

## A Microbial Fuel Cell Type Lactate Biosensor Using a Metal-Reducing Bacterium, *Shewanella putrefaciens*

KIM, HYUNG JOO, MOON SIK HYUN, IN SEOP CHANG, AND BYUNG HONG KIM\*

Water-Environment Research Center, Korea Institute of Science and Technology, P. O. Box 131, Cheongyang, Seoul 130-650, Korea

Received: December 11, 1998

**Abstract** A fuel cell type biosensor for lactate was developed using a metal-reducing bacterium, *Shewanella putrefaciens* IR-1. Under the operational conditions, the bacterial cell suspension generated the current without an electrochemical mediator in the presence of lactate. The current was proportional to the lactate concentration up to 30 mM.

**Key words:** Microbial sensor, mediator-less microbial fuel cell, metal-reducing bacteria, *Shewanella putrefaciens*

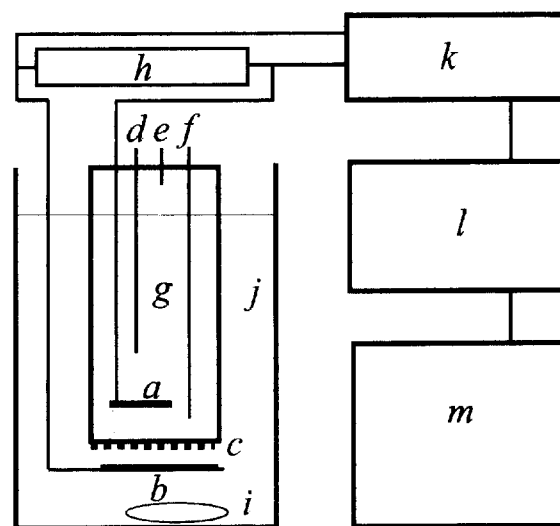
Microbial cells are used for the construction of a wide variety of biosensors [12]. These sensors exploit the microbial metabolism or their enzymes. Various ion selective electrodes and oxygen electrodes are used as transducers to convert the microbial or enzyme activities related to the concentration of the analytes to electrical signals, or electrochemical mediators are employed to facilitate the electron transfer from enzymes to electrodes [6, 13, 14]. Most of the mediators used are expensive and are toxic aromatic compounds [15].

The water-insoluble ferric iron is used as an electron acceptor by facultative and obligate anaerobes [2, 7, 10, 16]. One of them, *Shewanella putrefaciens* MR-1, was reported to have cytochromes in its outer membrane [9]. These electron carriers (i.e. cytochromes) are believed to participate in the reduction of the water-insoluble electron acceptor using electrons generated from the oxidation of electron donors in the cytoplasm. Recently, we have shown that anaerobically grown cells of a ferric iron reducing bacterium, *S. putrefaciens* IR-1, are electrochemically active [5], and that the bacterium can be cultivated in a microbial fuel cell without additions of any terminal electron acceptors such as oxygen or Fe(III) [3, 4]. This communication describes the use of this microbial fuel cell as a prototype lactate sensor.

The metal-reducing bacterium, *S. putrefaciens* IR-1, was used throughout the study. *S. putrefaciens* IR-1 was

isolated in this laboratory from a paddy field in Korea. The bacterium was cultivated in LB broth containing 10 g/l of FeOOH [11] for 72 h at 30°C, and cells were washed by centrifugation using oxygen-free 0.05 M phosphate buffer containing 0.1 M NaCl (pH 7.0). The same buffer was used to prepare a cell suspension (0.25 g l<sup>-1</sup> in dry weight).

A microbial fuel cell was used to construct a lactate sensor [1]. Figure 1 shows the schematic diagram of the electrochemical cell. The cathode was filled with approximately 20 ml of 0.05 M phosphate buffer (pH 7.0) containing 0.1 M NaCl and 0.1 M potassium ferricyanide. The cathode compartment was separated from the anode compartment by a cation exchange membrane (Asahi Chemical, Tokyo, Japan) held between two butyl rubber gaskets. The anode compartment contained 19 ml of bacterial suspension. The reaction mixture in the anode compartment was agitated and kept anoxic by sparging with oxygen-free nitrogen. The anode consisted of a plate of graphite



**Fig. 1.** Schematic diagram of the microbial lactate sensor. (a) Working electrode (anode). (b) Counter electrode (cathode). (c) Ion-exchange membrane. (d) Sample injection port. (e) N<sub>2</sub> outlet. (f) N<sub>2</sub> inlet. (g) Anode compartment. (h) Resistor. (i) Magnetic stirrer bar. (j) Cathode compartment. (k) Voltmeter. (l) Scanner. (m) Data acquisition system.

