



Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell

Byung Hong Kim^{1,*}, In Seop Chang¹, Geun Cheol Gil¹, Hyung Soo Park² & Hyung Joo Kim³

¹Water Environment and Remediation Research Centre, Korea Institute of Science and Technology, 39-1, Hawolgok-dong, Sungpook-ku, Seoul 136-791, Korea

Present addresses: ²Samsung Engineering Co. R&D Center, Yongin, Kyounggi, Korea; ³Korea BioSystems Co. Seoul, Korea

*Author for correspondence (Fax: +82-2-958-5839; E-mail: bhkim@kist.re.kr)

Received 20 December 2002; Revisions requested 20 January 2003; Revisions received 4 February 2003; Accepted 4 February 2003

Key words: biochemical oxygen demand, biosensor, microbial fuel cell

Abstract

A microbial fuel cell type of biosensor was used to determine the biochemical oxygen demand (BOD) of wastewater. The biosensor gave a good correlation between the BOD value and the coulomb produced. The BOD sensor has been operated for over 5 years in a stable manner without any servicing. This is much longer than that of previously reported BOD biosensors.

Introduction

Since the 5-d biochemical oxygen demand (BOD₅) test was adopted in 1936 by the American Public Health Association Standard Methods Committee, it has been widely used for measuring the concentration of biodegradable organics in wastewater. This method, however, is time-consuming (5 d) and usually requires experience and skill to achieve reproducible results. Therefore, alternative methods have been explored. Various BOD biosensors have been reported, mostly based on dissolved O₂ monitoring using different microorganisms such as yeast (Hikuma *et al.* 1979, Yang *et al.* 1997), *Bacillus subtilis* (Riedel *et al.* 1988), and *Serratia marcescens* (Kim & Kwon 1999). Single microorganisms used in these studies metabolize a limited range of contaminants in the samples, which may result in inaccurate estimation of BOD values. In order to alleviate this problem, mixed cultures (Tan *et al.* 1993) or activated sludge (Liu *et al.* 2000) have been used.

A luminous bacterium, *Photobacterium phosphoreum*, can also be used for a similar purpose. The intensity of luminescence, which is proportional to the amount of assimilable organic compounds in wastewa-

ter, can be measured using a photodiode (Hyun *et al.* 1993). Recently, rapid determination of BOD has been made using a fluorescence technique (Reynolds & Ahmad 1997) which gave a linear relationship between BOD of wastewater and the fluorescence intensities of the organisms growing in the wastewater at 340 nm.

Although there are good relationships between BOD concentration and their responses, biosensors based on the dissolved O₂ probe have problems such as long-term stability due to membrane fouling. In addition, BOD sensors based on a single organism, such as *P. phosphoreum* do not have a broad range of substrate utility. Certain non-biodegradable chemicals interfere BOD estimation through fluorescence techniques (Reynolds & Ahmad 1997).

Our previous studies showed that a microbial fuel cell could be operated without mediator using an electrochemically-active metal-reducing bacterium, *Shewanella putrefaciens* (Kim *et al.* 1999a, 2002). The electrochemically-active bacterium at the anode of the microbial fuel cell oxidises the substrate as a fuel and the resulting electrons are directly transferred to the electrode. The microbial fuel cell using *Shewanella putrefaciens* was tested as a lactate sensor (Kim *et al.* 1999b). We have shown that a fuel

Table 1. BOD sensors and their performance characteristics.

Biological recognition element	Transducer	Measuring range (ppm)	Reproducibility ($\pm\%$)	Operational stability	Reference
Enriched microbial consortium	Microbial fuel cell	< 206	3–12	Over 5 years	This study
<i>Trichosporon cutaneum</i>	DO electrode	4–100	3.3	48 d	Riedel <i>et al.</i> (1988)
<i>Trichosporon cutaneum</i>	DO electrode	0–110	4	7–30 d	Preininger <i>et al.</i> (1994)
<i>Bacillus subtilis</i>	DO electrode	2–22	5	30 d	Riedel <i>et al.</i> (1988)
<i>B. subtilis</i> (heat killed)	DO electrode	< 80	2.4–3.4	140 d	Qian & Tan (1998)
<i>Pseudomonas putida</i>	DO electrode	0.5–10	10	10 d	Chee <i>et al.</i> (1999)
Multi-species culture	DO electrode	0–45	8.5–12.4	20 d	Tan & Wu (1999)
Activated sludge	DO electrode	< 60	5	Very short	Sasaki <i>et al.</i> (1995)

