



A two-staged system to generate electricity in microbial fuel cells using methane

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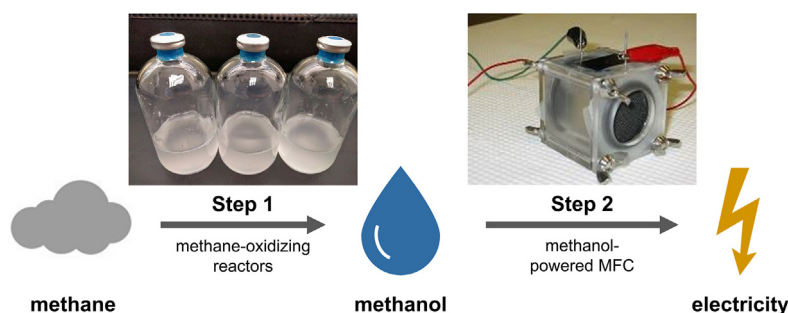
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HIGHLIGHTS

- A two-step process was used to produce bioelectricity using methane as a substrate.
- In the first step, methane-oxidizing culture oxidizes methane to methanol.
- In the second step, the MFC is supplied with methanol to generate power.
- Acetogens converted methanol into acetate, which was consumed by exoelectrogens.
- Power is generated without the need for engineered strains or aseptic techniques.

GRAPHICAL ABSTRACT



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ABSTRACT

Methane is an abundant and inexpensive feedstock that is available as natural gas and renewable biogas. However, methane has not been regarded as a good substrate for microbial fuel cells (MFCs) due to low power densities. To increase power, a two-step strategy was used based on conversion of methane into methanol, followed by electricity generation using methanol as the substrate in the MFC. To produce methanol, a methane-oxidizing culture was grown in a high phosphate buffer resulting in the accumulation of 350 ± 42 mg/L of methanol. The methanol-fed MFC produced a maximum power density of 426 ± 17 mW/m². It was also shown that the methanol-rich medium produced from the first step can directly be supplied to the MFCs, removing the need for purification of methanol. Analysis of the microbial community suggests that acetogens first converts methanol into acetate, which is then consumed by exoelectrogens for power generation.

1. Introduction

A microbial fuel cell (MFC) is a technology for harvesting electricity directly from organic matter, and thus it has great potential for treating wastewater economically without the use of energy derived from fossil fuels [1–3]. A variety of substrates can be used in MFCs for electricity production ranging from pure compounds such as acetate [4–7],

propionate [7], butyrate [4,7], glucose [6,8,9], ethanol [10], and xylose [6,11,12], to complex mixtures of organic matter present in wastewater [13–19]. However, few gaseous substrates have been examined other than hydrogen or methane [20–23].

Methane is a readily available from both natural and anthropogenic sources, and is a feedstock that does not compete with food demands [24]. Methane-utilizing bacteria (methanotrophs) have been used to

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convert methane into various bioproducts including biodiesel [25], propylene oxide [26], single cell protein [27,28], extracellular polysaccharides [29], human health supplements [30], and polyhydroxyalkanoate (PHA) bioplastics [31–36]. Use of methane as a substrate for electricity production in MFCs, however, has not been well examined. Electricity was produced first from methane using an uncultured anaerobic methane-oxidizing consortia isolated from oceanic sediment, but the power density was very low (0.65 mW/m^2) [37] compared to organic substrates such as acetate [38]. A recent study reported that an air-cathode MFC operated in continuous mode on a synthetic, methane-saturated medium generated approximately 62 mW/m^2 [20]. So far, the highest power density using a methane-powered MFC of 168 mW/m^2 was obtained using a genetically engineered archaeal strain that was capable of converting methane into acetate, which was then oxidized by exoelectrogens to generate electricity [21].

In this study, we examined a two-step process to utilize methane as a feedstock for bioelectricity generation based on enriching a natural microbial consortium with aerobic methanotrophs in the first step to oxidize methane to methanol. In the second step, the produced methanol solution was used in an MFC to produce bioelectricity from the methanol using a mixed-culture community. Methanotrophs use methane as a carbon and energy source, but are not known to be capable of electricity generation [39,40]. However, methanotrophs can convert methane to methanol using methane monooxygenase (MMO) enzymes that catalyze the single-step conversion of methane into methanol, which is then metabolized by methanotrophs to formaldehyde using methanol dehydrogenase (MDH), and finally formaldehyde is converted to formate by formaldehyde dehydrogenase. The accumulation of methanol can be achieved using various MDH inhibitors such as phosphate buffer, ethylenediaminetetraacetic acid (EDTA), sodium chloride (NaCl), and ammonium chloride (NH_4Cl) [41]. Here we examined the use of a phosphate buffer as a simple method to readily convert methane into methanol, with the methanol used in an MFC to produce bioelectricity by a mixed microbial exoelectrogenic and fermentative consortium.

