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On-Line Monitoring of Low Biochemical Oxygen Demand Through Continuous Operation of a Mediator-Less Microbial Fuel Cell

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Abstract Oligotrophic microbial fuel cells (MFCs) were tested for the continuous monitoring of low biochemical oxygen demand (BOD) by using artificial wastewater, containing glucose and glutamate, as check solution. Ten times diluted trace mineral solution was used to minimize the background current level, which is generated from the oxidation of nitrilotriacetate used as a chelating agent. The feeding rate of 0.53 ml/min could increase the sensitivity from 0.16 to 0.43 μA/(mg BOD/l) at 0.15 ml/min. The dynamic linear range of the calibration curve was between 2.0 and 10.0 mg BOD/l, and the response time to the change of 2 mg BOD/l was about 60 min. The current signal from an oligotroph-type MFCs increased with the increase in salts concentration, and the salt effect could be eliminated by 50 mM phosphate buffer.

Key words: Microbial fuel cell, biochemical oxygen demand, continuous monitoring, bioelectrochemistry

Real-time environmental monitoring is important for the process control of potable water production, wastewater treatment, and in aquaculture [9]. A standard method has been employed to measure the concentration of biodegradable compounds as biochemical oxygen demand (BOD). Alternatively, microbial sensors can be used [11]: The microbial BOD sensors have been developed for the determination of low BOD values [4, 5, 21], based on the microbial respirometric principle. These sensors employ dissolved oxygen electrode [4, 21] or optical fiber [5] to monitor the decrease in the dissolved oxygen concentration which is coupled to the oxidation of degradable compounds by the immobilized microbial cells. The BOD sensors showed the measuring range of 1–18 mg BOD/l, the response time of 2–20 min, and the detection limit of 0.2–0.5 mg BOD/l. However, the microbial BOD sensors, based on the microbial respirometric principle, have some limitations in their application to continuous on-line monitoring. They include influence of the DO concentration in the sample, requirement for careful maintenance and storage, a short lifetime ranging from a few days to a few months, narrow spectrum of applicable substrates, susceptibility to poisoning, and deactivation [1, 17, 20].

A microbial fuel cell (MFC) is a device that can directly convert chemical energy of organic or inorganic fuel into electricity. An MFC can be operated without mediators using an electrochemically active metal-reducing bacterium, *Shewanella putrefaciens* [14, 15], or microbial consortium [12, 19]. The mediator-less MFC was studied for the development of a BOD sensor [3, 8, 10, 13, 16, 18]. MFC offers several advantages including over 2 years of long-operational stability, low maintenance requirement, resistance to heavy metals, and broad specificity. Kang *et al.* [10] showed that an MFC enriched with oligotrophic microbes could be used as a low BOD sensor in batch operation. Continuous on-line monitoring of BOD with the range between 20 and 100 mg/l has been realized by an MFC enriched with copiotrophic microbes [3]. A good correlation was observed between the BOD_5 values of artificial wastewater and estimated BOD from steady-state current output. Thus, the present work was undertaken to test the performance of the MFC enriched with oligotrophic microbes as a microbial sensor for continuous and on-line monitoring of low BOD below 20 mg/l.

The MFCs used throughout the study were oligotrophic [10], designed to minimize the oxygen diffusion through a cation-specific membrane. Artificial wastewater (AW), containing glucose and glutamate, or surface water collected

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from Jungnang-cheon (Seoul, Korea, BOD_5: 11.3±0.99 mg/l), was used as the fuel throughout the present study [3, 10]. The MFCs were enriched with oligotrophic microbes [10]. All experiments were conducted by using more than 3 MFCs, and typical results were presented. The variations in current of the MFCs were within 10%. The AW was made and maintained under nitrogen atmosphere by connecting to a nitrogen-containing gas-tight bag with a volume of 5 l (Alltech, Deerfield, IL, U.S.A.). The AW was fed to the anode compartment through the injection port at the rates of 0.15-0.65 ml/min, using a peristaltic pump (505S, Watson-Marlow, Falmouth, Cornwall, U.K.) equipped with Marprene II tubing (Watson-Marlow). Air-saturated tap water was fed to the cathode compartment as the oxidant at the feeding rate of 1.0 ml/min throughout the study, using a peristaltic pump (505S, Watson-Marlow). The MFCs were installed in a temperature chamber controlled at 33°C. The external resistance was 10 Ω. The potential between anode and cathode was measured and converted to current as described previously [3, 10]. The conductivity was measured by using a conductivity meter (CON 5 Meter, LaMotte, ML, U.S.A.).

