한국환경분석학회지 제6권 (제1호) 29~34, 2003
J. of the Korean Society for Environmental Analysis

Electricity Production Coupled to Bacterial Oxidation of Organic Compounds of Sediment Isolated from Riverbed of Anyang-cheon: Simulation for Removal of Organic Compounds from Sediment of Water Stream

Doo Hyun Park, Woo Man Lee, Seong Jin Jeon, and Jae Seong Rhee*

Department of Biological Engineering, Seokyeong University, Jungneung-dong Seongbuk-gu, Seoul 136-704, Korea
**Environment & Process Technology Division, Korea Institute of Science and Technology, P.O. Box 131, Cheongyangri, Seoul 136-650, Korea*

The functional electrodes (graphite-Mn(IV) anode and graphite-Fe(III) cathode) were equipped in a simulated bioreactor of a natural water stream for testing the electricity production coupled to bacterial oxidation of organic compounds of sediment on riverbed. Anode was located under sediment and cathode was located over upper a water stream. Three-quarters of cathode was exposed to atmosphere. About 0.45 volt (4.5 mA) of electricity was produced and ORP between upper stream and sediment was increased to 0.65 volt in bioreactor used for test. The Decreasing rates of COD of both sediment and upper stream in bioreactor equipped with electrodes were 1.6−1.8 times higher than in bioreactor without electrodes. Results suppest that This serves a possibility that the sediment and upper stream of natural water stream contaminated with wastewater could be treated with biological method but without dredging.

Key words: natural water stream, sediment, wastewater, bioreactor, electricity production, oxidation-reduction potential, cyclovoltammetry

1. Introduction

The natural stream flowing through metropolis may be easily contaminated with wastewater from industries and households (apartments and independent houses), and air pollutant consisting of exhaust gas from vehicles and industries. Biodegradable organic compounds of contaminants can be aerobically and anaerobically oxidized in water stream and under sediment, respectively.[12)] For the biological oxidation of organic compounds under aerobic condition sufficient oxygen has to be in water stream but for that under anaerobic condition sufficient oxidant such as nitrate, sulfate, carbonate and ferric ion.[11)] Naturally, atmospheric air (oxygen) can be dissolved into water stream but the dissolved oxygen has to be insufficient for oxidation of organic compounds because amount of contaminants is much more than amount of dissolved oxygen. By which the organic compounds have to be accumulated on sediment and the environment of sediment has to be converted to anoxygenic or anaerobic condition.[4,23)] Under anaerobic condition, nitrate, sulfate, carbonate and ferric ion can be biologically reduced to ammonium (amines and amides), hydrogen sulfide, methane and ferrous ion, respectively. These reduced compounds coupled to bacterial oxidation of organic compounds under anaerobic condition can be another contaminant for atmosphere because those can emit various bad odors.[5,6,3)] For removal of sediment accumulated on riverbed, the dredging method has been used for a long time. The dredging method, however, was confirmed not to be helpful for quality improvement of water stream. And the sediment has to be accumulated again in a relatively short span of time. Accordingly, the best way for control or inhibition of sediment accumulation is completely stopping influx of wastewater into waster stream. At present, however, any water streams or rivers located in or near to Seoul metropolis are not free from contamination of wastewater or other contamination sources.

In this research, a experiment with a bioreactor was performed to remove organic compounds from sediment of water stream instead of dredging method. Most of bacterial metabolism for oxidation of organic compounds is coupled to reduce NAD^+ to NADH which is primary reducing power for production of free energy and biosynthesis of molecules for bacterial structure.[24] Generally, bacterial reducing power (NADH) can be oxidized coupled to reduction of artificial electron mediators (e.g., neutral red, thionin or hydroquinone) and the reduced electron mediator can be reoxidized coupled to electron transfer from electron mediator to anode. However, the electron mediators are difficult to be applied to biofuel cell equipped in natural water stream because all electron mediator is water-soluble, toxic and expensive. It is reason why the electron mediator has not to be continuously added to biofuel cell. Park and Zeikus[15,16,17] developed electrodes with electron transfer from bacterial cell to electrode (anode) and from electrode (cathode) to water, respectively. The electron flow from bacterial cell to anode and anode to cathode can be activated by function of electrodes, and the bacterial metabolism may be activated for oxidation of organic compounds for compensation of electrons (free energy) transferred from bacterial cell to electrode.[13,14,18] By this mechanism, the bacterial metabolism for oxidation of organic compounds in sediment my be activated and volume and weight of sediment may be decreased.

