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A Novel Biosensor for the Rapid Determination of Biochemical Oxygen Demand[1]

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Objective To investigate the function of a novel biosensor used for the rapid determination of biochemical oxygen demand (BOD) which is developed by our research group based on suspended immobilized microbial cell system in a completely mixed determining chamber as a substitute of the traditional membrane system. **Methods** Activated sludge was immobilized by PVA gel and used as a bio-sensing element. The novel biosensor was used to measure the short time BOD value and the conventional cultivation method was used for BOD_5 measurement. **Results** A linear relationship was observed for the difference between the current and the concentration of glucose-glutamic acid (GGA) solution below 200 mg/L with a correlation coefficient of 0.995. The optimal response of the sensor was obtained at pH 7.0 and 30℃. The sensor response was within 15 min and was reproducible within ±5% of the mean in a series of eight samples containing 75 mg/L BOD using standard GGA solution. The novel sensor response was found to be fairly constant over a period of 30 days, with ±5% fluctuations. **Conclusion** A relatively good agreement is found between BOD estimated by the novel BOD biosensor and that determined by the conventional 5-day BOD method. This novel BOD biosensor has good sensitivity, stability and reproducibility.

Key words: BOD; BOD biosensor; Wastewater; Water quality; Water pollution

INTRODUCTION

Biochemical oxygen demand (BOD) is the most important and widely used environmental index for monitoring organic pollutants in wastewater. The current international standard for measuring biodegradable organic levels in wastewater is the 5-day biochemical oxygen demand (BOD_5) described by the APHA[1]. However, the conventional BOD method requires not only 5 days, but also experience and skills. Therefore, the notion to develop a biosensor is most attractive because such a device would enable the BOD measurement to be completed within several minutes. BOD biosensors which generally consist of a bio-membrane (containing biological recognition element) and an oxygen electrode (transducer) offer a faster possibility for the estimation of a BOD_5-related parameter. The bio-membrane contains immobilized bioactive material to catalyze biochemical reactions. The change in the response of the oxygen electrode is measured in term of the current. The first report of such a microbial sensor was published by Karube *et al*.[2]. Since then, the research and development of BOD biosensors have made rapid progress.

BOD sensors using microorganisms, such as *Pseudomonas putida*[3], *Trichospwron cutaneum*[4], *S.marcescens* LSY4[5], *Arxula adeninivorans* LS3[6], a mixture of microorganisms[7] including *Enterobacter cloaca*, *Citrobacter amalonaticus*, *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, *Klebsiella oxytoca*, *Enterobacter Sakazaki*, and *Serratia liquefaciens*, have been developed. They are designed to immobilize living cells on porous cellulose membranes such as nitrate cellulose membrane, acetate cellulose membrane and the like. However, membrane-type BOD biosensors have several disadvantages: a great decline of DO resulting from the mass transfer resistance of the membrane; unstable biosensors and poor reproducibility of the measurement results because of the small amount of biomass immobilized in membrane; and high requirement for DO electrode.

Unlike other biosensors that require high

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sensitivity, selectivity and specificity, the BOD sensor is designed to be highly capable of analyzing a sample of complex constituents with a relatively low selectivity. BOD biosensors using mixed cultures which contain an efficient biodegrading agent for organic compounds have good kinetics, sensitivity, stability and reproducibility[8].

Most previously reported BOD sensors are biofilm-type whole-cell-based microbial sensors, which rely on measuring the bacterial respiration rate in close proximity to a suitable transducer. A common feature of these sensors is that they consist of a microbial film sandwiched between a porous cellulose membrane and a gas-permeable membrane as the biological recognition element. This microbial film is an immobilized microbial population, which can bio-oxidize the organic substrate to be quantified. The response is usually a change in concentration of dissolved oxygen (DO) or other phenomena such as light emission. A physical transducer is used to monitor this process. A change is found in an electrical or optical signal, which is amplified and correlated to the content of biodegradable material measured.

