B−65 Direct power generation from wastewater using continuous microbial fuel cells

Kyungmi Chung[1]* · Keiichi Kumano[1] · Satoshi Okabe[1]

[1] Division of Built Environment, Hokkaido University

* E-mail: wjdrudal@eng.hokudai.ac.jp

1. Introduction

The concerns of global fossil fuel depletion and environmental pollution from fossil fuel combustion are driving the search for carbon-neutral, renewable energy alternatives. Using electrochemically active bacteria as biocatalysts, microbial fuel cells (MFCs) are devices that use bacteria as the catalysts to oxidize organic and inorganic matter and generate current. In MFCs, anodophilic bacteria gain energy by transferring electrons to an anode, either via direct contact, soluble electron shuttles, or nanowires (Reguera *et al.*, 2006). In most MFCs the electrons that reach the cathode combine with protons that diffuse from the anode through a specific membrane and oxygen provided from air; the resulting product is water. Chemical oxidizers, such as ferricyanide can also be used although these must be replaced or regenerated.

MFCs operated using mixed cultures currently achieve substantially greater power densities than those with pure cultures. Community analysis of the microorganisms that exist in MFCs has so far revealed a great diversity in composition. Certain bacteria can oxidize organic matter and use an electrode as an electron acceptor.

Recently, many researchers interested in capabilities of the microorganisms in MFC biofilms because of limited maximum power densities (Logan and Regan, 2006). Achieving maximum power densities requires a better understanding of the type of bacteria that persist and become predominant in these communities, the mechanisms by which bacteria transfer electrons to the electrode and the ways in which bacteria interact in these systems. Thus, microbial populations in MFC biofilms have been studied by several groups. These studies suggested that electrochemically active bacteria such as *Shewanella putrefaciens* and *Geobacter sulfurreducens* had a significant potential for energy generation in the absence of electron acceptors. (Kim *et al.*, 2006; Pham *et al.*, 2003). In this study, we examined effects of various sizes and types of electrode on power generation using continuous systems. Also, we investigated the microbial community that developed in the biofilms of the anode compartment using 16S rRNA gene approaches.

2. Materials and Methods

MFCs were consisted of two chambers (9×7×9 cm) separated by proton exchange membrane (PEM; Nafion ™ 117, Dupont Co., DE). In our reactors, porous carbons (8×7×1 cm, 4 plates) were used as both anode and cathode electrodes and the number of porous carbon plate was changed to evaluate its effect on power generation. In addition, woven graphite sheets loaded with 0.5 mg/cm^2 and 4.0 mg/cm^2 of Pt were added to the cathode electrode.

The anode chamber was inoculated with activated sludge taken from membrane biioreactor (MBR) and fed with the synthetic medium containing glucose (10 mM) in a first reactor and fed with the effluent of the first reactor in a second reactor at a continuous mode at HRT between 4.2 and 4.5 h. The anode and cathode compartments were continuously sparged with nitrogen gas and air, respectively. These reactors were operated at room temperature.

The power output were measured and monitored by using an Agilent HP 34970 data acquisition unit. The power output of the cells (P) was calculated as follows: $P=I\times V$. The coulombic efficiency, E (%), was calculated as $E=C_T/C_{TH}\times 100$, where C_T is the total coulombs calculated by integrating the current over time and C_{TH} was calculated as $FbSv/M$, where F is Faraday's constant, b is the number of electrons of substrate, S is the substrate concentration, and M is the molecular weight of substrate (Oh et al., 2006).

Genomic DNA was extracted from biofilms attached on the anode electrode and amplified by PCR for 16S

rRNA gene cloning analysis. PCR products were purified using a purification kit prior to cloning into ONE SHOT *E.* coli using the pCR-XL-TOPO vector system. All sequences were compared with the similar sequences of the reference organisms by a BLAST search.

