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Amperometric screen-printed algal biosensor with flow injection analysis system for detection of environmental toxic compounds

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ABSTRACT

A screen-printed algal biosensor was fabricated for evaluation of toxicity of chemicals. An algal ink was prepared by mixing unicellular microalga *Chlorella vulgaris* cells, carbon nanotubes and sodium alginate solution. The algal ink was immobilized directly on a screen-printed carbon electrode surface using screen-printing technique. Photosynthetically generated oxygen of the immobilized algae was monitored amperometically. Responses of the algal biosensor to four toxic compounds, 6-chloro-*N*-ethyl-*N*-isopropyl-1,3,5-triazine-2,4-diamine (atrazine) and 3-(3,4-dichlorophenyl)-1,1-diethylurea (DCMU) were evaluated as inhibition ratios of the reduction current. The concentrations that gave 50% inhibition of the oxygen reduction current (IC_{50}) for atrazine and DCMU were 12 and 1 μmol dm^{-3}, respectively. In comparison with the conventional algal biosensors, in which the algal cells were entrapped in an alginate gel and immobilized on the surface of a transparent indium tin oxide electrode, the present sensor is much smaller and less expensive, with the shorter assay time.

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1. Introduction

The rapid and precise evaluation of water toxicity has been an important issue for environmental risk management. However, it is difficult to measure toxicity of individual chemicals contaminated in water, since a wide variety of chemicals exist in environmental water, and their mixture may exhibit complex toxicity. In these cases, it is more effective to evaluate the overall toxicity of a sample solution to living organisms rather than determination of individual chemicals. Bioassay has been one of the most useful methods for the comprehensive determination of toxicity in environmental and industrial waste water. It is also effective in screening and evaluation of chemicals in terms of toxicity.

Various bioassays based on algae [1–4], luminescence bacteria [5], plant tissues [6], and animal cells [7] have been developed in recent years. In particular, microalgae have been widely used for the toxicity tests because of their high sensitivity and reproducibility. For instance, an algal growth inhibition test is involved in organization for economic co-operation and development (OECD) guidelines for the testing of chemicals (OECD TG201). However, the growth inhibition tests take several days in general, and require large culture apparatus. Therefore, on-site monitoring of toxicity is difficult with the above-cited methods.

To reduce the assay time and apparatus size, electrochemical whole cell biosensors [8–13], in which photosynthetic activity of the microalgae is monitored by the Clark-type oxygen electrode, have been developed. These biosensors were found to allow rapid detection of herbicides and organic solvents within a few minutes. However, the oxygen electrode is not very compact.

Shitanda et al. [14] developed a compact and disposable device for rapid toxicity testing on the basis of amperometric monitoring of photosynthetically generated oxygen. Algal cells were immobilized directly on the surface of a transparent indium tin oxide (ITO) electrode using the polymer membranes. However, the biosensor is relatively expensive for on-site monitoring. In addition, the membrane thickness of the biosensor is slightly difficult to control since the polymer membranes are prepared by casting method. In order to make the algal biosensor for practical use, it is necessary to improve its disposability.

Screen-printing technique has been widely applied to the fabrication of enzyme electrodes [15–21], because it has the following merits: (a) drawing precise pattern of micron order, (b) a wide variety of inks, (c) high reproducibility, and (d) low cost. The screen-printed enzyme electrodes consist of several layers which were formed by depositing the inks successively. Screen-printed enzyme electrodes have been also applied for the detection of various toxic chemicals including herbicide [22–25].

Recently, we presented a preliminary report on a screen-printed algal biosensor [26]. An algal ink was prepared and immobilized on a carbon electrode by screen-printing. The photosynthetic oxygen evolution of the algal cell could be monitored on a flow injection analysis system. In the present study, we investigated the sensitivity of the screen-printed algal biosensor for toxic chemicals. Responses of the algal biosensor to toxic herbicide were evaluated as inhibition

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ratio of the reduction current. The present method allows establishment of a compact analytical system for water toxicity testing, and screening and evaluation of newly synthesized chemicals.

2. Experiment

2.1. Preparation of the algal biosensor

An unicellular alga *Chlorella vulgaris* strain C-27 (Institute of Applied Microbiology Culture Collection (IAMCC), Japan) was used throughout. The algae were grown in Kessler medium [27] (pH 7.5) in 1 L culture bottles at 25 °C. The culture bottles were aerated through a membrane filter and illuminated by dim fluorescent light periodically (12 h illumination, 12 h dark). An algal ink was prepared by mixing 4 wt.% sodium alginate solution containing algal cells (6×10^{12} cell mL^{-1}) and 1×10^{-2} wt.% multiwall carbon nanotubes (3–10 nm diameter, Wako Chemical).

A carbon electrode was fabricated by using a screen-printer (LS-150TV, Newlong Seimitsu Kogyo). A polyimide film (Capton, Toray-Dupont) was used for the substrate. A Silver lead was printed on the polyimide film with a silver ink (ECM-100AF500, Taiyo Ink). A carbon film was formed on the silver leads by a carbon ink (ECM-100AF500, Taiyo Ink). A resist ink (TF-200FR1, Taiyo Ink) was printed on the silver lead and carbon film in order to prevent the silver lead from corroding and define the surface area of the carbon electrode. The carbon electrode was dried at 100 °C for 30 min. The algal ink (1.2 μL) was printed on the screen-printed carbon electrode surface (surface area, 20 mm^2) using screen-printing technique. Afterward, the electrode was immersed in 200 mM $CaCl_2$ solution for 30 min. The screen-printed algal immobilized electrode prepared was used for the amperometric measurements on the same day except storage stability test.