There are two measuring techniques available for biofilm-type BOD sensors, *viz*. the steady-state (also entitled as end-point, dynamic) method and the initial rate (also entitled as quasi-kinetic, kinetic, dynamic transient) method. In the steady-state method, the current difference (ΔI) between the two steady states is used for the BOD_{st} estimation. The measuring time is normally 15-20 min followed by 15-60 min recovery time. In the initial-rate method, the initial current change ($\Delta I/\Delta t$) after sample addition is used instead as the sensor response. This parameter reflects acceleration of the bacterial respiration rate and, to a certain extent, is proportional to the substrate concentration. That is the general working principle of BOD sensors.

In recent years, our research group has studied a novel BOD sensor for the rapid measurement of BOD, which is based on the completely mixed determining chamber containing immobilized microorganisms beads instead of membrane element used in the conventional BOD sensor. The reactor-type BOD biosensor invented by our group can overcome the drawbacks of the membrane-type BOD biosensor[9-16].

The aim of this study was to investigate the response characteristics of the novel BOD biosensor to obtain the relationship between the results of BOD measurement obtained by the new approach and those obtained from the conventional BOD analysis, and to circumvent the limitations of BOD sensors reported previously. A series of experiments on the effect of microbial biomass, temperature, initial BOD concentration and activation time on the biosensor response, comparison of the sensor BOD measurements with the conventional BOD_5 analysis were carried out.

MATERIALS AND METHODS

Chemicals

PVA (polyvinyl alcohol), calcium chloride, glucose and glutamic acid were purchased from Beijing Chemical Reagent Company. Other reagents were commercially available analytical reagents of laboratory grade materials.

Microorganisms

Activated sludge was collected from Beijing Gaobeidian Sewage Treatment Plant and cultivated under aerobic conditions at 30℃. The growth medium consisted of 1 mg $CaCl_2{\cdot}2H_2O$, 19 mg $MgSO_4{\cdot}7H_2O$, 4.4 mg K_2HPO_4, 26.8 mg NH_4HCO_3, and 0.1031 g $C_6H_{12}O_6$. The pH of the medium was maintained at 7.0±0.2.

Immobilization of Microbial Cells

Ten g of polyvinyl alcohol (PVA, nominal degree of polymerization=1750, approx. molecular weight 75 000-80 000) was dissolved in 50 mL of distilled water and cooled to 40℃, then mixed thoroughly with 50 mL of cell suspension with concentration of ca. 4.0×107 cells/mL. The resulting mixture was dropped into saturated boric acid solution for 1 h to form spherical beads. The formed gel beads with an average diameter of 3 mm were then soaked in 0.5 mol/L sodium orthophosphate solution for 1 h. The particles were washed with physiological saline, then dried at 4℃ for 24 h and stored at 4℃ before use.

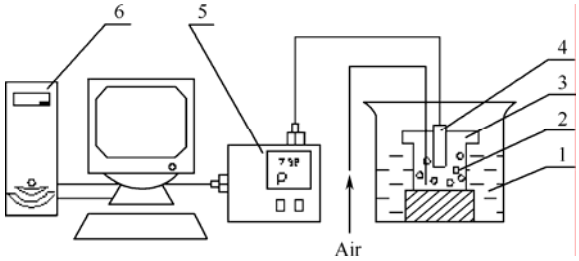
BOD_5 Standard Solution

The BOD standard solution (150 mg/L glucose and 150 mg/L glutamic acid) was prepared according to standard procedures (APHA, 1995). This solution has a known BOD value of 198±31 mg BOD5/L. Higher strength BOD standards were prepared by increasing the GGA loading and lower strength BOD standards were prepared by appropriate dilution with distilled water.

Schematics of BOD Sensor

The BOD sensor is shown in Fig. 1. The sensor response is expressed as the difference in the pseudo steady-state of change in the oxygen concentration before and after the addition of the water sample to

a buffer. The efficacy of this biosensor to detect BOD is described by the linear correlation between the sensor response and the BOD value of the water sample (linear range and sensitivity), response and recovery times, stability and reproducibility of the response, service and shelf life of the biosensor.



