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BIOTECHNOLOGY METHODS

Electron flux and microbial community in microbial fuel cells (open-circuit and closed-circuit modes) and fermentation

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Abstract A closed-circuit microbial fuel cell (C-MFC) was operated to investigate the electron flux under fed-batch mode, and the results were compared to those of open-circuit MFC (O-MFC) and a fermentation reactor (F-reactor). The current was the largest electron sink (52.7 % of influent SCOD) in C-MFC, whereas biomass and methane gas were the most significant electron sinks in O-MFC and F-reactor. Interestingly, some of the unknown sink may have accumulated in the electrode of O-MFC. Principal component analysis based on gradient gel electrophoresis profiles showed that the microbial communities were significantly affected by the growth conditions and the presence of electrode, regardless of the circuit connection. Therefore, the electrode and circuit mode might help to control the amount of biomass and enhance the MFC performance.

Keywords Closed-circuit · Electron flux · Fermentation · Microbial community · Open-circuit

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Introduction

Microbial fuel cells (MFCs) capable of producing electricity from organic compounds by electrochemically active bacteria (EAB) as catalyst have emerged as a promising technology [2, 8]. They can use a great variety of substrates from pure to complex for electricity generation [9].

However, electron loss takes place when complex substrates such as wastewater are used because complex biofilm are developed on the electrode for producing electricity [6] and fermenters and methanogens are involved in the electron flow from substrates to electricity [11]. Therefore, it is critical to understand this electron flow for enhancing MFC performance. Several research groups have reported the electron flow in MFC. In case of MFC fed with ethanol, acetate produced from ethanol fermentation can be used for electricity generation but hydrogen gas cannot be consumed and methane gas was a significant electron sink [14]. When MFC fed with glucose and acetate was operated under closed-circuit mode, the electricity was the largest electron sink and methane gas was only detected in glucose-fed MFC [7]. Electricity was the largest electron sink as chemical oxygen demand (COD) in glucose-, butyrate-, and acetate-fed MFCs. Methane gas was detected at low levels in MFCs fed with glucose and butyrate and was little detected in propionate- and acetate-fed MFCs [17]. Although the electron balances of MFC have been well studied, the electron flow from the anode to the cathode remains poorly understood.

In this study, therefore, the electron balances and microbial community were investigated in closed-circuit MFC (C-MFC) operated under fed-batch mode. For increased understanding of the electron flows, C-MFC was compared to open-circuit MFC (O-MFC) and a fermentation reactor operated without electrode (F-reactor).

Materials and methods

Reactor construction and operation

Three reactors (two MFCs and one F-reactor) were prepared. Two single-chamber MFCs (50 × 90 × 60 mm, working volume 225 mL) were constructed [17]. The anode and cathode electrode were used as previously described and a polypropylene nonwoven fabric (Korea Non-Woven Tech. Co, Ltd., Korea) was used as the separator [17]. For C-MFC, the anode and cathode were connected by a copper wire with an external resistance of 100 Ω. For O-MFC, the anode and cathode were not connected. For fermentation, an identically sized F-reactor without electrodes was set up and was operated under anaerobic conditions.

The anode compartments of MFC and F-reactor were inoculated with activated sludge (3000 mg/L) obtained from a domestic wastewater treatment plant (Busan, Korea). All reactors were fed with synthetic wastewater including glucose (2.7 mM and 510 mg-SCOD/L) as carbon source. The substrate distribution in the reactors was maintained uniform by mixing with magnetic stirrers. The synthetic wastewater contained K_2HPO_4, 3.40 g/L; KH_2PO_4, 4.35 g/L; NH_4Cl, 0.20 g/L; NaCl, 0.04 g/L; $MgSO_4{\cdot}7H_2O$, 0.01 g/L; $CaCl_2{\cdot}H_2O$, 0.02 g/L; $NaHCO_3$, 0.25 g/L; KCl, 0.02 g/L and yeast extract, 0.01 g/L, except for the carbon source. All experiments were performed in duplicate at room temperature (22 ± 4 °C) in fed-batch mode.

