Available online at www.sciencedirect.com

Biosensors and Bioelectronics 19 (2004) 607–613

BIOSENSORS & BIOELECTRONICS

www.elsevier.com/locate/bios

Continuous determination of biochemical oxygen demand using microbial fuel cell type biosensor

In Seop Chang[a], Jae Kyung Jang[a], Geun Cheol Gil[a], Mia Kim[a,1],
Hyung Joo Kim[a,1], Byung Won Cho[b], Byung Hong Kim[a,*]

[a] *Water Environment & Remediation Research Center, Korea Institute of Science and Technology,*
39-1, Hawolgok-dong, Sungpook-ku, Seoul 136-791, South Korea
[b] *Eco-Nano Research Center, Korea Institute of Science and Technology, 39-1, Hawolgok-dong, Sungpook-ku, Seoul 136-791, South Korea*

Received 3 January 2003; received in revised form 12 May 2003; accepted 8 July 2003

Abstract

A mediator-less microbial fuel cell (MFC) was used as a biochemical oxygen demand (BOD) sensor in an amperometric mode for real-time wastewater monitoring. At a hydraulic retention time of 1.05 h, BOD values of up to 100 mg/l were measured based on a linear relationship, while higher BOD values were measured using a lower feeding rate. About 60 min was required to reach a new steady-state current after the MFCs had been fed with different strength artificial wastewaters (Aws). The current generated from the MFCs fed with AW with a BOD of 100 mg/l was compared to determine the repeatability, and the difference was less than 10%. When the MFC was starved, the original current value was regained with a varying recovery time depending on the length of the starvation. During starvation, the MFC generated a background level current, probably due to an endogenous metabolism.

Keywords: BOD; Biosensor; Microbial fuel cell; Continuous monitoring

1. Introduction

Since the American Public Health Association Standard Methods Committee adopted the 5-day biochemical oxygen demand (BOD_5) test, this method has been widely used as the standard method for determining the concentration of biodegradable organics in wastewater. However, this conventional method is time-consuming (5 days of incubation) and usually requires experience and skill to achieve reproducible results. As such, studies have already been conducted to develop alternative methods for real-time or on-line BOD monitoring.

Since Karube et al. (1977) first reported on a BOD sensor that used immobilized microorganisms with an oxygen probe, various studies have developed BOD sensors based on the dissolved oxygen (DO) consumption by immobilized microorganisms, such as yeast (Hikuma et al., 1979; Kulys and Kadziauskiene, 1980; Yang et al., 1997; Sangeetha et al., 1996), *Bacillus subtilis* (Riedel et al., 1988) and *Serratia marcescens* (Kim and Kwon, 1999), using a DO electrode. Yet, such strains are unable to oxidize the entire range of organic contaminants in samples, thus the DO consumption is not always directly proportional to the concentration of biodegradable organics. Therefore, mixed cultures (Tan et al., 1993) or activated sludge (Liu et al., 2000) have been used to overcome this problem.

The luminous bacterium *Photobacterium phosphoreum* has been found to exhibit a luminescent intensity in proportion to the amount of assimilable organic contaminants in wastewater (Hyun et al., 1993), thereby enabling the BOD to be determined from the intensity difference measured using a photodiode. More recently, a fluorescence technique was developed for the rapid determination of the BOD, which is analyzed by reading the fluorescent intensities of microbial communities growing in wastewater excited by UV at 340 nm (Reynolds and Ahmad, 1997).

However, despite the strong relationship between the BOD concentration and its responses, the above-mentioned methods have all drawbacks. For example, membrane fouling is a serious problem in biosensors based on a DO

---

[*] Corresponding author. Tel.: +82-2-958-5831; fax: +82-2-958-5839.
E-mail address: bhkim@kist.re.kr (B.H. Kim).
[1] Present address: Korea BioSystems Co., Seoul, Korea.

0956-5663/$ – see front matter © 2003 Elsevier B.V. All rights reserved.
doi:10.1016/S0956-5663(03)00272-0

electrode and frequent maintenance is required to keep a high sensitivity, while in the case of sensors based on photometric or fluorescence techniques, some compounds interfere with the performance.