The AW with different BOD concentrations was continuously fed into oligotrophic MFCs to test their performance as a low BOD sensor. The current generated from the MFC was monitored with the fuel-feeding rate of 0.15 ml/min (Fig. 1A), and the correlation between the AW concentration and current difference was plotted (Fig. 1B). The dynamic linear range was observed up to 20 mg BOD/l. It took about 2 h to reach the new steady state after AW without glucose and glutamate was replaced with that containing glucose and glutamate at the concentration of 10 mg BOD/l. A response time of about 15 h was observed, when the BOD concentration was changed from 10 to 20 mg/l. This response time is too long for the real-time on-line BOD monitoring. Since an MFC enriched with copiotrophic microbes (copiotrophic MFC) can be used to measure the BOD values over 10 mg/l [3], AW of 0-10 mg BOD/l was used to optimize the oligotrophic MFCs as low BOD sensors.

Due to the high background current level, the detection limit was rather high, about 5 mg BOD/l. To lower the detection limit, the background signal generated from a biosensor should be identified and minimized [2]. The identification of background signal can be extremely important because the apparent current generated from oligotrophic MFCs is lower by several orders of magnitude than in copiotrophic MFCs [3, 10]. The AW used in the present study as a model analyte consisted of basal inorganic salt solution (BS), buffer, trace mineral solution (TMS), and

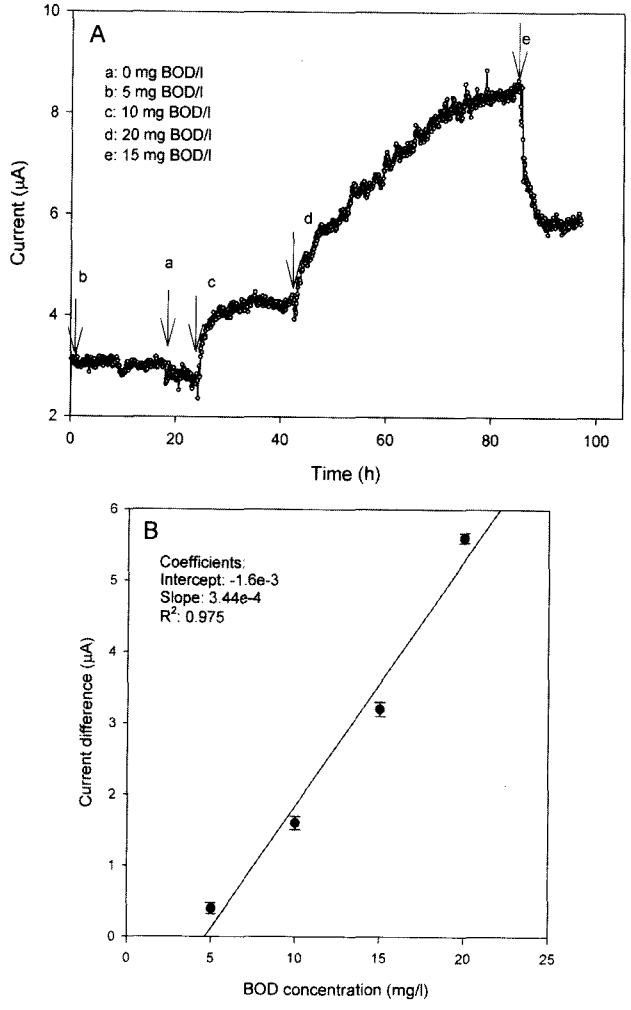

Fig. 1. Current generation from an oligotrophic MFC fed with artificial wastewater (AW) of different BOD concentration.
Ten ml of TMS were added to the AW. The fuel-feeding rate was 0.15 ml/min. (A) Time course of current generation. (B) Relationship between steady-state current and BOD concentration.

