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A novel whole-cell biosensor for the determination of trichloroethylene

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ABSTRACT

Trichloroethylene (TCE) is one of common pollutants in soil and groundwater. TCE has a recalcitrant pollutant that is uneasy to degrade by microorganisms. A novel microbial biosensor based on *Pseudomonas* sp. strain ASA86 was developed for the TCE detection. A chloride ion electrode was employed as a transducer to detect the released chloride ions from TCE degraded by the bacteria, which was immobilized on a porous cellulose nitrate membrane. The performance of the biosensor was evaluated at different TCE concentrations, with different concentrations of the immobilized bacteria, pH levels, temperatures, and with different interferents. There was a linear relationship between biosensor response and TCE concentration ranging up to 4 mg/L, and the response could be detected up to 0.05 mg/L TCE concentration. The biosensor response showed good reproducibility (relative standard deviation of 6.1%) at 0.5 mg/L TCE concentration. The detection time was about 5 min under the optimum conditions of the biosensor. The optimal response was obtained at pH 8.0 and 30 °C. Compounds that have molecular structures similar to that of TCE did not significantly affect the response of the biosensor. The biosensor response remained stable over five days. This TCE biosensor shows enough sensitivity for environmental monitoring.

1. Introduction

Water pollution affects living organisms in water systems. In particular, groundwater pollution is a very serious problem because groundwater is one of the sources of drinking water. Trichloroethylene (TCE) out of chlorinated ethenes is a major pollutant in groundwater pollution [1–3]. TCE has been widely used as an organic solvent, a dry cleaning fluid, and as a cleansing agent for electronic parts in industrial processes. Owing to its improper disposal and management, as well as its leakage from widespread use, TCE has become one of the most common and recalcitrant pollutants in soil and groundwater. TCE is toxic to humans as it causes an impairment in the central nervous system at high level of exposure, and has been classified as a carcinogenic substance [4].

Until about three decades ago, it was believed that microorganisms could not degrade TCE. However, Bouwer et al. [5] discovered that TCE could be biodegraded by methanogens. TCE is degraded by cometabolism by aerobic or anaerobic bacteria in the presence of a cosubstrate such as methane, ammonia, or aromatic compounds [6]. TCE is usually cometabolically degraded by aerobic bacteria, which have oxygenase enzymes such as soluble methane monooxygenases of *Methylosinus trichosporium* OB3b [7], toluene monooxygenases of *Burkholderia cepacia* G4 [8] and *Pseudomonas mendocina* KR1 [9], and toluene dioxygenases of *Pseudomonas putida* F1 [10]. *Pseudomonas* sp. strain ASA86 used in this study is an aerobic bacterium, and it degrades TCE to activate toluene dioxygenase (TOD) in the presence of toluene [11]. Of the four genes encoding TOD (*todC1*, *todC2*, *todB*, and *todA*), the *todC1* gene encodes the α subunit of TOD and responds to toluene [12,13]. *P. putida* F4, which is a mutant with a defective *todC1* gene in *P. putida* F1, is not able to catalyze TCE degradation [14]. Toluene is utilized as a growth substrate by the microorganism and is first decomposed into toluene *cis*-dihydrodiol by the TOD enzyme. During aerobic biodegradation of toluene, TCE is converted into a dioxetane intermediate by the TOD enzyme. The intermediate is further degraded to form the non-chlorinated compounds, glyoxylate and formate, and finally carbon dioxide [15]. TCE is a pollutant widespread in soil and groundwater, and therefore, bioremediation to remove TCE has been carried out by many researchers [16–20]. Bioremediation may be more efficient under aerobic conditions than under anaerobic conditions because anaerobic biodegradation of TCE results in the formation of harmful metabolites such as dichloroethylenes and vinyl chloride.

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Biosensors for the TCE detection have been relatively poorly studied compared to those for other pollutants. To date, only a few biosensors for the TCE detection have been developed, including the potentiometric biosensors based on *Pseudomonas aeruginosa* JI104 had been developed by Han et al. [21–23], and the conductometric and impedimetric biosensor based on *Pseudomonas putida* F1 were assembled by Hnaien et al. [24,25]. The reason for the scarcity of biosensors for the TCE detection is that TCE is a volatile, chlorinated, aliphatic compound and not directly degraded by microorganisms [21,22,25]. Moreover, from a practical standpoint, analysis of TCE requires lengthy sampling times, several preparatory steps, and expensive equipment. The solvent extraction and purge-trap steps are complex and troublesome because of poor detection limits. Thus, a simple and quick method for the TCE detection is strongly needed.

In this study, we developed a new microbial biosensor for the TCE detection based on *Pseudomonas* sp. strain ASA86, which was isolated and characterized in previous study [11]. Strain ASA86 had been found to be a suitable bioelement for the determination of TCE. The biosensor was characterized and the optimal conditions for the TCE detection were investigated.

2. Materials and methods

2.1. Chemicals

TCE and toluene were purchased from Wako Pure Chemical Industries, Ltd. (Japan). Chemicals used for analysis were obtained from Wako Pure Chemical Industries, Ltd., and Sigma Chemical Co. (Milwaukee, WI). Media for cell culture were purchased from Difco Laboratories (Detroit, MI).