2.2. Measurements

Amperometric measurements were performed by using a flow injection analysis system with an Ag/AgCl and a coiled platinum wire as reference and counter electrodes, respectively. Fig. 1 shows schematic illustration of experimental setup. First, 0.2 mol dm^{-3} $CaCl_2$ solution (20 °C, pH 7.5) was passed into the flow cell. Then, a solution containing 0.2 mol dm^{-3} $CaCl_2$ and a test toxic chemical was flowed thorough the flow cell. The potential of the algal electrode was set at −0.7 V vs. Ag/AgCl. Responses of algal biosensor were evaluated as inhibition ratio of photosynthetic oxygen

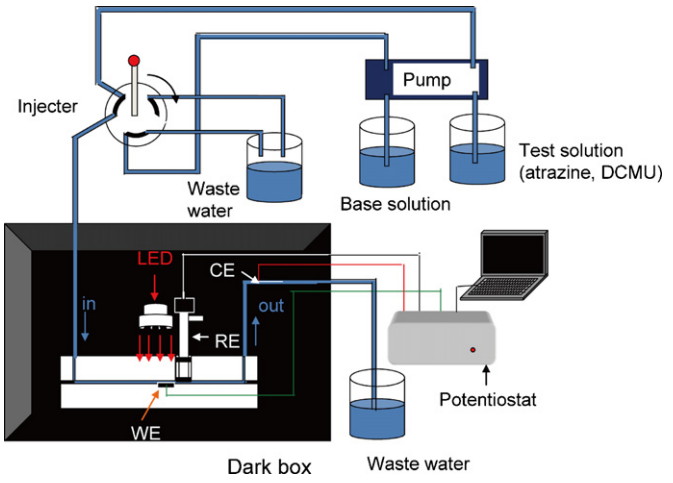

Fig. 1. Schematic illustration of the experimental setup. The electrochemical flow injection cell was equipped with working, reference and counter electrodes (WE, RE and CE). The working electrode was irradiated with red LED light periodically.

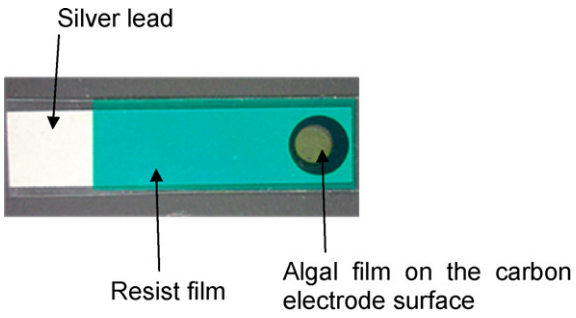

Fig. 2. Photograph of the screen-printed algal biosensor.

evolution under periodic red light illumination from a red LED. The electrochemical measurements were carried out in a dark box. 6-Chloro-*N*-ethyl-*N*-isopropyl-1,3,5-triazine-2,4-diamine (atrazine, Wako Chemical) and 3-(3,4-dichlorophenyl)-1,1-diethylurea (DCMU, Wako Chemical) were used as toxic test chemicals.

3. Results and discussion

Alginate is a copolymer consisting of 1,4-linked D-mannuronic acid and L-guluronic acid, and is known to form a gel with divalent ions such as calcium ion. Such gels can easily be formed without alteration of pH or temperature. For this reason, alginate gel was used as a support material for algal cells. Fig. 2 shows a photograph of the screen-printed algae-immobilized electrode. Obviously, an algal film was formed uniformly on the carbon electrode surface. The film thickness of the algal film was about 100 μm. In addition, stability of the algal film was also studied. The algae-immobilized electrode was immersed for 1 h in the identical solution to that used for the electrochemical measurements, with stirring. Then, changes in the absorbance of the solution were measured at 660 nm to detect the chlorophyll in the algae. The absorbance remained constant during the experiment, indicating that release of the algae from the alginate membrane was negligible in the solution at least for 1 h.

Fig. 3 shows typical current responses of the algal biosensor. The potential of the algae-immobilized electrode was set at −0.7 V vs. Ag/AgCl. This potential was chosen to reduce oxygen substantially, but not toxic test chemicals. As can be seen in Fig. 3, the oxygen reduction current was significantly increased when the algal biosensor was illuminated. This reflects that oxygen concentration increased at the electrode surface as a result of the algal photosynthesis. The steady state was reached within only 15 s upon illumination. This could be explained in terms of small film thickness. The concentration of oxygen accumulated in a thin algal film should take the shorter time to reach steady state. In addition, the

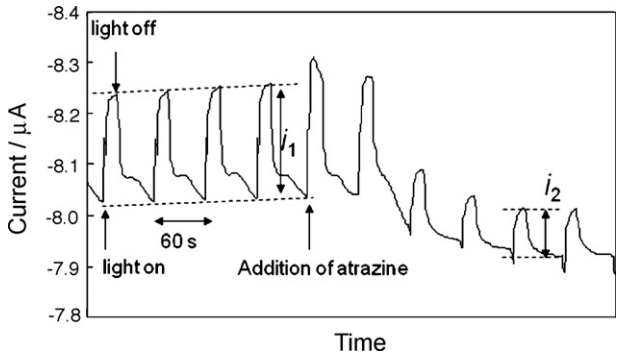

Fig. 3. Current changes of the algal biosensor in response to switching of light (15 s illumination followed by 45 s dark) and addition of atrazine (20 μmol dm^{-3}) at 0.7 V vs. Ag/AgCl in 0.2 mol dm^{-3} $CaCl_2$ solution (pH 7.5) at 20 °C.

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