Analysis and calculation

The voltage (V) in C-MFC was measured using a data acquisition system (Model 7700, Keithley Instruments Inc., USA) and recorded every 30 s onto a personal computer. The surface power density (PD; mW/m^2) was normalized by the anode projected surface area (4 × 3 cm, 12 cm^2). The maximum PD (PD_{max}) and open-circuit voltage (OCV) were acquired by the linear sweep voltammetry (LSV) method, which was performed at 10 mV/s using a potentiostat (KST-P1, Kosentech Co., Korea). The COD was measured using a COD_{Cr} test kit (HS-COD_{Cr}-LR, Humas Co., Korea). The composition of the volatile fatty acids (VFA) was analyzed using high-performance liquid chromatography (HPLC; HP-1100 series, Agilent Inc., CA, USA) and methane and hydrogen were measured using gas chromatography (GC; 7890A, Agilent Inc., CA, USA). Electron equivalents as soluble COD (SCOD; mg) were calculated as described by [7].

Microbial community analysis

The anodic biofilm and suspended growth bacteria (SGB) in MFC and SGB in F-reactor were collected and DNA was extracted using a Power Soil™ DNA extraction kit (Mo Bio Labs, Carlsbad, CA, USA). Bacterial 16S rRNA genes were amplified and denaturing gradient gel electrophoresis (DGGE) was conducted as previously described [17].

DGGE profiles were digitized using the Fingerprinting II Informatix software (Bio-Rad, Hercules, CA, USA) and principal components analysis (PCA) was performed to identify the relationships in the band profile using SPSS 14.0 software (SPSS Inc. Chicago, IL, USA) [17].

Results and discussion

VFA and methane production in MFCs and fermentation

VFA and methane were analyzed in MFC and fermentation process. Glucose was converted to acetate via lactate, propionate, and butyrate in all reactors (Fig. 1). In O-MFC, glucose was primarily converted in lactate and then decomposed into propionate, butyrate and acetate. C-MFC showed a similar glucose pathway but butyrate was not detected. In F-reactor, glucose was converted into acetate via propionate. Lactate and butyrate were not found in F-reactor. However, glucose in C-MFC was removed within 5 days, which was faster than in O-MFC (Fig. 1). Continuous consumption of protons and electrons at the cathode of C-MFC may have increased the substrate removal efficiency compared to O-MFC [13]. The substrate removal rate in F-reactor was similar that of C-MFC. Direct consumption of protons and electrons may have increased the substrate removal rate observed in the F-reactor compared to that in O-MFC.

After 10 days, F-reactor showed the highest accumulated methane gas production (7.2 mM), followed by O-MFC (6.3 mM) and C-MFC (5.5 mM). Carbon dioxide gas comprised over 70 % of total gas in all reactors, followed by methane, which was about 15 % of total gas in MFCs and 20 % of total gas in F-reactor. Hydrogen gas was only detected at low level in O-MFC and F-reactor (Fig. 2). This might indicate that methane production was limited by electron migration to the electrode. In MFC using glucose (1500 mg-SCOD/L), glucose was converted into lactate, propionate, and butyrate. Methane gas was only measured at low level and no hydrogen gas was detected [17]. Although the anode was not connected to the cathode, the electrode may have hindered methane production. In H-type MFC fed with glucose, EAB out-compete acetoclastic methanogens because methane gas was detected at low level [7]. In case of H-type MFCs using acetate,

