Articles

Comprehension of the Response Time in a Microbial BOD Sensor(II)

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A microbial BOD sensor using immobilized *Hansenula anomala* was prepared for the estimation of BOD. The sensor voltage was increased with time and increasing concentration of GGA when it was inserted in a sample solution. A linear relationship was obtained with a correlation coefficient, 0.998 between the concentration of standard GGA solutions and dV/dt by using the initial change of voltage, in which the response time was 20 min. It could be concluded that the oxidation of GGA conformed to a first-order kinetics. Therefore, the good linearities were also observed at various times. This sensor showed the best linearity at 30 min.

Introduction

Biochemical oxygen demand(BOD) implies the amount of dissolved oxygen required to oxidize organic pollutant by microorganisms. It is one of the important indexes which show the polluting level of waste water. In the estimation of BOD, the total process is divided into two stages. The first is due to the oxidation of carbonaceous matter while the second is due to the oxidation of nitrogenous organic compounds. A great number of studies on the biochemical oxidation of organic compounds have been reported up to date. They have been chiefly discussed with respect to a rapid estimation of carbonaceous matter, that is, 5-day BOD.[1] However these methods take 5 days and thus are unstable for process control. Therefore, a more rapid estimation of BOD was developed by using a microbial sensor containing whole cells immobilized on an oxygen electrode. A number of reports on the microbial BOD sensor have been published since 1977.[2]

To determine the response time in BOD sensors, there are two possibilities of measurement; (i) end-point(steady-state) measurement and (ii) the kinetic measurement. Most BOD sensors used the steady state measurement and showed the response time of 15-40 min. Karube *et al.*, estimated the BOD within 10-15 min[2] and 30-40 min[3] for *Clostridium butyricum*, and Hikuma and his coworkers[4] measured within 20 min when *Tricosporon cutaneum* was employed for the sensor.

On the other hand, Karube *et al.*[5] and Riedel *et al.*[6,7] used the kinetic measurement to determine the response time of the BOD sensor, and obtained the output signal with current.

The purpose of this research is to discuss the estimation of BOD kinetically and explain the response time showed in the previous work[8].

In that work, the voltage was employed as the output signal, and the response time was determined by reading the voltage at 30 min sharp after the BOD sensor was soaked in the sample solutions.

Experimental

Chemicals. Yeast extract and agar were obtained from Difco Lab. Glucose, glutamic acid, acrylamide, N,N'-methylenebisacrylamide, potassium persulfate, and dimethylaminopropionitrile were purchased from Sigma Chemical Co. Peptone(from casein) was purchased from Kyokuto Phamarceutical Co. Other reagents were commercially available analytical reagents or laboratory grade materials. A standard solution (5-day BOD; 220 ppm) containing glucose (150 mg/L) and glutamic acid (150 mg/L)(GGA) was employed as a model waste water according to Japan Industrial Standard (JIS, Japanese Industrial Standard Committee).[9]

Culture and Immobilization of Microorganisms. The culture and immobilization of microorganisms, *Hansenula anomala* (NRRL Y-7174) have been described in the previous work.[8] The microorganisms were grown in the solid peptone medium (1000 m*l*) containing 10 g peptone, 10 g glucose, 5 g yeast extract, 1 g NaCl, and 20 g agar.

0.3 g of intact cells directly removed from the solid medium and 0.1 g of a mixture of 90% acrylamide and 10% *N,N'*-methylenbisacrylamide were suspended in a syringe (5 m*l*) containing 1 m*l* of sterilized water. The suspension was saturated with nitrogen gas, and then 0.25 m*l* of 100 g/L dimethylaminopropionitrile and 12.5 mg of potassium persulfate as the polymerization initiators were added to the suspension. 0.5 m*l* of the suspension was dropped on the area of 8 cm^2 (thickness 1 mm). The plate was allowed to proceed anaerobically for 30 min at 30℃.

The schematic diagram of the microbial BOD sensor was illustrated in the previous work.[8] An oxygen electrode (Model 97-08, Orion) consisted of a gas-permeable membrane, a silver cathode and anode. The prepared microbial membrane was placed carefully on the gas-permeable membrane of the oxygen electrode so that it was fixed with a bored glass cap.

