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Microbial communities involved in electricity generation from sulfide oxidation in a microbial fuel cell

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ABSTRACT

Simultaneous electricity generation and sulfide removal can be achieved in a microbial fuel cell (MFC). In electricity harvesting from sulfide oxidation in such an MFC, various microbial communities are involved. It is essential to elucidate the microbial communities and their roles in the sulfide conversion and electricity generation. In this work, an MFC was constructed to enrich a microbial consortium, which could harvest electricity from sulfide oxidation. Electrochemical analysis demonstrated that microbial catalysis was involved in electricity output in the sulfide-fed MFC. The anode-attached and planktonic communities could perform catalysis independently, and synergistic interactions occurred when the two communities worked together. A 16S rRNA clone library analysis was employed to characterize the microbial communities in the MFC. The anode-attached and planktonic communities shared similar richness and diversity, while the LIBSHUFF analysis revealed that the two community structures were significantly different. The exoelectrogenic, sulfur-oxidizing and sulfate-reducing bacteria were found in the MFC anodic chamber. The discovery of these bacteria was consistent with the community characteristics for electricity generation from sulfide oxidation. The exoelectrogenic bacteria were found both on the anode and in the solution. The sulfur-oxidizing bacteria were present in greater abundance on the anode than in the solution, while the sulfate-reducing bacteria preferably lived in the solution.

1. Introduction

Microbial fuel cells (MFC) provide a new approach for electricity generation from biomass. By utilizing microbial metabolism an MFC produces an electrical current from the degradation of organic/inorganic matters. Different types of exoelectrogenic bacteria have been isolated from naturally colonized anodes. However, many of these strains generate low power densities when grown as pure cultures (Rabaey et al., 2004; Zuo et al., 2008). It is therefore proposed some synergistic interactions might exist among members of an exoelectrogenic community (Logan, 2009).

Molecular techniques have been widely applied to examine the composition of microbial communities in MFCs inoculated with mixed cultures (Kim et al., 2007; Lee et al., 2003; Phung et al., 2004). The communities in MFCs display great bacterial diversity, depending on the inoculum source or electron donors. An acetate-fed system inoculated with activated sludge exhibited a near-even distribution of α-, γ-, and δ-*Proteobacteria* (Lee et al., 2003). In an ethanol-fed two-chamber MFC, 83% of cloned 16S rRNA gene sequences were related to β-*Proteobacteria* (Kim et al., 2007). A β-*Proteobacteria*-dominated MFC community was reported with a river sediment inoculum and river water feeding, while in another MFC fed with glucose and glutamate the predominant clones were α-*Proteobacteria* (Phung et al., 2004).

Bacteria in MFCs can survive either through being electrochemically active or through interactions with other bacteria (Logan, 2007). A synergistic relationship between photosynthetic bacteria and heterotrophic exoelectrogenic bacteria was proposed to exist in a self-sustained phototrophic MFC (He et al., 2009). Glucose conversion in MFCs was demonstrated to be a complex process, in which different bacterial populations were involved. Hydrogen and acetate produced by fermentative bacteria could be used by exoelectrogenic bacteria for electricity generation (Freguia et al., 2008). The synergistic mechanism also plays an important role in electron transfer process. *Lactobacillus amylovorus* and *Enterococcus faecium* could make use of phenazine-based mediators produced by *Pseudomonas* sp. to transfer electrons (Rabaey et al., 2005).

Wastewaters usually contain not only organics but also inorganic matters, such as sulfide. Sulfide is a hazardous substance and needs to be removed from wastewater before discharged

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into environment. The MFC system was found to be effective for simultaneous sulfide removal and electricity generation (Rabaey et al., 2006). Recently, we have demonstrated that microbial catalysis played an important role in the electricity generation from a sulfide-fed MFC (Sun et al., 2009). Although the microbes involved in the oxidation of carbon-based compounds have been extensively studied (Kim et al., 2007; Lee et al., 2003; Phung et al., 2004), information about the microbial communities in a sulfide-fed MFC is not available yet. Therefore, in order to further elucidate the microbes involved and their roles in such a process, in the present work we examined the microbial community diversity and its catalytic activity in a sulfide-fed MFC anode. The microbial communities on the anode and in the solution were characterized by the 16S rRNA clone library analysis.

2. Materials and methods

2.1. MFC construction and operation

The single-chamber MFC with air cathode as described previously (Sun et al., 2008) was used in this study (Fig. S1). The anodic chamber was filled with 350 mL of medium consisted of (in 1 L of 50 mM phosphate buffer, pH 7.0): NH_4Cl, 310 mg; KCl, 130 mg; Na_2CO_3, 750 mg; $CaCl_2$, 50 mg; $MgCl_2{\cdot}6H_2O$, 100 mg; NaCl, 10 mg; $FeCl_2$, 25 mg; $CoCl_2{\cdot}2H_2O$, 5 mg; $MnCl_2{\cdot}4H_2O$, 5 mg; $AlCl_3$, 2.5 mg; $(NH_4)_6Mo_7O_{24}$, 15 mg; H_3BO_3, 5 mg; $NiCl_2{\cdot}6H_2O$, 0.5 mg; $CuCl_2{\cdot}2H_2O$, 3.5 mg; $ZnCl_2$, 5 mg. The MFC was inoculated with 10 mL of sludge collected from an anaerobic reactor fed with wastewater rich in sodium sulfide. Sodium sulfide was added to a final concentration of 2 mM after the anodic chamber was purged with N_2 to remove dissolved O_2. The fed-batch mode was adopted to incubate the active microbial communities. The MFC, designated as Reactor A, was operated with a fixed external resistance of 1 kΩ. Electricity was generated in the first cycle and sodium sulfide was amended when the current density was less than 20 mA m^{-2}. Two identical MFCs were operated, with one for electrochemical evaluation and another for microbial community analysis. An abiotic reactor with a sterile anode, designated as Reactor B, was operated as control under the same conditions as Reactor A. All experiments were conducted in duplicate at 25 °C.

