Original report

Electricity Generation by a Methanogen Cathode Microbial Fuel Cell

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Abstract Microbial fuel cells (MFCs) use microbial metabolism as a catalyst. The most immediate and useful application of MFCs is in wastewater treatment because they enable simultaneous sewage treatment and power generation; sewage is treated with minimal energy input and reduced sludge production. Electrons produced by anaerobic bacteria flow from the anode to the cathode, where they combine with protons and oxygen to form water in a reaction catalyzed by platinum. However, due to the high cost of platinum, there is a need for an alternative cathode catalyst. We developed a fed-batch, continuous MFC that uses a methanogen as the cathode catalyst to generate electricity. The hydrogenotrophic methanogen *Methanothermobacter thermautotrophicus* strain ΔH was inoculated onto the cathode to catalyze the reduction of CO_2. We confirmed that power generation and methane production in the cathode occurred simultaneously. In addition, the maximum power density during continuous operation of the microscale MFC was 385 mW/m^2. Thus, the methanogen received electrons directly from the cathode, and converted CO_2 to CH_4 as like the catalyst. Our MFC shows promise as an alternative to MFCs with a platinum catalyst.

Keywords: Microbial fuel cell, methanogen, biocathode, electrochemical analysis, methane

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Introduction

Microbial fuel cells (MFCs) convert organic matter to electricity using microorganisms as biocatalysts [1]. The most immediate and useful application of MFCs is in wastewater treatment [2, 3] because they enable simultaneous sewage treatment and power generation, and sewage can be treated using minimal energy input with reduced sludge production [4, 5]. Anaerobic microorganisms in the anode chamber of an MFC oxidize organic compounds and transfer electrons to the anode. Electrons move along a circuit to the cathode, where they combine with an electron acceptor to generate current. In conventional MFCs, the cathode transfers electrons to oxygen as the terminal electron acceptor because of the abundance of oxygen in the atmosphere; this approach also has the advantage of a low cost.

Due to the low oxygen reduction rate on the

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surface of carbon electrodes, catalysts or artificial electron mediators are typically required [6]. Platinum is the most frequently used catalyst for oxygen reduction in MFCs [7, 8] due to its excellent catalytic activity; however, platinum-cathode MFCs are impractical because of their high cost. In addition, oxygen flows into the anode chamber from the cathode chamber, decreasing operational sustainability by increasing the number of aerobic microbes in the cell [9]. Therefore, we developed a biocathode MFC that uses a methanogen as the cathode catalyst instead of a metal catalyst and a chemical mediator. Methanogens are strictly anaerobic autotrophs that produce methane by building symbiotic relationships with other syntrophs.

Some microorganisms produce pili, which have metal-like conductivity and perform direct interspecies electron transfer [10, 11]. Therefore, designing a biocathode MFC that does not use a metal catalyst but rather a methanogen and organic matter decomposition bacteria as a cathode catalyst and an anode catalyst, respectively, may be possible. Methanogenic reactions are reportedly unfavorable for electricity generation in MFCs because of the low redox potential [12], but MFCs that use methanogens as a cathode catalyst can remove CO_2 and generate CH_4. Furthermore, under anaerobic conditions, the anode (organic matter oxidation) and cathode (methanogenesis) reactions restrict diffusion of oxygen into the anode via the proton-exchange membrane, preventing loss of electrons to oxygen.

In this study, the cathode chamber of an MFC was inoculated with a hydrogenotrophic methanogen, *Methanothermobacter thermautotrophicus* strain ΔH. To examine the potential of the methanogen biocathode MFC, we evaluated its power output and degradation of organic acids.