cell-type electrochemical device can be used to enrich an electrochemically active microbial consortium, and that the device can be used as a microbial fuel cell to treat wastewater (Gil *et al.* 2003). This microbial fuel cell has been maintained over 5 years with stable current generation. The coulomb generated from the microbial fuel cell was directly proportional to the strength of the wastewater. This observation gave the possibility to use it as a BOD sensor.

Materials and methods

Wastewater and chemicals

Wastewater was collected from a starch processing plant (Samyang Genex Co., Korea). The chemical oxygen demand (COD_{Cr}) and biochemical oxygen demand (BOD_5) of the wastewater was 1 200 and 520 ppm, respectively. The wastewater contained 25 ± 7.7 mg total nitrogen l^{-1} and 10.7 ± 1.7 mg total phosphorus l^{-1} , respectively. Inorganic nitrogen was less than 5 mg l^{-1} . It was diluted using 50 mM phosphate buffer (pH 7) containing 100 mM NaCl to a designated concentration before being fed into the anode compartment of the fuel cell as fuel.

Microbial fuel cell system

Mediator-less microbial fuel cells enriched and maintained for over 4 years (Gil *et al.* 2003) were used in this study. The anode and cathode compartments (working volume of 25 ml each) were separated by a cation exchange membrane (Nafion, Dupont Co., USA). Graphite felt ($50 \times 50 \times 3$ mm, GF series, Electro-synthesis Co., USA) was used as electrodes with platinum wire connecting them through resistance of 10Ω and a multimeter (Keithley Co., USA). Injection ports were installed in each compartment of the fuel cell. The experimental set-up was shown previously (Gil *et al.* 2003). The anode compartment was kept anoxic by purging with nitrogen gas (100 ml min^{-1}). Air (100 ml min^{-1}) was purged into the cathode compartment in order to supply O_2 needed for the electrochemical reaction. The cathode compartment contained 50 mM phosphate buffer (pH 7) with 100 mM NaCl as the electrolyte, and the anode compartment wastewater diluted with the electrolyte. The microbial fuel cell was operated in a batch mode. The anode content (25 ml) was replaced by diluted wastewater as fuel. The microbial fuel cells were placed in a temperature-controlled chamber controlled at $30 \text{ }^\circ\text{C}$.

