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Study of BOD Microbial Sensors for Waste Water Treatment Control

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ABSTRACT

A microbial sensor consisting of immobilized yeast or bacterial cells and an oxygen electrode was developed for the estimation of biochemical oxygen demand (BOD). A flow-through system was used, and the response time was within 20 min. A linear relationship was observed between the relative current decrease and the BOD of the sample solution within the range of 1–45 mg/L. The storage lifetime was >1 yr. The reproducibility was quite good, within 6% fsd at a concentration of 20 mg/L BOD. Satisfactory results were attained when the biosensor was applied to the determination of BOD in brewery-plant and glutamate-plant wastewater and in a river.

Index Entries: BOD; dissolved oxygen electrode; biosensor; microbial sensor; wastewater analysis.

INTRODUCTION

Biochemical oxygen demand (BOD) is one of the critical indices that indicate the degree of pollution by biomaterial in water. Since the conventional 5-d method for BOD estimation is time-consuming, laborious, and tedious, there is an urgent need to develop a fast-response BOD-measuring system to show the results of sewage treatment. For this purpose, American and Japanese scientists have invented quick BOD-measuring biosensors using immobilized microbes and a dissolved-oxygen (DO) electrode (*1–3*). However, there is still much room for improvement in BOD biosensors. The aim of current research is to work out a fast BOD-

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measuring system that shows good reproducibility of performance, high sensitivity, a wide range of linearity, and a long storage lifetime. Various factors that affect the assay of BOD have been investigated.

MATERIALS AND METHODS

Strain and Culture Media

Hansennula anomala, which was provided by the Shanghai Research Institute of Industrial Microbiology, and *Pseudomonas* sp, which was isolated from sewage, were cultured in mediums A and B respectively. The mediums were composed of the following nutrients (per liter):

- Medium A: lactic acid (60%), 22.5 mL; NH_4Cl, 2 g; KH_2PO_4, 3 g; $CaCl_2$, 0.25 g; $MgSO_4 \cdot 7H_2O$, 0.25 g; pH 5.0.
- Medium B (g/L): beef extract, 5; peptone, 10; NaCl, 5; agar, 15; pH 7.4–7.6.

Preparation of Biomass

Biomass from *H. anomala* was prepared by the method presented elsewhere (4).

Isolation of *Pseudomonas* sp from Sewage

A sample from sewage disposal was allowed to stand for about 5 min, and the supernatant was then diluted and transferred onto a Petri dish containing the following medium (g/L): beef extract, 5; peptone, 10; NaCl, 5; agar, 15, pH 7.4–7.6, and incubated at 30°C for 1–2 d. A single colony was then inoculated into an agar slant with the same medium, and cultured at 30°C for 1 d.

Immobilization of Microbial Cells

A "sandwich" method was used in this study. A tiny amount (<1 mg dry cell wt) was sandwiched between fluorinated ethylene propylene (FEP) membrane and cellulose acetate membrane (pore size: 0.45 μm).

Configuration of the BOD Biosensor

The BOD biosensor was composed of a DO electrode and a thin-layer microbial membrane. The DO probe that was developed in our laboratory consists of a Ag cathode, a Pb anode, an electrolyte, and a FEP membrane (25 μm). The microbial cells were adhered closely to the surface of the FEP membrane and covered with a cellulose membrane. The two membranes were fixed together with a stainless steel device, the inner part of which served as a membrane fixing nut and the other part of which was a

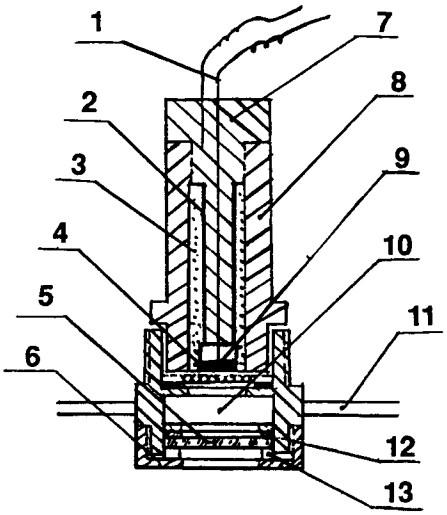

Fig. 1. The structure of the BOD electrode: (1) lead, (2) anode, (3) electrolyte, (4) biomembrane, (5) peep glass, (6) peep glass fixing nut, (7) interior electrode body, (8) Teflon™ housing, (9) cathode, (10) sample chamber, (11) stainless steel tube, (12) silicone washer, (13) stainless steel washer.