Materials and Methods

1. Preparation of the microbial electrode

The nutrient medium consisted of 0.14 g/L KH_2PO_4, 0.54 g/L NH_4Cl, 0.20 g/L $MgCl_2$•$6H_2O$, 0.075 g/L $CaCl_2$•$2H_2O$, 2.5 g/L $NaHCO_3$, 0.1 mg/L resazurin, 0.5 g/L cysteine-HCl, and 0.5 g/L Na_2S•$9H_2O$. As the cathodic medium, solutions of trace elements and of vitamins to promote growth of the methanogen [13] were added to the nutrient medium, and the pH was adjusted to 7.00 using HCl. To produce the methanogen cathode electrode, the hydrogenotrophic methanogen *Methanothermobacter thermautotrophicus* strain ΔH (NBRC 100330) and carbon felt (Carbon®; Nippon Carbon Co., Ltd., Japan) of φ 1 cm, 0.5 cm thickness were placed in a 50 mL vial closed with a butyl and aluminum cap. Cultivation was conducted at 55°C in an H_2/CO_2 (80/20, vol/vol) atmosphere without shaking. For the anodic medium, we added 10 mM propionate and solutions of trace elements and vitamins [14] to the nutrient medium and adjusted the pH to 7.00 using HCl. To produce the anode electrode, seed sludge from a thermophilic anaerobic digestion system (Miyagi, Japan) which treated food waste [15] was placed in a 50–75 mL vial containing carbon felt and cultivated at 55°C. After cultivation for 5 days, the carbon felt was dried under air at 55°C to decrease methanogen activity and was subsequently used as the anode electrode.

2. Batch operation of the MFC

The MFC reactor comprised two 500 mL bottles separated by a proton exchange membrane (Nafion®117, Sigma-Aldrich, St. Louis, MO, USA) (Fig. 1). The anode tank was filled with DSMZ Medium 960 (Leibniz Institute German Collection of Microorganisms and Cell Cultures GmbH, Braunschweig, Germany) containing 0.5 mM bromoethanesulfonic acid (BES) with propionate (10 mM) as organic matter. The gas phase of the

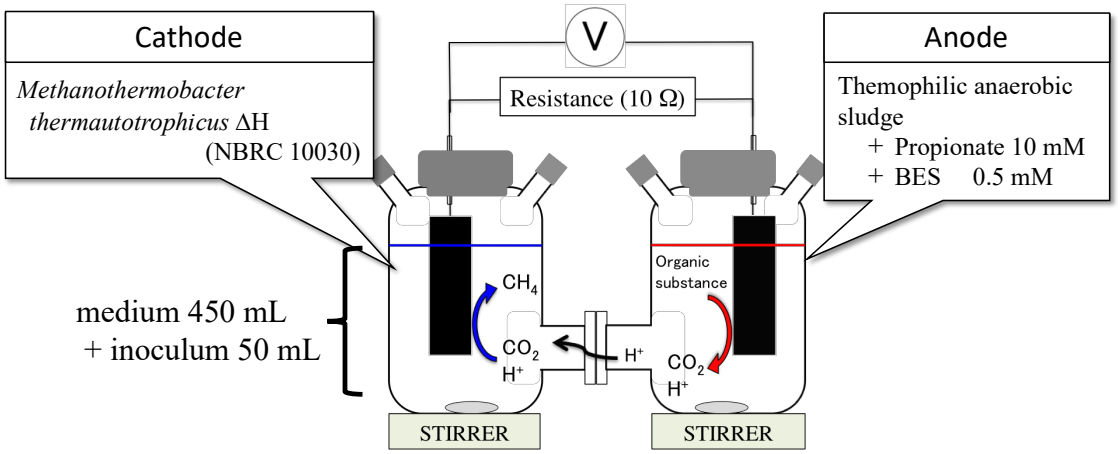

Fig. 1. Diagram of batch operation of the methanogen cathode microbial fuel cell (MFC).

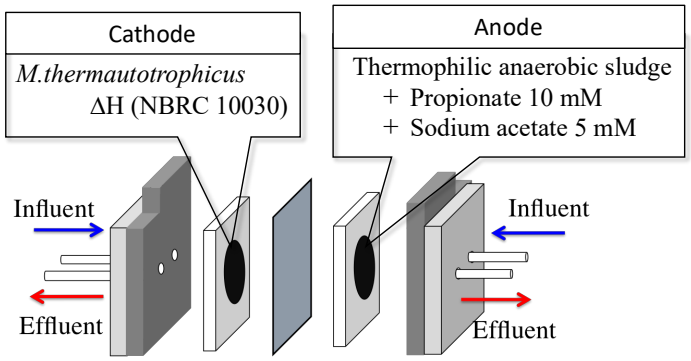

Fig. 2. Diagram of continuous operation of the methanogen cathode MFC.

anode bottle was exchanged with N_2 gas. The cathode bottle contained NBRC Medium 1067 (National Institute of Technology and Evaluation Biological Resource Center, Tokyo, Japan), and its gas phase was exchanged with CO_2 gas. Thermophilic anaerobic sludge as described in 2.1 preparation of the microbial electrode was used as seed sludge for the anode bottle. The thermophilic hydrogen-utilizing methanogen *M. thermautotrophicus* strainΔH (NBRC 100330) was added to the cathode bottle. The electrodes consisted of 8×3×0.5 cm carbon felt (Carboron® felt, Nippon Carbon Co., Ltd.). The reactor was operated at 55°C under anaerobic conditions for 73,120 min.