Microbial fuel cells (MFCs) have also been studied as a BOD sensor. Karube et al. (1977) developed a BOD sensor based on an MFC using the hydrogen produced by *Clostridium butyricum* immobilized on the electrode. MFCs with electron-mediators have also been studied as BOD sensors (Matsunaga et al., 1980; Striling et al., 1983; Thurston et al., 1985), where the mediators are used to facilitate electron transfer from the microbial cells to the electrode. Yet these sensors have a poor long-term stability, as the mediators are generally toxic to microorganisms.

Previous studies by the current authors have shown that an MFC can be operated without mediators using an electrochemically active metal-reducing bacterium, such as *Shewanella putrefaciens*, where the bacterial cells in the anode of the MFC consume the substrate as fuel and transfer electrons directly to the electrode (Kim et al., 1997, 1999b, 2002). Furthermore, due to its lactate oxidizing activity, *S. putrefaciens* can also be used as a model strain for testing a lactate sensor using an MFC (Kim et al., 1999c).

The current authors also showed that a fuel cell-type electrochemical cell could be used to enrich an electrochemically active microbial consortium using wastewater as the fuel and activated sludge as the microbial source (Kim, 1999a). This MFC was maintained for over 5 years with a stable current generation, plus the electricity generation was directly proportional to the strength of the wastewater, suggesting potential use as a BOD sensor (Gil et al., 2003; Kim et al., 2003).

Accordingly, the present study was conducted to test whether a mediator-less MFC could be used to continuously measure the BOD of wastewater for real-time monitoring.

2. Materials and methods

2.1. Microbial fuel cell and its operation

Fig. 1(A) shows a schematic diagram of the experimental set-up. The fuel cells (Fig. 1(B)) were constructed using transparent polyacrylic plastic and had anode and cathode compartments separated by a cation exchange membrane (Nafion® 450, Dupont Co., Wilmington, USA). Graphite felt, 20 mm × 120 mm × 5 mm (GF series, Electrosynthesis, Amherst, NY, USA), was used for the electrodes. The void volume of each compartment was 20 ml. Platinum wire (0.5 mm diameter) was used to connect the electrodes and the electronic port. The internal resistance between the electrodes and the platinum wire was less than 3 Ω measured by a multi-tester (Model 2000, Keythley, MA, USA). Throughout the study, the anode and cathode were connected through a voltameter (Won-A Tech Co., Seoul, Korea) and resistance (R_{load}) of 10 Ω to monitor the current under close circuit conditions. The current (I) was calculated as $I = V/R_{\text{load}}$, where V is the potential drop across R_{load}.

The wastewater was fed through the injection port of the anode compartment at a rate of 0.13–1.37 ml/min using a peristaltic pump (505S, Watson-Marlow, Falmouth, Cornwall, UK) equipped with Marprene II tubing (Watson-Marlow). Air-saturated tap water was fed into the cathode compartment as the oxidant at a feeding rate of 5 ml/min throughout the study, unless stated otherwise, using a peristaltic pump (505S, Watson-Marlow).

The fuel cells were installed in a temperature-controlled chamber at 35 °C. All experiments were conducted using three separate microbial fuel cells operated over 1 year, and the typical results are presented.