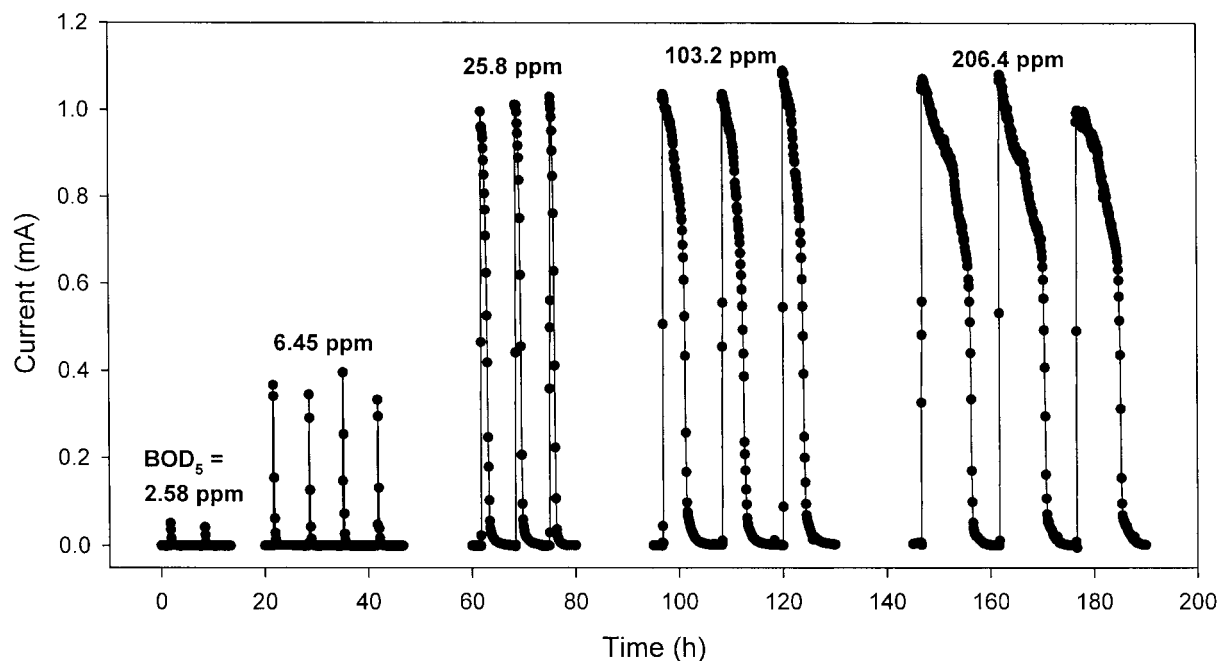


Fig. 1. Current generation patterns from the microbial fuel cell-type BOD sensor fed with samples of different BOD concentration. The microbial fuel cells were fed with wastewater diluted to BOD values indicated in the figure, and the current generated was monitored.

Instrumentation and analyses

The potential difference (PD) between anode and cathode was measured using a multimeter and recorded every 5 min through a data acquisition system (Testpoint, Capital Equipment Co., USA). The measured PD was converted to current according to the relationship of $PD = \text{current} \times \text{resistance}$. Coulomb, which is expressed as $\text{current} \times \text{time}$, was calculated by integrating the current over the time from the start point of experiment to the time where current was decreased to 5% of maximum current. All experiments were conducted using three separate microbial fuel cells, and results were presented as average values or a typical result. COD_{Cr} and BOD_5 were measured using standard methods (Eaton *et al.* 1995).

Results and discussion

Fuel cell operation with different BOD concentrations of wastewater

The microbial fuel cell was supplied with diluted wastewater to determine correlation between BOD concentration and coulomb generation. Figure 1 shows the current monitoring results of the operation.

Five different concentrations were tested, and each diluted was tried at least twice. After anode solution was replaced by new sample, the current increased very rapidly and reached to maximum value. The maximum value was kept for a while, and decreased. When sample BOD concentrations were higher than 25 ppm, the maximum current showed almost same value (Figure 2A). At the BOD values lower than 25 ppm, the maximum current was lower than those obtained from the higher strength fuel. Coulomb obtained in a similar experiment showed a good relationship with the strength of the wastewater (Figure 2B). This result shows that microbial fuel cell can be used as a BOD sensor.

Performance characteristics of microbial fuel cell-type BOD sensor

As shown in Figure 2B, the correlation between BOD concentration and coulomb generation showed a good linearity (regression coefficient, $r^2 = 0.99$) up to 206 ppm. The linearity of the sensor on higher concentrations was tested. The undiluted sample with BOD value of 520 ppm generated a Coulomb of 57.7 ± 1 C. The expected value was 70.8 C, calculated by linear correlation curve (Figure 2C). This low coulomb yield could be due to low buffering capacity in the sample

