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Microbial BOD Sensor Using *Hansenula anomala*

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A microbial sensor for BOD (Biochemical Oxygen Demand) measurement has been developed by immobilizing *Hansenula anomala* in a polyacrylamide gel. The optimum pH and temperature for BOD measurement using this sensor were pH 7.0 and 30℃, respectively. The response time was 30 min. A linear relationship was observed between the potential and the concentration below 44 ppm BOD. The potential was reproducible within ± 9% of the relative error when a sample solution containing 20 mg/*l* of glucose and 20 mg/*l* of glutamic acid was employed. The effect of various compounds on BOD estimation was also examined. The potential output of the sensor was almost constant for 30 days. The relative error in BOD estimation was within ± 10%.

Introduction

Biochemical Oxygen Demand (BOD) is one of the most widely used and important tests in the measurement of the organic pollution in waste waters, effluents, and polluted waters. The 5-day BOD test has remained a standard pollution monitoring tool since 1936[1]. In the 5-day BOD test, the bottle size, incubation temperature (20℃) and incubation period (5 days) are all specified as well as, furthermore, the skill of operators is also required. Therefore, because the 5-day BOD test is too long and complex for use in process control, rapid and reproducible methods are desirable. In an effort to develop a shorter test for a given sample, a bioelectrochemical sensor consisting of microorganisms immobilized and dissolved oxygen electrode has been developed.

The first BOD sensor was described by Karube *et al.*[2], in which *Clostridium butyricum*-collagen membrane and oxygen probe was used. Furthermore, various microbial BOD sensors using microorganisms, such as *Trichosporon cutaneum*[3,4], *Hansenula anomala*[5], *Pseudomonas* sp.[6], *Escherichia coli*[7], *Bacillus subtilis*[4], and thermophilic bacteria[8] have been developed by many authors.

In this paper, a microbial BOD sensor consisting of immobilized *Hansenula anomala* in a polyacrylamide gel and an

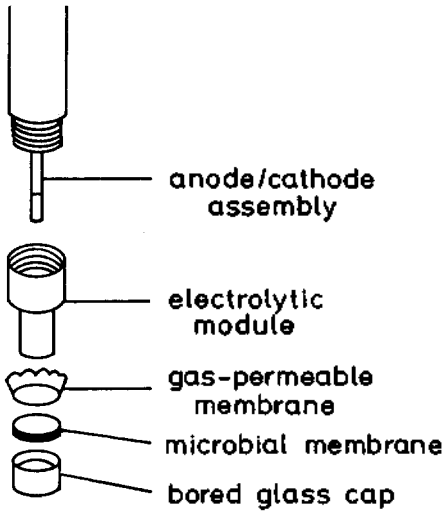

Figure 1. Construction of BOD sensor.

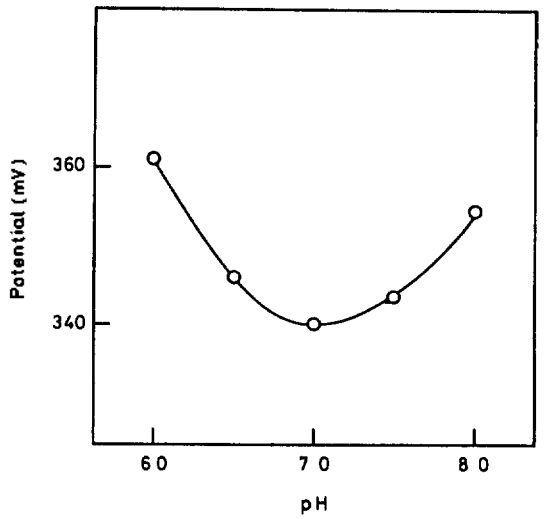

Figure 2. Relationship between the potential and pH when a standard solution (20 mg/*l*) was employed.

oxygen probe was prepared for the estimation of BOD. This sensor was investigated for the effects of pH, temperature and interferences. Then, actually, this sensor was employed for the BOD estimation of various waste waters.

Experimental

Apparatus. An Orion 97-08 oxygen electrode was used in the construction of a microbial sensor. The potential and pH of sample solutions were measured with an Orion Model SA 720 and a Beckman Century SS with a combined glass calomel electrode, respectively. The temperature of sample was controlled by Forma Scientific Bath and Circulator-2067.

Chemicals. Yeast extract was obtained from Difco Laboratories. 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 containing glucose (150 mg/*l*) and glutamic acid (150 mg/*l*) was employed as a model waste water according to Japanese Industrial Standard (JIS)[9].

Culture and Immobilization of Microorganisms. *Hansenula anomala* (NRRL Y-7174) was used for the microbial BOD sensor. It was cultured under aerobic conditions and 28℃ for 24 hr in a solid medium containing 1% glucose, 1% peptone, 0.5% yeast extract, 0.1% NaCl, and 2% 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'-methylenebisacrylamide were suspended in 1 m*l* of deionized water in a syringe (5 m*l*). 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℃.

