Compact Amperometric Algal Biosensors for the Evaluation of Water Toxicity

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Bioassay has been one of the popular methods for the determination of toxicity in environmental and industrial waste water. Microalgae have been widely used for the bioassay because of their high sensitivity. The conventional toxicity tests take several days since they are based on the monitoring of algal growth. To reduce the assay time, a new method, in which photosynthetic oxygen evolution is monitored by the Clark-type oxygen electrode, has been developed [1]. However, the oxygen electrode is relatively expensive and not very compact. The aim of the present study was to develop a compact and disposable device for rapid toxicity testing on the basis of amperometric monitoring of photosynthetically generated oxygen.

In a typical experiments, an unicellular alga *Chlorella vulgaris* strain NIES-227 (Microbial Culture Collection of an National Institute for Environmental Studies, Japan) was used throughout. An indium tin oxide (ITO) (surface area, 25 mm^2) as a working electrode was treated with a 1 mol dm^{-3} NaOH solution prior to use. Algal cells were entrapped in an alginate gel or polyion complex membrane coated on the ITO electrode surface. In the case of the alginate gel, 2 wt% sodium alginate solution containing algal cells (1×10^8 cell μL^{-1}) was cast on the electrode. Afterward, 1 wt% calcium chloride was cast as a gelling agent. On the other hand, a polyion complex containing algae was formed from poly(L-lysine) (PLL) as a polycation and poly(styrenesulfonate) (PSS) as a polyanion. PLL, PSS, and algae cells were successively cast on the ITO electrode, followed by additional dropping of PLL and PSS for covering the cells. The electrode was then dried at room temperature. Amperometoric measurements were performed in a sodium carbonate solution with a Ag/AgCl and a coiled plutinum wire as a reference and a counter electrodes, respectively. The potential of the algae electrode was set at -0.7 V vs. Ag/AgCl. Responses of the algal biosensor to various toxic compounds were evaluated as inhibition ratios of photosynthetic oxygen evolution under periodic visible light illumination (light intensity, 10 mW cm^{-2}). 6-Chloro-*N*-ethyl-*N*-isopropyl-1,3,5-triazine-2,4-diamine (atrazine), 3-(3,4-dichlorophenyl)-1,1-diethylurea (DCMU), and toluene were used as toxic chemicals.

Figure 1 shows typical current responses of the polyion complex-modified electrode containing algae. The reduction current of photosynthetically generated oxygen became constant within 5 sec upon illumination. The reduction current appeared to decrease upon the addition of atrazine. The dose-response curves obtained with the polyion complex based algal biosensor for atrazine, DCMU, and toluene are shown in Figure 2. Based on these results, the IC_{50} values (concentration that gives 50% inhibition of the oxygen evolution) were estimated for the toxic compounds, and compared with the EC_{50} values [2, 3] (concentration that gives 50% inhibition of the growth after 96 h) of the standard growth test (Table 1). The IC_{50} tests based on the polyion complex-modified electrode appeared to require a shorter measurement time compared to that based on the alginate gel and to be in a good accordance with EC_{50}.

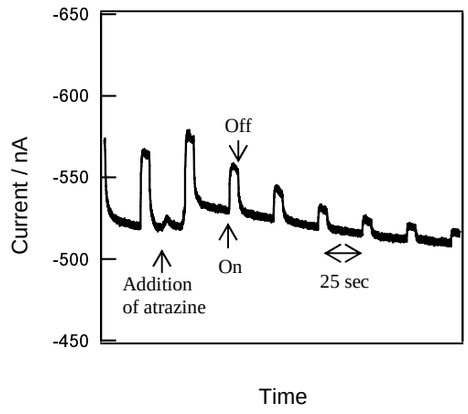

Fig. 1 Current changes of the polyion complex-based algal biosensor in response to switching of light (5 sec illumination followed by 20 sec dark) and addition of atrazine (2 mmol dm^{-3}).

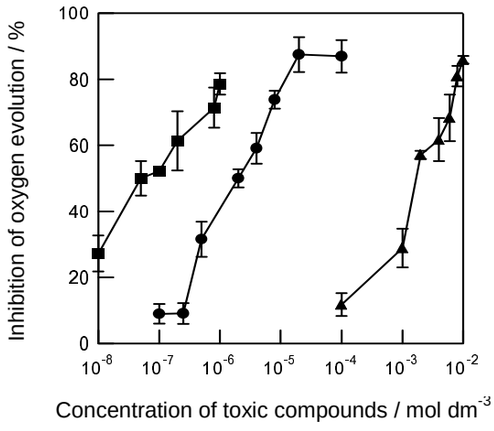

Fig. 2 Dose-response curves obtained with the algal biosensor for 5 min exposure to the toxic compounds, atrazine (●), DCMU (■) and toluene (▲).

Table 1 Sensitivities of the algal biosensors to different toxic compounds in comparison with the growth tests

Toxic compounds	Growth test EC_{50} (μmol dm^{-3})	Alginate gel IC_{50} (μmol dm^{-3})	Polyion complex IC_{50} (μmol dm^{-3})
Atrazine	1.9 [2)]	10	2.0
DCMU	0.02 [2)]	1	0.05
Toluene	2660 [3)]	3000	1550

[1] P. Pandard et al., *Environ. Toxicol. Water Qual.*, **8**, 323 (1993).
[2] M. Jianyi et al., *Ecotoxicol. Environ. Saf.*, **51**, 128 (2002).
[3] P. B Kauss et al., *Environ. Pollut.*, **9**, 157 (1975).