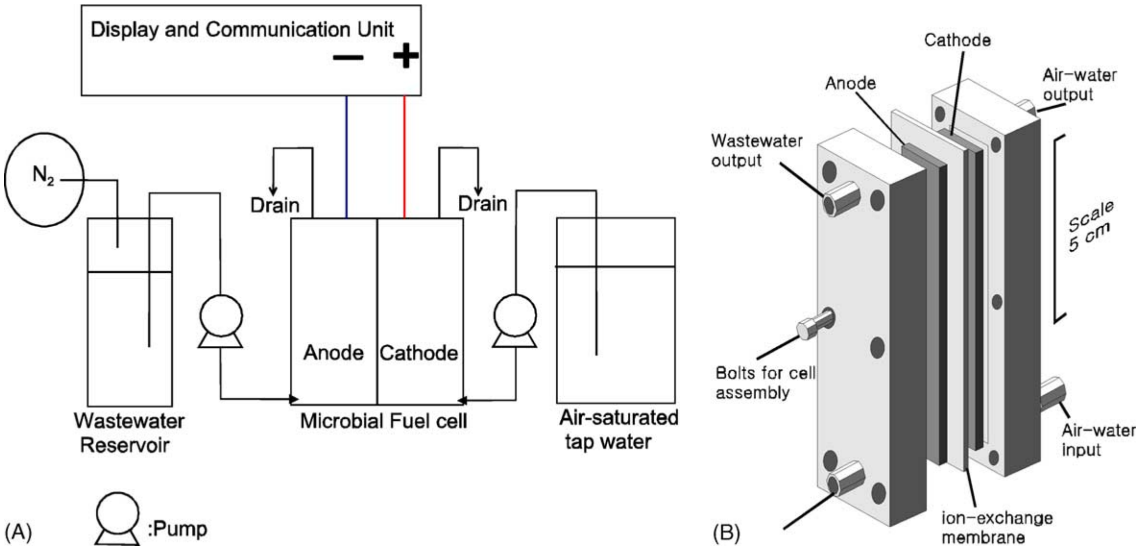

Fig. 1. Schematic diagram of biosensor system (A) and microbial fuel cell (B).

2.2. Wastewater

A glucose and glutamic acid check test solution for the BOD (American Public Health Association, 1995) was modified and used as the model wastewater throughout the study. This modified artificial wastewater (AW) contained $(NH_4)_2SO_4$, 0.56 g; $MgSO_4{\cdot}7H_2O$, 0.20 g; $CaCl_2$, 15 mg; $FeCl_3{\cdot}6H_2O$, 1 mg; $MnSO_4{\cdot}H_2O$, 20 mg; $NaHCO_3$, 0.42 g; a trace mineral solution (Diekert, 1991), 10 ml; phosphate buffer (1 M, pH 7.0), 50 ml; and distilled water, 940 ml. The wastewater was autoclaved at 110 °C for 1 h before use. The autoclaved wastewater was added along with filter-sterilized glucose and glutamic acid solutions. The BOD of the wastewater was varied from 20 to 200 mg/l. The artificial wastewater was made and maintained under a nitrogen atmosphere by connecting to a nitrogen-containing gas-tight bag with a volume of 15 l (Alltech, Deerfield, IL, USA), as shown in Fig. 1(A).

2.3. Enrichment

The MFCs were initially inoculated by activated sludge (10 ml) collected from the Jungryang Sewage Treatment Plant (Seoul, Korea). The inoculated MFCs were then fed with the AW (10 ml, 100 mg/l BOD) in a batch mode based on activating the feed pump when the current dropped below 0.1 mA at a rate of 1.42 ml/min for 60 min. The current was monitored during the enrichment.

2.4. Starvation and chloramphenicol experiments

The starvation test was conducted by inactivating the feeding pump. The AW (100 mg/l as BOD) was then fed into the anode compartment after a predetermined period of feeding disruption. In some experiments the starved MFC was fed with AW containing chloramphenicol at a concentration of 15.6 mg/l (Caccavo, 1999). The feeding rate was fixed at a rate of 0.35 ml/min, while the oxidant flow rate was 5 ml/min.

2.5. Analyses

The chemical oxygen demand (COD) was determined by the standard method (American Public Health Association, 1995) using chromate as the oxidant. The dissolved oxygen concentration was measured using a DO meter (Orion Model 850, Beverly, MA, USA). The current yield was calculated using the following equation: (observed current/theoretical current) × 100 (%), where the observed current was monitored and the theoretical current calculated based on the BOD consumption rate using following equation:

$$\text{current (A)} = \text{BOD consumption rate (mg/s)} \times 12\ (\text{C/mg BOD}) \qquad (1)$$

3. Results and discussion

3.1. Microbial fuel cell and typical current generation

The MFCs were inoculated with activated sludge and enriched for 4 weeks using AW with a BOD of 100 mg/l as fuel. The COD value decreased gradually during the enrichment. After 4 weeks of enrichment, a stable current of over 5 mA was generated, as reported elsewhere (Gil et al., 2003). The MFCs were operated for over 1 year before the present study was conducted.