Procedure. The microbial BOD sensor was immersed in a sample solution saturated with dissolved oxygen and stirred magnetically while measurements were taken. The

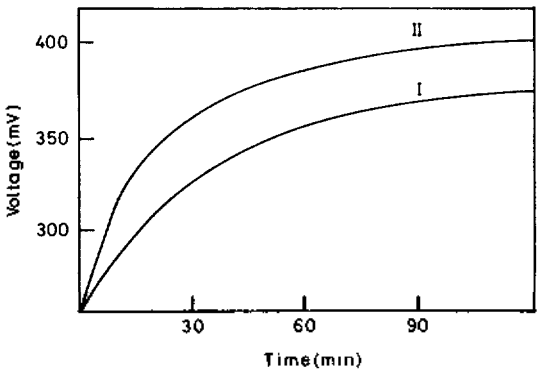

Figure 1. Response curves of the microbial sensor. 45 ml of 0.1 M phosphate buffer (pH 7.0) containing glucose and glutamic acid (I. 10 mg/L and II. 20 mg/L) was employed at 30℃.

sample solutions were diluted with 0.1 M phosphate buffer solution (pH 7.0) before use in the experiments. The temperature of the sample cell was maintained at 30℃ by circulating the temperature-controlled water through the jacket with Forma Scientific Bath and Circulator-2067.

The voltage and pH of the sample solutions were measured with an Orion Expandable ionAnalyzer EA 940 and a Beckman Century SS, respectively. The values of output voltage were recorded every one minute on a Seikosha SP-1600AS recorder(Japan). To compare the response of the microbial BOD sensor with the conventional 5-day BOD, the 5-day BOD of the sample was tested by the "standard method" according to JIS.[9]

Results and Discussion

Figure 1 shows typical response curves of the microbial BOD sensor using *Hansenula anomala*. When the BOD sensor was immersed in a sample solution, the consumption of oxygen by immobilized microorganisms began and caused a decrease in dissolved oxygen around the membrane. As a result, the output voltage of the sensor increased gradually with time until a steady state was reached within about 2 hr. The steady state indicates that the consumption of oxygen by a microbial membrane and the diffusion of oxygen to the membrane from bulk solution are in equilibrium. As shown in Figure 1, thus, the steady state voltage depended on the concentration of glucose and glutamic acid.

When the initial change of voltage was measured, instead of the steady state voltage, the time needed for one measurement could be shortened to 15 min. Figure 2 shows the relationship of dV/dt (corresponding to the initial change of voltage) and the concentration of standard solution. The correlation coefficient of this linearity is 0.998.

Howevere, because all of the sample solutions are saturated with dissolved oxygen, the initial partial pressure of oxygen is excessive in the case of the BOD sensor, and the oxygen electrode responds to the remained oxygen after the reaction by microorganisms. The reaction is described by the equation

$$n\text{GGA}+m\text{O}_2 \xrightarrow{\text{microorganisms}} \text{Product}+\text{O}_2\,(\text{remained}) \quad (1)$$

and this equation is expressed kinetically as follows:

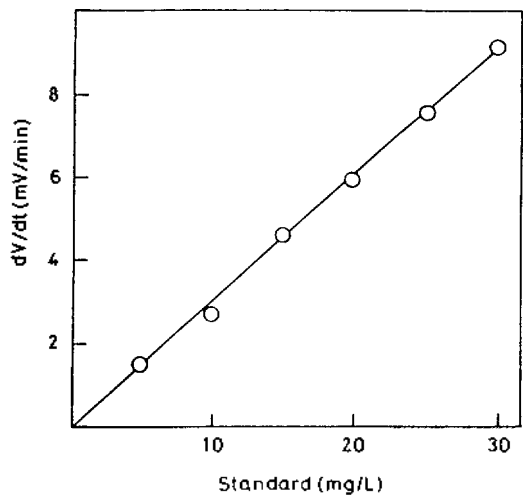

Figure 2. Relationship of the dV/dt and the concentration of glucose and glutamic acid.