3. Results and discussion

These reactors are operated in a continuous mode, which is more practical for further scale up than the batch-fed operation adopted by most researchers. Acetate of 20mM was rapidly produced by glucose fermentation such as $C_6H_{12}O_6 \rightarrow 2CH_3COO^- + 2CO_2 + 4H^+ + 4e^-$. The rapid appearance of acetate in this system indicated that electricity generation proceeded in a method related to glucose degradation via acetate in a ferment condition (Fig. 1, (A)). Electricity generation began to noticeably increase after 3 days, reaching 5 mW/m^2 after 8 days in the first reactor (Fig. 1, (B)).

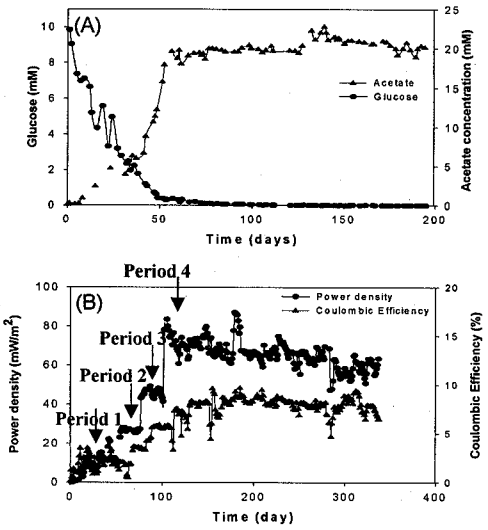

Fig.1 (A): Characteristics of the continuous MFC operated during degradation of glucose and production of acetate in the effluent of the first reactor. (B): Power density and Coulombic efficiency of the first reactor during one year.

The appropriate resistance obtained from a polarization curve, measured by varying the external resistance (1-1000 kΩ). The maximum power density, current density, and proper external resistance were determined to be 24 mW/m^2, 25 mA/m^2 and 300 Ω, respectively (Fig. 2, (A)). With a fixed anode surface area of 224 cm^2, increasing the cathode surface area of the first reactor from 56 to 299 cm^2 increased in the power from 5 to 90 mW/m^2. For the period 1, the power density was increased 5 mW/m^2, the anode effluents showed a DOC removal percentage of 7.9±3%, and the Coulombic efficiency was 3.4%. As well as, for the period 2, the power density was 49 mW/m^2, DOC removal rate of 24.8±2.5%, and the Coulombic efficiency was 3.6%. However, power density in period 3 and 4 was increased from 77 mW/m^2 to 86 mW/m^2. The DOC removal rate and Coulombic efficiencies in period 3 and 4 were 25.7±3.9% and 28.4±4.5% and 9.8% and 10.7%, respectively (Fig. 2, (B)).

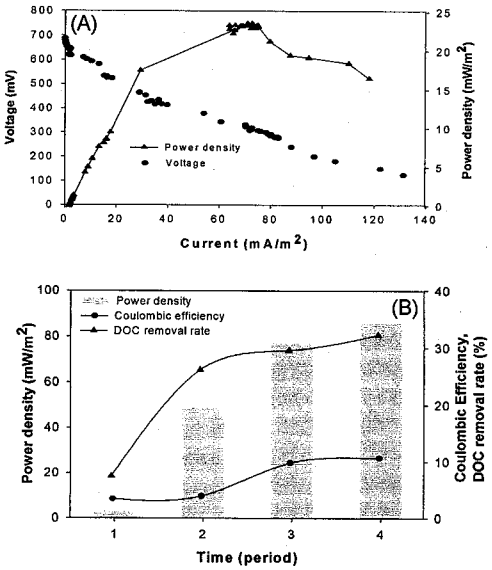

Fig.2 (A): Polarization curve of the MFC operated at various resistances (B): Effects of cathode surface area on power generation, DOC removal rate and Coulombic efficiency.

These results indicated that the power density was continuously generated during the long-term operation, however, it did not increase in proportion to cathode electrode surface area, indicating that other factors are limiting such as the low loading rate.

