Electrode System for Determination of Microbial Cell Populations in Polluted Water

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Microbial cell populations in polluted water were determined by using a fuel cell-type electrode. The electrode was composed of a Pt anode, a Pt-K4Fe(CN)6-K3Fe(CN)6 cathode, and a cation-exchange membrane for separating two electrode compartments. The principle of microbial cell number determination is based on sensing a redox dye reduced by microorganisms with the electrode. Sample solutions containing microorganisms, a redox dye (thionine), and peptone were purged with oxygen-free nitrogen during the determination. A linear relationship was obtained between the increasing rate of current and the number of microbial cells measured by the colony count method above 106 cells per ml. The determination time varied with the number of microbial cells determined from 20 to 60 min for 3.6 × 106 and 3.6 × 107 cells per ml, respectively.

The determination of microbial cell populations in polluted water is important for environmental protection. The colony count method is widely used to determine microbial cell numbers in polluted water. However, this method is time-consuming and not suitable for the continuous determination of cell numbers. Recently, several electrochemical methods, such as impedance measurement (1) and potentiometry (2, 7, 8), have been developed, but reproductions of the data were poor since these methods were based on bacterial metabolism.

Matsunaga et al. (3, 4) developed two fuel cell-type electrode systems for the continuous determination of cell numbers. However, these electrode systems were based on the direct oxidation of microorganisms on the surface of the anode, so they could be used only in instances of high cell populations such as fermentation industries. Nishikawa et al. (5) have developed another fuel cell-type electrode system coupled with a redox dye (2,6-dichlorophenol-indophenol). The electrode system was used to determine populations in the range of 104 to 106 cells per ml by preconcentration of microorganisms on a porous acetylcellulose membrane. In this paper a new fuel cell-type electrode system is described. We have used this system to determine microbial cell populations in polluted water.

MATERIALS AND METHODS

Culture of microorganisms. Escherichia coli AS 1.129 and Pseudomonas aeruginosa AS 1.512 were obtained from the Institute of Microbiology, Academy of Science of China. Each species was grown at 37°C for 24 h in 50 ml of medium (pH 7.0) containing 0.15 g of beef extract, 0.5 g of peptone, and 0.25 g of NaCl under aerobic conditions (water bath shaker, 180 rpm). Polluted water samples were taken from the Jinchuan River.

Apparatus. Figure 1 shows the electrode system for determining microbial cell populations. It consists of a platinum anode (2 by 3 cm), a platinum cathode (1 by 4 cm), and a cation-exchange membrane (1.5 by 2.5 cm). Saturated K4Fe(CN)6-K3Fe(CN)6 solution was used as the catholyte (45 ml). The current was measured with a digital multimeter (Univolt, model DT-830).

Procedure for determination of microbial populations. Microbial populations were determined as follows. The cultured cells were centrifuged at 3,000 × g for 20 min. The cells were suspended in an appropriate amount of physiological saline and kept at 0°C. The anode compartment was washed several times with sterilized water. Phosphate buffer (0.1 M) was used as the anolyte (150 ml). A saturated calomel electrode was inserted into the anolyte from the sample inlet. The anolyte containing 200 μM thionine and 0.3 g of sterilized peptone was purged continuously with oxygen-free nitrogen. When the anode potential reached ca. −130 mV (standard calomel electrode [SCE]), an appropriate amount of cell suspension was introduced into the anolyte and the current was measured at 37°C.

For the determination of microbial cell populations in polluted water, a polluted water sample diluted with an appropriate amount of sterilized water was used as the

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anolyte. Five milliliters of 6 M NaCl solution was added to the anolyte to increase conductivity. When the anode potential reached ca. -130 mV (SCE), 5 ml of thionine solution (6 mM) was introduced into the anolyte and the current was measured.

Measurement of microbial populations by the conventional method. The number of microorganisms was measured by plating suitable diluted portions of a sample and counting colonies that appeared after 48 h of incubation at 37°C.

RESULTS AND DISCUSSION

Variation of anode potential with time. Figure 2 shows the time course of the anode potential of the electrode. The potential decrease was slow in the initial 10 min and became rapid when the potential was below -136 mV (SCE). Obviously, a trace amount of dissolved oxygen in the anolyte affected the reduction of the redox dye by microorganisms at the initial stage. Microorganisms can rapidly reduce the redox dye only when dissolved oxygen is exhausted. Therefore, the experiments were performed at an anode potential below -130 mV (SCE).

