We are IntechOpen, the world's leading publisher of Open Access books Built by scientists, for scientists

6,900
Open access books available

185,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities



WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Numbers displayed above are based on latest data collected.
For more information visit www.intechopen.com



11

Algal Biosensor-Based Measurement System for Rapid Toxicity Detection

Thi Phuong Thuy Pham, Chul-Woong Cho and Yeoung-Sang Yun
Chonbuk National University
Republic of Korea

1. Introduction

Microalgae are now widely used as relevant biological indicators in the field of environmental impact studies. Owing to their ubiquity, short life cycles, easiness of culture and high sensitivity to a number of pollutants, these organisms are frequently utilized in ecotoxicological screening of contaminated freshwater (Lewis, 1995). As primary producers, either directly or indirectly, of organic matter required by small consumers in aquatic food webs, microalgae serve an important role in nutrient recycling and equilibrium of aquatic ecosystems (Raja et al., 2008). The most important common biochemical attribute that unites algae is their ability to split water, producing molecular oxygen during photosynthesis and concomitantly assimilating carbon dioxide. Furthermore, the rest of biotic communities are strictly dependent upon the photosynthetic activity of these organisms. Perturbations of microalgal photosynthesis might lead to alterations of their primary production, which in turn causes severe repercussions on the aquatic biota (Morris, 1981).

Nowadays, the development of convenient methods or parameters for assessment of the presence of pollutants and their toxicity has become a major goal in environmental monitoring research. Growth rate, fluorescence induction and photosynthetic activity (through oxygen evolution or incorporation of ^{14}C) are the most popular endpoints studied (Jensen, 1984; Puiseux-Dao, 1989). Particularly, photosynthesis inhibition is a reliable indicator that rapidly demonstrates the toxic effect of hazardous contaminants (Overnell, 1976). Table 1 displays a brief review on the literature concerning research on application of microalgal photosynthesis to detect the effects of pollutants. The advantage of photosynthesis inhibition assay is the short duration of the test, usually 2–4 h compared to 48–96 h of chronic exposure (Hall et al., 1996). Bioassays involving photosynthesis process; however, are contingent upon light intensity and initial algal cell concentration. In this regard, light is the most critical element influencing phytoplanktonic photosynthetic activity (Aiba, 1982). In most cases, microalgae-based biosystems are restricted by light, which is easily absorbed and scattered by the microalgal cells (Yun & Park, 2001). It is therefore crucial to figure out and monitor the light dependence of microalgal activity for the sake of designing an efficient algal biosensor-based measurement system for toxicity assessment. Other than irradiance, algal cell concentration and initial dissolved oxygen level applied in the test are also important and should be evaluated to give a better performance of the proposed algal biosensor.

Organism	Test substances	Modulation	Ref.
Chlorella vulgaris	Potassium cyanide, methyl parathion, diuron (DCMU), paraquat	The detection of toxic agents in sunlight-exposed primary-source drinking waters based on fluorescence induction	Rodriguez et al. (2002)
Chlorella vulgaris	DCMU, simazine, atrazine, alachlor, glyphosate	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 optical algal biosensor based on alkaline phosphatase activity	Durrieu & Tran-Minh (2002)
Chlorella vulgaris	DCMU, atrazine, toluene, benzene	Development of a compact and disposable device for rapid toxicity testing on the basis of amperometric monitoring of O_2 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, phenol, chlorophenol, toluene, chlorobenzen,	Detection of the toxic effects of both organic and metallic toxicants using a closed-system algal toxicity test based on measuring dissolved oxygen production, algal growth rate and cell density	Lin et al. (2005)
Selenastrum capricornutum	Phenol. chlorophenol	Evaluation of the toxicity of chlorophenols using a closed-system algal toxicity test based on measuring dissolved oxygen production and growth rate	Chen & Lin (2006)
Selenastrum capricornutum	Zinc, cadmium	Development of a short-term (4 h) test based on uptake and assimilation of radio-labelled carbon in *S. capricornutum*	Pardos et al. (1998)
Selenastrum capricornutum	Copper	Determination of the response of phytoplankton to Cu in natural, soft lake waters to different dissolved organic carbon 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 & Ekelund (2001)
Chlamydomonas reinhardtii	DCMU	The report on a biosensor system in which pH changes caused by the uptake or production of CO_2 by *Chlamydomonas* cells	Schnubnell et al. (1999)
Scenedesmus obliquus	Atrazine, metribuzin, diuron, paraquat, terbuthylazine	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 & 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 signalling 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 & 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 labelled glucose	Nalewajko (1995)
Phytoplankton, periphyton, epipsammon	Paraquat, simazine	Comparison of sensitivities of three marine microalgal communities to two herbicides using photosynthesis (incorporation of ^{14}C) as a test parameter	Bonilla et al. (1998)

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

2. Objective of the study

The inhibition of microalgal photosynthetic activity induced by different contaminants has been extensively investigated in the literature. Chen & Lin (2006) reported the investigation on hazardous impact of volatile organic compounds (VOCs) using an air-tight algal toxicity assay. Nonetheless, there has been no discussion on the effect of irradiance, although the tested BOD bottles could be influenced by shading effect at high algal densities. Also, the duration of the bioassay was not very short (i.e., 48 h) in their study. With regard to algal biosensor, a device for the monitoring of water toxicity in estuarine environments was reported in the work of Campanella et al. (2000). The developed biosensor provides a new approach to the research on harmful effects of heavy metals, herbicides and insecticides; however, no information on toxicity of volatile organic solvents was addressed using this system. Additionally, a biosensor with an oxygen electrode containing *Chlorella* cells immobilized on the membrane was well established to detect VOCs in the form of aerosols (Naessens & Tran-Minh, 1999). Nevertheless, one major drawback is that a controlled atmosphere chamber is required for the operation of this biosensor. Podola et al. (2004) described a non-selective sensor chips for the detection and identification of VOCs using different algal strains. Perhaps the disadvantage of the proposed multiple-strain biochip system is related to the complicate and expensive equipment, which might be regarded as limitations for practical utilization. Consequently, there is a need to design a simple and cost-effective indicator system that supports rapid toxicity detection of volatile and/or hazardous substances.