sample chamber (V=1.72 mL) composed of a silicone washer, a viewing glass, a stainless steel washer, and a viewing-glass fixing nut. Two stainless steel tubes with 3-mm inner diameter, were soldered at two sides of the sample chamber. The inlet tube was connected to a peristaltic pump by a silicone tube (Fig. 1). The configuration of the biosensor possesses the following advantages:

1. High sensitivity, by virtue of the small volume of the chamber;
2. Easy-to-clean chamber; and
3. Being equipped with a viewing glass, so that bubbles can be easily observed and driven out in time.

Establishment of the BOD Electrode Measuring System

A flow-through measuring system was designed in this study. Fig. 2 is a schematic diagram of a BOD measuring system. A beaker, which contains buffer or sample, was put into the thermostated water bath and saturated with air. The buffer was then pumped into the chamber at a flow rate of 40 mL/h. When the DO in the buffer permeated through the porous membrane and diffused into the microbial cell at a rate equal to the endogenous respiration rate of the organism, a steady state of current output could be obtained. Then the sample or standard BOD solution (consisting of equal parts of glucose and glutamic acid, 150 mg/L) was pumped into the chamber, and organic compounds permeated through

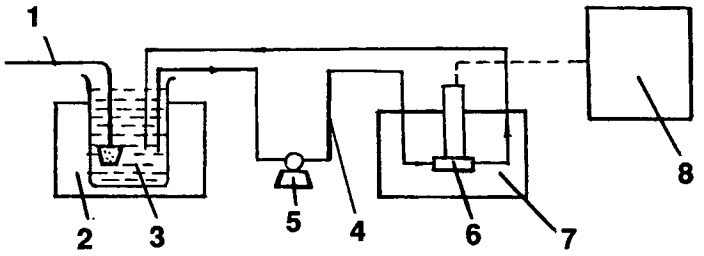

Fig. 2. Schematic diagram of the BOD measuring system: (1) air pump, (2) thermostated water bath, (3) beaker, (4) silicone tube, (5) microperistaltic pump, (6) BOD electrode, (7) super-thermostated water bath, (8) potential difference recorder, model XWC-100.

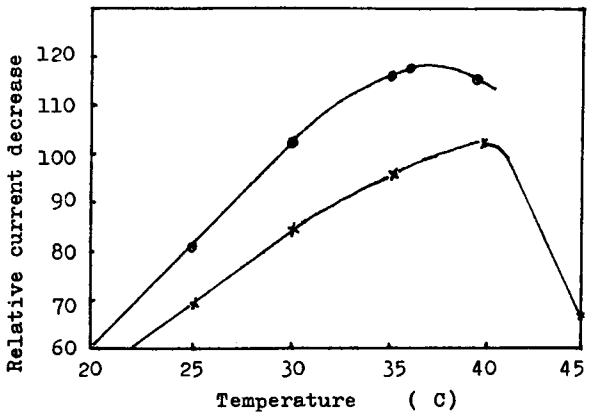

Fig. 3. The influence of temperature on the response value of BOD electrode: O——O: *Hansenula anomals; x——x: Pseudomonas* sp.

the porous membrane and were assimilated by the immobilized microorganisms. As a result, the current of the electrode decreased markedly with time until another steady state was reached; thus, the difference of the monitored current outputs can be used to represent the BOD value.

RESULTS AND DISCUSSION

Factors Influencing the Response of a BOD Sensor

Temperature

The results shown in Fig. 3 indicate that the response value of the biosensor reaches its maximum at 37 and 40°C for *H. anomala* and *Pseudomonas* sp., respectively. In order to prolong the activities of the microbes,

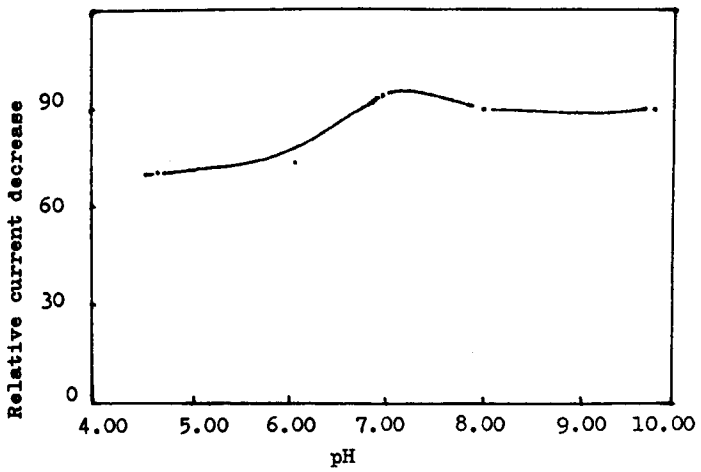

Fig. 4. The influence of pH on the response value of the BOD electrode.