Construction of Microbial BOD Sensor. The scheme of the microbial BOD sensor is illustrated in Figure 1. The membrane immobilized the microorganisms was set onto a gas-permeable membrane of an oxygen electrode, and fixed with a bored glass cap.

Assay Procedure. The microbial BOD sensor was immersed in 45 m*l* of sample solution, which was saturated with dissolved oxygen and stirred magnetically while measurements were taken. The potential values were read at 30 min sharp after the BOD sensor was soaked in the sample solutions. A solution containing glucose (150 mg/*l*) and glutamic acid (150 mg/*l*) with a BOD value of 220 ppm in 0.1 M phosphate buffer was employed as a standard solution for the calibration curve of the BOD sensor according to the JIS[9].

Results and Discussion

Response Properties of the Sensor. When the microbial BOD sensor was immersed in a sample solution, organic compounds permeated through the porous membrane and then were assimilated by the immobilized microorganisms. The consumption of oxygen by the immobilized microorganisms began and caused a decrease in dissolved oxygen around the membrane. After the sensor was immersed, the output potential gradually increased and a steady state potential was observed within about 2 hr. Recently, several papers have appeared dealing with the response time of ion-selective electrode (ISE) in solutions. In these cases, various definitions such as t_{50}, t_{90}, t_{95}, t_{99} and t^* have been proposed[10-15]. t_r is defined as the time required for the ISE potential to reach r% of its equilibrium potential after a step change in sample activity[10,11,13,14]. Also, t^* is defined as the length of time required for the ISE potential to become equal to its steady value within 1 mV[12]. But in this study, the response time for calibration curve was determined by elapsed time, that is, the potential values were read at 30 min sharp after the BOD sensor was soaked in the sample solutions.

On the other hand, no matter how the sample solution was concentrated, the potential was below *ca.* 400 mV. There-

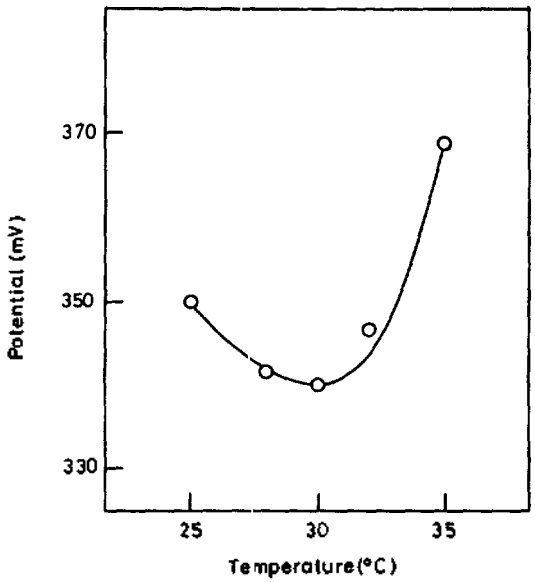

Figure 3. Influence of temperature.

Table 1. Sensor Response to Various Substrates. The Measurements were Carried Out at pH 7.0, 30℃ in the Presense of 20 mg of Each Substrate

Substrate	Sensor response (mV)
glucose	340
glutamic acid	339
maltose	301
mannose	313
galactose	260
sucrose	338
fructose	337
lactose	262
ethanol	260

fore, to obtain the wider linear range, the sensor was investigated to find the conditions with the lowest potential value.

Effects of pH and Temperature. Figure 2 shows the effects of pH on the sensor response, and it was tested at different pH values in a standard solution (20 mg/*l* glucose and 20 mg/*l* glutamic acid) at 30℃. As the result, the activity of the microorganisms is markedly dependent on pH. This microbial sensor showed the best response at pH 7.0, because it gave the widest linear range in that pH. Immobilized microorganisms may be inactivated at lower pH or higher pH. Therefore the pH of the sample was adjusted to pH 7.0.

The influence of temperature on the sensor response is shown in Figure 3. The dependence of temperature of the sensor was studied in the same solution as in the effects of pH. Because, as shown, the optimum temperature was 30℃, the temperature was set in all experiments.

Interferences. The effect of various pure substances, such as sugars and ethanol, on BOD estimation by the microbial sensor was examined. The results are summarized in Table 1. The response values were expressed as the absolute potential in the presence of 20 mg/*l* of each substrate.

