Proc. KMB Annual Meeting
June 24-26, 2003, Muju, Korea

Mediator-Less Microbial Fuel Cell Using Electrochemically Active Microbes

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A microbial fuel cell is a two-compartment structure divided by a cation specific membrane. The electrons available through the metabolism of the electron donors by microorganisms are transferred to the anode of the fuel cell, and then to the cathode through the circuit, where they reduce the oxidant consuming protons available through the membrane from the anode (Allen and Benetto 1993). At least two types of microbial fuel cells have been studied. One involves the utilization of electrochemically active metabolites such as hydrogen sulfide produced by microbial metabolism (Haberman and Pommer 1991), and another involves the use of mediators (Park and Zeikus 2000).

A number of bacteria have been isolated with the ability to use Fe(III) as a terminal electron acceptor (Lovley 1991; Nealson and Saffarini 1994). Though there is some evidence that a soluble electron carrier is involved in the electron transfer to the water-insoluble electron acceptor (Newman and Kolter 2000; Turick et al. 2002), direct contact between the bacterial cells and the electron acceptor is required for the dissimilatory Fe(III) reduction (Lovley 1991; Nevin and Lovley 2000). Among the Fe(III)-reducers, *Shewanella putrefaciens* (Myers and Myers 1992) and *Geobacter sulfurreducens* (Lloyd et al. 2000) are known to localize the majority of their membrane-bound cytochromes on the outer membrane, and the former is electrochemically active (Kim et al. 1999a; Kim et al. 1999b). *S. putrefaciens* grew on lactate in the absence of electron acceptors in an electrochemical fuel cell, but did not metabolize lactate when the anode was disconnected from the cathode (Kim et al. 1999b). A mediator-less microbial fuel cell has been successfully operated using *S. putrefaciens* (Kim et al. 2002). An electrochemically active strain of *Clostridium butyricum* has been isolated from the mediator-less microbial fuel cell described in this communication (Park et al. 2001). A strain of *Aeromonas hydrophila* has been isolated from an acetate-fed microbial fuel cell. This strain reduces Fe(III) and is electrochemically active (Pham et al. 2003).

When activated sludge was inoculated, a fuel cell-type electrochemical cell developed an open circuit potential around 0.7 volt and generated current with continuous wastewater feeding at a closed circuit arrangement. The current increased for 2-4 weeks depending on the fuel used before stabilized. Electron microscopic observation of the anode revealed biofilm developed onto the electrode, and microbial clumps loosely associated with the electrode. The current generation was inhibited by rotenone, 2-heptyl-4-hydroxyquinolone-*N*-oxide, and *p*-CMPS; *p*-chloromercuriphenylsulphonate, but not by azide and cyanide. These results show that electrochemically active microbes had been enriched in the fuel cell, and that electrons are transferred from NADH to coenzyme Q before the electrode accept them.

Enrichment cultures were made using different fuels including wastewater and artificial wastewater containing acetate, and glucose + glutamate. Also made were oligotrophic culture with artificial wastewater or surface water. The colony forming units were several orders of magnitude lower than the estimated bacterial cells through chemical analyses of the microbial community of the enriched electrodes. The enriched electrodes showed different bacterial community from that of activated sludge used as the inoculum when analyzed with denaturant gradient gel electrophoresis of segments of 16S rDNA PCR products. Nearly complete 16S rDNA sequence analyses revealed that the enriched electrodes contain many unknown and uncultured bacteria.

This technology is applied to develop a biochemical oxygen demand sensor based on the fact that the current is directly proportional to the strength of wastewater fed, and a novel wastewater treatment process. It is expected that the process generate less sludge than the aerobic process since a large part of energy contained in the organic contaminants is recovered as electricity.

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