$$-\frac{d([\text{O}_2]_i-[\text{O}_2]_r)}{dt}=k_1'[\text{GGA}]^n[\text{O}_2]_i^m \quad (2)$$

where k_1' is a rate constant, $[\text{O}_2]_i$ is the initial concentration of oxygen, and $[\text{O}_2]_r$ is the concentration of oxygen passed through the microbial membrane. The initial concentration of oxygen is a constant because it is saturated in the sample solution. Thus Eq. (2) gives

$$\frac{d[\text{O}_2]_r}{dt}=k_1[\text{GGA}]^n \quad (3)$$

where k_1 is a new rate constant. According to the experimental results (Figure 1) and the theory (Eq. (3)), the output voltage is proportional to the decreasing rate of glucose and glutamic acid concentration, and the remained partial pressure of oxygen. Consequently, the change of voltage produced on the oxygen electrode with the lapse of time is as follows:

$$\frac{d[\text{O}_2]_r}{dt}=\frac{dV}{dt}=k_1[\text{GGA}]^n \quad (4)$$

where V is the output voltage by the sensor.

As the results, n is one in Eq. (4) because the initial increasing rate of voltage is proportional to the concentration of glucose and glutamic acid. Thus it can be concluded that the process of the oxidation of GGA (Eq. (1)) conforms to first-order kinetics. Therefore, in order to obtain the BOD values more precisely, the vertical axis was plotted to the absolute voltage, which was read on the recorder directly instead of dV/dt, as shown in Figure 3, and the good linearities, thus, were given also for all samples. The voltage values were read at 25, 30, 35 and 40 min sharp, respectively, after the BOD sensor was immersed in sample solutions. The correlation coefficients of the calibration curves are 0.996, 0.999, 0.998 and 0.997 at 25, 30, 35 and 40 min, respectively, in Figure 3.

The response time is one of the important properties for the electrodes. The various definitions of the response time for the ion selective electrodes have been proposed.[10-12] However in the biosensor, the steady state and the kinetical signal have been accepted for the determination of response

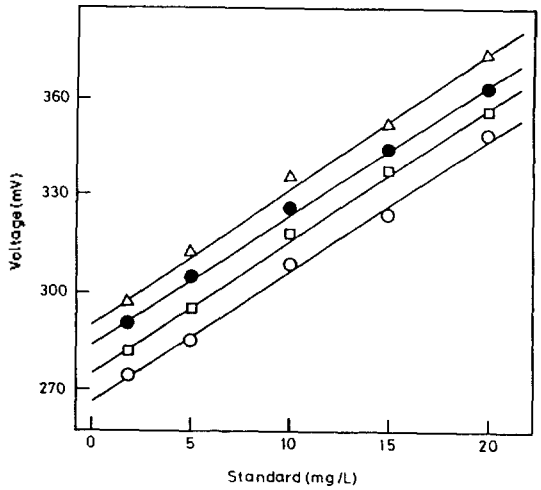

Figure 3. Calibration curves of the microbial sensor at 25(○), 30(□), 35(●) and 40 min(△).

time. As the result of the investigations concerning the kinetics of the biochemical oxidation of waste water, Streeter and Phelps concluded that the rate of oxidation of carbonaceous matter at any instant during the first stage accordingly conformed to first-order kinetics.[13]

Stones[14] proposed that the rate of oxidation of carbonaceous matter in waste water was proportional to both its unsatisfied BOD and the residual dissolved oxygen(DO) concentration, and that the process therefore conformed to a second-order kinetics.

In addition, when the size of inocula and the microbial activities in the system is considered, the third-order kinetics was proposed for the oxidation of carbonaceous organic compounds on the basis of analysis of BOD experimental results. In this sensor, the size of inocula and the microbial activities are under the same condition for all samples as well as it also was proposed that the process can be converted into a first-order kinetic equation according to the experimental results.[15]

Consequently, the calibration curve of 30 min sharp after the sensor was immersed in the sample conforms to a first-order kinetics and has the best linearity, and thus it can be employed for the estimation of BOD. This point coincides with the result to previous report.[8]

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