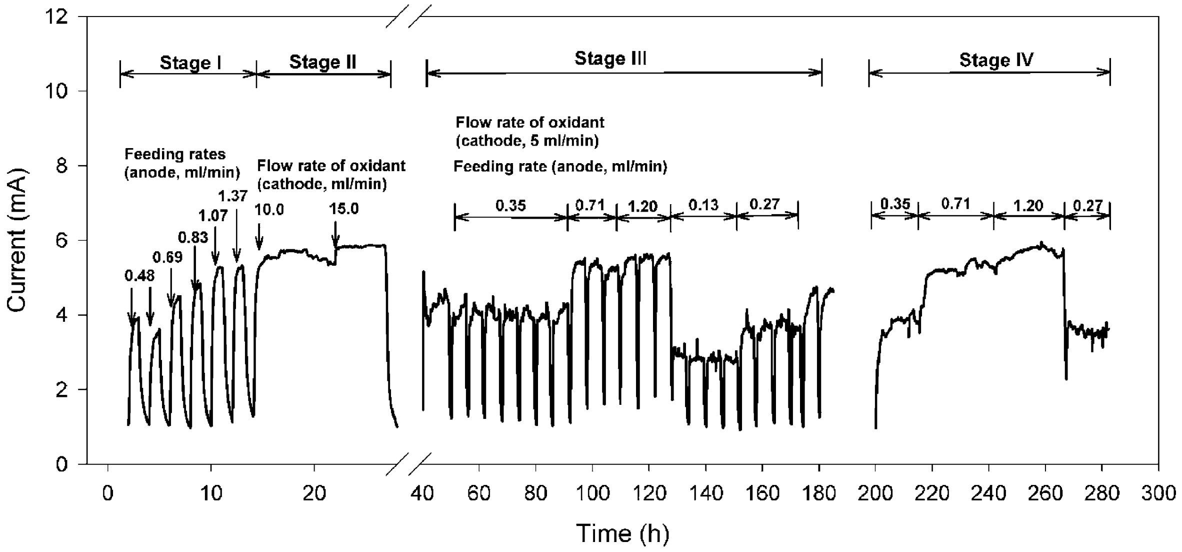

Fig. 2. Effect of feeding rate and oxidant flow rate on current generation by BOD sensor. Modified artificial wastewater (102.4 mg/l as BOD) and air-saturated tap water were used as the fuel and oxidant, respectively.

Table 1
BOD removal rate in MFCs fed with AW (BOD of 102.4 mg/l) at various feeding rates

Feeding speed (ml/min)	BOD loading rate (mg/h)	BOD removal rate (mg/h)	Yield[a]
0.13	0.79	0.74 ± 0.10	97.2 ± 14.0
0.27	1.65	1.17 ± 0.20	87.0 ± 14.5
0.35	2.15	1.28 ± 0.16	95.5 ± 11.6
0.71	4.36	1.81 ± 0.52	88.4 ± 25.4
1.20	7.37	2.27 ± 0.30	77.5 ± 10.6

[a] The yield was calculated by dividing the recorded current with the theoretical current calculated from the BOD consumed, as specified in Section 2.5.

When the MFCs were operated under various conditions, the current generated was recorded (Fig. 2), along with a COD analysis of the effluent (Table 1). Fig. 2 shows the typical current generation from one of the MFCs. In Stage I, the MFC was operated in a batch mode based on feeding 102.4 mg/l BOD AW at different feeding speeds for 1 h followed by an hour break. The oxidant was supplied continuously at a constant feeding rate of 5 ml/min. The current increased gradually as the flow rate increased from 3.7 mA at a feeding rate of 0.48 ml/min to 5.2 mA at a feeding rate of 1.07 ml/min. However, no significant increase in the current was observed when the feeding rate was raised from 1.07 to 1.37 ml/min. At this fuel feeding rate, the oxidant (air-saturated tap water) flow rate was then increased from 5 to 10, and 15 ml/min to test whether the oxidant was limited in the cathode compartment (Stage II in Fig. 2). The maximum current was 5.3, 5.7, and 5.9 mA at an oxidant flow rate of 5, 10, and 15 ml/min, respectively, indicating that the cathode reaction was limited by oxidant supply at a high fuel feeding rate, as reported earlier (Gil et al., 2003). Accordingly, the following experiments were conducted at a fuel feeding speed of less than 1.37 ml/min.

