Direct Biological Conversion of Electrical Current into Methane by Electromethanogenesis

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New sustainable methods are needed to produce renewable energy carriers that can be stored and used for transportation, heating, or chemical production. Here we demonstrate that methane can directly be produced using a biocathode containing methanogens in electrochemical systems (abiotic anode) or microbial electrolysis cells (MECs; biotic anode) by a process called electromethanogenesis. At a set potential of less than −0.7 V (vs Ag/AgCl), carbon dioxide was reduced to methane using a two-chamber electrochemical reactor containing an abiotic anode, a biocathode, and no precious metal catalysts. At −1.0 V, the current capture efficiency was 96%. Electrochemical measurements made using linear sweep voltammetry showed that the biocathode substantially increased current densities compared to a plain carbon cathode where only small amounts of hydrogen gas could be produced. Both increased current densities and very small hydrogen production rates by a plain cathode therefore support a mechanism of methane production directly from current and not from hydrogen gas. The biocathode was dominated by a single Archaeon, Methanobacterium palustre. When a current was generated by an exoelectrogenic biofilm on the anode growing on acetate in a single-chamber MEC, methane was produced at an overall energy efficiency of 80% (electrical energy and substrate heat of combustion). These results show that electromethanogenesis can be used to convert electrical current produced from renewable energy sources (such as wind, solar, or biomass) into a biofuel (methane) as well as serving as a method for the capture of carbon dioxide.

Introduction

Increasing competition for fossil fuels, and the need to avoid the release of carbon dioxide from combustion of these fuels, has increased the search for new and sustainable approaches for energy production. Two new methods of bioenergy production from biomass include electricity production using microbial fuel cells (MFCs) and hydrogen production by electrohydrogenesis using microbial electrolysis cells (MECs) (1, 2). Electricity generation in an MFC is spontaneous with oxidation of organic matter such as acetate by electrogenic bacteria on the anode (\(E_{\text{an}} = -0.2 \ \text{V} \text{ vs standard hydrogen electrode}\)) and oxygen reduction at the cathode (\(E_{\text{cat}} = 0.2 \ \text{V}\)), with a working cell potential of approximately 0.4 V and a theoretical potential as high as 1.1 V under neutral pH conditions (1). The MEC is a type of modified MFC that has been used to efficiently store electrical energy as a biofuel (hydrogen gas) (2). Hydrogen gas evolution from the cathode, however, is not spontaneous (3–5). The voltage produced by electrogenic bacteria on the anode using a substrate such as acetate \(E_{\text{an}} = -0.2 \ \text{V}\) is insufficient to evolve hydrogen gas at the cathode \(E_{\text{cell}} = -0.414 \ \text{V, pH}=7\). By adding a small voltage, hydrogen gas can be produced using MECs at very high energy efficiencies evaluated in terms of just electrical energy alone (200–400%) or both electrical energy and substrate heat of combustion energy (82%) (3). One disadvantage of electrically assisted method of hydrogen production (electrohydrogenesis) is that a precious metal catalyst such as platinum is usually used on the cathode. Hydrogen compression is also an energy-intensive process, and hydrogen storage can be problematic (6).

Renewable biomethane is typically produced by methanogens from a few substrates such as acetate, formate, and biohydrogen gas in anaerobic digesters (7). Based on thermodynamic calculations, methane could also be produced electrochemically through carbon dioxide reduction at a voltage of 0.169 V under standard conditions, or −0.244 V under more biologically relevant conditions at a pH=7, by the reaction

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\text{CO}_2 + 8\text{H}^+ + 8\text{e}^- \rightarrow \text{CH}_4 + 2\text{H}_2\text{O}
\] (1)

This suggests that methane could be produced without an organic fuel, at about the same potential needed for hydrogen production with an organic fuel (such as acetate). Methane production by eq 1 has the added advantage of CO2 capture into a fuel (but not sequestration). A purely electrochemical route of methane production in practice is energy intensive due to high electrode potentials and the lack of suitable catalysts able to efficiently reduce this overpotential (8). The required voltage could theoretically be eliminated when using an organic substrate due to a positive electrochemical cell potential for methane production \(E_{\text{an}} = 0.071 \ \text{V} \text{ with acetate}\), compared to a negative potential for hydrogen production. This thermodynamically favorable reaction of acetate conversion to methane is the reason why aceticlastic methanogenesis occurs in nature and in engineered anaerobic digesters.