During culture, voltage and power density were measured continuously. During the operation period, the solution and the gas were sampled twice. The gas phase of the anode and cathode was replaced with N_2 and CO_2 gas, respectively. Organic acids were analyzed by high-performance liquid chromatography (HPLC) and the gas composition by gas chromatography.

3. Continuous operation of the MFC

The pore size of the cathode and anode was set to 1 cm φ using a silicon rubber sheet. A proton-exchange membrane (Nafion® 117; Sigma-Aldrich) was placed between the two electrodes using a microcell (Tsukuba Materials Information Laboratory, Ltd., Tsukuba, Japan) (Fig. 2). The

medium flowed to the anode and cathode at 80 μL/min. The volume of each electrode chamber was 0.3925 mL. After setup, the MFC was subjected to a pre-run for 7,000 min. To start the analysis, new medium was introduced for each electrode and 5 mM acetate was added to the anode medium. The experiment was run for approximately 8,000 min.

To investigate the performance of the methanogen cathode electrode, methanogens were not added to the cathode in the control MFC, which was autoclaved at 120°C for 20 min. The anode in the control MFC was cultured with anaerobic digestion seed sludge, similar to the methanogen MFC. For the control MFC, the experiment was run for 4,000 min. Both MFCs were incubated at 55°C in a water bath.

4. Electrochemical measurements

The methanogen cathode and control MFCs used a 10 Ω external resistor (R), except where noted. The voltage (V, mV) between the electrodes was recorded at 5-min intervals using a voltmeter (Midi Logger GL 240; Graphtec, Tokyo, Japan). The current (I, mA) was calculated as I = 98 V ÷ R. The power density (P = VI ÷ anode electrode surface area [A], mW/m^2) was calculated from the measured V, I, and A values. During continuous operation, electrochemical analysis was performed by periodically discontinuing the connection between the voltmeter and external resistance and using a potentiostat (ECstat-101; EC Frontier Co., Ltd., Japan). Electrochemical analysis was performed using linear voltammetry at a scan rate of 1 mV/s, and the data were recorded on a personal computer connected to the potentiostat.

5. Analysis

We collected the initial influent and effluent from the anode at regular intervals for chemical analysis. The samples were centrifuged at 8,000 × *g* for 20 min and filtered (0.2 μm pore diameter, Dismic-25CS; Advantec, Tokyo, Japan) prior to analysis. Short chain fatty acid (SCFA) concentrations were analyzed by HPLC (Jasco, Tokyo, Japan) with an ion-exchange column (RSpak KC-811; Shodex, Tokyo, Japan) operating at 60°C using 3 mM $HClO_4$ as the eluent at a flow rate of 1.0 mL/min, and an ultraviolet detector (870-UV; Jasco). We calculated the coulombic efficiency (CE) by measuring the theoretical number of coulombs available from acetate (i = a) and propionate (i = p) oxidation in the anode effluent of the methanogen cathode MFC. For continuous flow through the system, the CE based on the current generated under steady-state conditions was calculated according to Logan *et al.* [1], where Mi is the molecular weight of the SCFA (Ma = 60, Mp = 74), ni is the number of electrons exchanged per mole SCFA (na = 8, np = 6), F is the Faraday constant (F = 98,4856 C/mol per e-), Q is the volumetric influent flow rate (Q = 80 μL/121 min = 1.33 Å 10^{-6} L/s), and Si (g/L) is the difference in the SCFA concentration between the influent and effluent.

6. DNA extraction

We could not extract DNA directly from the carbon felt before operating the MFC because this would have required crushing the MFC. Therefore, at the time of MFC construction, a cathode carbon felt from a culture vial was used to quantify methanogens on the cathode electrode. For the cathode, we extracted DNA directly from the electrode after operation of the MFC. DNA was extracted using a Power Soil DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA) according to the manufacturer's instructions.

