Variations of electron flux and microbial community in air-cathode microbial fuel cells fed with different substrates
Jaecheul Yu, Younghyun Park, Haein Cho, Jieun Chun, Jiyun Seon, Sunja Cho and Taeho Lee

ABSTRACT
Microbial fuel cells (MFCs) can convert chemical energy to electricity using microbes as catalysts and a variety of organic wastewaters as substrates. However, electron loss occurs when fermentable substrates are used because fermentation bacteria and methanogens are involved in electron flow from the substrates to electricity. In this study, MFCs using glucose (G-MFC), propionate (P-MFC), butyrate (B-MFC), acetate (A-MFC), and a mix (M-MFC, glucose:propionate:butyrate:acetate = 1:1:1:1) were operated in batch mode. The metabolites and microbial communities were analyzed. The current was the largest electron sink in M-, G-, B-, and A-MFCs; the initial chemical oxygen demands (CODini) involved in current production were 60.1% for M-MFC, 52.7% for G-MFC, 56.1% for B-MFC, and 68.3% for A-MFC. Most of the glucose was converted to propionate (40.6% of CODini) and acetate (21.4% of CODini) through lactate (80.3% of CODini) and butyrate (6.1% of CODini). However, an unknown source (62.0% of CODini) and the current (34.5% of CODini) were the largest and second-largest electron sinks in P-MFC. Methane gas was only detected at levels of more than 10% in G- and M-MFCs, meaning that electrochemically active bacteria (EAB) could out-compete acetoclastic methanogens. The microbial communities were different for fermentable and non-fermentable substrate-fed MFCs. Probably, bacteria related to Lactococcus spp. found in G-MFCs with fermentable substrates would be involved in both fermentation and electricity generation. Acinetobacter-like species, and Rhodobacter-like species detected in all the MFCs would be involved in oxidation of organic compounds and electricity generation.

Key words | electron flux, electron sink, microbial community, microbial fuel cell, substrate

INTRODUCTION
Microbial fuel cells (MFCs) can convert chemical energy to electricity using microbes as catalysts and a variety of organic wastewaters as substrates (Kim et al. 2004; Logan & Regan 2006; Torres et al. 2007; Lovley 2008). Bacteria can use various types of substrate. The substrate type affects not only the microbial community but also aspects of the MFC performance, e.g., electricity generation and coulombic efficiency (CE) (Chae et al. 2009; Zhang et al. 2010). It is generally known that glucose, a fermentable substrate, is converted to electrical current via volatile fatty acids (VFAs) such as butyrates, propionates, and acetates. However, electron loss occurs when fermentable substrates are converted to their metabolites, because fermentation bacteria and methanogens are involved in electron flow from the substrates to electricity (Parameswaran et al. 2010).

Some previous studies have electron pathways and microbial ecologies for reducing electron loss and increasing electricity generation in MFCs. Glucose was fermented to hydrogen and acetate, which were used as substrates for electricity generation and methane production in glucose-fed MFCs (Freguia et al. 2008). The electrical current was the largest electron sink in both glucose-fed MFCs (49% of initial chemical oxygen demand (CODini) applied) and acetate-fed MFCs (71% of CODini applied) but methane gas was only detected in glucose-fed MFCs. This indicated that electrochemically active bacteria (EAB) could out-compete acetoclastic methanogens (Lee et al. 2008). Glucose-fed MFCs showed the widest microbial diversity and Betaproteobacteria was relatively abundant in acetate-fed, butyrate-fed, and glucose-fed MFCs, but not in propionate-fed MFCs (Chae et al. 2009).
However, it is unclear whether non-fermentable substrates are really better than fermentable substrates for electricity generation, and how EAB, fermenters, and methanogens relate to electron flow from the substrate to the electricity. In this study, single-chamber MFCs using fermentable (glucose, mixed) and non-fermentable (acetate, butyrate, and propionate) substrates were operated under batch mode. We investigated the effects of substrate type on electricity generation and microbial communities in MFC.

**MATERIALS AND METHODS**

**MFC construction and operation**

Ten identical air-cathode MFCs (225 mL working volume) with a rectangular geometry were constructed (Figure 1). The anode and cathode electrodes (12 cm²) were made of graphite felt and 50% wet-proof carbon cloth (E-Tek, BASF Fuel Cell, Inc., USA), respectively. The air cathode was made by applying a Pt/C catalyst (0.5 mg/cm²) in a NaF ion solution (5%) to the solution side of the cathode. A polypropylene non-woven fabric (Korea Non-Woven Tech. Co, Ltd., Korea) was used as the separator. The anode and cathode were connected using a copper wire.