Oxygen supply limitation was also observed when comparing the current generations between batch and continuous runs of the MFCs. In addition, a slightly higher current was repeatedly obtained with a batch operation of the microbial fuel cell compared to a continuous run with a low cathode flow rate (Fig. 2). As such, in a batch run, it was assumed that the DO diffused into the porous cathode space during the pause period was used during the feeding period, while in a continuous run, the DO was used before being diffused into the porous space.

3.2. *Effect of feeding rate during continuous operation*

In Stages III and IV, the AW (102.4 mg/l as BOD) was fed into the MFCs at different feeding rates and the currents monitored at a fixed cathode feeding rate of 5 ml/min. In Stage III, the continuous feeding was interrupted for 1 h every 5 h to determine the recovery time. The maximum current was obtained within 30 min after the feeding was resumed. In addition, the microbial fuel cells generated a reasonably stable current value when the system was continuously operated without a stop mode (Stage IV), indicating that MFCs can be used as a biosensor for real-time BOD monitoring.

The MFCs generated a current with a coulomb yield of over 90% at a fuel feeding rate lower than 0.71 ml/min. When the feeding rate was 1.20 ml/min the yield fell to 77% probably due to incomplete fuel consumption (Table 1). As such, these results suggest that the MFC should be operated at a feeding rate well below the fuel saturation conditions.

3.3. *Continuous monitoring of different concentrations*

A series of AWs with different concentrations ranging from 20 to 200 mg/l as the BOD was prepared and fed into the MFCs to monitor the steady-state response time under a fixed feeding rate of 0.35 ml/min. Fig. 3 shows the current generation that was monitored using two different MFCs. It took about 60 min to reach a new steady-state current after changing the AW concentration, yet this is too long for real-time monitoring. The long response time was believed to be due to the small initial contact area of the anode, thus altering the configuration of the fuel cell to increase the initial contact area may reduce the response time.

The feeding sequence did not affect the current generation. The AW generated a similar current after either an up-shift or down-shift in the strength. The current generated from the MFCs fed with an AW with a BOD of 100 mg/l was compared to determine the repeatability, and found to be 3.73 ± 0.36, 3.92 ± 0.25 (MFC1) and 4.09 ± 0.17 mA (MFC2). As such, the difference was less than 10%.

The MFCs were run using AW with different BOD at a feeding rate of 0.35 ml/min, then the current was plotted against the BOD concentration to find the reliability (Fig. 4). Linearity was observed up to 100 mg/l (dotted line). However, the relationship ceased to be linear when the BOD concentration was higher than 100 mg/l (solid line in Fig. 4).

Some of the factors that affect current generation may be limited at BOD concentrations higher than 100 mg/l. As discussed elsewhere (Gil et al., 2003), several factors affect the performance of an MFC, including (1) fuel oxidation in the anode compartment, (2) electron transfer from the microbial cells to the anode, (3) proton permeability across the membrane, and (4) oxygen supply and consumption in the cathode compartment. Consequently, these limitations should be avoided when running an MFC under limited conditions as a BOD sensor.

An MFC can be operated at a low anode feeding rate to monitor high BOD values, or the sample can be diluted. Although the present MFCs monitored the BOD concentration as a linear relationship, model fitting (Monod, 1942) has also been applied to predict a BOD concentration higher than 100 mg/l. The solid line in Fig. 4 exhibited a good fitting with higher BOD concentrations as well as lower than critical concentrations.