1. water bath; 2. immobilized microbial beads; 3. reactor; 4. oxygen electrode; 5. DO meter; 6. computer.

FIG. 1. Schematics of the BOD sensor system.

BOD Measurement Procedure of the Biosensor

BOD measurement with the biosensor was carried out in a reactor maintained at 25℃ with constant temperature water bath. The temperature of the bioreactor was maintained at 30℃ by a water bath. The oxygen electrode was inserted into the bioreactor containing 50 mL of a certain sample with the air flow rate of 100 mL/min.

The current output of the oxygen electrode was measured using a digital multi-meter and a computer. When the current output of the oxygen electrode reached an initial steady state by the consumption rate of oxygen by endogenous respiration of the immobilized microbe diffusivity of oxygen in a sample containing no beads, the current output of the oxygen electrode decreased and reached another steady state in a few minutes after adding the beads. The difference between the two currents was defined as change in current (ΔI). The magnitude of ΔI is proportional to a concentration of immediately biodegradable organic compounds in a certain range of BOD.

Conventional BOD Measurement

The BOD_5 of wastewater samples indicates the amount of biochemically degradable organic material and oxidizable inorganic material, and the method is described in the Standard Methods[1].

RESULTS

Effect of Amount of Microbial Beads on Biosensor Response

The effect of the amount of immobilized activated sludge on the response of the reactor-type biosensor was investigated. The relationship between the variation of current output and the amount of immobilized activated sludge in the PVA beads is shown in Fig. 2.

Effect of the Activation Time of Immobilized Cell on Sensor Response

The response of the biosensor in terms of DO (mg/L) using the immobilized microbial beads stored for one month at 4℃ is shown in Fig. 3. Before use, the microbial cells were activated for different time. The response was assessed according to the variation of DO value using 50 mg/L GGA solution. The immobilized microbial cells exhibited their maximum response during the period of time from 3 to 4 hours. This could be attributed to the fact that cells cultivated for 3 to 4 h after one month storage were operating at their maximum rate and efficiency.

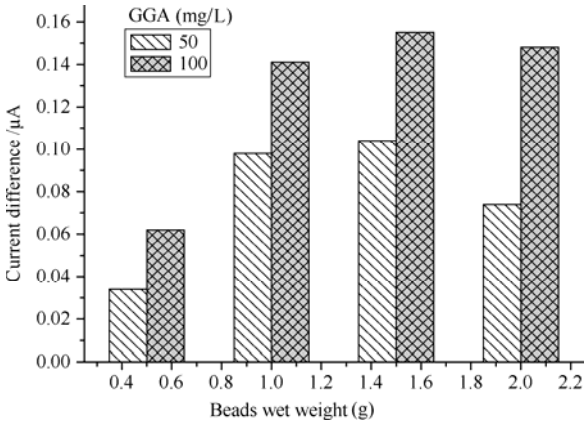


FIG. 2. Effect of microbial amount on biosensor response.

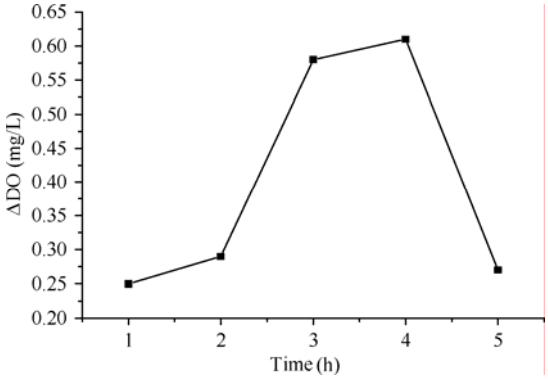


FIG. 3. Effect of the activation time on biosensor response.

