evolution rates were evaluated using data in a linear range (closed symbol). The algal cell densities were 0 g cell/l, 0.048 g cell/l, 0.095 g cell/l and 0.182 g cell/l. The intensity of stimulated daylight was constantly fixed at 1,000 $\mu Em^{-2}\ s^{-1}$ and the concentration of toxicant ([BMIM] [BF_4]) applied was 101.5 μM

6.68 mg O_2/l. Based on the preliminary studies, the light intensity, [BMIM] [BF_4] concentration and cell concentration were fixed at 1,000 $\mu Em^{-2}\ s^{-1}$, 101.5 μM and 0.095 g cell/l, respectively. As being displayed in Fig. 6, volumetric oxygen evaluation rate was observed as 0.2045 ± 0.0063, 0.1987 ± 0.0058, 0.2027 ± 0.0177 and 0.1315 ± 0.0169 mg O_2/l min for initial DO levels of 0.78, 3.37, 5.26 and 6.68 mg O_2/l, respectively. It was worth noting that initial DO concentration caused no effect to the algal photosynthetic activity except the case of the highest DO value, in which CO_2 gas was not utilized. From this

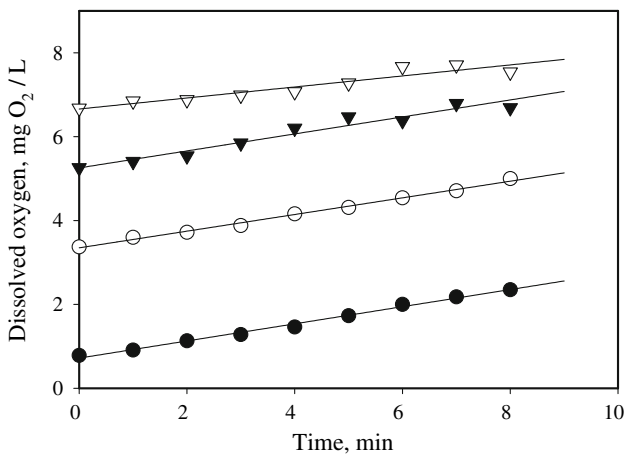

Fig. 6 Oxygen production by alga in different initial dissolved oxygen concentrations in the presence of toxicant. Volumetric oxygen evolution rates were evaluated using data in a linear range. The initial dissolved oxygen concentrations were (●) 0.78 mg O_2/l, (○) 3.37 mg O_2/l, (▼) 5.26 mg O_2/l and (▽) 6.68 mg O_2/l. The intensity of stimulated daylight was 1,000 $\mu Em^{-2}\ s^{-1}$ and the concentration of toxicant ([BMIM] [BF_4]) applied was 101.5 μM

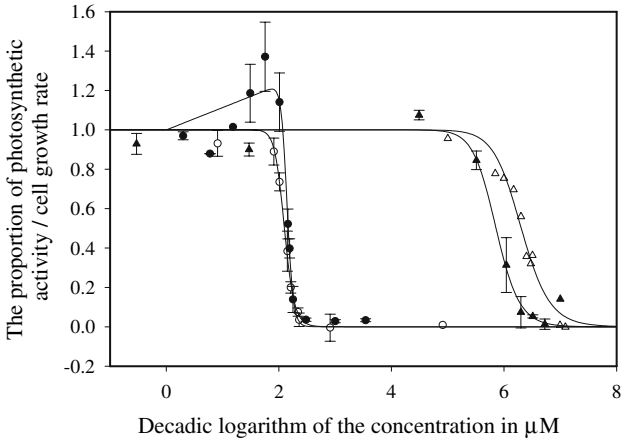

Fig. 7 Dose-response curves of algal toxicity test with respect to [BMIM] [BF_4] (○) and methanol (Δ) based on photosynthetic activity measurement whereas [BMIM] [BF_4] (●) and methanol (▲) based on growth rate

observation, it was obvious that CO_2 play an important role for algal photosynthetic activity in the studied system.

Toxicity testing

According to the obtained parameters, a short-term algal test based on photosynthetic activity was employed for assessing the toxic effects of imidazolium salt and methanol as representatives of ionic liquids and organic solvents, respectively. Figure 7 illustrates the dose-response curves of *S. capricornutum* for [BMIM] [BF_4] and methanol in both cases of short-term and traditional algal assays. The results coincided well for imidazolium salt with the EC50 values of 0.115 mM and 0.126 mM for photosynthetic activity measurement and cell growth experiments, respectively. For methanol, the corresponding results were 2,089 mM and 759 mM, which indicated that the methanol was 2.75 times more toxic towards algal growth rate than photosynthetic activity. This can be explained by the longer exposure time in algal growth assay compared to short-term test (96 h and 2 h, respectively), thus resulted in more injury to the algal cells.

Conclusions

In this paper, a biosensor was specifically designed to assess the toxicity of pollutants by measuring the oxygen evolution during microalgal photosynthesis. In contrast to most biosensors previously reported, the present sensor was designed for rapid detection. In order to evaluate the suitability of the system, the photosynthetic activity was measured as a function of light intensity, cell concentration and initial dissolved oxygen. According to the results, the

algal photosynthetic activity was dependent only on light intensity and cell concentration whilst initial dissolved oxygen caused effects only in condition where CO_2 was not supplied to the test samples. At constant light intensity (1,000 $\mu Em^{-2}\ s^{-1}$) and algal cell density (0.095 g cell/l), the device was investigated on the basis of toxicity with [BMIM] [BF_4] and methanol as model toxicants. It was obviously observed that toxicants affected the oxygen evolution rate related to microalgal photosynthetic response. A conventional algal test based on growth rate was performed in cases of [BMIM] [BF_4] and methanol in order to compare the inhibition rates. Although the EC50s coincided well only for [BMIM] [BF_4], the algal toxicity test based on this system can be useful for evaluating the toxicity of chemicals more rapidly than a conventional test. Algal photosynthesis is one of the major physiological phenomena that contribute to algal growth, which might affect the structure and functioning of the whole aquatic ecosystems. Therefore, the present system, which deals with the photosynthetic response of algae, can be served as a useful tool in preliminary screening toxicity methods. It should be recognized that simple acute ecotoxicity measurements do not completely characterize the full impact of solvents released to the environment but are only part of the environmental impact assessment. In general, this method is applicable to the toxicity assessment of not only organic solvents, ionic liquids but also heavy metals, pesticides as well as other pollutants. Nonetheless, it is premature to make this claim based on the data obtained in the present study. Whether this system can be expanded as above or not remains to be further tested. If the system is valid for other toxicants, this rapid test can be used as an alternative for real time bio-monitoring, where immediate toxicity evaluation is required.

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