2. Materials and methods

2.1. Methane-oxidizing cultures

All methane-oxidizing cultures were grown in 2.38 g/L (25 mM) of a phosphate buffer solution (PBS; contained the following chemicals per liter of solution: 2.283 g Na_2HPO_4 , 1.226 g $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, 0.155 g NH_4Cl , 0.065 g KCl) amended with 12.5 mL/L minerals and 5 mL/L vitamins [42]. Activated sludge was obtained from the aeration basin at the Penn State University Wastewater Treatment Plant (State College, PA, USA). Large particles were removed by filtration through a 100- μm pore-diameter cell strainer (BD Falcon Biosciences, Lexington, TN, USA). The dispersed cells were centrifuged ($10,000 \times g$) for 5 min to produce a pellet, resuspended in 50 mL of PBS medium, and then shaken to disperse the cells. Cell suspensions were incubated in 160 mL serum bottles (Wheaton, Millville, NJ, USA) capped with thick butyl-rubber stoppers and crimp-sealed under a $\text{CH}_4\text{:O}_2$ headspace (molar ratio 1:1.5, > 99% purity). Cultures were incubated horizontally on orbital shaker tables at 150 revolutions per minute (rpm) at 30°C . The headspace of each bottle was flushed daily with a $\text{CH}_4\text{:O}_2$ mixture (molar ratio of 1:1.5), and every 48 h, 40 mL of the suspensions were replaced with 40 mL of fresh PBS medium. The methane-oxidizing enrichments were allowed to reach a steady-state condition (based on their maximum cell densities) for the first 16 d, and data were collected starting on day 17. In order to find the concentrations of phosphate and ammonium that resulted in the maximum methanol concentrations, the methane-oxidizing enrichment was subjected to a PBS medium containing different concentrations of phosphate (0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0 g $\text{PO}_4\text{/L}$) and ammonium (34, 68, 102, 136, 170, 255, 340 mg $\text{NH}_3\text{-}$

N/L).

2.2. MFC construction and operation

MFC tests were conducted in triplicate using single-chamber, cubic-shaped air-cathode MFC reactors containing a cylindrical anode chamber 4-cm long and 3-cm in diameter [43]. The graphite fiber brush anode (2.5 cm in both diameter and length) was heat treated at 450°C in air for 30 min before use and was placed horizontally in the middle of MFC chambers. Cathodes were prepared using a hot-pressing method as previously described [44]. The catalyst layer was prepared by mixing activated carbon (AC, Norit SX plus, Norit Americas Inc., TX, USA) with a 60% polytetrafluoroethylene (PTFE) emulsion (Sigma Aldrich, MO, USA) at a mass ratio of AC:PTFE (6:1). The cathode current collector was a stainless steel mesh (42×42 , type 304, McMaster-Carr, IL, USA). A hydrophobic polyvinylidene fluoride (PVDF) membrane (0.45 μm , Millipore, MA, USA) was used as a diffusion layer to prevent water leakage. The AC:PTFE, current collector and diffusion layer were pressed at 3×10^7 Pa for at least 15 s at 60°C until the membrane surface became dry [44,45]. The pressed cathodes were then taken out and dried in a fume hood for later use.

Reactors were inoculated using anaerobic sludge collected from the Penn State University Wastewater Treatment Plant and operated in batch mode (State College, PA, USA), with a 1000Ω resistor in the circuit. The MFCs were emptied and refilled daily with a fresh 8.0 g/L PBS medium amended with 320 mg/L methanol, 12.5 mL/L minerals, and 5 mL/L vitamins for 30 d until the reactors reached steady state based on repeatable cycles of voltage production. In some tests, MFCs were refilled with a methanol-rich medium produced from the methane-oxidizing reactors instead of the PBS medium.

Voltage (U) across the external resistor in the MFC circuit was measured at 20 min intervals using a data acquisition system (2700, Keithley Instrument, OH, USA) connected to a personal computer. Current ($I = U/R$) and power ($P = IU$) were calculated as previously described [2], and normalized by the projected surface area of the cathode (7 cm^2). Power density curves were obtained by varying external circuit resistance using the single cycle polarization method, with a single resistor used for a full batch cycle. An Ag/AgCl reference electrode (BASi) was placed in the middle of the MFC chamber to obtain anode potentials (reported versus Ag/AgCl electrode, +210 mV vs. a standard hydrogen electrode), with the cathode potential calculated using the anode potential and the whole cell voltage. Coulombic efficiency (ϵ_c) was calculated by dividing the total coulombs transferred to the anode by the theoretical maximum number of coulombs (total coulombs produced by complete methanol oxidation to carbon dioxide).

2.3. Analytical methods

The gas composition of methane-oxidizing reactors were analyzed using a gas chromatograph (SRI Instruments, models 8610B and 310, CA, USA) as previously described [46].

Methanol and acetate concentrations were analyzed using a gas chromatograph (Agilent, model 6890, CA, USA) equipped with a FID and a DB-FFAP fused-silica capillary column with helium as carrier gas (constant pressure of 103 kPa). The oven temperature of the GC was started at 60°C and programmed at $20^\circ\text{C}/\text{min}$ to 120°C , and then $30^\circ\text{C}/\text{min}$ to a final temperature of 240°C held constant for 3 min. The injector and detector temperature were both 250°C [42].

To analyze total suspended solids (TSS), 0.5–5.0 mL of cell suspension was filtered through pre-washed, dried, and pre-weighed 0.2 μm pore-diameter membrane filters (Pall, Port Washington, NY, USA). The filtered cells and membrane filters were dried at 105°C for 24 h, then weighed.

For all data, arithmetic mean values and standard deviations were calculated for triplicate samples. Statistical differences between sample

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