2. Experimental

2.1. Sampling

The sediment was collected at 12 sites of Anyang-cheon and transferred to laboratory by using plastic sampling bottles. The mixture of sediment collected from different sites was not stored but immediately applied to bioreactor prepared for experiment for maintenance of anaerobic condition.

2.2. Preparation of electrodes

A metallic (Fe^{3+}) cathode(graphite-Fe(III)) was made from mixture of 60% (w/w) fine graphite powder (mean particle size was below 600 mesh), 37% (w/w) inorganic binder (white clay mainly composed of Kaolin for porcelain of which mean particle size was below 400 mesh), 3.0% (w/w) ferric ion, respectively. And one side of cathode was coated with 2 mm thickness porcelain septum made from 100% clay, through which proton can transfer from anolyte (bacterial culture) to cathode. The A metallic (Mn^{4+}) anode (graphite-Mn(IV)) was made from mixture of 60% (w/w) fine graphite powder, 37% (w/w) inorganic binder, 3.0% (w/w) manganese ion, respectively. An unmodified electrode was made from mixture of 60% (w/w) fine graphite powder and 40% (w/w) inorganic binder (Kaolin, White clay), respectively. Proper amount of distilled water was added to the mixture for making a graphite paste, and the paste was configured to square-shaped plate (20 cm×20 cm×1 cm thickness) by pressing at 1.0 kg/cm^2, drying on air for one to two weeks at room temperature and solidified by baking at 1200°C for 12 hr under anaerobic condition using an electric Kiln (Red Corona Model 50L, USA).

2.3. Composition of bioreactor

The bioreactor was composed of functional electrodes which are modified graphite with Mn(IV) and Fe(III), respectively. Graphite-Mn(V) was used as an anode and graphite-Fe(III) was used as a cathode. The anode was equipped at bottom of bioreactor and cathode was equipped on water stream. Especially, the three-quarter of cathode was exposed on air for being maintained contact with oxygen but one-qurater was immerged in water stream for transfer of proton produced from bacterial metabolism as shown in Figs. 1 and 2. The total volume of bioreactor was 50 liter and flow of water stream was maintained at 10 liter/hr. No extra nutrition was added to bioreactor. The temperature of bioreactor was maintained from 18 to 20°C.

2.4. Measurement of electricity production

The electricity produced from anode and cathode equipped in bioreactor of water stream was measured with automatic data acquisition system. The external variable resistance was connected between anode and cathode for control of electron flow rates and 100 ohm

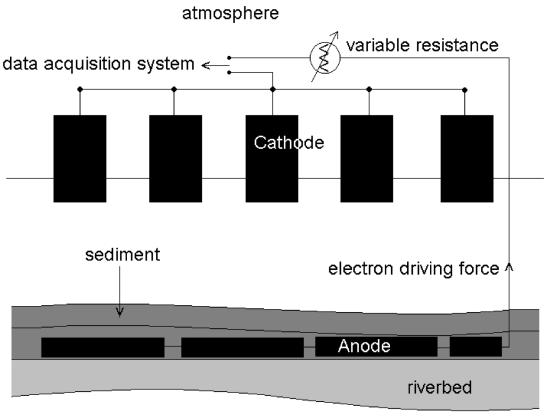

Fig. 1. Schematic structure of anode and cathode installed in water stream. Both anode and cathode have micropore inside itself.