The anode compartment was inoculated with activated sludge obtained from a domestic wastewater treatment plant (Busan, Korea). MFCs fed with glucose (G-MFC), propionate (P-MFC), butyrate (B-MFC), acetate (A-MFC), and mixed substrates (M-MFC, glucose:propionate:butyrate:acetate = 1:1:1:1) were operated in batch mode at room temperature (20 ± 3 °C). All the MFCs were operated under two sets of conditions (Table 1). The MFCs were operated at carbon-source concentrations of 8 mM (glucose: 1,540 mg/L, propionate: 890 mg/L, butyrate: 1,280 mg/L, acetate: 510 mg/L and mixture: 1,050 mg/L) with an external resistance of 1,000 Ω for intermediate metabolite analysis during six batch cycles (phase I). The MFCs were then operated at COD concentrations of 510 mg/L (glucose: 2.7 mM, propionate: 4.6 mM, butyrate: 3.2 mM, acetate: 8 mM, and a mixed substrate: 3.9 mM) with an external resistance of 100 Ω for electron sink analysis during two batch cycles (phase II). The experimental conditions were changed when the MFC showed stable electricity generation in each phase. The MFCs were refreshed with a basal medium with each carbon source when the voltage dropped below 50 mV. The basal medium contained K₂HPO₄, 3.40 g/L; KH₂PO₄, 4.35 g/L; NH₄Cl, 0.20 g/L; NaCl, 0.04 g/L; MgSO₄·7H₂O, 0.01 g/L; CaCl₂·H₂O, 0.02 g/L; NaHCO₃, 0.25 g/L; KCl, 0.02 g/L and yeast extract, 0.01 g/L except carbon source. The initial pH of all solutions was adjusted to 7.0. Other MFCs were operated in open circuit (disconnected electrodes) mode in a similar way.

**Chemical and electrochemical analysis**

Voltages were measured using a data-acquisition system (Model 7700, Keithley Instruments Inc., OH, USA), and recorded every 270 s on a personal computer. The COD was measured using a CODₐ₂₃ test kit (HS-CODₐ₂₃-LR, Humas Co., Korea). For metabolite analysis, VFAs and glucose were analyzed using HPLC (HP-1100 series, Agilent Inc., CA, USA) and methane and hydrogen were analyzed using GC (7890A, Agilent Inc., CA, USA).

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Substrate type</th>
<th>G-MFC</th>
<th>P-MFC</th>
<th>B-MFC</th>
<th>A-MFC</th>
<th>M-MFC*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase I</td>
<td>Concentrations (mg/L)</td>
<td>1,540 (8 mM)</td>
<td>890 (8 mM)</td>
<td>1,280 (8 mM)</td>
<td>510 (8 mM)</td>
<td>1,050 (8 mM)</td>
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<td></td>
<td>External resistance (Ω)</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
<td>1,000</td>
</tr>
<tr>
<td>Phase II</td>
<td>Concentrations (mg/L)</td>
<td>510</td>
<td>510</td>
<td>510</td>
<td>510</td>
<td>510</td>
</tr>
<tr>
<td></td>
<td>External resistance (Ω)</td>
<td>100</td>
<td>100</td>
<td>100</td>
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</tbody>
</table>

*A mixed substrate contains glucose, propionate, butyrate and acetate mixed at the same ratio.*
Microbial community analysis

The attached growth bacteria in the anode compartments of the MFCs were collected and DNA was extracted using a PowerSoil™ DNA extraction kit (Mo Bio Lab., CA, USA). Bacterial 16S rRNA genes were amplified as described by Yu et al. (2010). Denaturing gradient gel electrophoresis (DGGE) was conducted and visualized as described by Yu et al. (2010, 2012). The band profile was visualized using an ultraviolet transilluminator (Uvitec, Cambridge, UK) and photographed using a digital camera (Olympus 720 UZ, Olympus Optical Co., Ltd., Japan). The band positions and intensities in the DGGE profiles were determined using the Fingerprinting II Informatix software (Bio-Rad, Hercules, CA, US). Principal component analysis (PCA) was performed to identify relationships in the band profile using the SPSS 14.0 software (SPSS Inc. Chicago, IL, USA). DNA fragments extracted from the DGGE band profile were polymerase chain reaction (PCR) amplified using the same primers as those used for PCR amplification in the DGGE experiments. The fragments were then sequenced using an ABI 3730XL capillary DNA sequencer (Applied Biosystems, NJ, USA) by a professional company (Solgent Co., Korea). The sequence results were analyzed using the GenBank database, and phylotype identification was performed on the basis of the 16S rDNA sequence homology.

RESULTS AND DISCUSSION

Electrical performance

The voltage generation varied with substrate type. After enrichment (about four cycles, 70 d), stable voltages were generated in the G-, P-, B-, A-, and M-MFCs during phases I and II (Figure 2). All the MFCs showed similar peak voltages of 580 ± 17.30 mV at an external resistance of 1,000 Ω during phase I (Figure 2(a)). However, in phase II, the MFC showed differences in their peak voltages, depending on the substrate type: G-, A-, and M-MFC produced similar voltages of 289 ± 39.38 mV; P-MFC showed the lowest peak voltage, 167 mV (Figure 2(b)). These results indicated that glucose or acetate would be suitable substrates for electricity generation in MFCs.