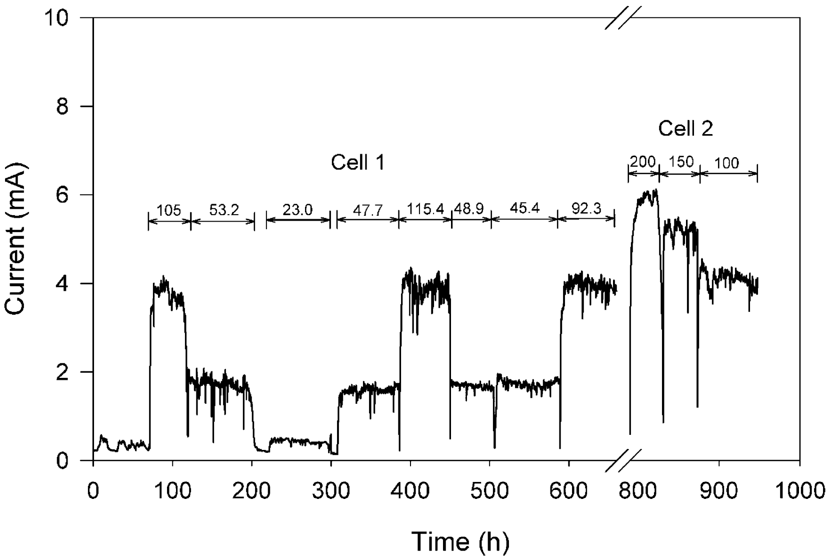

Fig. 3. Continuous monitoring of AW with different BOD values. Two MFCs were independently operated under a substrate feeding rate of 0.35 ml/min and oxidant flow rate of 5 ml/min. The numbers above the arrows indicate the BOD values of the AW in mg/l.

3.4. Starvation and recovery

Tests were conducted to determine the relationship between starvation and recovery. Fig. 5 shows the current generation patterns after starvation for varying periods. The longer the MFC was starved, the longer the recovery time. The current returned to the original level within 30 min with the resumption of feeding after interruption of the feeding for 3 h, whereas it took 3.5 and 80 h to reach the original current level after starvation for 15 and 270 h, respectively.

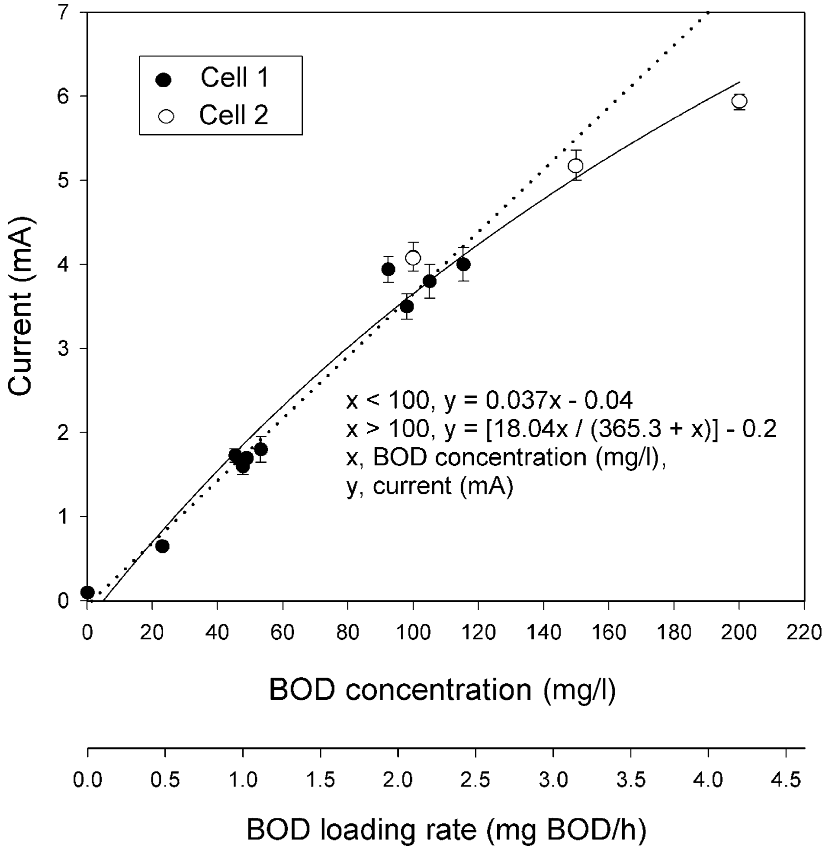

Fig. 4. Relationship between BOD value and steady-state current.