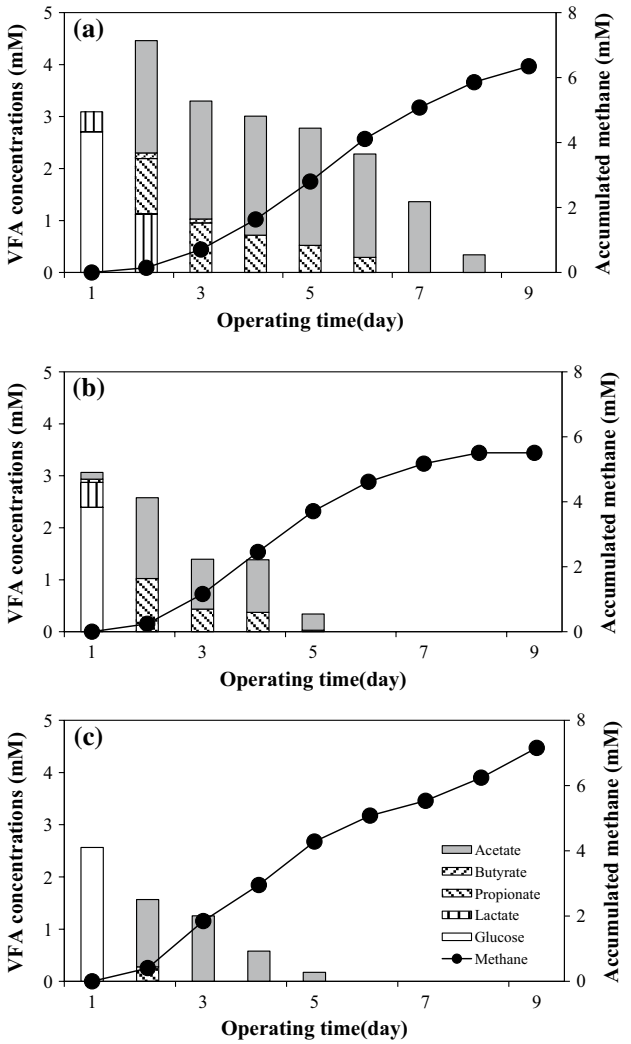

Fig. 1 Glucose, and volatile fatty acid (VFA) concentrations and methane gas production for O-MFC (**a**), C-MFC (**b**) and F-reactor (**c**)

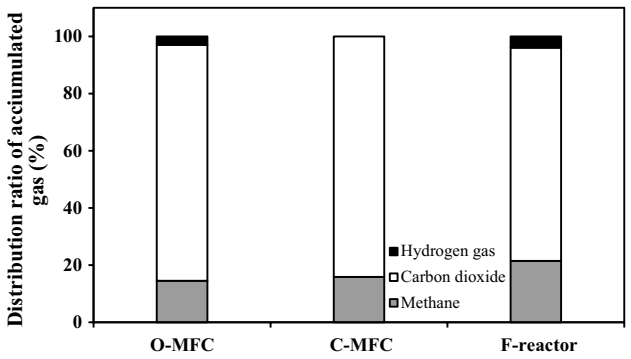

Fig. 2 The distribution ratio of accumulated gas produced for O-MFC, C-MFC and F-reactor

butyrate and propionate, the methane production in C-MFC was lower than that in O-MFC because methanogens were impeded by current flowing through an external circuit in closed-circuit MFC [5]. Open-circuit and closed-circuit MFCs operated at 30 °C showed different microbial communities due to difference in redox potential, which may have suppress methane production [4].

Table 1 Electron distribution (%) as SCOD (mg) in open-circuit MFC (O-MFC), closed-circuit MFC (C-MFC) and fermentation (F-reactor)

Electron sinks	O-MFC	C-MFC	F-reactor
Initial SCOD	100	100	100
Final SCOD	23.4	9.7	8.5
Current	No current	52.8	No current
Biomass[a]	17.6	20.8	25.6
CH_4 gas	15.1	12.9	22.1
H_2 gas	0.8	Not detected	1.1
Unknown[b]	44.0	3.9	42.6

[a] Biomass was assumed 'the fraction of electrons invested in biomass' for fermenters, methanogens, electrochemically active bacteria (EAB), and homo-acetogens of 0.1, 0.08, 0.05, 0.1 (Parameswaran et al. [10])

[b] Unknown sinks were calculated by e^-(Initial SCOD) − e^-(Final SCOD) − e^-(current) − e^-(biomass) − e^-(CH_4 gas) − e^-(H_2) = e^-(unknown sinks)

Electron distribution in MFCs and fermentation

After several fed-batch cycles, C-MFC showed reproducible voltage generation (peak value: 0.25 V) at an external resistance of 100 Ω and the power curves were obtained by LSV. C-MFC showed a PD_{max} of 1.5 W/m^2, OCV of 0.58 V and coulombic efficiency of 62 %.

The electron distribution as SCOD (mg) was analyzed at the end of the operation (Table 1). The current (52.7 % of SCOD influent) was the largest electron sink in C-MFC, followed by biomass (20.8 %), methane gas (12.9 %), final SCOD (9.7 %), and unknown sink (3.9 %), which indicated that most of the electrons were used in electricity generation. Methane gas (22.1 %) was the third largest electron sink in F-reactor, which was higher than that in O-MFC (15.1 %) and C-MFC (12.9 %). Therefore, electrode itself might suppress methane production although circuit was disconnected.

