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Compact amperometric algal biosensors for the evaluation of water toxicity

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Abstract

Unicellular microalga *Chlorella vulgaris* was entrapped in an alginate gel or a polyion complex membrane immobilized directly on the surface of a transparent indium tin oxide electrode. 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), 3-(3,4-dichlorophenyl)-1,1-diethylurea (DCMU), toluene and benzene, were evaluated as inhibition ratios of the reduction current. The concentrations that give 50% inhibition of the oxygen reduction current (IC'_{50}) for atrazine, DCMU, toluene and benzene were 2.0, 0.05, 1550 and 3000 μmol dm^{-3}, respectively. There was a good correlation between these data and those of the conventional standard growth test. In comparison with the conventional algal biosensors based on the Clark-type oxygen electrode, the present sensor is much smaller and less expensive, and its assay time is much shorter (≤200 s).

Keywords: Amperometric biosensor; *Chlorella vulgaris*; Algae; Alginate gel; Polyion complex; Herbicides

1. Introduction

The rapid and precise evaluation of water toxicity has been an important issue for environmental water safety. However, it is difficult to measure toxicity of individual chemicals contained in water, since a wide variety of chemicals exist in environmental water, and a mixture may exhibit complex toxicity. Bioassay has been one of the most useful methods for the determination of toxicity in environmental and industrial waste waters. Many 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. Microalgae are also ubiquitous and can grow year-round. However, the toxicity tests take several days in general since they are based on monitoring of algal growth, and require large culture apparatus. Therefore, on-site monitoring of the toxic compounds is difficult with those methods.

To reduce the assay time, whole cell biosensors, in which photosynthetic activity of the microalgae is monitored by optical or electrochemical means, have been developed. Most of these biosensors detect the toxic compounds on the basis of inhibition of photosynthetic activity. The optical sensors are generally based on fluorescence of chlorophyll contained in chloroplast [8–12], and the electrochemical sensors are usually based on photosynthetic oxygen evolution measured by the Clark-type oxygen electrode [13–19].

Although the optical biosensors have better detection limit than the electrochemical biosensors, some of them take a few hours until fluorescence intensity is stabilized before toxicity measurements [19]. In addition, the former requires a spectrophotometer, which is generally large and expensive. On the other hand, the electrochemical biosensors respond faster, but the oxygen electrode is relatively expensive and

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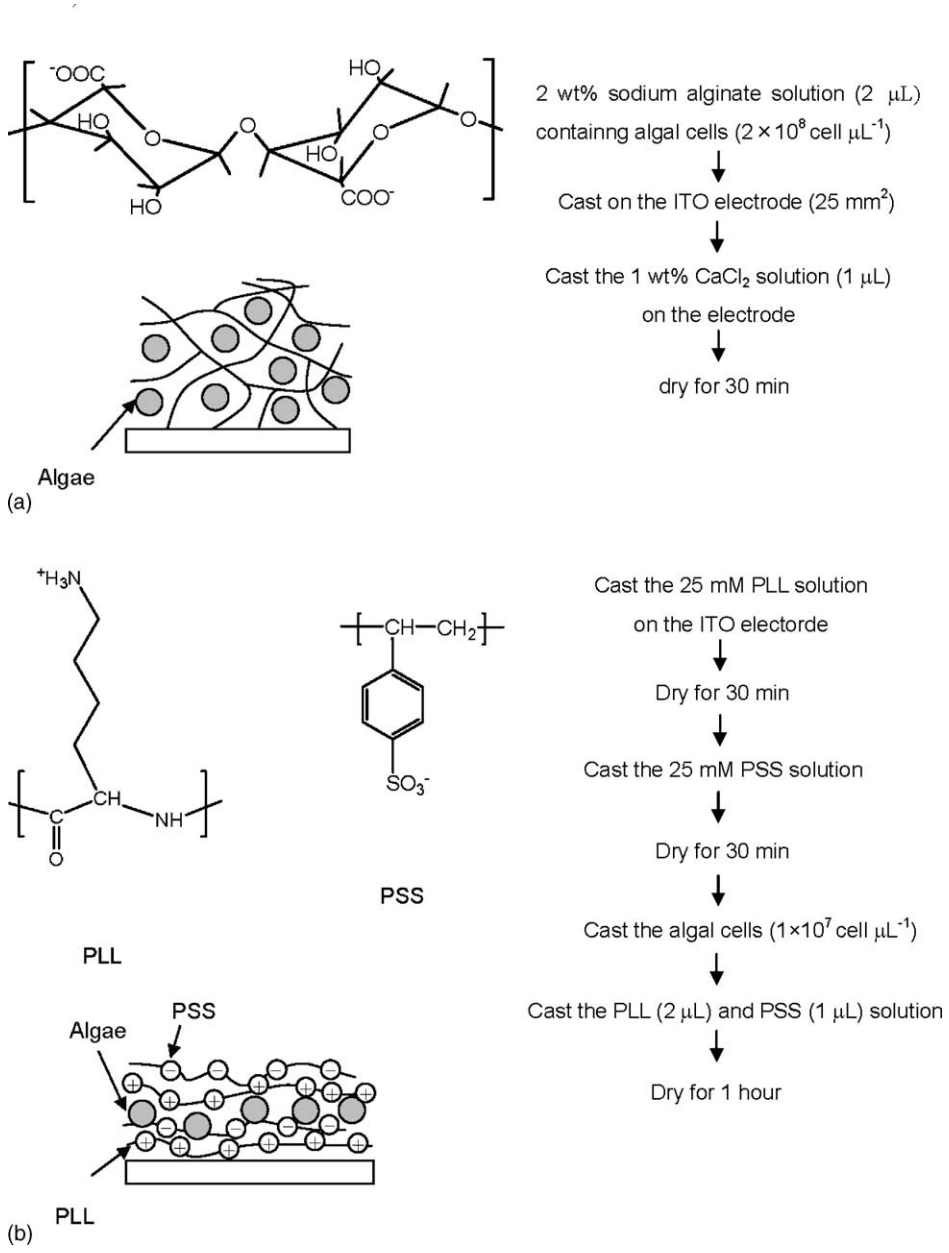

Fig. 1. Molecular structures and schematic illustrations of membranes immobilizing algae on the ITO electrode: (a) alginate and (b) poly(L-lysine) (PLL, left) and poly(styrenesulfonate) (PSS, right).

not very compact. For these reasons, these devices are also not suitable for the on-site monitoring.

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. Unicellular microalga *Chlorella vulgaris* was entrapped in an alginate gel (Fig. 1(a)) or a polyion complex (Fig. 1(b)) and immobilized directly on the surface of a transparent indium tin oxide (ITO) electrode. The present method allows establishment of a compact analytical system for water toxicity testing, and screening and evaluation of newly synthesized chemicals including herbicides, in terms of toxicity or the herbicidal effect.

2. Experimental

2.1. Preparation of the algal biosensor

Unicellular alga *Chlorella vulgaris* strain NIES-227 (Microbial Culture Collection of National Institute for Environmental Studies, Japan) was used throughout. The algae were grown in Kessler medium [20] (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 ITO (surface area, 25 mm^2) as a working electrode was treated with a 1 mol dm^{-3} NaOH solution prior to use.