The aim of the present study is to design, construct and validate a new algal biosensor-based measurement system that provides a rapid toxicity determination of pollutants. The apparatus allows the monitoring of photosynthetic efficiency of the green alga *Selenastrum capricornutum* cells in the absence and presence of toxic agents by recording the oxygen produced. The new point of the work is that the biosensor was air-tight, with no headspace, thus prevents volatile organic toxicants from escaping into the environment as well as partitioning from the aqueous phase into the headspace until equilibrium was reached. In this aspect, the designed measurement system supports toxicity screening of volatile organic substances.

In this chapter, six common organic solvents including methanol, ethanol, isopropanol, acetone, acetonitrile, dimethylformamide and one ionic liquid (i.e., 1-butyl-3-methylimidazolium tetrafluoroborate, [BMIM] [BF_4]), a representative of non-volatile pollutants, were selected to check the system performance. The response of the proposed algal biosystem was studied in terms of light intensity, cell density and initial dissolved oxygen level. It was concluded that only 2 h was required to predict EC_{50} values (concentrations which result in a 50% reduction of the exposed organisms relative to controls) as compared to 96 h in a conventional algal assay based on algal growth rate.

3. Experimental Methods and Procedures

3.1 Microalgal strain and cultivation

The freshwater green alga *Selenastrum capricornutum* ATCC-22662 was used as the test organism and was obtained from the National Institute Environment Research (Incheon, Korea). Cells of *S. capricornutum* routinely have been propagated in a 250 mL Erlenmeyer flask containing 200 mL of Bold's Basal medium (Bold, 1950), which was nitrate-enriched by adding 58.8 mM $NaNO_3$ to avoid nitrogen limitation in a high-density culture (Yun & Park,

1997). Culture flask was shaken continuously at 170 rpm on a rotary shaking apparatus with air bubbling (1 vvm) without a sparger. Continuous illumination was provided at an average of 30 ± 5 $\mu Em^{-2}s^{-1}$ by warm-white fluorescent tubes (Korea General Electric, Yongin, Korea). The alga was subcultured every week with fresh medium (200 mL) and 10 mL of the cultured alga in order to keep algal cells in linear growth with doubling time of approximately 1 day at a controlled temperature of 25 ± 2°C.

3.2 Test reagents

The chemicals employed in the present study included an ionic liquid 1-butyl-3-methylimidazolium tetrafluoroborate ([BMIM] $[BF_4]$) and six common organic solvents (e.g. methanol, ethanol, isopropanol, acetone, acetonitrile and dimethylformamide). The ionic liquid was obtained at 98% of purity from C-Tri Company (Korea) whereas organic solvents (with purity > 99.5% for all compounds) were purchased from Samchun Pure Chemical Company (Korea).

3.3 Design of the algal biosensor-based measurement system and operating procedure

The system was constructed with a reaction cell, which was a double-jacket cylinder made of Pyrex® glass, an illuminator (A3200, Donan-Jenner, Boxborough, MA, USA), a quantum sensor (LI-190A, Licor, Lincoln, NE, USA), a light meter (LI-250, Licor), a dissolved oxygen meter (Hach, Loveland, CO, USA) and a computer for data acquisition using Hach software (Fig. 1). During experiments, microalgal suspension along with toxicants was injected into the reaction vessel with working volume of 3.58 mL and light path length of 1.8 cm. This mixture was made homogeneous by magnetic stirring with a small bar (0.5 cm in length). Opposite to the reaction vessel, a light beam was provided from a duck neck-like optical fiber connection to facilitate the algal photosynthesis. A convex lens was located on the head of optical fiber connection and was oriented to make the light beam parallel to the axial direction without dispersion. It was confirmed that the oxygen probe, inlet/outlet gates and stirring bar had minor effects on light penetration. A 150-W quartz halogen lamp (EKE, Tokyo, Japan) as a light source was equipped inside the optical fiber illuminator. The irradiance was controlled *via* the scale of illuminator aperture. The light absorption by Pyrex® glass, thermostating water and distilled water was negligible compared to absorption by microalgal cells. The quantum sensor connected to a light meter was positioned opposite to the illumination side in order to measure the transmitted light. Since algal photosynthesis is known to be temperature sensitive, cooling water of 25 ± 2°C from a water bath was circulated continuously through the double-jacket of the reaction cell. The oxygen probe was placed in the circular top of the reaction cell and used for measuring the concentration of dissolved oxygen generated by the algal photosynthesis.

Prior to the test, cell suspension was prepared by centrifuging algal cells in the late exponential phase at 3,000 × *g* for 5 min at room temperature and resuspending them in the fresh medium to yield different cell densities (0.048, 0.095 and 0.182 g cell/L). The estimation of cell densities based on algal dry cell weight was done by passing 5 mL of each suspension through a pre-dried and pre-weighted 0.45 μm cellulose nitrate membrane filter (Whatman, Ann Arbor, MI, USA), then drying in an oven at 70°C for 24 h. A correlation between algal dry cell weight versus optical density, DCW (g cell/ mL) = 0.139 × OD_{438}, was

established to facilitate the measurement of cell density. Mixture of the earlier prepared algal broth and toxicant then was loaded to the reaction vessel after being exposed for 10 min and passed through a gas mixture at a rate of 75 ± 10 mL/min for 10 min to control the initial dissolved oxygen level. The gas combination used in this experiment included 99% N_2 and 1% CO_2 as an extra carbon source for algal growth. The photosynthetic oxygen released by algal cells was recorded every minute throughout a 10-min illumination period by the personal computer directly linked to the system. It took almost 2 h to conduct the entire experiment in order to obtain complete dose-response curves. A similar procedure was applied for controls in which deionized water rather than toxicants was used. In each experiment, the volumetric oxygen evolution rate was obtained from the slope of linearity between dissolved oxygen and time. The specific oxygen evolution rate was achieved by dividing the volumetric oxygen evolution rate by the cell concentration (Jeon et al., 2005).