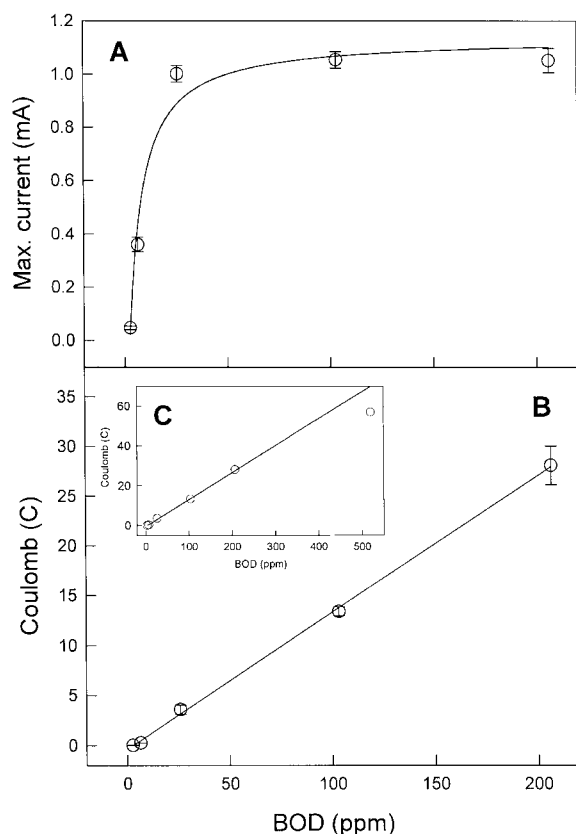


Fig. 2. The correlation curves between BOD value of wastewater and maximum current (A), and between BOD value and coulomb (B and C). The maximum current and coulomb were measured using wastewater diluted to different BOD values. The correlation between BOD concentration and coulomb showed a good linearity ($r^2 = 0.99$) up to 206 ppm, but the coulomb value was lower than expected with BOD values higher than 206 ppm probably due to acidification in the anode compartment.

without phosphate buffer. Our previous result showed that the higher current was generated from an experiment which employed phosphate buffer with NaCl as the electrolyte than the control without the electrolyte (Gil *et al.* 2003).

The higher the strength of the sample, the longer the microbial fuel cell-type BOD sensor took to measure the BOD values. The response time for two low concentration samples, 2.58 and 6.45 ppm, was shorter than 30 min. However, the sample with a BOD value of 206.4 ppm took about 10 h. Although this BOD sensor has good linearity up to this high concentration, response time may be too long. High strength samples may therefore have to be diluted to be analysed within a reasonable time.

Table 2. Comparison of BOD values of wastewater samples measured by the sensor with those determined by BOD₅.

Wastewater	BOD (ppm)	
	Microbial fuel cell sensor	BOD ₅
Sample 1	19.3 ± 2.2	17.2 ± 2.5
Sample 2	67 ± 2.1	68.8 ± 10.3
Sample 3	138.5 ± 9.4	137.6 ± 20.5

The microbial fuel cell can be used to measure BOD values either by reading the maximum current or by calculating the coulomb. The low BOD values might be obtained from the maximum current as shown in Figure 2A. This mode may be used for a real-time determination of BOD values. Since the current increased so fast after the addition of samples that it was impractical to use the initial current increase rate to measure BOD values.

BOD values measured by the microbial fuel cell showed the standard deviation from ±3% to ±12% during repeated experiments over a year (Table 1). The reproducibility of previously reported biofilm-type BOD sensors varied from ±2.4% to ±10% for single strain sensors, and slightly wider range for multi-strains based sensors (Liu & Mattiasson 2002). According to the Eaton *et al.* (1995), ±15.4% reproducibility is acceptable in BOD₅ test. Table 2 shows the comparison of BOD values of wastewater samples measured by the sensor and those determined by BOD₅.

Operational stability is one of the important factors to be considered in biosensors. A stable sensor performance over a desired period is essential for a reliable sensor system. A typical BOD sensor based on DO monitoring has a limited stability due to the nature of a DO probe. DO probe (Clark-type) is a two-electrode system consisting of a silver anode and a gold or platinum cathode, and anode metal can be easily oxidized (Liu & Mattiasson 2002). Therefore, it is necessary to change the electrolyte and clean the anode surface of DO probe regularly. However, microbial fuel cell-type BOD sensor has been operated over 5 years without any services. If fuel (wastewater) supply is not limited, the operational stability could be maintained up to 5 years. This period is much longer than that of previously reported BOD biosensors, 7 to 140 d (Liu & Mattiasson 2002).