7. Quantitative real-time polymerase chain reaction

Real-time polymerase chain reaction (RT-PCR) was performed to quantify the number of copies of methanogen 16S rDNA on the cathode. RT-PCR was performed using Chromo 4™ and Opticon

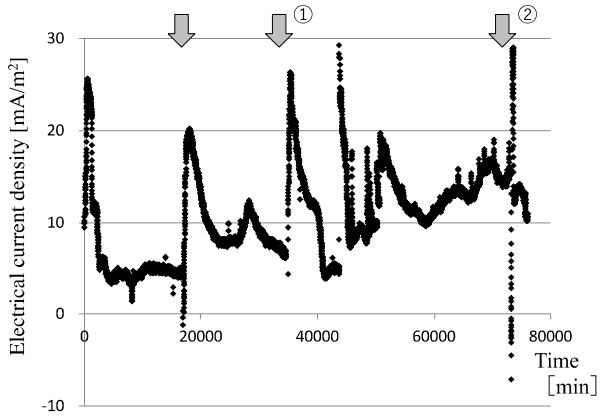

Fig. 3. Changes in current during batch operation of the methanogen cathode MFC. Arrow showed sampling time for analysis of water quality in the reactor. ①, ② showed the time for electrochemical analysis. ① showed 36000 times. ② showed 72000 times.

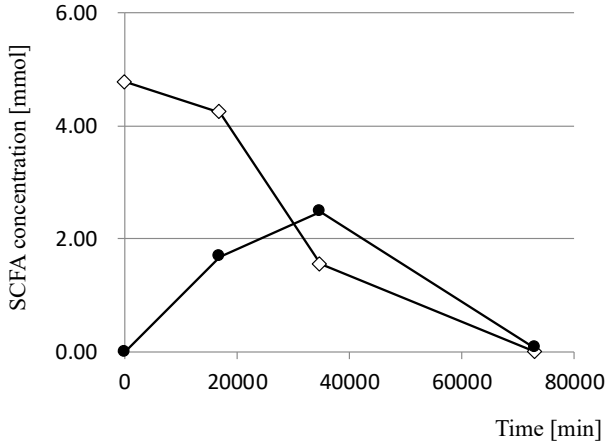

Fig. 4. SCFA concentrations in the anode bottle during batch operation of the methanogen cathode MFC. White rhombus showed the propionic acid concentration, black circle showed the acetic acid concentration.

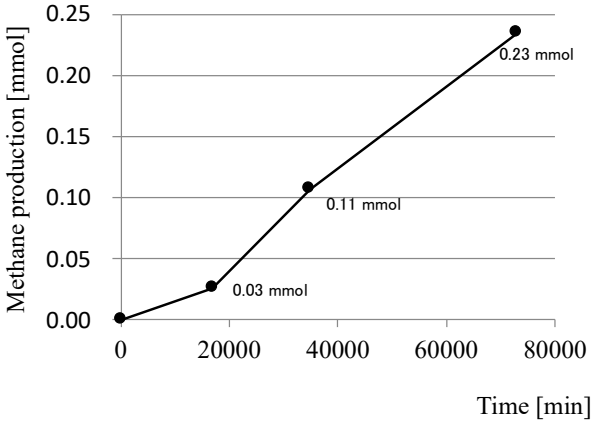

Fig. 5. Methane production in the cathode bottle during batch operation of the methanogen cathode MFC.

Monitor™ software (ver. 3.1; Bio-Rad) and Mighty Amp for Real Time (SYBR Plus) (TaKaRa, Japan). The reaction mixture (25 μL) was prepared according to the manufacturer's protocol, and consisted of 2 ~ Mighty Amp for Real Time (SYBR Plus, 12.5 μL), primers 1106F (5′-139 TTW AGT CAG GCA ACG AGC-3′) and 1378R (5′-TGT GCA AGG AGC AGG GAC-3′; 140 μL each) [16], extracted DNA (1 μL), and Milli-Q water. The RT-PCR program consisted of an initial denaturation at 95°C for 10 s, followed by 50 cycles of denaturation at 95°C for 10 s, annealing at 57°C for 10 s, and extension at 72°C for 6 s [17].