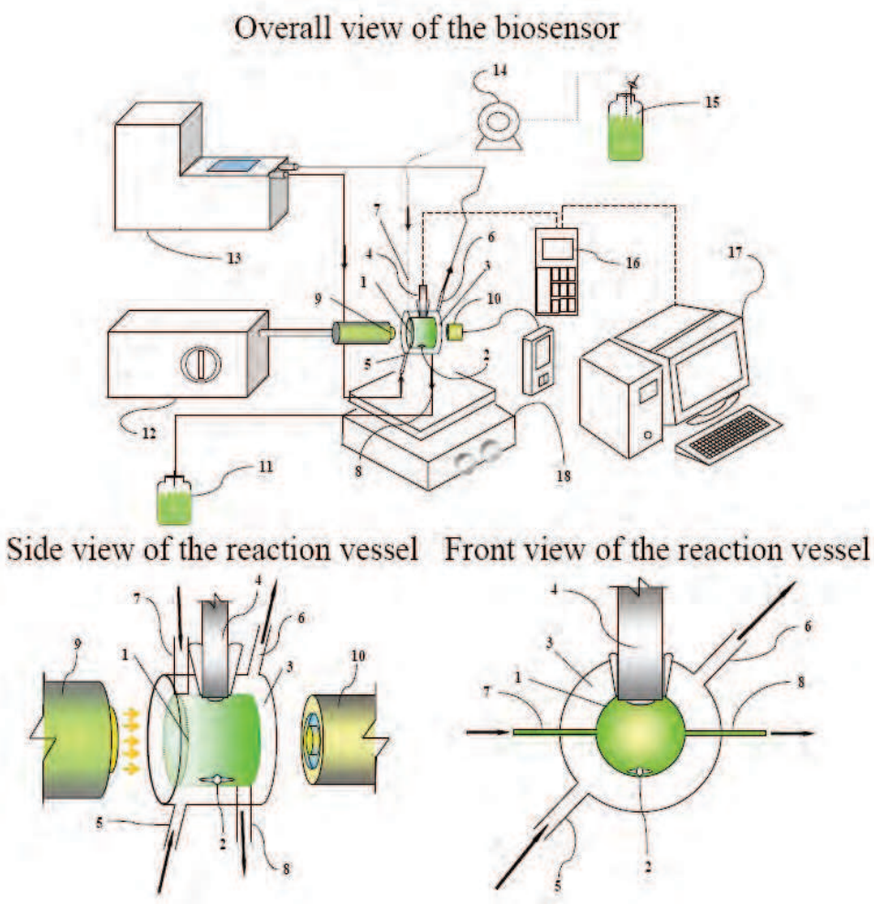


Fig. 1. Schematic diagram of the photosynthetic activity measurement system. *1* reaction cell, *2* magnetic bar, *3* cooling water jacket, *4* dissolved oxygen electrode, *5* inlet of cooling water, *6* outlet of cooling water, *7* inlet of sample, *8* outlet of sample, *9* convex lens, *10* quantum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

3.4 Photosynthesis-irradiance model and parameter estimation

For estimation of algal photosynthetic activity, a general photosynthesis-irradiance model (Yun & Park, 2003) can be applied

$$A_X = \frac{A_m I_O}{K + I_O} - R_X \tag{1}$$

where A_X stands for the specific photosynthetic activity (g O_2/g cell min), A_m, K and R_X denote maximum specific activity (g O_2/g cell min), half constant (μEm^{-2}s^{-1}) and specific respiration rate (g O_2/g cell min), respectively and I_O corresponds to the incident light intensity (μEm^{-2}s^{-1}). The respiration rate (R_X) was obtained by measuring the specific oxygen consumption rate of algal broth in the dark. The maximum photosynthetic activity (A_m) and the half constant (K) were calculated based on the nonlinear regression with Marquardt-Levenberg algorithm (Marquardt, 1963).

3.5 Cell growth effect test

The conventional algal chronic toxicity assay was done according to the procedures set out in the U.S. Environmental Protection Agency (1996) and Organization for Economic Cooperation and Development (2002) guidelines. In this experiment, the algal cells were exposed to different concentrations of toxicants for 96 h and growth of cultures relative to optical density of algal suspension was determined at wavelength of 438 nm *via* a spectrophotometer (UV mini-1240, Shimadzu, Kyoto, Japan). The growth rate inhibition (I) was calculated from the below equation.

$$I(\%) = \frac{A_c - A_t}{A_c} \times 100 \tag{2}$$

where A_c and A_t indicate the mean value of area under the curve of the control and treatment groups, respectively.