Based on the study, microbial fuel cell-type biosensor can be used to determine BOD concentration in wastewater with the advantage of long stability.

Acknowledgements

This work was supported partly by Korea Institute of Science and Technology (2E15471) and the Ministry of Science and Technology in Korea (Bioproducts and Biotechnology Research Programme and National Research Laboratory Programme).

References

- Chee GJ, Nomura Y, Karube I (1999) Biosensor for the estimation of low biochemical oxygen demand. *Anal. Chem. Acta* **379**: 185–191.
- Eaton AD, Clesceri LS, Greenberg AE (1995) *Standard Methods for the Examination of Water and Wastewater*, 19th edn. Washington, DC: American Public Health Association.
- Gil GC, Chang IS, Kim BH, Kim M, Jang JK, Park HS, Kim HJ (2003) Operational parameters affecting the performance of a mediator-less microbial fuel cell. *Biosens. Bioelectron.*, in press.
- Hikuma M, Suzuki H, Yasuda T, Karube I, Suzuki S (1979) Amperometric estimation of BOD by using living immobilized yeast. *Eur. J. Appl. Microbiol. Biotechnol.* **8**: 289–297.
- Hyun CK, Tamiya E, Takeuchi T, Karube I, Inoue N (1993) Novel BOD sensor based on bacterial luminescence. *Biotechnol. Bioeng.* **41**: 1107–1111.
- Kim BH, Kim HJ, Hyun MS, Park DH (1999a) Direct electrode reaction of Fe(III) reducing bacterium, *Shewanella putrefaciens*. *J. Microbiol. Biotechnol.* **9**: 127–131.
- Kim HJ, Hyun MS, Chang IS, Kim BH (1999b) A microbial fuel cell type lactate biosensor using a metal-reducing bacterium, *Shewanella putrefaciens*. *J. Microbiol. Biotechnol.* **9**: 365–367.
- Kim HJ, Park HS, Hyun MS, Chang IS, Kim M, Kim BH (2002) A mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. *Enzyme Microb. Technol.* **30**: 145–152.
- Kim MN, Kwon HS (1999) Biochemical oxygen demand sensor using *Serratia marcescens* LSY 4. *Biosens. Bioelectron.* **14**: 1–7.
- Liu J, Mattiasson B (2002) Microbial BOD sensors for wastewater analysis. *Water Res.* **36**: 3786–3802.
- Liu J, Björnsson L, Mattiasson B (2000) Immobilised activated sludge based biosensor for biochemical oxygen demand measurement. *Biosens. Bioelectron.* **14**: 883–893.
- Preininger C, Klimant I, Wolfbeis OS (1994) Optical fiber sensor for biological oxygen demand. *Anal. Chem.* **66**: 1841–1846.
- Qian Z, Tan TC (1998) Response characteristics of a dead-cell BOD sensor. *Water Res.* **32**: 801–807.
- Reynolds DM, Ahmad SR (1997) Rapid and direct determination of wastewater BOD values using a fluorescence technique. *Water Res.* **31**: 2012–2018.
- Riedel K, Renneberg R, Kühn M, Scheller F (1988) A fast estimation of biochemical oxygen demand using microbial sensors. *Appl. Microbiol. Biotechnol.* **28**: 316–318.
- Sasaki Y, Abe N, Takeuchi S, Takahashi F (1995) BOD sensor using magnetic activated sludge. *J. Ferment. Bioeng.* **80**: 300–303.
- Tan TC, Wu C (1999) BOD sensors using multi-species living or thermally killed cells of a BIOSEED microbial culture. *Sensor. Actuator.* **B54**: 252–260.
- Tan TC, Li F, Neoh KG (1993) Measurement of BOD by initial rate of response of a microbial sensor. *Sensor. Actuator.* **B10**: 137–142.
- Yang Z, Suzuki H, Sasaki S, McNiven S, Karube I (1997) Comparison of the dynamic transient- and steady-state measuring methods in a batch type BOD sensing system. *Sensor. Actuator.* **B45**: 217–222.