Table 1
Influence of Phosphate Concentration
on the Response Value of a BOD Electrode Using *Pseudomonas* sp. Strain

Phosphate concn., mol/L	0.005	0.01	0.02	0.05	0.10	0.20	0.50
Relative current decrease	59.6	62	68.2	80	94	102	70.8

25 and 30°C were chosen to be the operating temperature for *H. anomala* and a *Pseudomonas* sp. strain, respectively.

pH

As shown in Fig. 4, the response value of the biosensor using *Pseudomonas* sp. reaches its peak value at pH 7.0. Hence, pH 7.0 was chosen to be the measuring pH.

Concentration of Phosphate Buffer

Table 1 shows that, with the increase of phosphate concentration, the response of the electrode using a *Pseudomonas* sp. strain increased up to 0.2 mol/L. Since a phosphate concentration of 0.01 mol/L can meet the needs of the buffer capacity, that concentration was used throughout the study.

Heavy-Metal Ion

The results shown in Table 2 reveal that Fe^{2+}, Zn^{2+}, Mn^{2+}, and Cu^{2+} (50 mg/L each) have virtually no effect on the response of the biosensor; on the contrary, Hg^{2+} and Ag^{2+} (50 mg/L each) and Cu^{2+} (100 mg/L) show apparent effects on *Pseudomonas*. In spite of the toxic effect of Cu^{2+} at 100 mg/L, the microbe restored its activities after 2 d of multiplication.

Table 2
The Influence of Heavy-Metal Ions
on the Response Value of BOD Electrode

Heavy-metal ion 50 mg/L	Response of a BOD electrode[a] %		Maximum allowable concn. during the biochemical process mg/L
	Hansenula sp.	*Pseudomonas* sp.	
Fe^{3+}	95.6	96	2.6
Mn^{2+}	100	101	30
Cu^{2+}	94.1	95	0.5
Cu^{2+}, 100 mg/L	—	30	0.5
Zn^{2+}	105.9	98	50
Cr^{3+}	94.9	—	6
Co^{3+}	92.3	—	6
Hg^{2+}	—	70	—
Ag^{2+}	—	37	—
Control	100	100	—

[a]The response of a BOD electrode of a GGA solution with a BOD value of 20 mg/L, in which no heavy-metal ions are added, is regarded as 100%.

Response of the BOD Electrode to Various Substrates

Organic Compounds

Twelve kinds of pure organic compounds were tested. The results shown in Table 3 indicate that the current BOD biosensor with *Pseudomonas* sp. can be applied only to certain kinds of sewage, containing the substrates that can be utilized normally. It is evident that current BOD biosensor is inappropriate to those sewages containing methanol, ethanol, and acetone: perhaps the strain used needs a longer period to adapt such compounds.

In view of its restricted application, we succeeded in expanding the range of application of the current BOD microbial sensor by using the strain newly isolated from the sampling spot. As shown in Table 4, the microbial sensor employing the new isolate shows much better results than that using *Pseudomonas* when applied to the wastewater discharged from a print-and-dye works.

Standard BOD Solution

Phosphate buffer (0.01 mol/L, pH 7.0) was used to prepare standard solutions with various BOD values. Figure 5 shows that there is good linearity between the relative current decrease and the BOD value tested using the biosensor with *Pseudomonas* sp. within the range of 1–40 mg/L. For the biosensor with *H. anomala*, the range of linearity was 1–45 mg/L. When the BOD of real wastewater is located near the standard curve, it can be measured quickly and accurately with such a BOD electrode.