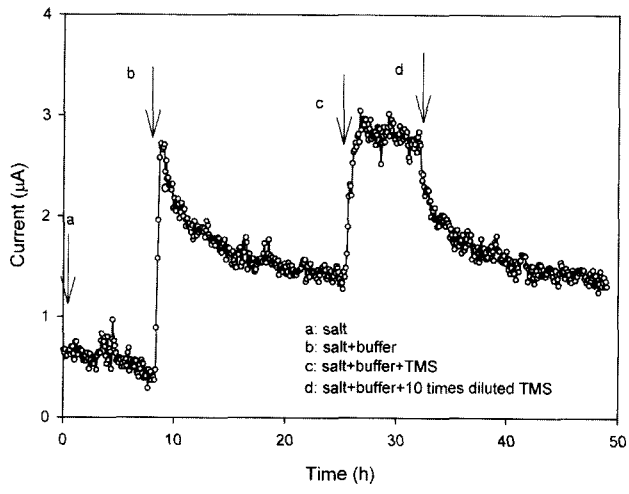

Fig. 2. Effects of components of artificial wastewater on current generation.
The feeding rate was 0.15 ml/min.

fuels (glucose and glutamate) [3, 6]. To explore whether the inorganic constituents were responsible for the background current generation, the oligotrophic MFCs were fed with the BS, followed by BS added with each constituent of AW (Fig. 2). When the BS was fed, a current of 0.5 μA was observed. The current increased to about 2.8 μA with the feeding of the BS containing 50 mM phosphate buffer, and then the current decreased gradually to around 1.5 μA. The current generated with the buffer addition might be due to the membrane potential exerted by proton, which was provided with the buffer solution, or to an increase in conductivity, which is stabilized through the continuous feeding. The conductivity of the BS containing buffer was 6.66 mS/cm, which was much higher than 0.0055 mS/cm of the BS alone, suggesting that the changes in the current with the buffer is due to increase in conductivity. When the BS containing buffer was substituted with the BS containing buffer and TMS, the current increased to a similar level and was maintained. The addition of TMS did not change the conductivity. These results show that, when the MFC was fed with the BS containing buffer and TMS, the increase in current was due neither to the membrane potential nor to the increase in conductivity. Nitrilotriacetate (NTA) of 15 mg/l was used to dissolve the metal ions [3]. The current generated from the MFC fed with the BS containing buffer and TMS is believed to be due to the oxidation of NTA as fuel. NTA is known to be oxidized under anaerobic conditions [7]. Recently, it was observed that NTA is oxidized in MFCs with concomitant current generation (Jang *et al.*, unpublished work). When MFCs were fed with 1 ml/l TMS containing AW instead of the usual 10 ml/l, the current decreased to about 1.5 μA, similar to that observed with BS containing buffer alone. TMS at a concentration of 1 ml/l appears to be enough to support oligotrophic microbes with the reduced background signal by NTA. Indeed, TMS was added to AW of 200 mg BOD/l (10 ml TMS/l AW) to support anaerobes in a copiotrophic MFC [3].

The increase in feeding rate could improve the sensitivity of an MFC as a BOD sensor [18]. The steady-state current

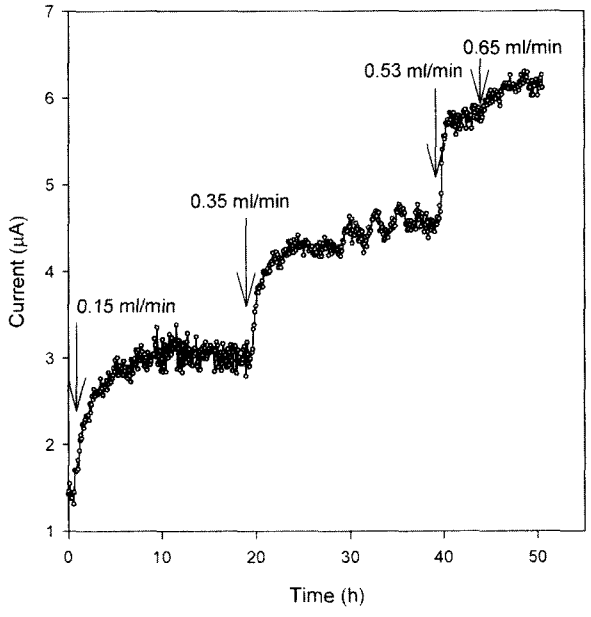

Fig. 3. Effects of AW feeding rate on the current generation.
One ml of TMS was added to the AW. The AW concentration was 10 mg BOD/l.