3.6 Effect data modeling

The dose-response curves, where feasible, were fitted to the multinomial data with the nonlinear least-squares method adopting for the logistic model to determine the relationship of cell viability and inhibition to the decadic logarithm of the examined dosages, which can be written as:

$$P = \frac{1}{1 + (x / x_0)^b} \tag{3}$$

where x is the substrate concentration to which the cells are exposed, P represents the physiological response, normalized with negative controls to the interval from 1 (x = 0) to 0 (negative control), x_0 indicates the EC_{50} value whereas b stands for the slope of the function on a logit-log-scale. All calculations were performed using Sigmaplot® 10.0 (SPSS, Chicago, IL, USA). In particular cases, algal growth rate increased at low concentrations of toxicants instead of the expected decrease in response that was observed at higher doses. Therefore, the concentration-response curves were fitted with the linear-logistic model proposed by

Brain & Cousens (1989) and modified by van Ewijk & Hoekstra (1993) for the case of a subtoxic stimulus.

$$P = \frac{1 + fx}{1 + (2 \times fx_0 + 1)(x / x_0)^{b'}} \tag{4}$$

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

4. Results and Discussion

4.1 Effect of light intensity

It is well-known that physiological response to changes in light intensity is an important factor determining alteration in photosynthetic activity of microalgae in nature. In general, the photosynthetic performance of phytoplankton is enhanced as the light increase up to the point where photosynthetic apparatus comes to be saturated at higher photon flux densities. In the present study, various light incidents were adjusted to estimate the influence of light intensity on microalgal photosynthetic process in the presence and absence of a representative pollutant ([BMIM] [BF_4]).

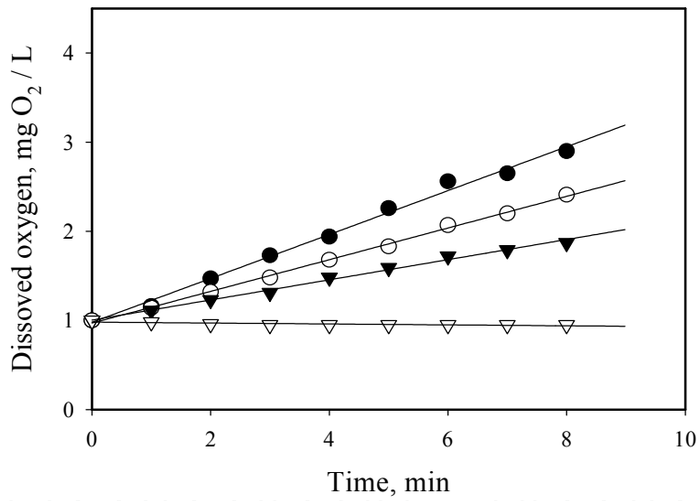


Fig. 2. Oxygen production by alga in different light intensities in the presence of toxicant. Volumetric oxygen evolution rates were evaluated using data in a linear range. The alga concentration was 0.095 g cell/L and the concentration of stimulated toxicant ([BMIM] [BF_4]) was 22.94 mg/L in all cases. The intensities of stimulated daylight were (▽) 0 μE m^{-2} s^{-1}, (▼) 100 μE m^{-2} s^{-1}, (○) 500 μE m^{-2} s^{-1} and (●) 1,200 μE m^{-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.

As can be obviously observed in Figs. 2 and 3, the stronger the light intensity, the more oxygen will be produced. However, when no illumination was provided, dissolved oxygen concentration comparatively decreased as a result of algal respiratory process. In addition, the volumetric oxygen evolution rates were found to be lower in the presence of pollutant

compared to the results of test medium without pollutant. This can be explained by the toxic effects of pollutant towards microalgal respiratory function. Also, the algal photosynthetic response was significantly different when exposed to toxicant at different light intensities with 55, 6.5, 31.7 and 25% of oxygen was generated at illumination power of 0, 100, 500 and 1,200 $\mu Em^{-2}s^{-1}$, respectively. This variability in the toxicity of the tested compound implies that the results of an algal photosynthesis inhibition assay can differ considerably under different light conditions. This of course makes the comparability more complicated and should be avoided by controlling stringent rationales for a light regime during the test. For this purpose, the optimum light intensity for phytoplankton photosynthetic efficiency was obtained by plotting the specific oxygen evolution rate against the light intensities. Figure 4 demonstrates that the generated oxygen initially increased with light intensity and attained a plateau at higher photon flux densities. As light intensities ranged between 1,000 and 1,200 $\mu Em^{-2}s^{-1}$, specific oxygen evolution rates were noticed to be almost constant. Therefore, the light intensity between 1,000 and 1,200 $\mu Em^{-2}s^{-1}$ was considered to be appropriate for examining the photosynthetic performance of *S. capricornutum* in the present apparatus.

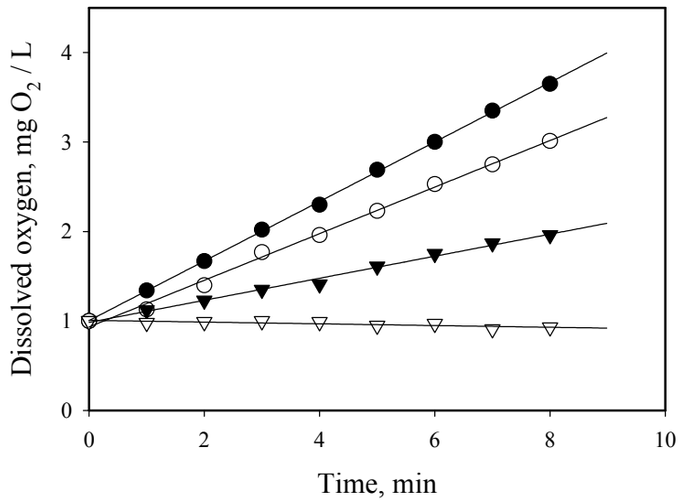


Fig. 3. Oxygen production by alga in different light intensities in the absence of toxicant. Volumetric oxygen evolution rates were evaluated using data in a linear range. The algal concentration was 0.0095 g cell/L in all cases. The intensities of stimulated daylight were (▽) 0 μE m^{-2} s^{-1}, (▼) 100 μE m^{-2} s^{-1}, (○) 500 μE m^{-2} s^{-1} and (●) 1,200 μE m^{-2} s^{-1}. Estimated volumetric activities were (▽) -0.0097 ± 0.0030, (▼) 0.1232 ± 0.0039, (○) 0.2603 ± 0.0075 and (●) 0.3327 ± 0.0025 mg O_2/L min.