Effect of oxidizable substrates. The effect of oxidizable substrates on the time courses of the current of the electrode is shown in Fig. 3. The lag period was shorter and the increasing rate of current was higher when peptone or yeast extract was added. It was impossible for the cells that were in the steady period of growth to grow in such a short time under the oxygen-free condition. Therefore, peptone was used as an oxidizable substrate.

Time course of current for various cell populations. Figure 4 shows the time courses of the current for various cell populations. It can be seen that after the lag period the current was linear with time and the higher the cell populations, the higher the increasing rate of current.

It has been confirmed that the anodic reaction of the electrode is diffusion controlled (9). Therefore, the current of the electrode can be expressed as \( i = nFDC_{red}\delta \), where \( i \) is the current density of the electrode, \( n \) is the number of electrons transferred per molecule of thionine, \( F \) is the Faraday constant, \( D \) is the diffusion coefficient, \( C_{red} \) is the concentration of reduced thionine, and \( \delta \) is the thickness of the diffusion layer.

The speed of magnetic agitation being fixed, \( \delta \) is a constant. The increasing rate of current depends on the rate of dye reduction by microorganisms:
where \( t \) is the time.

It has also been confirmed that the kinetics of dye reaction by microorganisms conforms to a Michaelis-Menten type of expression after the lag period (9):

\[
di \cdot nFD = \frac{1}{\delta} \cdot \frac{dC_{\text{red}}}{dt}
\]

(1)

where \( C_{\text{oxi}} \) is the concentration of oxidized thionine, \( V_{\text{max}} \) is the maximum specific dye reduction rate, \( K_s \) is the semisaturation constant, and \( x \) is the cell population.

Because \( C_{\text{oxi}} \approx K_s \) in the experiments, equation 2 can be written as

\[
\frac{dC_{\text{red}}}{dt} = V_{\text{max}} \cdot x
\]

(3)

Substituting equation 3 into equation 1, we obtain \( di/dt = kx \), where \( k = nFDV_{\text{max}}/\delta \) is a proportionality constant.

It can be seen that the increasing rate of current is directly proportional to the cell population.

**Effects of temperature and dye concentration.** Figure 5 shows the effect of the thionine concentration on the increasing rate of current. The rate increased linearly with increasing concentrations of thionine below 40 \( \mu \)M, but it hardly increased with increasing concentrations of thionine above 140 \( \mu \)M. To ensure that the dye was sufficient for higher cell populations, the thionine concentration was fixed at 200 \( \mu \)M.

The effect of temperature on the increasing rate of current is shown in Fig. 6. The rate increased with rising temperatures. A further rise in temperature was not examined because the ion-exchange membrane could not be used at temperatures above 40°C. The temperature was fixed at 37°C in order to obtain a higher increasing rate of current.

**Calibration curves and determination of cell populations in polluted water.** Figure 7 shows the calibration curves for the determination of *E. coli* and *P. aeruginosa*. Figure 8 is the calibration curve for the determination of microorganisms in polluted water from the Jinchuan River (1 to 140 ml of polluted water samples was used). A linear relationship was obtained between the increasing rate of current and the number of cells measured by the colony count method above \( 10^4 \) cells per ml.

The determination of cell populations in polluted water from the Jinchuan River is shown in Fig. 9. The solid line is the calibration curve determined on 3 July. The points show the data measured after 3-, 6-, and 9-day intervals. Although over a long time the proportion of different species of cell number may vary, the fact that the points are located near
the solid line shows that the calibration curve is available for a week or longer.

The following conclusions can be derived from above: microorganisms significantly reduce thionine only when the anode potential is below ~136 mV (SCE); a higher increasing rate of current can be obtained with peptone or yeast extract as an oxidizable substrate than with glucose; the increasing rate of current is linear with microbial populations above 4 \times 10^4 cells per ml; the thionine-coupled electrode system can be applied to determine rapidly and simply microbial cell populations in polluted water; and the determination time varies with the number of cells determined from 20 to 60 min for 3.6 \times 10^6 and 3.6 \times 10^4 cells per ml, respectively.

It should be noted that this microbial electrode system has a much lower detectable limit and a much wider detectable range for the determination of microbial cell populations as compared with non-dye-coupled microbial electrode systems (3, 4). In the present study the microbial cell populations are correlated with the increasing rate of current. So long as the thionine is increased properly, the high detectable limit can be raised and the low detectable limit can also be lowered by prolonging the determination time. Therefore, the detectable range of this system is wider than that of the microbial electrode systems of the fuel cell type with 2,6-dichlorophenol-indophenol and phenazine ethosulfate as electron transfer mediators (5, 6).

LITERATURE CITED