Electron flux and sink

Glucose was converted into lactate (80.3% of COD ini), propionate (7.8% of COD ini) and butyrate (6.1% of COD ini) during the first day. After that, propionate (40.6% of COD ini) and acetate (21.4% of COD ini) were accumulated as a result of lactate decomposition. Butyrate and propionate were converted to acetate with 12.0 and 2.0% of COD ini, respectively. G-MFC showed the highest methane gas production, and no hydrogen gas was detected in any of the MFCs. Phase I showed that lactate was an important intermediate metabolite in the G-MFC, and acetate was accumulated in G-, P-, and B-MFCs. Only the A-MFC produced electricity through VFA oxidation (Figure 3). Acetate was therefore used for direct electricity generation in all the MFCs.

Current generation was independent of the substrate type. All substrates were directly or indirectly converted to current in the MFCs. Table 2 describes the electron sinks as COD (mg) in G-, P-, B-, A-, and M-MFCs at the end of batch operation (phase II). The current was the largest electron sink in M-, G-, B-, and A-MFCs: 60.1% of COD ini for M-MFC, 52.7% for G-MFC, 56.1% for B-MFC, and 68.3% for A-MFC. However, an unknown source (62.0% of COD ini) and the current (34.5% of COD ini) were the largest and second-largest electron sinks in the P-MFC. Lee et al. (2008) reported that electricity was the largest electron sink (acetate, 71%; glucose, 49%) and no hydrogen gas was detected in glucose- and acetate-fed MFCs. Methane gas was only detected at levels above 10% in G- and M-MFCs, the A-MFC (2.7% of total electrons) showed little methane production. This
means that EAB can out-compete acetoclastic methanogens (Lee et al. 2008).

**Microbial community analysis**

The attached growth bacteria in the anodes of the MFCs at phase II were analyzed. The DGGE profile showed the microbial communities were affected by the substrate type and circuit mode (open or closed) (Figure 4(a)). PCA results more clearly indicated that microbial communities were varied according to substrate type and circuit mode, especially with regard to whether the supplied substrate was fermentable or non-fermentable (Figure 4(b)).

Bacteria similar to *Rhodobacter gluconicum*, *Xanthomonas axonopodis* and an uncultured gamma proteobacterium were detected in all the MFCs of both closed and open circuits, indicating the possible involvement of fermentation. *Rhodobacter* sp. can use a variety of organic compounds as carbon and electron sources under anoxic conditions (Garrity et al. 2005a). *Xanthomonas* sp., which is chemoorganotrophic, is able to use various organic acids as sole carbon sources (Garrity et al. 2005b). A band sequence with 99% similarity to *Pseudomonas* sp. was only detected in closed-circuit M-, B-, and P-MFC, and this would be involved in electricity generation. A bacterial sequence with 99% similarity to *Pseudomonas putida* was
observed in M-, B-, P-, and G-MFC, and a sequence with 97% similarity to *Acinetobacter* sp. was detected in G-, B-, and A-MFCs; both would be involved in fermentation and electricity generation in closed-circuits, because previous studies have demonstrated that several *Pseudomonas*-like species can transfer electrons to electrodes (Logan 2009). A band sequence with 98% similarity to *Acinetobacter johnsonii* and a sequence with 96% similarity to uncultured *Bacteroidetes* bacteria were detected in closed circuit B- and P-MFCs, respectively; these were involved in electricity generation. They can use acetate or lactate as carbon and energy sources (Garrity et al. 2008b). A bacterial sequence with 96% similarity to a *Propionibacteriaceae* bacterium was observed in closed- and open- circuit P-MFC; these bacteria can ferment propionate to acetate, and would be related to fermentation (Garrity et al. 2009b). A band sequence with 99% similarity to *Lactococcus* sp. was discovered in G-MFCs with fermentable substrates, and these would be involved in both fermentation and electricity generation.

CONCLUSION

This study shows the variations of electron flux and microbial community in MFCs using fermentable and non-fermentable substrates. An MFC can directly or indirectly use both fermentable and non-fermentable substrates for electricity generation. The glucose and acetate-fed MFCs showed higher voltage than other substrate-fed MFCs. Most of the glucose was converted to propionate and acetate through lactate for electricity generation. Acetate is used for direct electricity generation in all the MFCs. Current was the most significant electron sink in all the MFCs, except in the case of propionate-fed MFCs, and EAB were more competitive than acetoclastic methanogens. There were differences between the microbial communities in fermentable and non-fermentable substrate-fed MFCs. Probably the bacteria related to *Lactococcus* sp. discovered in G-MFCs with fermentable substrates would be involved in both fermentation and electricity generation. *Acinetobacter*-like species, and *Rhodobacter*-like species, which were detected in all the MFCs, would be involved in oxidation of organic compound and electricity generation.

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REFERENCES