4.2 Effect of cell concentration

Regarding the effect of cell concentration, the experiment was performed with various concentrations of algal broth under light intensity set at 1,000 $\mu Em^{-2}s^{-1}$. The rates of photosynthetic oxygen evolution were observed to be very well correlated with the algal cell densities. It is apparent in Fig. 5 that the oxygen production was inhibited in the presence of [BMIM] [BF_4]. Also, the levels of inhibitory effects were different at different algal cell densities with 46, 34 and 34% of photosynthetic activity were hampered by this compound at cell concentrations of 0.048, 0.095 and 0.182 g cell/L, respectively. These data inferred that

at low algal cell densities, the inhibitory percentages were rather higher. A possible explanation for this might be the relationship between toxicity and photoinhibition (Göksan et al., 2003), which is more likely to occur at low concentration due to the influence of mutual shading at high algal concentration (Contreras-Flores et al., 2003; Evers, 1991; Richmond, 2000). Though concentrations exceeding 0.182 g cell/L were not evaluated in the present study, it is supposed that mutual shading might be involved in high-density algal culture (Grobbelaar & Soeder, 1985). Taken together, 0.095 g cell/L was selected and employed for the subsequent experiments.

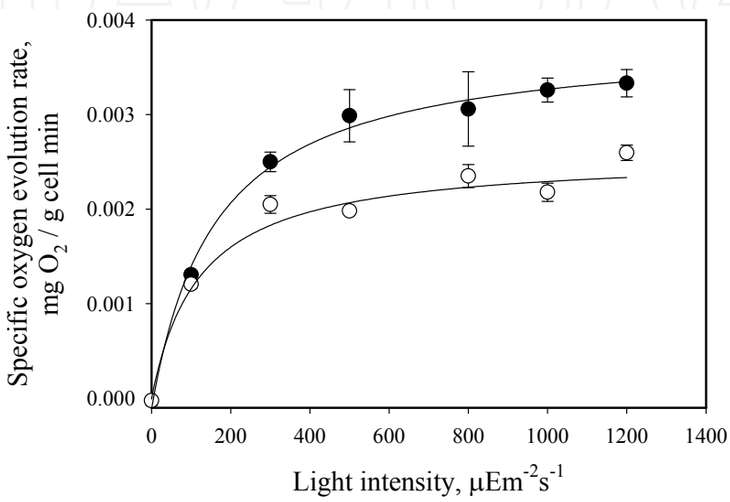


Fig. 4. Specific oxygen evolution rate as a function of incident photon flux density in the presence (○) and absence (●) of toxicant. Data points and error bars were average values and standard deviation of two or three replicated experimental results. Solid lines represent the calculated results from the photosynthesis-irradiance model (Eq. 1). The alga concentration was 0.095 g cell/L in all cases and the concentration of toxicant ([BMIM] [BF_4]) applied was 22.94 mg/L.

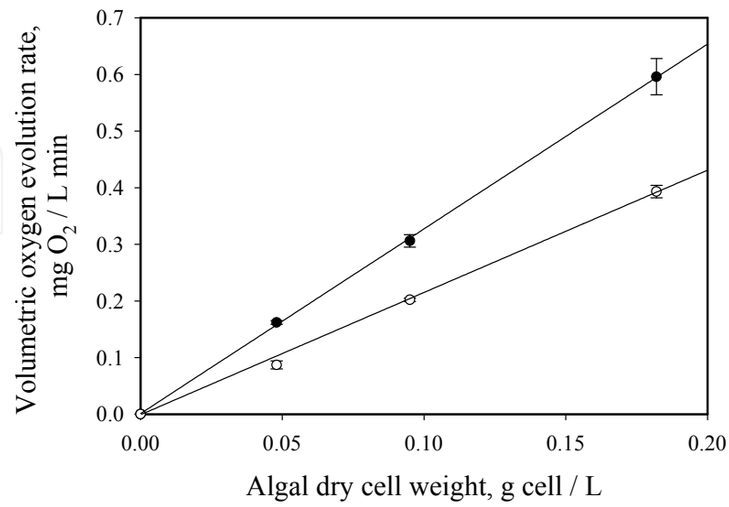


Fig. 5. Oxygen production by alga in different cell concentrations in the presence (○) and absence (●) of toxicant ([BMIM] [BF_4]).

4.3 Effect of initial dissolved oxygen concentration

Figure 6 depicts the influence of initial dissolved oxygen concentration on algal photosynthesis process with initial DO levels varied between 0.78 and 6.68 mg O_2/L. These concentrations (excluding the highest concentration of 6.68 mg O_2/L) were selected randomly by stripping with controlled amount of gas mixture containing N_2 and CO_2. Through preliminary studies, other conditions including light intensity, algal cell density and concentration of pollutant were fixed at 1,000 $\mu Em^{-2}s^{-1}$, 0.095 g cell/L and 22.94 mg/L, respectively. The data revealed that volumetric oxygen evolution rates were 0.2045 ± 0.0063, 0.1987 ± 0.0058, 0.2027 ± 0.0177 and 0.1315 ± 0.0169 mg O_2/L min corresponding to initial DO levels of 0.78, 3.37, 5.26 and 6.68 mg O_2/L. It should be pointed out that there was no effect of initial dissolve oxygen concentration towards algal photosynthetic response apart from the case of the highest DO value, in which CO_2 gas was not utilized. It can therefore be assumed that CO_2 plays an important role for microalgal photosynthesis process in the studied system.