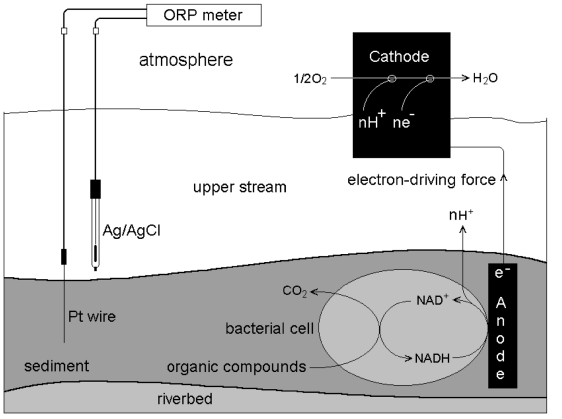

Fig. 2. Mechanism of biofuel cell of which anode and cathode were installed under sediment and over stream. In this fuel cell, no membrane between anode and cathode and no electron mediator are required.

of resistance was maintained for this experiment. No electrodes were equipped in bioreactor used for control test.

2.5. Measurement of COD

Chemical oxygen demand was measured with Haak (DR-2100 model, German) COD measurement system and kit, all procedure for pre-treatment of samples was being done in accordance with Standard Method.

2.6. Measurement of oxidation-reduction potential

Oxidation-reduction potential (ORP) can be a key indicator for estimate of water condition. ORP between sediment and upper stream of water stream was measured with Ag/AgCl reference electrode and platinum wire (0.5 mmϕ×10 cmm) working electrode. As shown in Fig. 2. Pt wire was located in sediment and reference electrode was located on upper stream. It is useful method for measurement of ORP difference between upper stream and sediment.

2.7. Cyclic voltammetry

The cyclic voltammograms was obtained using graphite working electrode modified with Mn^{4+} and Fe^{3+} which was transformed into rod type (diameter 5 mm, length 4 cm), platinum wire counter electrode and an Ag/AgCl reference electrode in 50 mM phosphate buffer (pH 7.0). Cyclic voltammetry was performed using a cyclic voltammetric potentiotat (model CV50W, BAS, USA) linked to an IBM personal computer data acquisition system. Prior to use, the electrodes were cleaned using ultrasonic cleaner. The scanning rate used was 25 mVs^{-1} over the range of +2.0 volt to 2.0 volt.

3. Results and Discussion

The electron mediators have been used for making electron channel from bacterial cell to electrode, which have to be continuously added to bacterial culture in bacterial fuel cell because only some of them can be contacted to both bacterial cell and electrode surface.[21] In bacterial fuel cell with soluble electron mediator, the current production was reported not to be very enough for useful energy production because current production is proportional to amounts of reduced electron mediators coupled to oxidation of bacterial reducing power and of transfer efficiency of electron mediator from bacterial cell to electrode across bacterial membrane.[14,19,1,22] Generally, the bacterial fuel cell with two-compartment-anode and cathode compartment-has been used and cation-selective membrane has to be used for separation of anode compartment from cathode compartment. As shown in Fig. 1, the electron mediator can't be applied to natural water stream, and the cation-selective membrane also can't be used for natural water

stream. Instead, anode and cathode with different function have to be used for maintenance of separated oxidation and reduction reaction. Only oxidation reaction of organic compounds on surface of anode but only reduction reaction of oxygen with proton and electron transferred from anode on cathode is possible because the redox potential of anode and cathode is different as shown in Fig. 2. The difference of redox potential between anode and cathode is electron-driving force by which the electron can flow only from anode to cathode. The potential difference between anode and cathode is electron-driving force that was increased by modification of anode and cathode with Mn(IV)-ion and Fe(III)-ion, respectively. As shown in cyclic voltammograms of Figs. 3 and 4, the *Eh*(half redox potential) difference between Mn(IV)-graphite anode of which *Eh* is 0.35 volt vs. NHE and Fe(III)-graphite cathode of which *Eh* is 0.693 volt vs. NHE was about 0.34 volt. In close-circuited bacterial fuel cell the potential difference must be lower than maximal potential of open-circuited fuel cell because the electron density on anode surface produced coupled to oxidation of bacterial reducing power is quickly dissipated through cathode to oxygen, however, the dissipation of electron density can be controlled by using an external resistance between anode and cathode[16] as shown in Fig. 1. Various anaerobic bacterial consortium such as *Clostridium* sp., *Shewanella* sp., sulfate-reducing bacteria and denitrifying bacterial consortium have been reported to reduce metal ion such as manganese ion (Mg^{4+}), ferric ion (Fe^{3+}), uranium ion (U^{2+}), selenium ion (Se^{2+}) and cupric ion (Cu^{2+}).[2,7,8] The metal-reducing bacteria have been reported to spend their reducing power coupled to reduce water-insoluble metallic ion located outside of bacterial cell.[9,10,19] If these metallic ions can be immobilized to electrode, the cation-selective membrane between anode and cathode can be excluded from bacterial fuel cell. As shown in Fig. 5, the 0.45 volt of