Substantial methane production has been reported in recent MEC studies (5, 9–13), but the source of this methane appears to be acetate via aceticlastic methanogenesis and hydrogenotrophic methanogenesis using hydrogen gas produced in the process. Methane generation in anaerobic digesters originates mostly from acetate (70%) with a smaller portion from hydrogen gas (14). In contrast, methane generation in MECs may be more likely from hydrogen gas than acetate. Clauwaert et al. (10) measured a methane production rate of 0.28–0.75 L/L-d in several MEC experiments at an applied voltage of −0.8 V but found only 0.17 L/L-d with no applied potential, suggesting that 23–61% of the methane was derived from acetate. Liu et al. (15) found no methane generation in a reactor consistently operated in an open-circuit mode compared to otherwise identical MECs operated at −0.7 V, suggesting hydrogen was needed for methane production. Eliminating bicarbonate from the medium has helped to reduce methanogenesis in some studies (16) but not in others (10). Organic loading is clearly a factor in methane production, with long reaction times and increased substrate concentrations increasing methane production (2, 5). Most evidence suggests that in these...
previous studies the methane generation was primarily from hydrogen gas, likely by microorganisms on the anode. For example, little methane was found in the gas produced using a gas phase cathode (12), and in another MEC study there was little cathodically produced methane (11). Rather than avoid methane production, it has been suggested that combining MECs and anaerobic digestion may form a viable method of bioenergy production primarily due to methane from hydrogen gas (9, 10). In all of these studies (even those without a catalyst on the cathode), both methane and hydrogen gases have been present, indicating that methanogens could not completely remove hydrogen gas when it was being formed in the reactor.

There is evidence in the literature that suggests the possibility of direct electron transfer to methanogens rather than from hydrogen or acetate. Methane was produced using microorganisms and zerovalent iron (Fe⁰) or iron in mild steel, but methane was generated at rates consistent with hydrogen evolution produced by corrosion (17). Dinh et al. (18) observed that growth of a methanogenic isolate (strain IM1) was slow with H₂ + CO₂ but that methane generation rates with iron were much higher than expected based on hydrogen evolution. Thus, they speculated that this microbe could accept electrons from iron without the need for hydrogen evolution. However, it is possible that the microbe enhanced corrosion rates (and thus hydrogen evolution rates), and there was no further electrochemical analysis of the corrosion or examination of the growth of this microorganism on an electrode. The ability of microorganisms to donate electrons to iron does not necessarily mean they can use a carbon electrode as an electron acceptor. Many dissimilatory iron respiring bacteria (DIRB) are capable of growth using an electrode (1, 19, 20), for example, but some strains cannot grow using an electrode while others capable of growth with an electrode cannot use iron (21-23). Therefore, while thermodynamic data and these experiments suggest that direct electron transfer to methanogens is possible, it has not been previously demonstrated using electrodes in a bioelectrochemical system. It is shown here, however, that microorganisms can be used on the cathode in an MEC to produce methane gas from electrical current at rates much greater than those possible via hydrogen gas evolution from a noncatalyzed electrode.

**Methods**

**Reactors.** A single-chamber MEC (SCMEC) (400 mL) was used here that contained a single graphite fiber brush anode (5 cm in diameter and 7 cm long) (24) and several carbon cloth cathodes (14 cm² each) each coated only with a carbon layer on one side (2.5 mg/cm², Nafion as binder) and no metal catalyst. Titanium wires were used to connect the electrodes to the circuit. The chamber was sparged with ultra high purity nitrogen gas (99.999%) for 30 min before applying a constant voltage −0.7 V (vs Ag/AgCl) to the cathode (working electrode) using a multichannel potentiostat (WMPG100, WonATech, Korea), with the counter and reference poles connected to the anode and reference electrode, respectively.