Results

1. Power generation by the methanogen cathode MFC during batch operation

Figure 3 shows the current generated during batch operation of the methanogen cathode MFC. Based on the sum of the current over 7,300 min, 2.71 mmol electrons were transferred from the anode to the cathode. The concentration of propionic acid decreased slowly until 16885 minutes and then decreased rapidly to 34895 minutes. Furthermore, the concentration of propionic acid decreased to the detection limit after 73,000 min. Acetic acid, a byproduct of propionic acid, concentrations increased to 34895 minutes with the decrease of propionic acid, but it decreased after that, then was not detected at 73,000 min. (Fig. 4).

The amount of methane generated

Table1 Percentage of electrons used for methane production.

Electrons as electric current (mmol)	2.71
Electrons as methane from the cathode bottle (mmol)	1.84
Percentage of electrons used for methane production (%)	67.9

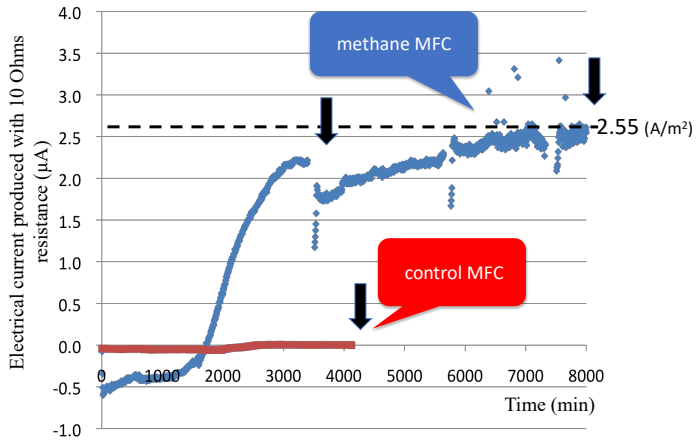

Fig. 6. Changes in current during continuous operation of the methanogen cathode MFC and the control cathode MFC (without methanogens).

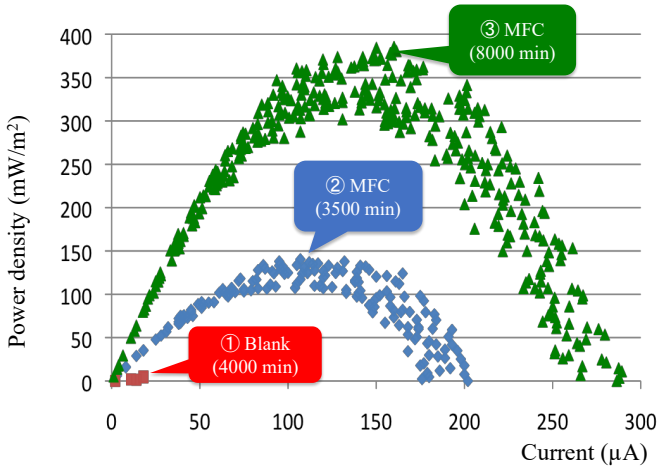

Fig. 7. Power density during continuous operation of the methanogen cathode MFC and the control cathode MFC (without methanogens).

from the cathode was 0.23 mmol (Fig. 5). Eight electrons were required to produce 1 mol CH_4 from CO_2 and protons. Based on the volume of methane, 1.84 mmol electrons were received by the cathode. The number of methanogens on the cathode was 2.3×10^6 copies/cm^3. The efficiency of the current converted to CH_4 was 67.9% at the cathode (Table 1).

2. Electricity generated by the methanogen cathode MFC during continuous operation

The electricity generated by the control and methanogen cathode MFCs is shown in Fig. 6. In the control MFC, which used a sterile cathode, little electricity was generated during operation for 4,000 min. In contrast, in the methanogen cathode MFC, electricity was generated before and after operation for 2,000 min. Furthermore, the methanogen cathode MFC achieved a maximum current density of 2.20 A/m^2 at 3,200 min during operation for 4,000 min. The level of current generated was maintained continuously, and the methanogen cathode MFC produced a maximum current density of 2.55 A/m^2 after operation for 8,000 min.