2.2. Microorganism and cultures

In this study, *Pseudomonas* sp. strain ASA86 was used [11]. The composition of the medium growing the strain ASA86 per liter of distilled water was as follows: toluene, 0.5 g; TCE, 1 mg; $NaNO_3$, 2.2 g; NaH_2PO_4, 0.2 g; Na_2HPO_4, 0.4 g; $MgSO_4$, 0.2 g; KCl, 0.04 g; $CaCl_2$, 0.02 g; yeast extract, 0.01 g. The pH of the medium was 8.0. *Pseudomonas* sp. strain ASA86 was grown under aerobic conditions in a rotating shaker at 30 °C for 20 h in the medium described above. After 20 h, toluene of 0.5 g/L and TCE of 1 mg/L were added to the medium and continuously cultured until the stationary phase.

2.3. Preparation of the biofilm

Cells used in the biofilm were harvested by centrifugation at 6000 rpm for 10 min at 4 °C and washed twice with 10 mM phosphate buffer (pH 8.0). Suspension cells were immobilized onto a porous cellulose nitrate membrane (20 mm diameter, 0.45 μm pore size; Advantec, Japan) by suction using a syringe filter holder. After the cells had adsorbed onto the membrane, another cellulose nitrate membrane was placed on the immobilized microorganism membrane to make a sandwich.

2.4. Biosensor system

TCE is decomposed by *Pseudomonas* sp. strain ASA86, releasing chloride ions. The released ions are detected by a chloride ion electrode which is an ion selective electrode, and then exchange into TCE level corresponding to its ion level. The biosensor system included a chloride ion electrode (7024L, DKK, Japan), an ion meter and an electronic recorder (EPR-200A, TOA electronics, Japan) (Fig. 1). The chloride ion electrode consisted of a working electrode with an ion-selective $AgCl/Ag_2S$ membrane, and an Ag/AgCl reference electrode. The biofilm was placed on the working electrode and fixed in place using 200-mesh nylon and an 'O'-ring. The microbial biosensor was preconditioned by immersing in 10 mM phosphate buffer (pH 8.0).

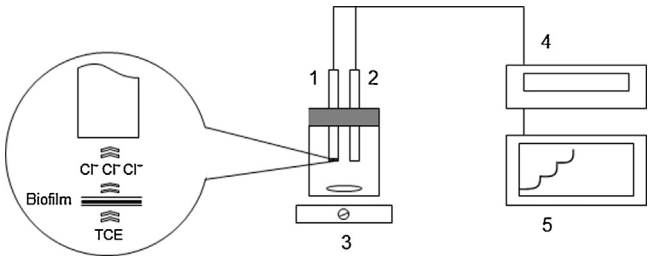

Fig. 1. Schematic representation of the TCE biosensor assembly. (1) a working electrode, (2) a reference electrode, (3) a stirrer, (4) an ion meter, and (5) a recorder.

2.5. Experimental procedure

The microbial biosensor was inserted into the reaction cell containing 50 mL of 10 mM phosphate buffer. The reaction cell was maintained at 30 °C using a control temperature water bath. During the measurements, the buffer solution was continuously stirred with a magnetic bar. The vapor pressure of TCE is approximately 65 mm Hg at 30 °C [21,26]. We made sure that the reaction cell hardly had headspace to suppress volatility of TCE and thereby promote the performance of the reaction cell through higher reproducibility and lower limit of detection. The output signal was recorded using an ion meter and an electronic recorder (EPR-200A, TOA electronics, Japan). The biosensor response was evaluated using the steady-state method [11]. After the output signal reached equilibrium, TCE, tetrachloroethylene (PCE), 1,1-dichloroethylene (1,1-DCE), 1,2-dichloroethylene (1,2-DCE), 1,2-dichloroethane (1,2-DCEA) or vinyl chloride (VC) were injected into the reaction cell containing the phosphate buffer solution. The output signal was continuously monitored until it reached a steady state. The sensor response was defined as the difference between the signal before and after injection of the sample.

3. Results and discussion

3.1. Characterization of TCE biosensor response

As shown in Fig. 1, the microbial biosensor for the TCE detection employs a chloride ion electrode. Chloride ions are released from TCE, which is degraded by the immobilized bacteria on the biofilm. The concentration of chloride ions, determined using an ion electrode, was exchanged for TCE level. The characteristics of the biosensor response are shown in Fig. 2. The relationship between the biosensor response and TCE concentration was determined at a TCE concentration range of 0.05–4 mg/L. Three measurements were conducted at each concentration level. The response was linear over the above concentration range. The correlation coefficient (R^2) was 0.9913, which shows a good relationship between the biosensor response and TCE concentration. The limit of detection (LOD), calculated as 3σ, was 0.05 mg/L TCE. This value is close to that of the microbial biosensor using *Pseudomonas aeruginosa* JI104 [22], and extremely lower than that of the *Pseudomonas fluorescens* A506 biosensor, which had an LOD of 0.2 mg/L [26], but slightly higher than that of the *Pseudomonas putida* F1 biosensor, which had an LOD of 0.02 mg/L [24]. The relative standard deviation (RSD) of reproducibility of the biosensor was 6.1% at 0.5 mg/L TCE, which indicates high reproducibility. The response time of the biosensor at the TCE concentration range measured was about 5 min, which is fast, considering that microbial biosensors generally have a response time of about 10 min.