The SCMEC was inoculated with the solution from an anode chamber of an existing two-chamber MEC reactor (3) (containing a Pt-catalyzed cathode) that was producing methane. This reactor was then operated for one month (two cycles in batch-mode) with acetate (1 g/L) in a buffered nutrient medium (100 mM phosphate buffer solution; PBS; pH=7) containing (per liter) NaH₂PO₄·H₂O, 9.94 g; Na₂HPO₄·H₂O, 5.5 g; NH₄Cl, 310 mg; KCl, 130 mg; and a minerals solution (12.5 mL) and vitamins solution (5 mL) (composition in the Supporting Information). All experiments were conducted in a constant temperature room (30 °C).

The two-chamber MEC (300 mL each bottle) (24, 25) contained an anion exchange membrane (AMI-7001, Membrane International Inc., U.S.) placed between the anode and cathode chambers (2.9 cm in diameter) (duplicate tests). Each chamber was filled with 250 mL of PBS, acetate (1 g/L) was added to the anode chamber, and the cathode chamber was initially sparged with CO₂. All voltages reported are with respect to Ag/AgCl reference electrode (+201 mV vs standard hydrogen electrode) that was placed in the chamber to obtain cathode potentials.

**Electrochemical Measurements.** Gas production was quantified using a respirometer and gas chromatography as previously described (2). Methods for calculating current and energy efficiencies are the same as those described elsewhere for tests with hydrogen gas production, except that here 8 electrodes were used for a mole of methane (3). Linear sweep voltammetry was conducted in the potential range from −0.5 to −1.0 V at a low scan rate of 1 mV/s.

**Analysis of the Biofilm.** Two pairs of universal primers of domains Archaea and Bacteria were used for PCR amplification of 16S rRNA gene: Arc341F, 5′-CTAYGGGYG-CASCAGGGC-3′ or Bac968F, 5′-AACCGGAACCTTAC-3′ which were attached a GC clamp (GCGCCCCCGCCG-GCCCGCCGCGTCCCCGCCGCCCCCGCCGC) at the 5′-termini, and Arc915R: 5′-GTGCTCCCCGAAATCCT-3′ or Bac1401R, 5′-CGGTGTGTACAGCC3′. Denaturing gradient gel electrophoresis (DGGE), sequencing, and phylogenetic analyses were carried out as previously described (26). The 16S rRNA gene sequences from this study were deposited in the GenBank database under accession numbers EU812208-EU812220.

A genus-specific probe for *Methanobacterium* (MB1174, 5′-TACCGTGCTCCACTTCTCCT-3′) was synthesized and labeled with Alexa Fluor 488 (Invitrogen, Molecular Probes). The cathodes were rinsed twice by phosphate-buffered saline (PBS; 0.13 M NaCl and 10 mM Na₂HPO₄ at pH 7.2), and then cells were extracted using a sterile razor and fixed with 4% formaldehyde for 30 min. Hybridizations were performed at 46 °C for 10 h with buffer (0.9 M NaCl, 20 mM Tris-HCl [pH 7.2], 0.01 sodium dodecyl sulfate and 35% formamide) containing 5 ng probe per microliter and then washed with buffer (30 min at 48 °C). FISH was performed on an Olympus FV 1000 confocal laser scanning microscope. The percent of *Methanobacterium* in the total cells was calculated by comparison between fluorescent and differential interference contrast (DIC) pictures by the ImageJ (http://rsb.info.nih.gov/ij/).

**Pure Culture Tests.** *Methanobacterium palustre* was purchased from the American Type Culture Collection (ATCC BAA-1077). The strain was cultured anaerobically [H₂/CO₂ (80:20, vol/vol)] in the ATCC specified medium using 125 mL serum bottles with thick rubber stoppers. Prior to inoculation into MECs, 75 mL of culture solution after incubation was centrifuged and resuspended in sterile phosphate buffered nutrient medium lacking electron donor and acceptor. This cell suspension was inoculated into the anaerobic cathodic chamber and immediately sparged with CO₂.