\*Corresponding author  
Phone: 82-2-958-5831; Fax: 82-2-958-5839;  
E-mail: bhkim@kistmail.kist.re.kr

felt (0.8×4×0.3 cm) cemented to a platinum wire using conducting cement (Graphite Epoxy EPOX-4, Electrosynthesis, New York, U.S.A.). The electrochemical cell was placed in a water bath (25°C). The circuit was completed by connecting the anode through a 500 ohm resistor to the cathode (reticulated vitreous carbon, 3×3×0.3 cm, Electrosynthesis).

After the background current had reached a steady value, 1 ml lactate solution of known concentration was added to the anode compartment, and the change of current was recorded using a digital voltmeter (Model 2000, Keithley, Cleveland, U.S.A.). Readings were taken (5 sec intervals) using a scanner (Model 2000-scan, Keithley) which was connected to the voltmeter. The data were collected on an IBM compatible PC linked to the IEEE 488 input/output system of the scanner and voltmeter (Model CTMGPIB-1, Keithley).

When lactate was added to the microbial fuel cell, the current increased to a plateau value (Fig. 2). The rates of current increment were proportional to lactate concentration over the range of 2–25 mM with the correlation coefficient factor of 0.84 (Fig. 3). Relatively large deviations in the current output were observed in the microbial fuel cell using cell suspensions exposed to air, or prepared from old

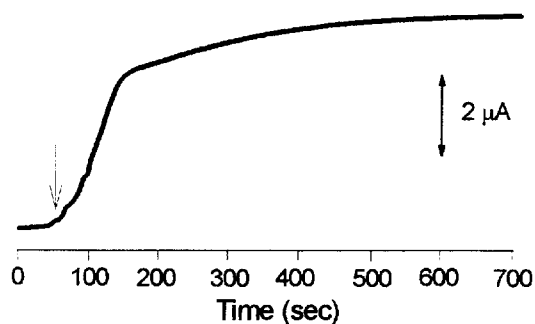


Fig. 2. A typical trace of the current change of the microbial sensor with addition of lactate (indicated by the arrow).

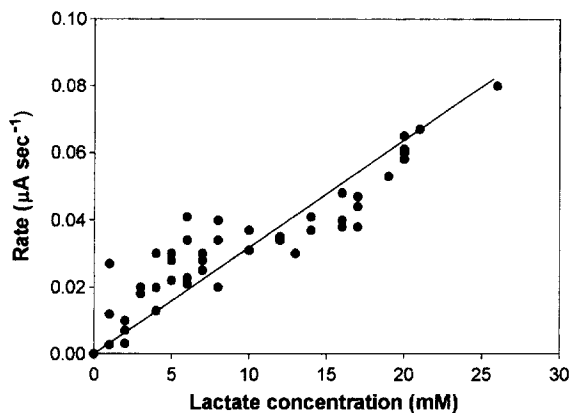


Fig. 3. Correlation between lactate concentrations and the rate of current increase.

cultures, especially at low lactate concentrations. Lactate concentrations lower than 1 mM produced unstable results due to the increased noise in the electrochemical signal. Glucose up to 20 mM did not produce any signal, but pyruvate and formate produced positive signals (results not shown). These results are expected because pyruvate and formate are used by the bacterium as electron donors [8], as is lactate. Since a current was produced by the addition of the natural electron donors of the bacterium, but not by glucose or by acetate to the system, it could be concluded that the bacterial oxidation of the electron donors and the direct electron transfer from the cell surface to electrode produced the electrochemical signals.

Several Fe(III)-reducers have been isolated based on their ability to use various electron donors [2, 16]. In this laboratory, electrochemical activities were observed in more than 80% of all 200 Fe(III)-reducers isolated (unpublished data). Since this fuel cell type microbial sensor is simpler and less expensive than those employing mediators or specific electrodes such as dissolved oxygen or pH sensors, a wide range of applications is possible. This might be the first application of a direct electrochemical reaction by an intact bacterial cell for the construction of a biosensor. Application of metal-reducing bacteria and the construction of a more accurate sensing device will be explored in future work. Currently, studies are also being made to construct a BOD sensor using a metal-reducing bacteria.