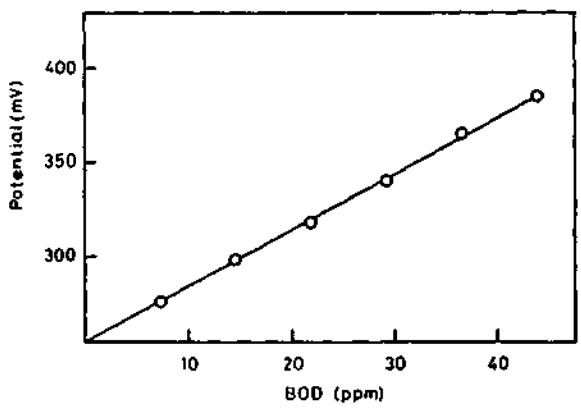

Figure 4. Calibration curve of the microbial sensor.

Table 2. Comparison of BOD Values by BOD Sensor and Those Determined by Conventional Method

Sample No	BOD sensor*	Conventional method*	Difference (%)
1	22	20	10
2(1 : 10)	29	34	10
3(1 : 20)	31	34	10
4(1 : 200)	28	27	4

*Unit: ppm.

Sucrose and fructose were responded similarly compared with glucose and glutamic acid, and galactose, lactose and ethanol were similar to the blank (0.1 M phosphate buffer, *ca.* 260 mV).

Calibration and Stability. Figure 4 shows a calibration curve of the microbial sensor when the diluted standard solutions were employed for experiments. A linear relationship was observed between the potential and 5-day BOD of the standard solution below 44 ppm BOD with a correlation coefficient of 0.999. The potential values after the lapse of 30 min were reproducible within ±9% of the relative error, when the standard solution (20 mg/*l* glucose and 20 mg/*l* glutamic acid) was measured repeatedly.

To measure the reproducibility with elapsed time, the measurement was repeated every day. No change in potential output was observed for 30 days, and the sensor was stored in 0.05 M phosphate buffer at 4℃ when not in use.

Applications. Actually the BOD sensor prepared in this experiment was employed for the determination of the BOD of untreated samples. The conventional BOD values of the samples were determined by the JIS method. As shown in Table 2, although the differences were obtained within 10% between the JIS method and the BOD sensor values, this method showed good agreement with a conventional method. The relative error of measurement was less than 10%, whereas the error in the 5-day test is more than 10%.

Consequently, the microbial BOD sensor using immobilized microorganisms appears very promising and attractive for the estimation of the 5-day BOD of various waste waters. That is to say, it is not necessary to pretreat for this system, thus making it easier, quicker less expensive and more exact to use for the estimation of the BOD of waste waters. As

the disadvantage of this method, it will be difficult to employ this sensor for the estimation BOD of the waste waters which contain organic compounds which can not be degraded or which can be assimilated very quickly by the microorganisms.

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Expert System Approach for Vapor-Phase Infrared Spectra of Aromatic Compounds

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Computerized interpretation of vapor phase infrared spectra using a novel expert system approach for spectra/structure correlation for vapor phase spectra is introduced. Rapid identification of aromatic functional groups of components in gaseous mixture can be achieved using this expert system.

Introduction

The need for the identification of compounds present in complex gaseous mixture or after separation by gas chromatography (GC) using vapor-phase infrared (VPIR) spectrometry is becoming increasingly important. This is primarily due to advances in Fourier transform infrared (FT-IR) spectrometry allowing the acquistion of complete spectra in a few tenths of a second with detection limits of less than 1 ppb in long path infrared gas cells and below 10 nanograms in the GC/FT-IR intenface. With a GC/FT-IR system hundreds of spectra can be generated per chromatogram. Clearly, the limiting processes are the spectral searching and in cases where the searching results are ambiguous, spectral interpretation steps. A powerful digital computer is needed to control the interferometer and perform the Fourier transformation from the time domain to the frequence domain in order to obtain the spectrum. This makes the incorporation of automatic spectral interpretation in the same computer with an obvious and sensible capability. It should be kept in mind that the ultimate goal of the chemist is not simply to produce a collection of spectra but rather to determine what chemical compounds are present in the unknown samples and what their siggnificance is to the problem at hand.

The laborious task of spectral identification has been greatly simplified by the use of computer based library searching routines. Two general classes of algorithms have been developed: procedures that seek to make an exact identification of an unknown by direct comparison with spectra of known compounds (which require the spectrum of the unknown to be in the reference database), and more general approaches striving to identify all the functional groups of the unknown. A wide range of encoding schemes has been proposed[1] to represent spectra in a digital form suitable for library searching and interpretation. The ideal storage format retains the minimum amount of information necessary for correct identification of unknown compounds in a form permitting rapid numerical comparison. The optimum instrumental parameters for acquiring spectra for spectral searching and identification are a function of the sample, so that practical considerations require some degree of standardization in sampling and measurement. In addition, specific computer characteristics such as word size, amounts of memory, and types of mass storage devices also be taken into account.

Buechi *et al.*[2] have discussed the compilation of spectral libraries in terms of five operations: selection, digitization,