Letters to the Editor

Biological Oxygen Demand Sensor Using an Arsenic Resistant Bacterium

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Various microbial biosensors, including BOD (biological oxygen demand) sensors, have been reported.[1,2] These are usually constructed by holding intact microorganisms, such as bacteria and yeasts, near the tip of an oxygen electrode. Since microorganisms consume organic compounds quantitatively, their concentrations can be determined by estimating the respiration activity of the immobilized microorganisms with the oxygen electrode.

Many microorganisms have variants which show a resistance to toxic substances.[3] We have studied the bioaccumulation of arsenic (As) by freshwater algae isolated from arsenic-polluted enviroments.[4] In the course of our studies we isolated some bacteria contaminated with an algae culture containing As(V); one of them was identified as *Pseudomonas putida*. This bacterium is an aerobic heterotroph which exhibits extreme resistance to As(V); it can even multiply at concentrations above 5000 mg l^{-1} of inorganic As(V).[5] It is known that ordinary bacteria become extinct when the surrounding As(V) level exceeds 1000 mg l^{-1}.[3] *P. putida* ingests carbohydrates and proteins. Therefore, if the bacterium is empolyed in microbial sensors, BOD biosensing can be conducted in the presence of high levels of As(V).

To our knowledge, microbial sensors have not yet been studied regarding the use of resistant microorganisms. The use of such microorganisms is advantageous because long-term sensor stability can be envisaged, since other microorganisms hardly contaminate a microbial sensor when the latter is continuously used in a medium containing toxic substances; such contamination frequently causes serious problems in the operation of microbial sensors.[6]

Experimental

The sources, isolation and culture of the arsenic reistant bacterium, *P. putida*, have been described elsewhere.[5] The bacterium was grown in a peptone medium (100 ml) containing 1 g peptone, 0.5 g NaCl and 0.1 g yeast extract in the presence of 100 mg l^{-1} As(V) (Na_2HAsO_4). The bacterium was separated by centrifugation and washed several times with sterilized water; 0.8 g (wet weight) of the bacterium was suspended in 25 ml of sterilized water. Two milliliters of the suspension was filtered through a porous acetyl cellulose membrane (Millipore Co., type HA, 0.45 μm pore size, 16 mm diameter, 150 μm thickness) attached with a doughnut-shaped PVC patch (16 mm o.d., 10 mm i.d., 250 μm thickness) under slight suction. Another acetyl cellulose membrane was attached; as a result, the bacterium (*ca.* 8×10^9 cells) was sandwiched by the two membranes.

The bacterial membrane was soaked in a buffered solution (0.01 M (1 M=1 mol dm^{-3}) KH_2PO_4-Na_2HPO_4, pH 7.0) for one day under air bubbling, and was then placed on the tip of an oxygen electrode in a biosensor kit (Denki Kagaku Keiki Co. (DKK) 7842) equipped with a DKK FLC-41 flow cell and a water jacket (30°C). The current output of the oxygen electrode was measured with a DKK PHL-40 voltage meter connected to a current-voltage converter (IVC-200). Tap water saturated with dissolved oxygen was transferred into the flow cell at a rate of 4 ml min^{-1}; after the current output of the electrode reached a steady state, BOD standard solutions contatining various concentrations of As(V) were injected into the flow cell for 5 min. The BOD standard solution was prepared according to JIS 0120-1974 by the use of glucose, glutamic acid and a phosphate buffer (0.01 M KH_2PO_4-Na_2HPO_4, pH 7.0).

Results and Discussion

Figure 1 shows typical response curves of the microbial sensor to various concentrations of BOD standard solutions containing 1000 mg l^{-1} As(V). When the organic compounds dissolved in a BOD standard solution permeate through the bacterial membrane, each immobilized bacterium ingests the organic compounds and, thereby , consumes dissolved oxygen. The amount of oxygen around the tip of the oxygen electrode thus decreases with time. As can be seen in Fig. 1, the current output of the electrode decreased

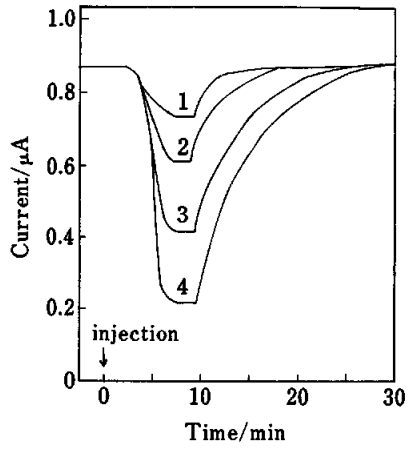

Fig. 1 Response curves of the microbial sensor. 11 mg l^{-1} (1), 22 mg l^{-1} (2), 44 mg l^{-1} (3) and 66 mg l^{-1} (4) of the BOD standard solutions containing 1000 mg l^{-1} As(V) were injected.

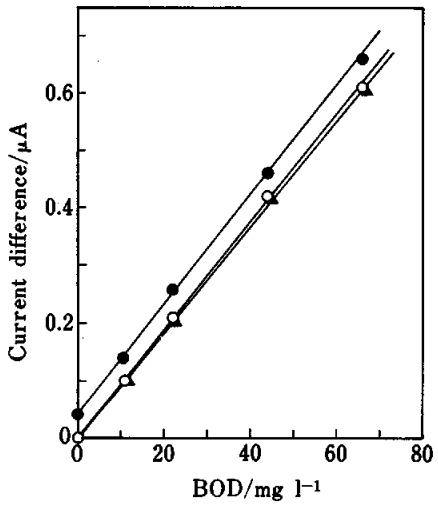

Fig. 2 Calibration curves of the microbial sensor in the presence of 0 mg l^{-1} (○), 100 mg l^{-1} (▲) and 1000 mg l^{-1} (●) of As(V).

and reached a steady state within 8 min after injecting the standard solution. The difference in the current output between the initial and the steady state stages was in proportion to BOD in the solution.

Figure 2 shows calibration curves of the microbial sensor under various concentrations of coexisting As(V). Both in the presence and in the absence of As(V), the plot exhibits good linearity within the range 0–66 mg l^{-1} BOD.

The current difference is systematically larger in the presence of 1000 mg l^{-1} of As(V) than in its absence. The oxygen concentration in the standard solution was independent of the concentration of coexisting As(V). Silver *et al.* reported that a variant of *Escherichia coli* showing As(V) resistance possesses a specific As(V) efflux pump.[3] Such an efflux pump presumably also exists in As-resistant *P. putida*; the action of the pump under high concentrations of As(V) would promote the oxygen consumption.

When a commercial microbial membrane (in which a yeast *Trichosporon cutaneum* is immobilized) was used[2], BOD sensing was not possible in the presence of 1000 mg l^{-1} of As(V).

The BOD values for several organic compounds were assayed in the absence of As(V); 0.78 g g^{-1} for glucose, 0.41 g g^{-1} for glutamic acid, 0.85 g g^{-1} for acetic acid and 0.50 g g^{-1} for ethanol. These values are comparable to those found by the use of the commercial microbial membrane mentioned above. These BOD values were almost independent of the concentration of coexisting As(V), except that a slight increase and decrease were noted in the values for glucose and for glutamic acid, respectively, with an increase in the As(V) concentration.

The current output of the microbial sensor was nearly constant during 50 runs which took place over a period longer than a week. Sensors using organisms resistant to other toxic substances are currently under study.

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