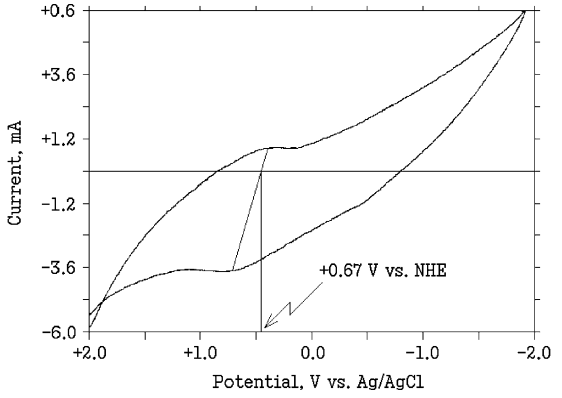

Fig. 3. Cyclovoltammogram of cathode modified with Ferric ion (Fe^{3+}) during successive 2 cycles following the introduction or the electrode into a phosphate buffer (50 mM, pH 7.0). The scan rate was 10mV/s, the working electrode was the modified electrode with Fe(III) ion, the reference electrode was Ag/AgCl and the counter electrode was platinum wire.

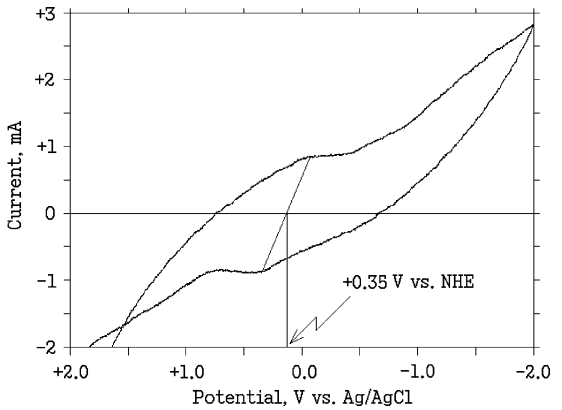

Fig. 4. Cyclovoltammogram of cathode modified with Mn-ion (Mn^{4+}) during successive 2 cycles following the introduction or the electrode into a phosphate buffer (50 mM, pH 7.0).

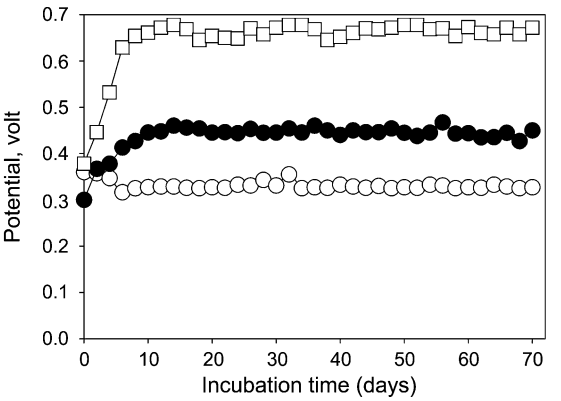

Fig. 5. Potential difference (●) between anode and cathode installed on the simulated water stream (bioreactor). 100 ohm of external resistance was connected between anode and cathode for maintenance of electron flow from anode to cathode.