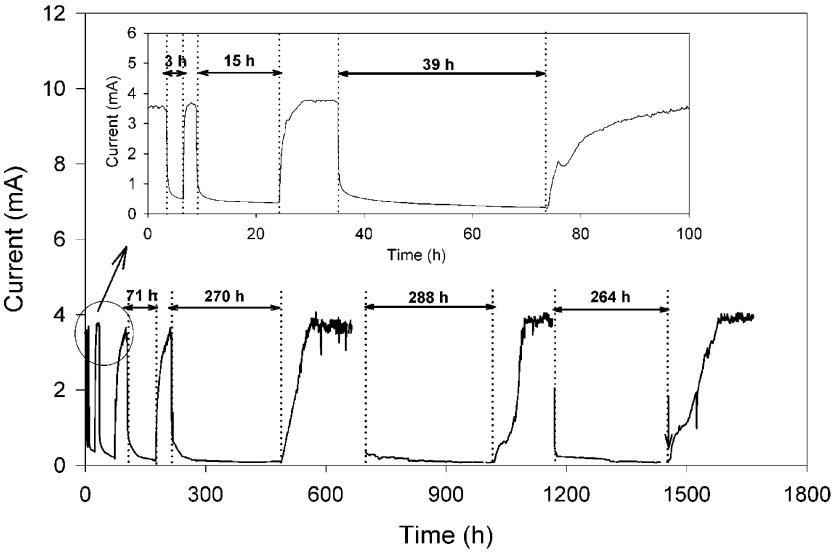

Fig. 5. Effect of starvation and chloramphenicol on recovery of current generation by MFCs. The MFCs were starved and fed with AW (BOD of 100 mg/l) at a rate of 0.35 ml/min. The duration of the starvation is shown in the figure above the double-headed arrows. AW (BOD of 100 mg/l) containing chloramphenicol (15.6 mg/l) was fed to test the effect of protein synthesis during the recovery period after the MFC was starved for 264 h as indicated by the arrow.

After starvation for 288 h, the MFC was fed with AW containing 15.6 mg/l chloramphenicol to test the effect of inhibiting protein synthesis during recovery. The MFC fed with AW containing chloramphenicol still generated the original current level, suggesting that new protein synthesis was not necessarily required for recovery.

During the starvation period, the MFC generated a background current of 0.08 mA, indicating that the microorganisms in the MFC maintained an endogenous metabolism during starvation.

4. Conclusions

A mediator-less microbial fuel cell was tested as a continuous BOD sensor. At a feeding rate of 0.35 ml/min (HRT = 1.05 h), BOD values of up to 100 mg/l could be measured based on a linear relation. Higher BOD values were then measured using either a model fitting method or a lower feeding rate. About 60 min was required to reach a new steady-state current after changing the strength of the AW. When the MFC was starved, the original current value was regained with varying recovery periods depending on the length of the starvation. During starvation, the MFC generated a background level current, probably through an endogenous metabolism. New protein synthesis was not required for the recovery.

Acknowledgements

This work was supported partly by the “Bioproducts and Biotechnology Research Program” and “National Research Laboratory Program” of the Ministry of Science and Technology, Korea.

References

American Public Health Association, 1995. Standard Methods for the Examination of Water and Wastewater, 19th ed. Washington, DC.

Caccavo Jr., F., 1999. Protein-mediated adhesion of the dissimilatory Fe(III)-reducing bacterium *Shewanella alga* BrY to hydrous ferric oxide. Appl. Environ. Microbiol. 65, 5017–5022.

Diekert, G., 1991. The acetogenic bacteria. In: Balows, A., Trüper, H.G., Dworkin, M., Harder, W., Schleifer, K.-H. (Eds.), The Prokaryotes, second ed. Springer, New York, pp. 517–533.

Gil, G.C., Chang, I.S., Kim, B.H., Kim, M., Jang, J.K., Park, H.S., Kim, H.J., 2003. Operational parameters affecting the performance of a mediator-less microbial fuel. Biosens. Bioelectron. 18, 327–334.