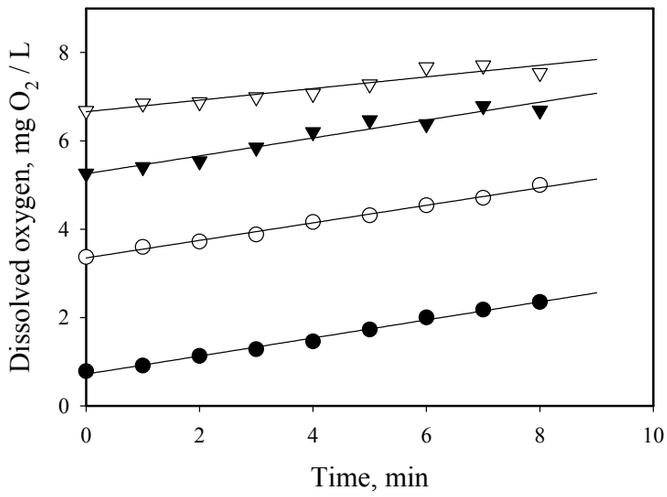


Fig. 6. Oxygen production by alga in different initial dissolved oxygen concentrations in the presence of toxicant. Volumetric oxygen evolution rates were evaluated using data in a linear range. The initial dissolved oxygen concentrations were (●) 0.78 mg O_2/L, (○) 3.37 mg O_2/L, (▼) 5.26 mg O_2/L, (▽) 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 22.94 mg/L.

4.4 Toxicity testing

As a development of the research work carried out by our group on this topic, here we present the results of a short-term algal photosynthesis inhibition tests performed on a representative of imidazolium-based ionic liquids and commonly used organic solvents. For checking the validity of the present system, a conventional algal growth assay was conducted in cases of [BMIM] [BF_4] and methanol. According to the data obtained, the effective concentrations of [BMIM] [BF_4] were identical in both cases of short-term and traditional assays. From Fig. 7, the EC_{50} values of [BMIM] [BF_4] were determined to be 0.115 mM and 0.126 mM for inhibition of algal photosynthesis process and growth rate, respectively. For methanol, the corresponding results were 2,089 and 759 mM suggesting the hazardous impact of this compound was 2.75 times higher towards algal growth than

photosynthetic activity. It seems possible that these results are owing to the longer exposure in growth rate assay compared to short-term test (96 h and 20 min, respectively), thus led to more critical injury to algal cells. Concerning the test data of the other commonly used organic solvents, it was found that all of these pollutants effectively inhibited algal photosynthesis with EC_{50} values varied between 589 and 2,089 mM (Table 2). Consequently, the toxicities of the tested organic compounds decreased in the order of isopropanol > acetone > acetonitrile > ethanol > dimethylformamide ≈ methanol.

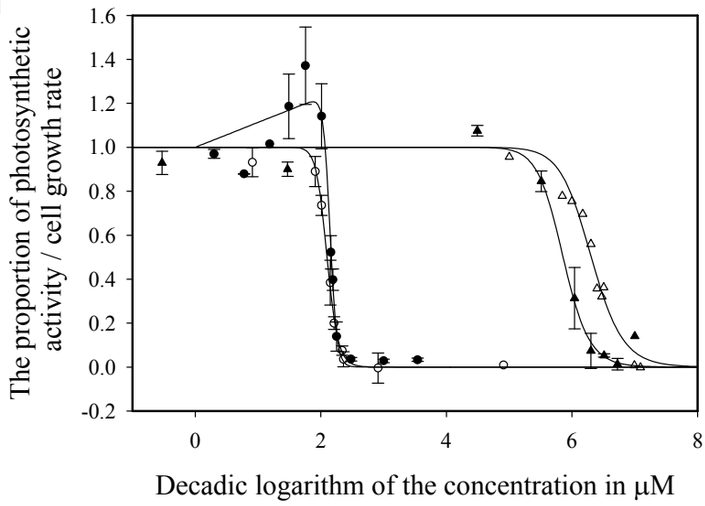


Fig. 7. Dose-response curves of algal toxicity test with respect to [BMIM] [BF_4] (○) and methanol (△) based on photosynthetic activity measurement whereas [BMIM] [BF_4] (●) and methanol (▲) based on growth rate.

Chemicals	$Log_{10}EC_{50}$/μM[a]	EC_{50}/mM	95% confidence interval/mM
Methanol	6.32 ± 0.11	2089	1623 – 2692
Ethanol	5.94 ± 0.24	871	501 – 1514
Dimethylformamide	6.32 ± 0.25	2089	1175 – 3715
Acetone	5.85 ± 0.03	708	661 – 759
Isopropanol	5.77 ± 0.15	589	417 – 832
Acetonitrile	5.92 ± 0.11	832	646 – 1072