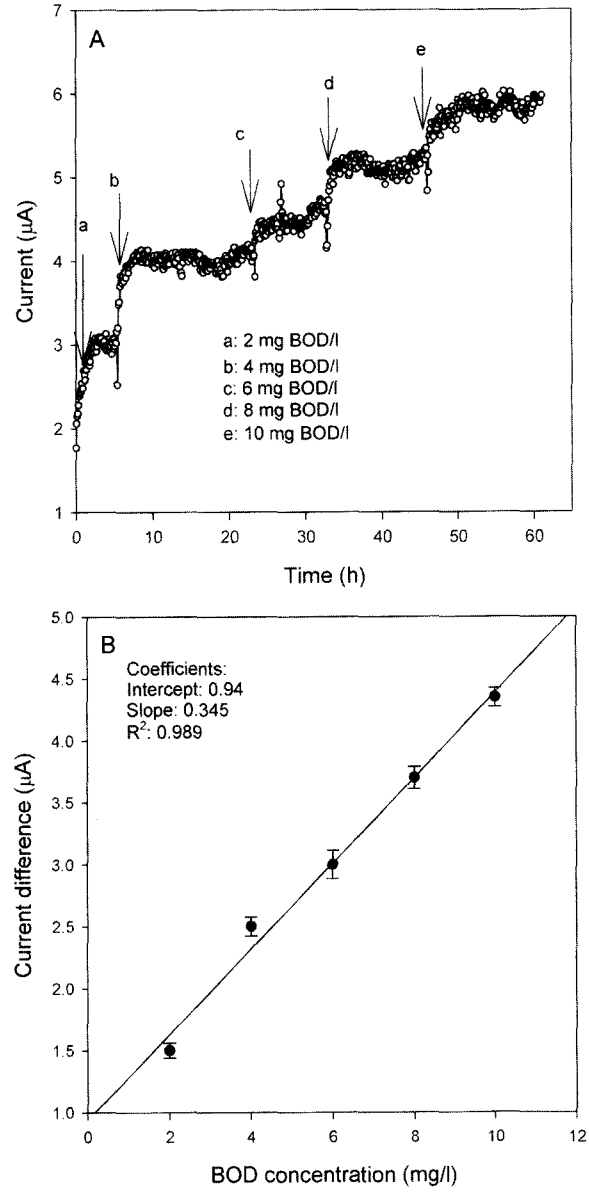

Fig. 4. Current generation from an oligotrophic MFC fed with artificial wastewater (AW) of different BOD concentrations.
One ml of TMS was added to the AW. The fuel-feeding rate was 0.53 ml/min. (A) Time course of current generation. (B) Relationship between steady-state current and BOD concentration.

linearly increased as the feeding rate increased up to 0.53 ml/min, using AW of 10 mg BOD/l; however, as shown in Fig. 3, the increase was not significant when the feeding rate was increased from 0.53 to 0.65 ml/min. These results suggest that the oligotrophic MFCs were nearly saturated with the fuel, when AW of 10 mg/l was fed at the feeding rate of 0.53. Thus, the feeding rate of 0.53 ml/min was chosen as the optimum feeding rate to measure BOD values less than 10 mg/l with the sensitivity of 0.43 μA/(mg BOD/l) that is higher than 0.16 at the feeding rate of 0.15 ml/min.

AW containing 1 ml TMS was used to determine the steady-state current at different BOD concentrations at the fuel-feeding rate of 0.53 ml/min (Fig. 4). The BOD concentration was increased stepwise from 0 to 10 mg/l by 2 mg/l. As shown in Fig. 4A, the current increased stepwise as the concentration increased. The response time to the change of 2 mg BOD/l was about 1 h. The linear relationship between BOD concentration in the range of 2 to 10 mg/l and the current was shown with the correlation coefficient (R^2) of 0.99 (Fig 4B). For practical reasons, BOD concentration below 2 mg BOD/l was not tried.