## Acknowledgments

A part of this study was supported by grants from the Ministry of Science and Technology, Korea. H. J. Kim was a recipient of a post-doctoral fellowship from the Korea Science and Engineering Foundation.

## REFERENCES

1. Allen, R. M. and H. P. Bennetto. 1993. Microbial fuel cell. *Appl. Biochem. Biotechnol.* **39**: 27–40.
2. Greene, A. C., B. C. Patel, and A. J. Sheehy. 1997. *Deferribacter thermophilus* gen. nov, a novel thermophilic manganese- and iron-reducing bacterium isolated from a petroleum reservoir. *Int. J. Syst. Bacteriol.* **47**: 505–509.
3. Hyun, M. S., H. J. Kim, and B. H. Kim. 1998. Use of a fuel cell to enrich electrochemically active Fe(III)-reducing bacteria, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., Atlanta, U.S.A., p. 309.
4. Kim, B. H., H. J. Kim, M. S. Hyun, and D. H. Park. 1999. Direct electrode reaction of Fe(III) reducing bacterium, *Shewanella putrefaciens*. *J. Microbiol. Biotechnol.* **9**: 127–131.

5. Kim, B. H., T. Ikeda, D. H. Park, H. J. Kim, K. Kano, K. Takagi, and H. Hatsumi. 1997. Electrochemical reactions of an iron-reducing bacterium, *Shewanella putrefaciens*, IR-1, Abstr. 97th Gen. Meet. Am. Soc. Microbiol., Miami, U.S.A., p. 343.
6. Kim, H. J., H. P. Bennetto, and M. A. Halablab. 1995. A novel liposome-based electrochemical biosensor for the detection of haemolytic microorganisms. *Biotechnol. Tech.* **9**: 389-394.
7. Lovley, D. R. 1993. Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiol. Rev.* **55**: 259-287.
8. Lovley, D. R., E. J. P. Phillips, and D. J. Lonergan. 1989. Hydrogen and formate oxidation coupled to dissimilatory reduction of iron or manganese by *Alteromonas putrefaciens*. *Appl. Environ. Microbiol.* **55**: 700-706.
9. Myers, C. R. and J. M. Myers. 1992. Localization of cytochromes to the outer membrane of anaerobically grown *Shewanella putrefaciens* MR-1. *J. Bacteriol.* **174**: 3429-3438.
10. Nealson, K. H. and D. Saffarini. 1994. Iron and manganese in anaerobic respiration: Environmental significance, physiology, and regulation. *Annu. Rev. Microbiol.* **48**: 311-343.
11. Phillips, E. J. P. and D. R. Lovley. 1987. Determination of Fe(III) and Fe(II) in oxalate extracts of sediment. *Soil Sci. Soc. Am. J.* **51**: 938-941.
12. Racek, J. 1995. *Cell-Based Biosensor*, pp. 3-21. Technomic Publication, Lancaster, Pennsylvania, U.S.A.
13. Rawson, D. M. and A. J. Willmer. 1989. Whole-cell biosensors for environmental monitoring. *Biosensors* **4**: 299-311.
14. Richardson, N. J., S. Gardner, and D. M. Rawson. 1991. A chemically mediated amperometric biosensor for monitoring of eubacterial respiration. *J. Appl. Bacteriol.* **70**: 422-426.
15. Siebel, D., H. P. Bennetto, G. M. Delaney, J. R. Mason, J. L. Stirling, and C. F. Thurston. 1984. Electron-transfer coupling in microbial fuel cells: 1. Comparison of redox-mediator reduction rates and respiratory rates of bacteria. *J. Chem. Tech. Biotechnol.* **34B**: 3-12.
16. Slobodkin, A., A.-L. Reysenbach, N. Strutz, M. Dreier, and J. Wiegel. 1997. *Thermoterrabacterium ferrireducens* gen. nov., sp. nov., a thermophilic anaerobic dissimilatory Fe(III)-reducing bacterium from a continental hot spring. *Int. J. Syst. Bacteriol.* **47**: 541-547.