2.2. Electrochemical analysis

Voltage across a 1 kΩ resistor was continuously recorded with an electrochemical workstation (660C, CH Instruments Inc., USA) and used to calculate the circuit current. Current density was then normalized by the projected surface areas of the two sides of anode (45 cm^2). After the MFC was operated for half a year, polarization curves were obtained by applying a linear potential decrease of 1 mV s^{-1} from the open circuit voltage to 0 mV.

2.3. 16S rRNA clone library construction

After the MFC had been operated for half a year, microbial analysis of the anode-attached and planktonic communities was performed. The anode was removed from the anodic chamber and rinsed with sterile distilled water to remove debris and loosely attached bacteria. Anodes were then cut and fragmented using sterile scissors. Planktonic bacteria were collected by centrifugating 10 mL of anode solution at 12,000 × *g* for 15 min at 4 °C. Genomic DNA was extracted using FastDNA® SPIN Kit for Soil (MP BIO Qbiogene Inc., USA) according to the manufacturer's instructions. The DNA was amplified using the 27f (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′) universal primer sets (Suzuki and Giovannoni, 1996) with a GeneAmp PCR System PCT-200 (MJ Research Co., USA). The PCR products amplified using a 27f/1492r primer pair were cloned into the pGEM-T vector system (Promega Co., USA) and transformed into competent *Escherichia coli* TOP10 competent cells (TianGen Biotech. Co. Ltd., China). The transformants were plated on Luria-Bertani agar medium containing 5 μg mL^{-1} ampicillin and 5-bromo-4-chloro-3-indolyl-β-galactopyrano-side (X-gal). Ampicillin-resistant and β-galactosidase-negative white clones were selected and transferred to a liquid medium of the same composition. Plasmids were then extracted from the colonies using the Plasmid Mini Kit (Huashun Inc., China). PCR amplification was performed twice according to the program. The first PCR amplification was performed with T7 forward (5′-TAA TAC GAC TCA CTA TAG GG-3′)/SP6 reverse (5′-ATT TAG GTG ACA CTA TAG AAT-3′) primers. The second PCR amplification was performed using the amplicons of the first PCR as a DNA template with 27f forward/1492f reverse primers.

2.4. Phylogenetic analysis

The clones were sequenced and analyzed in the Genbank database (http://www.ncbi.nlm.nih.gov) and Ribosomal Database Project II (RDP II, http://rdp.cme.msu.edu). All sequences were checked for chimeras using the CHECK_CHIMERA program (http://rdp8.cme.msu.edu/cgis/chimera.cgi?su=SSU). Nonchimeric sequences were aligned with Clustal X v.1.8 (Thompson et al., 1997) and manual adjustment was performed with the alignment-visualizing tool of MEGA4 (Kumar et al., 2004). Operational taxonomic units (OTUs) from clone libraries were defined with Mothur v.1.6.0 program at a cutoff value of 0.03 (http://schloss.micro.umass.edu/wiki/Main_Page). Phylogenetic trees were constructed with MEGA4 by using the UPGMA method and bootstrapping with 1000 repetitions.

Mothur v.1.6.0 program was used to generate richness (Chao1 and ACE) (Chao, 1984; Chao and Lee, 1992) and diversity estimators (Mills and Wassel, 1980) for individual libraries. Clone libraries were compared using LIBSHUFF based on Jukes-Cantor pairwise distance matrices (Schloss et al., 2004). Nonparametric estimators of the fraction and richness of OTUs shared between two communities were analyzed using Mothur v.1.6.0 program.

Partial 16S rRNA gene sequences have been deposited in the Genbank database under accession numbers FJ347713-FJ7719, GQ472933–GQ472959, and GU247454–GU247471.

3. Results

3.1. Enrichment of microbial community in sulfide-fed MFC

The potential of sulfide as a substrate to generate electricity was examined by monitoring current evolution during the culture enrichment in the sulfide-fed MFC (Fig. 1A). After inoculation, electricity was generated immediately after sulfide dose. In the 1st feed cycle, the current density jumped to 75 mA m^{-2}, and then rapidly dropped to zero after 25 h. In the subsequent three cycles, the current densities maximized at ca. 75 mA m^{-2} and stabilized above 17 mA m^{-2} after 25-h cultivation. In the 5th and 6th cycles, the current density reached the same maximum level (78 mA m^{-2}) as before, but declined more slowly. It took 60 h for the current density to decrease to 20 mA m^{-2}. The total charges produced by the MFC increased by 4 times from 4.9 C in the 1st cycle to 21.4 C in the 6th cycle. The polarization and power–current curves of this sulfide-fed MFC are shown in Fig. 1B. The maximum power density of 13 mW m^{-2} was achieved with a current density of 96 mA m^{-2}.