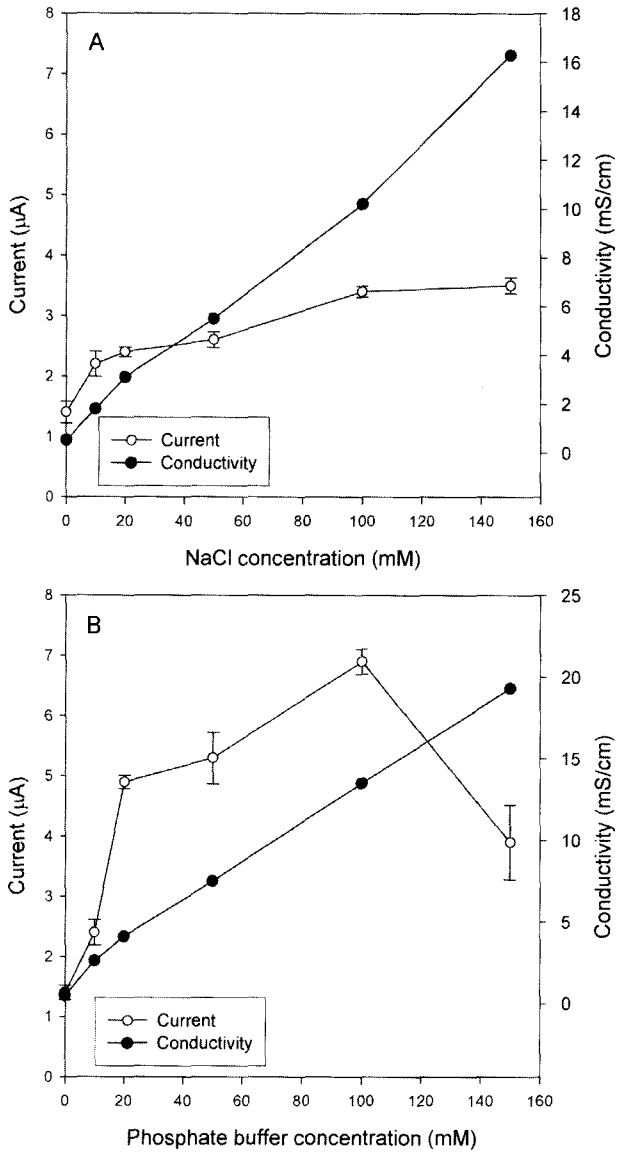

Fig. 5. Effects of NaCl (A) and phosphate buffer (B) addition to surface water sample on the current generation from MFCs enriched with surface water (n=120).
The surface water with the BOD value of 11.3 mg/l was fed at the rate of 0.53 ml/min.

The variation of inorganic salt concentration in surface water can influence the current signal from the oligotrophic MFCs by changing the conductivity. A BOD sensor should be developed to analyze various samples including those with varying salt concentrations. In order to test the effects of salt, the surface water was diluted with 1/10 volume of concentrated NaCl solution to obtain the final concentration of up to 150 mM before being fed to the oligotrophic MFCs enriched with surface water (Fig. 5A). The conductivity increased linearly with the salt concentration. The current increased up to 3.5 μA as the salt concentration increased, which is much lower than that of AW of 10 mg/l BOD. The increase of current with the increase in salt concentration might be due to the increase in conductivity, as shown in Fig. 5A. The conductivities of surface water collected from various sites of the Han River and its tributaries varied from tens to hundreds μS/cm (data not shown), indicating that buffer is needed to minimize the effects of salt and to increase the sensitivity.

Similarly, the surface water was diluted with 1/10 volume of concentrated phosphate buffer (pH 7.0) to obtain the final buffer strength of from 20 to 150 mM, and fed to the MFCs with the current recording. The conductivity increased linearly with the increase in buffer strength, in a magnitude similar to the increase in NaCl concentration. The current increased steeply from 1.3 μA to 5.0 μA with the addition of 20 mM buffer, and the increase was less significant with higher buffer strength. With the buffer strength of 150 mM, the current decreased to around 4.0 μA (Fig. 5B). The low current with 150 mM buffer might have resulted from reduced proton transfer through the membrane due to the increased cation concentration. The current was higher with surface water added with phosphate buffer than that with NaCl, although the conductivity was similar. These results suggest that the increase in current with buffer was not due to the increase in conductivity, and that the buffer might have other functions such as increasing proton availability and microbial activity, among others. Although the maximum current signal was obtained at 100 mM phosphate buffer, 50 mM is recommended for suitable buffer strength, since the current change was smaller at lower buffer strength. It is expected that the possible fluctuation of current signal, due to the conductivity variation of surface water sample with the similar BOD, can be reduced with 50mM phosphate buffer. Conductivity

monitoring is recommended for the reliable low BOD value measurement of water sample using an oligotrophic MFC.

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