electricity (current = 4.5 mA) was produced from electrode equipped in water stream and ORP was increased to 0.65 volt vs. Ag/AgCl between uppper stream and sediment but the ORP was about 0.32 volt vs. Ag/AgCl between uppper stream and sediment in bioreactor used for a control test. It is a clue that bacterial oxidation of organic compounds is coupled to electron generation on anode from which electron can flow to cathode on which oxygen can be reduced to water by reaction with electron and proton from anode. In this system, bacterial cell functions as an electron donor and oxygen functions as an electron acceptor. And anode and cathode are electron mediator for electron transfer from bacterial cell to oxygen and also function as a catalyst for oxidation of organic compounds and reduction of oxygen, respectively. When applied graphite-Mn(IV) anode and graphite-Fe(III) cathode to water stream as shown in Fig. 1, the chemical oxygen demand was decreased in both upper stream and sediment as shown in Figs. 6 and 7, and decreasing rates of COD of both sediment and upper stream in bioreactor equipped with electrodes was 1.6–1.8 times higher than in bioreactor without electrodes. Under anaerobic condition without electron acceptor, the energy production for bacterial growth is dependant on the fermentative metabolism but the energy metabolism can be changed to the respiratory metabolism by addition of electron acceptor such as Mn(IV), Fe(III),

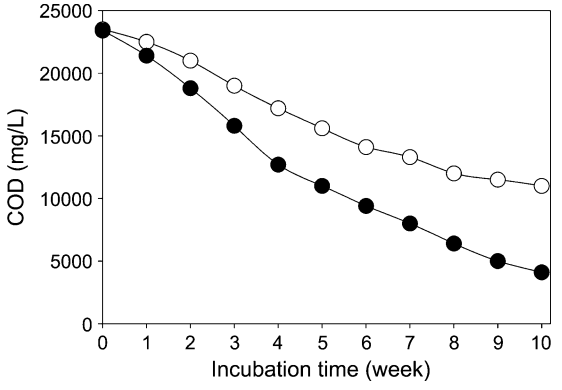

Fig. 6. Variation of chemical oxygen demand of sediment in bioreactor equipped with graphite-Mn(IV) anode and graphite-Fe(III) cathode (●) and without electrodes (○).

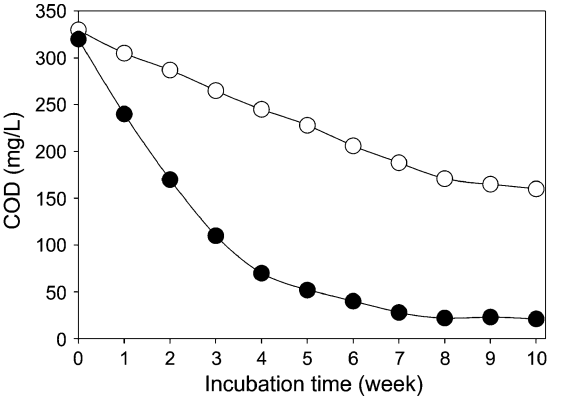

Fig. 7. Variation of chemical oxygen demand of upper stream in bioreactor equipped with graphite-Mn(IV) anode and graphite-Fe(III) cathode (●) and without electrodes (○).

NO^{3-} or SO_4^{2-}. This is shown that the Mn(IV)-graphite anode acts as an electron acceptor and Fe(III)-cathode acts as a oxidizer for re-oxidation of Mn(II) reduced coupled to bacterial metabolism, by which the substrate consumption may be increased. The potential difference between Mn(IV)-anode and Fe(III)-cathode is enough to activate the electron-driving from anode to cathode. The bacterial fuel cell composing of functional anode and cathode equipped in water stream serves a possibility that the electricity production may inhibit or control methane producted from sediment. The methane is 20 times stronger than carbon dioxide for green house effect.