Biomass was a significant electron sink because it was the second largest electron sink in all reactors except O-MFC. Unremoved SCOD (23.4 %) was the second largest electron sink in O-MFC, which indicated that the cut-off of current was unfavorable for the oxidation of organic matter. Unknown sink was the largest electron sink in O-MFC (44 %) and F-reactor (42.6 %). However, some of the unknown sink may have accumulated in the electrode (in case of O-MFC) because a peak OCV value of 0.8 V

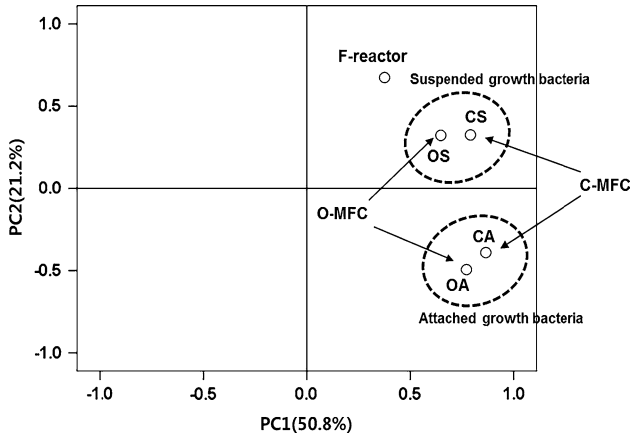

Fig. 3 Principal components analysis (PCA) based on DGGE band positions and intensities for attached growth bacteria and suspended growth bacteria (SGB) in O-MFC and C-MFC, and SGB in F-reactor

was measured in O-MFC. Among glucose- and acetate-fed MFCs, current (49 % and 71 %, respectively) was the largest electron sink and biomass (26 % and 15 %, respectively) was the second largest electron sink in both MFCs [7]. Among O- and C-MFCs (anode polarized at −100 mV and −200 mV), O-MFC exhibited much lower biomass growth rate (0.054 mM-C/h) than that in C-MFC (0.124−0.271 mM-C/h) [15].

Microbial community analysis

DGGE was performed to analyze the microbial community at the end of the operation. PCA based on DGGE profiles (Fig S1) revealed that microbial communities were significantly affected by the growth conditions and the presence of electrode, regardless of the circuit connection (Fig. 3).

In MFCs, interestingly, the microbial community in identical growth condition (either attached growth or suspended growth condition) showed a higher correlation than that in identical circuit mode (closed- and open-circuit modes), which indicated that the community was more significantly affected by the growth condition than by the circuit mode. The microbial community in F-reactor was different from that in O-MFC and C-MFC. Especially, the attached growth bacteria communities of both MFCs were very different, which indicated that the presence of electrode affected the microbial community.

Several studies reported differences in the microbial community between O-MFC and C-MFC. The fore showed higher diversity than that the latter but C-MFC was better for EAB than O-MFC because there were more EAB in C-MFC than in O-MFC [3]. When MFC fed with propionate as substrate was operated under closed- and open-circuit modes, C-MFC was more enriched with bacteria related to EAB and dissimilatory Fe(III)-reducing bacteria [1]. When C-MFC, O-MFC, and O-MFC with the seal off the cathode were operated with acetate in a fed-batch mode, bacteria similar to *Geobacter* (72 % of total sequences), *Azoarcus* (42−47 %), and *Dechloromonas* (17 %) were predominant in three MFCs, respectively [12].

In our experiments, EAB could be developed on the electrode even when operating under open-circuit mode, due to the lack of any significant difference in the microbial community between O-MFC and C-MFC, compared to that of F-reactor. When O-MFC inoculated with *Shewanella decolorationis* S12 was operated with lactate as the substrate, the biofilms viability in O-MFC decreased to 72 % compared to that of C-MFC (98 %). After switching into closed-circuit mode, the biofilm viability of O-MFC was increased from 72 to 97 % and the voltage was increased from 0.12 to 0.29 V [16].

Conclusions

MFCs with either open- or closed-circuit mode and a fermentation reactor were operated under fed-batch mode and their electron distribution and microbial community were analyzed. Current was a significant electron sink for C-MFC. The presence of the electrode significantly affected the electron flux and microbial community. Therefore, the electrode and circuit mode may have helped control the amount of biomass and enhanced the MFC performance. However, further studies are needed to elucidate the unknown sink in open-circuit mode and detailed microbial community in MFCs and fermentation reactor.

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