Table 3
Comparison between BOD Values of Various Compounds
Determined by Current Biosensor and Conventional 5-d Method

Organic compound[a]	BOD biosensor,[b] g/g	BOD_5, g/g[c]
Glutamic acid	0.47	0.52–0.64
Glucose	0.53	0.50–0.64
Lactic acid	0.593	0.64
Acetic acid	0.556	0.34–0.88
Acetyl acetate	0.429	0.29–1.13
Butyl acetate	0.444	—
Starch	0.525	0.51–0.68
Sucrose	0.33	—
Ethanol	0.38	0.93–1.67
Ether	0.204	—
Methanol	0.25	0.77–0.93
Acetone	0.213	0.54–1.39

[a]The concentration used was 30 mg/L.
[b]*Pseudomonas* sp. was used.
[c]Values from ref. 5.

Table 4
Comparison between the Results
Determined by a Current BOD Microbial Sensor Using Different Strains

Strain used	*Pseudomonas* sp.	Strain isolated from a print-and-dye works' sewage[a]
BOD_5, mg/L	126	126
BOD mg/L biosensor	50	115
Recovery, %	39.7	91.3

[a]Sample was drawn from a velvet print-and-dye works' wastewater.

Application of the Current BOD Sensor to the Assay of BOD in Wastewater

As shown in Table 5, satisfactory agreements were attained when the BOD biosensor was applied to the determination of BOD in a glutamic acid plant, a starch plant, a brewery plant, and a river.

Specifications of the Current BOD Sensor

Reproducibility

Under 30°C, standard BOD solutions (10 mg/L) were determined repeatedly by the BOD sensor. The average relative deviation was ±6,

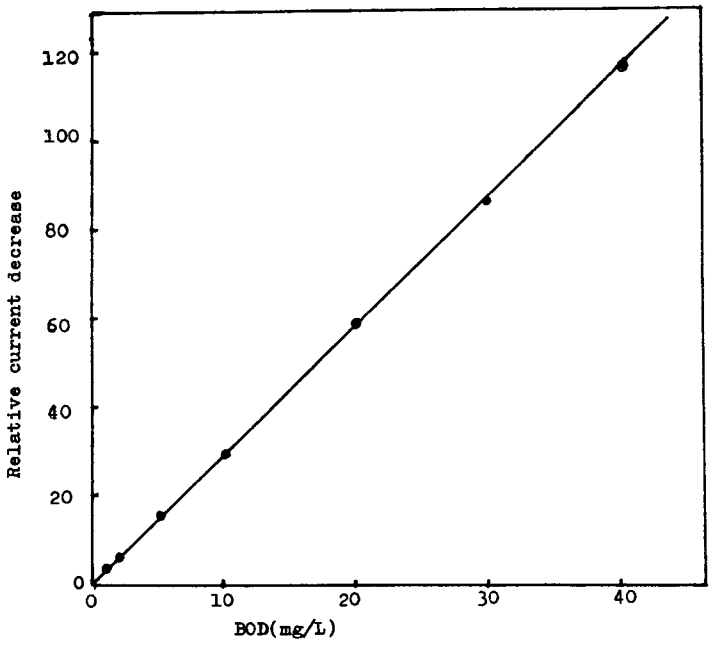

Fig. 5. BOD standard curve of the BOD biosensor.

Table 5
Applications of BOD Electrode to Various Water

Wastewater	BOD_5, mg/L	Measured BOD, mg/L	Recovery, %
River 1[a]	27.6	28.6	103.5
River 2[a]	6.94	7.5	108.0
River 3[b]	11.9	11.3	95.0
Yi Ming brewery[a]	479	484	101.0
Tian Chu glutamate plant[a]	52250	49750	95.2
Tian Ghu glutamate plant[b]	39800	38300	96.2
Shanghai starch plant[b]	1954	1740	89.0

[a]BOD sensor with *Hansenula anomala*.
[b]BOD sensor with *Pseudomonas* sp.

the storage life was over 1 yr, the response time was 13–20 min, and the range of BOD measurement was 1–45 mg/L.

CONCLUSIONS

The BOD sensor, characterized by its thin biomembrane and simple structure, was very useful in shortening the response time of the electrode. The storage method for the biosensor is simple and reliable. The chamber, which is equipped with a viewing glass, is very useful in eliminating air bubbles, which can cause trouble during the measurement. The measuring system of this type of electrode can be applied to other

biosensors in which a DO electrode is used as a basic transducer. The restricted application of the current BOD biosensor can be expanded, provided that the strain isolated from the sampling spot is used.

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