Table 2. Inhibition of photosynthetic activity induced by various pollutants

5. Conclusions

In this chapter, an algal biosensor-based measurement system for rapid toxicity detection was designed, constructed and validated for the use in detecting several toxic chemicals. The system provides a possibility to toxicological investigation by monitoring the photosynthetic activity of algal cells through the oxygen produced. In contrast to most biosensors previously reported, the present sensor offers some advantages such as fast response time, simple and low-cost instrument, and totally air-tight to prevent underestimation of VOCs due to their volatility. The validity of the system was verified in terms of light intensity, algal cell concentration and initial dissolved oxygen dosage. It was observed that illumination condition and algal cell density significantly affected the photosynthesis process, whereas initial oxygen level only caused effect when no CO_2 was supplied to the test suspension. At fixed light intensity of 1,000 $\mu Em^{-2}s^{-1}$ and algal broth concentration of 0.095 g cell/L, the device performance testing was conducted with an ionic liquid and six common organic solvents. Furthermore, for comparison, a standard assay based on algal growth rate was carried out for two representative toxicants (ionic liquid and methanol). Although there was a good correlation between the data obtained from the system and those of the conventional standard growth test only in case of ionic liquid, the proposed system can be considered as potential approach for rapid assessment of toxicants. For practical use, further improvement in sensitivity can be obtained by increasing the exposure time of algal cells to toxicants or using more sensitive photosynthetic strains to specific pollutants. Algal photosynthesis is one of the essential physiological phenomena that contribute to algal viability, which might affect the structure and functioning of the whole aquatic ecosystems. Therefore, the present system, which deals with the photosynthetic activity of phytoplankton, can serve as a beneficial tool in preliminary screening toxicity methods. It should be noticed that simple acute ecotoxicity measurements do not completely identify the full impact of pollutants released into the environment but are only part of the environmental impact assessment. In general, this system is applicable to the toxicity assessment of not only organic solvents, ionic liquids but also heavy metals, pesticides as well as other hazardous substances. Nonetheless, it is premature to make this claim based on the data obtained in this chapter. Whether the system can be expanded as mentioned or not remains to be further tested. If the system is valid for other polluted compounds, this rapid test can be used as an alternative for real time bio-monitoring, where immediate toxicity evaluation is required.

6. Acknowedgements

This work was supported by National Research Foundation of Korea Grant funded by the Korean Government (KRF-2007-521-D00106, NRL 2009-0083194).

7. References

Aiba, S. (1982). Growth kinetics of photosynthetic organisms. *Advances in Biochemical Engineering*, 23, 85–156.

Bold, H. C. (1950). Problems in the cultivation of algae, In: *The Culturing of Algae*, Brunel, J., Prescott, G. W. & Tiffany, L. H. (Eds.), pp. 11–17, Antioch, Yellow Springs, OH, USA.

Bonilla, S., Conde, D. & Blanck, H. (1998). The photosynthetic responses of marine phytoplankton, periphyton and epipsammon to the herbicides paraquat and simazine. *Ecotoxicology*, 7, 99–105.

Brain, P. & Cousens, R. (1989). An equation to describe dose responses where there is stimulation of growth at low doses. *Weed Research*, 29, 93–96.

Campanella, L., Cubadda, F., Sammartino, M. P. & Saoncella, A. (2000). An algal biosensor for the monitoring of water toxicity in estuarine environments. *Water Research*, 35, 69–76.

Chen, C.-Y. & Lin, J.-H. (2006). Toxicity of chlorophenols to *Pseudokirchneriella subcapitata* under air-tight test environment. *Chemosphere*, 62, 503–509.

Contreras-Flores, C., Peña-Castro, J. M., Flores-Cotera, L. B. & Cañizares-Villanueva, R. O. (2003). Advances in conceptual design of photobioreactor for microalgal culture. *Interciencia*, 28, 450–456.

Danilov, R. A. & Ekelund, N. G. A. (2001). Effects of Cu^{2+}, Ni^{2+}, Pb^{2+}, Zn^{2+} and pentachlorophenol on photosynthesis and motility in *Chlamydomonas reinhardtii* in short-term exposure experiments. *BMC Ecology*, 1, 1.

Durrieu, C. & Tran-Minh, C. (2002). Optical algal biosensor using alkaline phosphatase for determination of heavy metals. *Ecotoxicology and Environmental Safety*, 51, 206–209.

Endo, R. & Omasa, K. (2004). Chlorophyll fluorescence imaging of individual algal cells: effects of herbicide on *Spirogyra distenta* at different growth stages. *Environmental Science & Technology*, 38, 4165–4168.

Eullaffroy, P. & Vernet, G. (2003). The F684/F735 chlorophyll fluorescence ration: a potential tool for rapid detection and determination of herbicide phytotoxicity in algae. *Water Research*, 37, 1983–1990.

Evers, E. G. (1991). A model for light-limited continuous cultures : growth, shading, and maintenance. *Biotechnology and Bioengineering*, 38, 254–259.

Göksan, T., Durmaz, Y. & Gökpinar, S. (2003). Effects of light path lengths and initial culture density on the cultivation of *Chaetoceros muelleri* (Lemmermann, 1898). *Aquaculture*, 217, 431–436.

Grant, A. J., Graham, K., Frankland, S. & Hinde, R. (2003). Effect of copper on algal-host interactions in the symbiotic coral *Plesiastrea versipora*. *Plant Physiology and Biochemistry*, 41, 383–390.

Grobbelaar, J. U. & Soeder, C. J. (1985). Respiration losses in planktonic green algae cultivated in raceway ponds. *Journal of Plankton Research*, 7, 497–506.

Hall, N. E., Fairchild, J. F., La Point, T. W., Heine, P. R., Ruessler, D. S. & Ingersoll, C. G. (1996). Problems and recommendations in using algal toxicity testing to evaluate contaminated sediments. *Journal of Great Lakes Research*, 22, 545–556.

Jensen, A. (1984). Marine ecotoxicological tests with phytoplankton. In: *Ecotoxicological testing for the marine environment*. *Proceedings of the International Symposium on*

Ecotoxicological testing for the marine environment. Persoone, G., Jaspers, E. & Claus, C. (Eds.), pp. 195–213, Ghent, Belgium.

Jeon, Y.-C., Cho, C.-W. & Yun, Y.-S. (2005). Measurement of microalgal photosynthetic acitivity depending on light intensity and quality. *Biochemical Engineering Journal*, 27, 127–131.

Lewis, M. A. (1995). Algae and vascular plant tests, In: *Fundamentals of Aquatic Toxicology: Effects, Environment Fate, and Risk Assessment*, Rand, G. M. (2nd Ed.), pp 135–170, Taylors & Francis, Washington, DC, USA.

Lin, J.-H., Kao, W.-C., Tsai, K.-P., Chen, C.-Y. (2005). A novel algal toxicity of both metallic and organic toxicants. *Water Research*, 39, 1869–1877.

