Enrichment of Electrochemically Active Bacteria and Development of a Novel Energy Producing System using Mediatorless Microbial Fuel Cell

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Construction and application of a novel mediator-less microbial fuel cell were studied using Fe(III) reducing bacteria. Direct electron transfer was examined from different *Shewanella putrefaciens* strains and its mutants to an electrode. On the basis of these results, the microbial fuel cell was used to enrich electrochemically active microbes using starch-processing wastewater as the electron donor and with sludge of the anaerobic digester as the bacterial source. Within 4 weeks a current of 0.2 mA was generated with a resistance of 1 kΩ in semi-continuous mode. The COD of anode reaction mixture treated with anaerobic sludge decreased from 1900 ppm to 55 ppm. Coulomb has been maintained almostly 10 Coulomb(C). The current generation decreased in the presence of various metabolic inhibitors. Most of the Fe(III)-reducing isolates from the fuel cell were electrochemically active. And, the signal generated showed linear relationship with wastewater organic concentration and can apply to the BOD sensor.

Introduction

Electrochemical techniques can be used in various fields of biology, for example in biochemistry to characterize redox proteins and in biotechnology to develop biosensors, bioelectrochemical synthetic processes and biofuel cells (1). In many cases, intact microbial cells that contain active redox proteins are electrochemically inactive as their cell walls and other surface structures are electrically non-conductive. Mediators are used to facilitate the transfer of electrons between microbial cells and an electrode (2). Alternatively, the bacterial cells can be modified with hydrophobic conducting compounds to increase electrochemical activity (3). Recently a number of bacteria have been isolated with the ability to use Fe(III) as a terminal electron acceptor (4). Direct contact between the bacterial cells and the electron acceptor is required for the dissimilatory Fe(III) reduction. Amongst the Fe(III)-reducers, *Shewanella* putrefaciens (5) was known to localize the majority of their membrane-bound cytochromes on the outer membrane, and was electrochemically active(6). In this communication a fuel cell-type electrochemical cell was used to enrich electrochemically active microbes from activated sludge using wastewater as fuel.

Methods

Strains. *S. purtrefaciens* IR-1, MR-1, SR-21, *E.coli* NCIB10772.

Wastewater and activated sludge. Wastewater was collected from a starch processing plant(S co, Korea). The chemical oxygen demand (CODcr) value of the wastewater was around 1,700200 mg l^{-1}. Anaerobic digester sludge was collected from the wastewater treatment streams of the same plant.

Microbial fuel cell. Transparent polyacrylic plastic was used to construct the fuel cell with the anode compartment capacity of 25 ml. The anode compartment was kept anoxic by gassing oxygen-free nitrogen (15ml min^{-1}), and the cathode compartment oxic with air (15ml min^{-1}), respectively. The change in potential was recorded using a digital voltameter (Model 2000, Keithley) linked to a multichannel scanner. Coulomb was obtained by integrating the current over the time.

Inhibitors. The fuel cells were added with metabolic inhibitors. They are rotenone, 2-heptyl-4-hydroxyquinolone-*N*-oxide(HQNO), antimycin A, cyanide, azide, *p*-chloromercuri-phenylsulphonate(*p*-CMPS), 2,4-dinitrophenol(DNP) and dicyclohexylcarbodiimide (DCCD).

Cyclic voltammetry. Cyclic voltammograms were obtained using a potentiostat (CV-50W, BAS) interfaced to a personal computer with software supplied by the manufacturer.

Microscopy. Low vacuum electron micrographs were taken using JSM 5410(Jeol). Scanning electron micrographs were taken after the enriched electrode samples were coated with gold sputtering. Bacterial suspensions were prepared by vigorous shaking before fixed, embedded and stained according to the standard method for transmission electron microscopy. The electrode samples were stained with LIVE BacLight Bacterial Gram stain kit (Molecular Probes) using a Carl Zeiss LSM-410 confocal scanning laser microscope.

Results

An open circuit potential of around 0.6-0.8 volt was developed immediately after the addition of the sludge. When the fuel cell was discharged with a resistance of 1 kΩ the potential dropped to 20 mV, which corresponds to a current of 20 μA. When a portion of the anode content (5 ml) was replaced with fresh wastewater, the current increased to 0.2 mA before falling to a background level of 20 μA. Repeated wastewater replacements were coupled to current generation together with a stepwise fall in COD from 1,900 mg l^{-1} to 55 mg l^{-1} in 30 days. The current generation might be the results of electrons transferred to the electrode by the electrochemically active bacteria after they metabolizes organic compounds in the wastewater in the absence of electron acceptors other than carbonate. Similar results were obtained from fuel cells fed by other wastewater including artificial wastewater and effluent from septic tanks (data not shown).

The fuel cells were treated with various metabolic inhibitors. The current generation was reduced in the presence of the inhibitors to varying degrees depending on the inhibitor used. Current generation fell gradually by increasing the concentration of NADH reductase inhibitors, hydroxyquinoline-*N*-oxide (HQNO) and rotenone, and an iron-sulfur protein inhibitor, *p*-chloromercuriphenylsulphonate (*p*-CMPS). Antimycin A (0.12 mM) and azide (1.2 mM) did not show any effects. The current generation increased slightly with cyanide at low concentration up to 1.5 mM. The ATPase inhibitor, *N,N*-dicyclohexyl-carbodiimide (0.6 mM, DCCD) and uncoupler, dinitrophenol (0.24mM) inhibited the electrochemical activity completely.

A piece of the electrode was taken from the anode compartment of the fuel cell operated over a year and observed directly using a low vacuum electron microscope (LVEM). The observation showed biofilm formed on the electrode surface and clumps scattered around the electrode. A similar sample

was used for scanning electron microscopy (SEM) after coated with gold. SEM showed a thick film on the electrode surface with particles of different sizes on its surface. Some particles were about the same size as normal bacteria, and others were smaller (0.2-0.3 μm). The biofilm was separated from the electrode by vortexing and collected on a nucleopore filter (0.2 μm). SEM observation confirmed the existence of small particles in the size range of 0.2-0.3 μm. The enriched electrode was observed using confocal scanning laser microscope (CSLM) after fluorescent staining to distinguish the Gram reactions of the bacteria. The micrographs showed that Gram negative and positive bacteria formed microcolonies throughout the electrode surface. Colonies with clear halos on plates containing ferric pyrophosphate were picked up and cultivated to measure their Fe(III) reduction and electrochemical activities. Most (21 of 24) isolates had Fe(III) reduction and electrochemical activities determined by cyclic voltammetry.

Reference

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