Effect of Temperature on Biosensor Response

The influence of temperature on the microbial

activity and response of the biosensor was investigated. The effect of the temperature which was controlled at 20℃, 25℃, and 30℃, respectively, is shown in Fig. 4. The biosensor gave its maximum response at a reactor temperature of 30℃, as compared to that at 20℃ and 25℃. Moreover, when the temperature was above 30℃, the electrode response decreased slightly, which might be due to the inactivation of the microbial cells at higher temperature.

Effect of GGA Concentration on Biosensor Response

The effect of GGA concentration on biosensor response at different temperature was studied. The DO in the solution decreased gradually over the time until a steady state was reached. The response time of the novel biosensor depended on the BOD level, taking 5 to 15 min to reach its steady state, as shown in Fig. 5. The response time was measured by using a range of GGA solution (25-200 mg/L) at different temperatures. In addition, the response time decreased with the increase of temperature. Therefore, a response time of 15 min was employed for further research work.

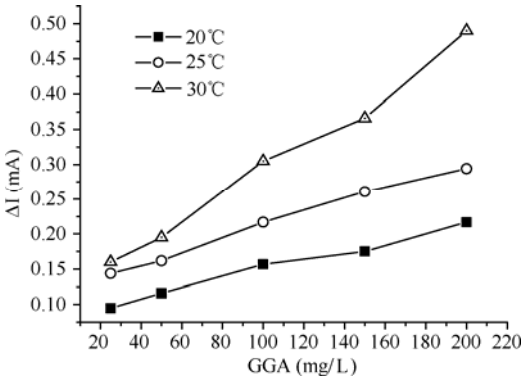


FIG. 4. Effect of temperature on biosensor response.

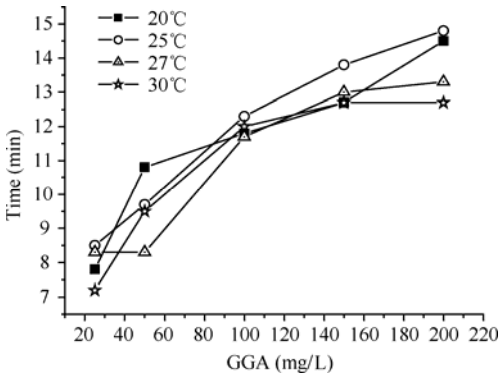


FIG. 5. Effect of GGA concentration on biosensor response at different temperatures.

Calibration Curve

The novel BOD biosensor was used to analyze the BOD values of a certain range of concentration of the standard GGA solution. Figure 6 shows a calibration curve of the BOD biosensor when the diluted standard solution of GGA was employed for the experiment. A linear relationship was observed between the current difference and the 5-day BOD of the GGA solution below 200 mg/L with a correlation coefficient of 0.995. The current was reproducible within ±5% of the mean in a series of eight samples containing 75 mg/L BOD using standard GGA solution.

Relationship Between the Novel BOD Sensor and BOD_5

The novel BOD biosensor was used to analyze real wastewater samples collected from the Beijing Gaobeidian Sewage Treatment Plant. The samples were diluted appropriately with deionized water depending on their BOD value. As a comparison, BOD_5 values of these samples were also measured. The BOD values measured by biosensor and conventional 5-day method are shown in Table 1.

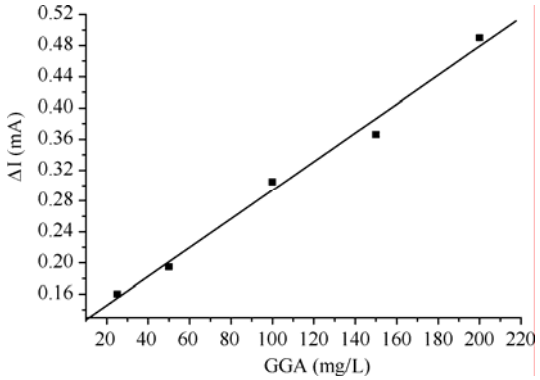


FIG. 6. Calibration curve for BOD estimation.