3. Maximum power density

The maximum power density was reached after ~4,000 min in the control MFC, and after 3,500 and 8,000 min in the methanogen cathode MFC (Fig. 7). The maximum power density of the control MFC was negligible. In the methanogen cathode MFC, a power density of 140 mW/m^2 was obtained after operation for 3,500 min, and the maximum power density of 385 mW/m^2 was achieved after operation for 8,000 min.

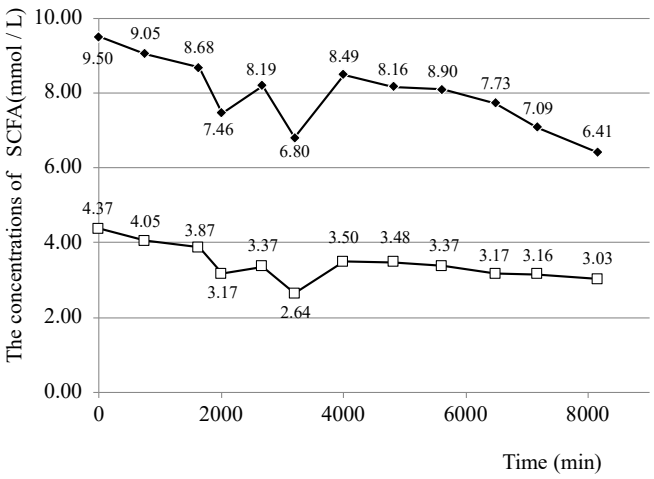

Fig. 8. SCFA concentrations in the anode bottle during continuous operation of the methanogen cathode MFC. Black rhombus showed the propionic acid concentration, and white square showed the acetic acid concentration.

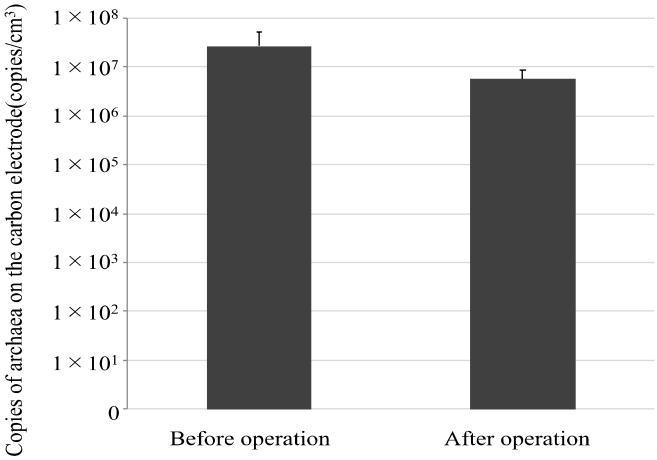

Fig.9 Archaeal copy numbers on the cathode electrode before and after continuous operation.

Table2. Overview of the performance of mediator-less MFCs.

Anode (Inoculum)	Cathode	max power density (mW/m^2)	References
Graphite brushes (wastewater)	Pt and PTFE coated carbon cloth (Pt 0.4 $mg/cm^{2)}$	262	Sciarria *et al.* 2015
Ccarbon paper (Domestic wastewater)	Carbon cloth (Pt 0.5 mg/cm^2)	309	Min and Logan 2010
Carbon paper (Domestic wastewater)	Carbon cloth (Pt 0.5 mg/cm^2)	320	Liu and Logan 2004
Carbon felt (Anaerobic sludge**)**	**Carbon felt(***M.thermautotrophicus)*	**385**	**This study**
Carbon cloth (mixed bacterial culture that was maintained in MFCs)	Pt and PTFE coated carbon cloth (Pt 0.5 mg/cm^2)	2160	Catal *et al.* 2008

4. Degradation of organic matter in the anode chamber

The initial concentrations of SCFA in the influent and effluent from the anode are shown in Fig. 8. After operation for 8,000 min, the concentrations of acetic acid and propionic acid decreased by ≥30%. Because these SCFAs were the only substrate in the MFC, the electrons generated by their degradation were likely involved in power generation.

5. Microbial population on the cathode during continuous operation

The number of methanogens adhering to the carbon felt before use as an electrode was 2.7×10^7 copies/cm^3, and that after continuous operation was 5.7×10^6 copies/cm^3 (Fig.9). Therefore, the number of methanogens after continuous operation was reduced by about 21%.