**Results and Discussion**

A methanogenic bioelectrode was developed in a single chamber MEC lacking precious metal catalysts on the electrodes. After one month of operation, this MEC produced only methane gas at a rate consistent with current generation. Because this was a single-chamber MEC, however, it was possible that some of the methane produced in this system was from acetoclastic methanogenesis. We therefore transferred the anode and one cathode from the reactor into a two-chamber MEC and added acetate to the anode chamber and buffer to both chambers. At a set voltage of −0.7 to −1 V, gas produced in the cathode chamber contained only methane with no detectable hydrogen gas (<1%). No methane was produced using a cathode lacking a biofilm at set
The rate of methane. (slope of 8.33; inset), indicating 96% current capture into methane. Electron consumption was proportional to methane production (slope of 8.33; inset), indicating 96% current capture into methane.

Using the single-chamber MEC at a set voltage of -1 V, we achieved an overall energy recovery of 80% based on electrical energy and the acetate (heat of combustion). It is possible that this overpotential could be reduced in future studies by using more optimized electrode materials.

**Characterization of the Biocathode.** Scanning electron micrographs (SEMs) of the cathode biofilm showed that it was composed of cells with a relatively homogeneous morphology (Figure 3). Based on phylotypes with a 99% minimum similarity threshold, denaturing gradient gel electrophoresis (DGGE) analysis indicated the populations were composed of several phylotypes of the *Archaeum* domain consisting of *Methanobacterium palustre*, *Methanoregula bonneti*, and *Methanospirillum hungatii* (see the Supporting Information). There were also several phylotypes of the *Bacteria* domain also present in the biofilm which were all gram-positive bacteria, consisting of *Sedimentibacter hongkongensis*, *Clostridium sticklandii*, *Clostridium aminobutyricum*, and an uncultured bacterium with was most closely related to *Caloramator coolthaiasi*. Staining the biofilm using fluorescent in situ hybridization (FISH) showed that *Methanobacterium* accounted for 86.7 ± 2.4% (n = 5) of the total cells (Figure 3). Based on the dominance of the DGGE bands by one species and FISH results, we concluded that the main microorganism responsible for methane generation was *Methanobacterium palustre*, a strain that uses hydrogen but not acetate for growth.

**Evidence for Direct Electron Transfer.** Additional electrochemical and pure culture experiments were conducted to support our hypothesis that methane production by the mixed culture biofilm was sustained primarily from electrons released at the cathode and not by hydrogen gas. In the absence of catalysts or microorganisms, current that can be transferred through a circuit is limited by the rate that electrons can be removed from the cathode. The only way to increase the current density is to catalyze the formation of a product, which then allows the current density to increase due to product formation. We used linear sweep voltammetry (LSV) to determine the current densities possible in this MEC in the absence and presence of the biofilm on the cathode. LSV using a plain carbon electrode (no Pt) showed that there was little current compared to that obtained with a bio-cathode, until potentials were more negative than -0.95 V, compared to -0.65 V with the biocathode (Figure 4). There was some hydrogen gas evolution from an abiotic cathode, but it was too low (Figure 5) to account for observed rates of methane production by the biocathode. Thus, only in the presence of the biofilm was current generation enhanced. This enhancement could not occur without microorganisms catalyzing the release of these electrons. Stirring the solution did not substantially affect current generation, demonstrating that current generation was not substantially affected by mass transfer. Thus, the increase in current generation provides electrochemical evidence of direct electron transfer from the cathode to the biofilm.

In order to further examine the effect of microorganisms on current generation, current and methane generation were examined in pure-culture MEC tests using the type strain. Methane was produced using a biocathode of a pure culture of *M. palustre* ATCC BAA-1077. However, the current density and methane production were much lower than those with the mixed culture biofilm (Figure 5). Even under these lower current conditions, however, methane was still produced by *M. palustre* at a rate (14×) greater than that expected from the hydrogen evolved in the absence of microorganisms. It is not unexpected that the mixed culture, consisting primarily of *M. palustre*, would have different characteristics than a culture collection strain. For example, *Rhodopseudomonas palustris* DX-1 was recently isolated from an MFC produced high power densities in an MFC, but *R. palustris* ATCC 17001...
did produce any current (29). There are other reports demonstrating that pure cultures of *Shewanella oneidensis* and *Geobacter sulfurreducens* do not produce as much power in MFCs as the mixed cultures in air-cathode MFCs (30–32). Isolates have so far not been obtained for biocathodes demonstrating oxygen and nitrate reduction or hydrogen evolution (16, 33, 34). We are in the process of isolating microorganisms from this methanogenic biocathode.