The performance of the second reactor with the effluent of glucose-fed first reactor was observed in order to utilize contained acetate in effluent of the first reactor. Characteristics of the continuous MFC operated during depletion of acetate and DOC removal rate in the effluent. About 10±3.8% of DOC was removed and approximately 13 mM of acetate was remained in effluent of the second reactor (Fig. 3, (A)). It should be noted that the low DOC removal rate and high acetate concentration are probably due to the short retention time. This organic carbon removal efficiency can be improved

by switching the retention time. When using the optimal external resistance (R=100 Ω) showed a maximum power density of 55 mW/m^2 at a Coulombic efficiency of 16% (Fig. 3; (B), (C)).

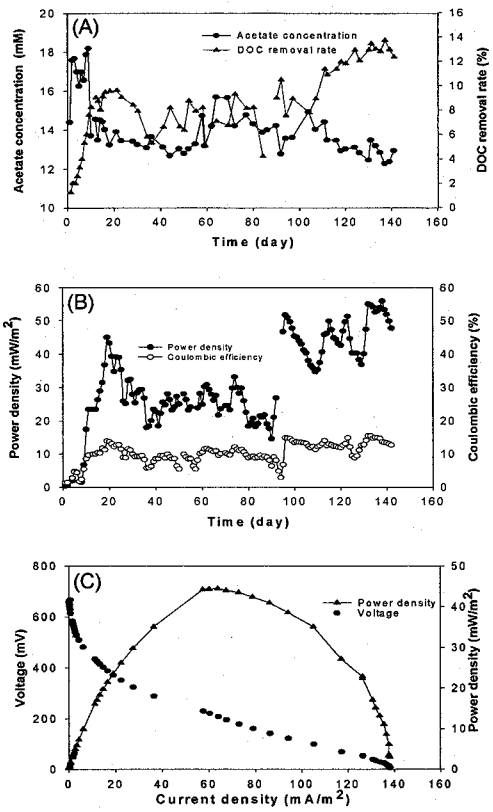

Fig. 3 (A): Characteristics of the continuous MFC operated during degradation of acetate and DOC removal rate in the effluent of second reactor. (B): Power density and Coulombic efficiency of the second reactor. (C): Polarization curve of the MFC operated at various resistances.

In these MFCs, the anode potential is unfavorable for the growth of methanogens and therefore no methane formation was observed during the first stage (within 50 days and 30days, respectively) in these reactors with consistently high Coulombic efficiencies. However, during the operation of these reactors, we found a significant amount of methane production (2.7%), because the electron transfer bacteria were incapable of converting all of the available organics into electricity by methanogens instead of the electron transfer bacteria.

The 16S rRNA gene clone libraries were constructed from the anode biofims taken from the period 1, period 3 of the first reactor, the second reactor and control reactor of open circuit system to determine dominated bacterial community. The *Proteobacteria* dominated in the anode biofilms of the glucose-fed first reactor and the most *Gammaproteobacteria* was closely related to *Aeromonas* species (97-99%) however, the ratio of *Firmicutes* was higher about 60%, and the most *Firmicutes* was closely related to *Lactococcus* species (98-99%) in the second reactor. In the control reactor of same energy source, materials and retention time with open circuit, the ratio of *Firmicutes* was 69% and observed more diverse community than the first reactor. The results of the 16S rRNA gene sequences provided evidences that there were clear differences in the bacterial community structure between the different energy sources and condition of reactors. FISH images clearly showed *Archaea* in the mixed bacterial sampled form the surface of the anode biofilms. Relative abundance by FISH using specific probes with EUB and *Arehaea* probes clearly showed methanogens of about 20% in the anode biofilms of both the first and second reactors.

In this study, two-chamber MFCs fed with glucose (10 mM) continuously produced up to 135 mW/m^2 (80 mW/m^2 in the first reactor and 55 mW/m^2 in the second reactor). However, Coulombic efficiency was low (10-16%) due to high internal resistances and presence of methanogens. The *Gammaproteobacteria* and *Firmicutes* dominated in the first reactor and the second with effluent of the first reactor, respectively. FISH analysis showed that *Aeromonas* (potential electrochemically active bacteria) were present throughout the biofilms (accounting for 40% of total bacteria), while, *Archaea* mainly existed in the deeper parts of the biofilms (accounting for 20% of total abcteria).

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