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, C.K., Tamiya, E., Takeuchi, T., Karube, I., Inoue, N., 1993. Novel BOD sensor based on bacterial luminescence. Biotechnol. Bioeng. 41, 1107–1111.

Karube, I., Matsunga, T., Mitsuda, S., Suzuki, S., 1977. Microbial electrode BOD sensors. Biotechnol. Bioeng. 19, 153–1547.

Kim, B.H., 1999a. Microbial fuel cell using wastewater as fuel. In: Proceedings of the 99th General meeting of the American Society for Microbiology, Chicago, 586 pp. (Abstract Book).

Kim, B.H., Kim, H.J., Hyun, M.S., Park, D.H., 1999b. Direct electrode reaction of Fe(III) reducing bacterium, *Shewanella putrefaciens*. J. Microbiol. Biotechnol. 9, 127–131.

Kim, H.J., Hyun, M.S., Chang, I.S., Kim, B.H., 1999c. Microbial fuel cell type lactate biosensor using a metal-reducing bacterium, *Shewanella putrefaciens*. J. Microbiol. Biotechnol. 9, 365–367.

Kim, M.N., Kwon, H.S., 1999. Biochemical oxygen demand sensor using *Serratia marcescens* LSY 4. Biosen. Bioelectron. 14, 1–7.

Kim, B.H., Ikeda, T., Park, D.H., Kim, H.J., Kano, K., Takagi, K., 1997. Electrochemical reactions of an iron-reducing bacterium, *Shewanella* *putrefaciens* IR-1. In: Proceedings of the 97th General meeting of the American Society for Microbiology, Miami Beach, 343 pp. (Abstract Book).

Kim, H.J., Park, H.S., Hyun, M.S., Chang, I.S., Kim, M., Kim, B.H., 2002. Mediator-less microbial fuel cell using a metal reducing bacterium, *Shewanella putrefaciens*. Enzyme Microb. Technol. 30, 145–152.

Kim, B.H., Chang, I.S., Gil, G.C., Park, H.S., Kim, H.J., 2003. Novel BOD (biological oxygen demand) sensor using mediator-less microbial fuel cell. Biotechnol. Lett. 25, 541–545.

Kulys, J., Kadziauskiene, K., 1980. Yeast BOD sensor. Biotechnol. Bioeng. 22, 221–226.

Liu, J., Bjornsson, L., Mattiasson, B., 2000. Immobilised activated sludge based biosensor for biochemical oxygen demand measurement. Biosen. Bioelectron. 14, 883–893.

Matsunaga, T., Karube, I., Suzuki, S., 1980. A specific microbial sensor for formic acid. Eur. J. Appl. Microbiol. Biotechnol. 10, 235–243.

Monod, J., 1942. Recherces sur des croissanres des cultures bacteriennes. Hermass et Cie, Paris.

Reynolds, D.M., Ahmad, S.R., 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.

Sangeetha, S., Sugandhi, G., Murugesan, M., Murali Mudhav, V., Berchmans, S., Rajasekar, R., Rajasekar, S., Jeyakumar, D., Prabhakara, R.G., 1996. *Torulopsis candida* based sensor for the estimation of biochemical oxygen demand and its evaluation. Electroanalysis 8, 698–707.

Striling, J.L., Bennetto, H.P., Delaney, G.M., Mason, J.R., Roller, S.D., Tanaka, K., Thurston, C.F., 1983. Microbial fuel cells. Biochem. Soc. Trans. 11, 451–453.

Thurston, C.F., Bennetto, H.P., G, M., Mason, J.R., Roller, S.D., Striling, J.L., 1985. Glucose metabolism in a microbial fuel cell: stoichiometry of product formation a thionine-mediated *Proteus vulgaris* fuel cell and its relation to coulombic yields. J. Gen. Microbiol. 131, 1393–1401.

Tan, T.C., Li, F., Neoh, K.G., 1993. Measurement of BOD by initial rate of response of a microbial sensor. Sens. Actuators 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. Sens. Actuators B45, 217–222.