Further evidence to support methane production without the need for hydrogen evolution was obtained by chemically removing hydrogen gas and examining current generation using LSV. When the cathode was coated with a hydrogen scavenger (1,4-diphenyl-butadiyne) (35), current densities were not increased compared to those obtained with an uncoated electrode. More detailed information on the hydrogen scavenging results and additional control experiments that were conducted to eliminate the possibility that hydrogen evolution supported methane generation can be found in the Supporting Information.

If direct current transfer is indeed occurring to methanogens, the mechanism(s) by which this occurs will need to be discovered. Electron transfer mechanisms to dissimilatory iron respiring bacteria (DIRB) are still being debated, with most strains having been found to be capable of growth using an electrode (1, 19, 20). However, some DIRB cannot grow using an electrode, while others capable of growth with an electrode cannot use iron (21–23). Moreover, some iron reducing bacteria such as *Shewanella oneidensis* may have multiple mechanisms of electron transfer that include self-produced flavins and nanowires (36, 37). Electron transfer also appears to be possible in both directions (i.e. to and from the cell). The iron reducing bacterium *Geobacter sulfurreducens* can transfer electrons to an anode, for example, but it can also accept electrons from the cathode for cell respiration with nitrate (38). Whether the same pathway is used for the cell to donate and accept electrons is not known. Gram positive bacteria capable of glucose fermentation to hydrogen have been found to respire in an MFC using the anode (39), and recently it was found that a cathodic biofilm accepted electrons and released hydrogen gas (16). Bacterial biofilms on MFC cathodes have also been
shown to enhance oxygen reduction (34, 40, 41), presumably through accepting electrons into the cell and then releasing them to oxygen. The capacity for electron transfer into and out of cells has of course evolved in microorganisms for reasons not related to current flow in MFCs or MECs. We believe that the capacity for exogenous electron transfer was developed to enable interspecies and interspecies electron transfer. S. oneidensis MR-1 grow on an electrode connected by a network of uninsulated nanowires (37), and G. sulfurreducens produce highly conductive and thick biofilms (42). A conductive appendage was shown to connect a fermentative bacterium (Pelotomaculum thermopropionicum) to a methanogen (Methanotrophomonas thermophiliotrophicus) (37). The use of nanowires or other methods to transfer electrons between cells avoids the need to form hydrogen and eliminates the possibility of accumulation of hydrogen gas that could inhibit fermentation. Our findings here that suggest that methanogens can directly accept electrons builds further support for interspecies electron transfer postulated by others (18, 37).

Outlook. The use of Archaea for producing methane via electromethanogenesis provides an additional route for biofuel production accompanied with carbon dioxide capture, without the need for precious metal catalysts. The use of a methanogenic biocathode enables methane production from any electrical source, although renewable energy sources would provide the greatest advantages for truly sustainable energy systems. For example, the use of excess solar or wind energy or an MFC could provide current for an MEC, producing methane that could be later reused to generate electricity or used as a transportation fuel. Existing industrial waste gases could provide CO₂ sources for capture.

Transforming electrons into methane has the advantage of producing a fuel that can easily be stored or transported. Compression, transport in pipes, and storage of methane involves mature technologies, and thus methane production by electromethanogenesis could immediately be integrated into an existing infrastructure. The efficiency of carbon dioxide capture demonstrated here by electromethanogenesis is high compared to other methods and thus may be useful for carbon capture. Electrochemical reduction of CO₂ using electromethanogenesis has an electron capture efficiency of 96%, compared to 10–57% using metal catalyzed electrodes for methane production (8).

The production of methane by electromethanogenesis will likely not displace existing methods of biomethane production from organic matter using anaerobic digesters, especially for high-strength wastewaters. MECs will likely be more appropriate for treatment of relatively dilute wastewaters. The overall advantages of MECs for wastewater treatment or biofuel production (including both methane and hydrogen gases) could make them an important method for bioenergy production in the near future.

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Supporting Information Available
Community analysis information, control experiments demonstrating the lack of hydrogen generation from the cathode, and composition of the medium. This material is available free of charge via the Internet at http://pubs.acs.org.

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