4. Conclusion

Sediment can be accumulated in any natural water stream, lake or artificial reservoir to which organic contaminants influx. Major components of sediment are mixture of various bacterial cells, organic acids and organic polymers. Inside of sediment has to be anaerobic condition because oxygen is difficult to penetrated into sediment. Various anaerobic bacterial consortium has to produce various metabolites such as organic acids, reduced inorganic compounds (nitrite, sulfite, sulfide, ammonium, ferrous ion and hydrogen) and methane. Accordingly, ecological circulation of organic compounds in sediment has to be very slowly

going. The graphite-Mn(IV) anode can be an electron acceptor for anaerobic respiratory metabolism instead of oxygen in sediment and graphite-Fe(III) cathode can be an oxidant for Mn(II) reduced coupled to bacterial oxidation of organic compounds. The anode equipped in sediment and cathode equipped over water stream can be a biofuel cell for making electricity and for activating oxidation of organic compounds in sediment.

References

1. Allen, M. J. **1972**, Cellular electrophysiology, P.247-283. *In* J. R. Norris and D. W. Ribbons (ed.), *Methods in microbiology*. Academic Press, New York, N.Y.
2. Eric, E. R. and Lovely, D. R., *Appl. Environ. Microbiol.* **1993**, 59, 734-742.
3. Hansen, T. A. Odom. J. M., and Singleton, R. (Eds). *The sulfate-reducing bacteria: contemporary perspective*. Springer-Verlag. **1993**, New York. Pp21-40.
4. Knowles, R., *Microbiol. Rev.* **1982**, 46, 43-70.
5. Lovely, D. R., Baedecker, M. J., Lonergan, D. J., Cozzarelli, I. M., Phillips, E. J. P., and Siegel, D. I., *Nature*, **1989**, 339, 297-299.
6. Lovely, D. R. *Microbiol. Rev.* **1991**, 55, 259-287.
7. Lovely, D. R. *J. Bacteriol*. **1993**, 170, 2136-2142.
8. Lovely, D. R., Givannoni, S. J., White, D. C., Champine, J. E., Phillips, E. J. P., Gorby, Y., and Goodwin, S., *Arch. Microbiol*. **1993**, 159, 336-344.
9. Lovely, D. R. and Philip, E. J. P. *Appl. Environ. Microbiol.* **1988**, 51, 683-689.
10. Myer, C. R. and Nelson, K. H. *J., Bacteriol.* **1990**, 172, 6232-6238.
11. Nedwell, D. B., *Adv. Microbial Ecol.* **1984**, 7, 233-235
12. Park, D. H., Kim, B. H., Lim, S. K., and Choi, Y. H., *Kor. J. Appl. Microbiol. Biotechnol.* **1996**, 24, 364-370.
13. Park, D. H. and Kim, B. H., *J. Microbiol.* **2001**, 39, 273-278.
14. Park, D. H., Kim, B. H., Moore, B., Hill, H. A. O., Song, H. K., and Rhee, H. W., *Biotechnol. Tech*. **1997**, 11, 145-148
15. Park, D. H. and Zeikus, J. G., *J. Bacteriol*. **1999**, 181, 2403-2410.
16. Park, D. H. and Zeikus, J. G., *Appl. Microbial. Biotechnol.* **2002**, 59, 58-61.
17. Park, D. H. and Zeikus, J. G., *Appl. Environ. Microbiol*. **2000**, 66, 1292-1297.
18. Park D. H., Kim, S. K., Shin, I. H. and Jeong, Y. J., *Biotech. Lett.* **2000**, 22, 1301-1304.
19. Park, D. H. and Zeikus, J.G., *J. Bacteriol*. **1999**, 181, 2403-2410.
20. Park, D. H. and Kim, B. H., *J. Microbiol.* **2001**, 39, 273-278.
21. Roller, S. D., Bennetto, H. P., Delaney, G. M., Mason, I. R., Stirling, I. L., and Thurston, C.F. *J., Chem. Tech. Biotechnol.* **1984**, 34B, 3-12.
22. Tanaka, K., Tamamushi, R., and Ogawa, T., *Chem. Technol. Biotech*. **1985**, 35B, 191-197.
23. Thauer, R. K. and Morris, J. G., *Gen. Microbiol. Symp*. **1984**, 36, 123-168.
24. Thauer, R. K., Jungermann, K., and Decker, K., *Bacteriol. Rev.* **1977**, 41, 100-180.