TABLE 1

Comparison of Sensor BOD and BOD_5 for Municipal Wastewater

Sample No.	BOD Sensor (mg/L)	BOD_5 (mg/L)	Difference (%)
1	24.1	26.9	10.4
2	85.4	90.0	5.1
3	129.6	135.0	4.0

As shown in Table 1, a good agreement between the results of the sensor BOD measurement and BOD_5 analysis was observed.

DISCUSSION

The novel BOD biosensor was developed by our research group, which could improve the sensitivity by separating oxygen electrode and immobilized microbial membrane. In the novel device, the immobilized microbial beads are used instead of the conventional membrane, thus causing a great decline of DO value, and the stability and reproducibility can be enhanced. In our novel biosensor system, the oxygen electrode measures the DO of wastewater, however, in the conventional biosensor, the sensor measures the DO which is not consumed by microbial biomass and penetrated through the membrane. The presence of the membrane contributes to an additional mass transfer resistance equivalent to a reduction of about 31%-27% of the oxygen-sensing sensitivity of the unmodified probes[17]. The DO in the solution decreases gradually over time until a steady state is reached. As Fig. 5 shows, the response time of the novel biosensor is quite rapid, only taking 5 and 15 min to reach its steady state, depending on the initial BOD concentration.

The BOD value as observed by the novel BOD biosensor reflects the concentration of the dissolved organic substances which are assimilated/metabolized by the immobilized microorganisms[18]. Figure 2 shows the variation of response of the biosensor with different concentrations of GGA solution, and the maximum response was observed when the amount of immobilized microbial biomass was 1.5 g. Beads wet weight less than 1 g did not give appreciable response. The sensor response declined when the biomass was higher than 1.5 g, which may be due to the fact that the density of the beads was too high and caused too much DO consumption.

The novel BOD sensor was used to determine the BOD of the wastewater collected from the Beijing Gaobeidian sewage treatment plant. The BOD values obtained by the biosensor were slightly lower than that obtained by using the conventional 5-day BOD method. This can be explained by the fact that the presence of compounds in real municipal wastewater, which are not easily assimilable to the novel sensor in a short time. In other words, the rate of oxidation for some substrates is lower than that of the standard substrates: glucose and glutamic acid[19].

The purpose of developing a BOD sensor was to find an alternative faster analytical approach that has at least an equally good performance (precision and bias) as the existing conventional test. Under defined conditions it is possible to deduce the BOD_5 values from the BOD_{st} with the help of specific conversion coefficient. It is essential that the qualitative composition remains relatively constant and that only the concentration is altered. The coefficient of conversion is applicable only to particular stages of an individual sewage treatment plant.

However, there are still some limitations for rapid estimation of BOD. The BOD biosensor is only suitable for measuring wastewater with a high content of fast and easily assimilable compounds, such as wastewater from food and fermentation industry, usually resulting in a more accurate BOD_{st} value than other types of wastewater, such as municipal wastewater and wastewater from chemical and pharmaceutical industry. This is due to the high concentration of polymers and/or recalcitrant compounds and low concentration of fast and easily assimilable compounds in such wastewater. Therefore, the BOD biosensors are more applicable to specific wastewater with high concentration of fast and easily assimilable organic compounds.

CONCLUSIONS

In conclusion, the novel BOD biosensor developed by using completely mixed reactor can be used to measure the BOD value of the standard solution and the real municipal wastewater. The novel BOD biosensor shows good reproducibility in the measure process and calibration procedure, which gives the response within 15 min. A linear relationship can be observed between the variation of the current and the 5-day BOD of the GGA solution below 200 mg/L with a correlation coefficient of 0.995. The optimum response of the sensor can be obtained at pH 7.0 and 30℃. The current is reproducible within ±5% of the mean in a series of eight samples containing 75 mg/L BOD, using standard GGA solution. The novel sensor response is fairly constant over a period of 30 days, with about ±5% fluctuations. This novel BOD biosensor has good sensitivity, stability and reproducibility.

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