Marquardt, D. W. (1963). An algorithm for least square estimation of parameters. *Journal of the Society for Industrial and Applied Mathematics*, 11, 431–441.

Morris, I. (1981). Photosynthetic products, physiological state, and phytoplankton growth, In: *Physiological Bases of Phytoplankton Ecology*, Platt, T. (Ed.), *Canadian Bulletin of Fisheries and Aquatic Sciences*, 210, 83–102.

Naessens, M., Leclerc, J. C. & Tran-Minh, C. (2000). Fiber optic biosensor using *Chlorella vulgaris* for determination of toxic compounds. *Ecotoxicology and Environmental Safety*, 46, 181–185.

Naessens, M. & Tran-Minh, C. (1999). Biosensor using immobilized *Chlorella* microalgae for determination of volatile organic compounds. *Sensors and Actuators B*, 59, 100–102.

Nalewajko, C. (1995). Effects of cadmium and metal-contaminated sediments on photosynthesis, heterotrophy, and phosphate uptake in Mackenzie River Delta phytoplankton. *Chemosphere*, 30, 1401–1414.

Organization for Economic Cooperation and Development. (2002). Freshwater alga and cyanobacteria growth inhibition test, 2002 update. OECD guideline 201. Paris, France.

Overnell, J. (1976). Inhibition of marine algal photosynthesis by heavy metals. *Marine Biology*, 38, 335–342.

Pardos, M., Benninghoff, C. & Thomas, R. L. (1998). Photosynthetic and population growth response of the test alga *Selenastrum capricornutum* Printz to zinc, cadmium and suspended sediment elutriates. *Journal of Applied Phycology*, 10, 145–151.

Podola, B., Nowack, E. C. M. & Melkonian, M. (2004). The use of multiple-strain algal sensor chips for the detection and identification of volatile organic compounds. *Biosensors and Bioelectronics*, 19, 1253–1260.

Puiseux-Dao, S. (1989). « Phytoplankton model » in ecotoxicology. In: *Aquatic Ecotoxicology : fundamental, concepts and methodologies*, Boudou, A. & Ribeyre, F. (Eds.), pp. 163–185, CRC, Boca Raton.

Raja, R., Hemaiswarya, S., Kumar, N. A., Sridhar, S. & Rengasamy, R. (2008). A perspective on the biotechnological potential of microalgae. *Critical Reviews in Microbiology*, 34, 77–88.

Richmond, A. (2000). Microalgal biotechnology at the turn of the millennium: a personal view. *Journal of Applied Phycology*, 12, 441–451.

Rodriguez, M., Sanders, C. A. & Greenbaum, E. (2002). Biosensors for rapid monitoring of primary-source drinking water using naturally occurring photosynthesis. *Biosensors and Bioelectronics*, 17, 843–849.

Schubnell, D., Lehmann, M., Baumann, W., Rott, F. G., Wolf, B. & Beck, C. F. (1999). An ISFET-algal (*Chlamydomonas*) hybrid provides a system for eco-toxicological tests. *Biosensors and Bioelectronics*, 14, 465–472.

Shitanda, I., Takada, K., Sakai, Y. & Tatsuma, T. (2005). Compact amperometric algal biosensors for the evaluation of water toxicity. *Analytica Chimica Acta*, 530, 191–197.

U.S. Environmental Protection Agency. (1996). Ecological effect test guidelines. Algal Toxicity, Tiers I and II. OPPTs 850.5400. Washington, DC, USA.

van Ewijk, P. H. & Hoekstra, J. A. (1993). Calculation of the EC_{50} and its confidence interval when subtoxic stimulus is present. *Ecotoxicology and Environmental Safety*, 25, 25–32.

West, L. J. A., Li, K., Greenberg, B. M., Mierle, G. & Smith, R. E. H. (2003). Combined effects of copper and ultraviolet radiation on a microscopic green alga in natural soft lake waters of varying dissolved organic carbon content. *Aquatic Toxicology*, 64, 39–52.

Yun, Y.-S. & Park, J. M. (1997). Development of gas recycling photobioreactor system for microalgal carbon dioxide fixation. *Korean Journal of Chemical Engineering*, 14, 297–300.

Yun, Y.-S. & Park, J. M. (2001). Attention of monochromatic and polychromatic lights in *Chlorella vulgaris* suspensions. *Applied Microbiology and Biotechnology*, 55, 765–770.

Yun, Y.-S. & Park, J. M. (2003). Kinetic modeling of the light-independent photosynthetic activity of the green microalgal *Chlorella vulgaris*. *Biotechnology and Bioengineering*, 83, 303–311.

IntechOpen



Advances in Measurement Systems
Edited by Milind Kr Sharma

ISBN 978-953-307-061-2
Hard cover, 592 pages
Publisher InTech
Published online 01, April, 2010
Published in print edition April, 2010

How to reference
In order to correctly reference this scholarly work, feel free to copy and paste the following:

Thi Phuong Thuy Pham, Chul-Woong Cho and Yeoung-Sang Yun (2010). Algal Biosensor-Based Measurement System for Rapid Toxicity Detection, Advances in Measurement Systems, Milind Kr Sharma (Ed.), ISBN: 978-953-307-061-2, InTech, Available from: http://www.intechopen.com/books/advances-in-measurement-systems/algal-biosensor-based-measurement-system-for-rapid-toxicity-detection

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821

IntechOpen

© 2010 The Author(s). Licensee IntechOpen. This chapter is distributed under the terms of the Creative Commons Attribution-NonCommercial-ShareAlike-3.0 License, which permits use, distribution and reproduction for non-commercial purposes, provided the original is properly cited and derivative works building on this content are distributed under the same license.

IntechOpen

IntechOpen