Discussion

To our knowledge, there is no previous report of power generation using a methanogen as a cathode catalyst without adding a chemical mediator or applying an external voltage. During batch operation, only *M. thermautotrophicus* strain ΔH was attached to the cathode electrode. As a result, methane was generated from the cathode, and current flowed. Based on the amount of methane generated and the quantity of electrons, 67.9% of the current produced was due to methane conversion.

Our results indicate that methane conversion by methanogens catalyzes the reaction between CO_2 and protons at the cathode electrode. In addition, the control MFC showed negligible power generation during continuous operation, suggesting that methanogens on the cathode are involved in power generation.

The maximum power density of 385 mW/m^2 of the methanogen MFC during continuous operation is comparable to that of a platinum-containing

cathode MFC that does not use a mediator (Table 2). Changing the distance between the anode and cathode electrodes can increase the power generation efficiency by enhancing proton transfer efficiency and reducing electrolyte resistance [18]. However, in MFCs that require oxygen for the cathode reaction, such as platinum-containing cathodes, the smaller the distance between the electrode and the anode, the wider the distribution of oxygen, which inhibits anaerobic reactions and the microflora in the anode chamber. Min and Logan created a flat-plate MFC with an anode and platinum-containing air cathode integrated with a proton exchange membrane; the power generation efficiency decreased as the amount of oxygen supplied to the cathode increased [19]. Therefore, use of an anaerobic biocathode may enhance the efficiency and longevity of MFCs.

During continuous operation of the MFC, the number of methanogenic bacteria on the cathode was reduced by about 20%. This may be because the medium flow rate (80 μL/min) inhibited adherence of methanogens, or because the methanogens were unable to proliferate on the cathode.

Methanothermobacter thermautotrophicus strain ΔH has not been reported to produce extracellular polysaccharides, and its adhesion is weak in the absence of other microorganisms. In biocathodes, the number of attached microorganisms is important, and the performance of the methanogen cathode MFC was improved by increasing the amount of *M. thermautotrophicus* strain ΔH attached to the electrode. Cheng and Logan reported that anode performance was improved by increasing its affinity for microorganisms by treating the anode surface with ammonia [20]. Therefore, modification of the cathode surface may increase its affinity for methanogens and thus improve the performance of the MFC.

Conclusions

We constructed an MFC with a thermophilic hydrogenotrophic methanogen, *M. thermautotrophicus* strain ΔH, attached to a methanogen cathode electrode. In the batch experiment, 67.9% of the current produced was due to methane conversion by methanogens on the cathode electrode. The current in the control cathode, which lacked methanogens, was negligible during continuous operation. These results suggest that methanogens on the cathode electrode catalyzed the reaction of protons with CO_2. During continuous operation, the MFC of the methanogen cathode electrode had a maximum power density of 385 mW/m^2, comparable to a previously reported platinum-containing MFC. Therefore, the product cathode electrode has potential for use in MFCs.

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原　著

メタン菌カソード電極の微生物燃料電池による発電

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微生物燃料電池(MFC)は、微生物代謝を触媒として利用するものである。MFC の最も有用な利用先は排水処理であり、排水の処理と発電を同時に行うことが可能であり、最小限のエネルギー投入で排水処理とスラッジ生成の減少が可能である。通常、アノード側の嫌気性細菌によって生成された電子はカソードに流れ、カソード側の触媒である白金によってプロトンと酸素と結合して水ができる。しかし、白金のコストが高いため、その代替触媒が必要である。

そこで本研究では、メタン菌をカソード触媒とするメタン菌カソード電極 MFC によるバッチ運転と連続運転において発電実験を行った。水素資化性メタン菌の *Methanothermobacter thermautotrophicus* strain ΔH をカソード電極上に培養し、CO_2の還元反応の実験を行った。その結果、カソードリアクター内で、発電とメタンガス生成が同時に起こったことを確認した。さらに、最大電力密度は連続培養 MFC において 385 mW/m^{2}となった。これらの結果より、メタン菌はカソード電極から直接的に電子を受け取り、二酸化炭素からメタンを生成する触媒反応を行うことがわかり、白金触媒カソード電極の代替触媒として有望であることが示された。

Key words：微生物燃料電池, メタン生成, 生物カソード, 電気化学分析, メタン

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