Effect of pre-acclimation of granular activated carbon on microbial electrolysis cell startup and performance

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ABSTRACT

Microbial electrolysis cells (MECs) can generate methane by fixing carbon dioxide without using expensive catalysts, but the impact of acclimation procedures on subsequent performance has not been investigated. Granular activated carbon (GAC) was used to pre-enrich electrotrophic methanogenic communities, as GAC has been shown to stimulate direct transfer of electrons between different microbial species. MEC startup times using pre-acclimated GAC were improved compared to controls (without pre-acclimation or without GAC), and after three fed batch cycles methane generation rates were similar ($P > 0.4$) for GAC acclimated to hydrogen (22 ± 9.3 nmol cm^{-3} d^{-1}), methanol (25 ± 9.7 nmol cm^{-3} d^{-1}), and a volatile fatty acid (VFA) mix (22 ± 11 nmol cm^{-3} d^{-1}). However, MECs started with GAC but no pre-acclimation had lower methane generation rates (13 ± 4.1 nmol cm^{-3} d^{-1}), and MECs without GAC had the lowest rates (0.7 ± 0.8 nmol cm^{-3} d^{-1} after cycle 2). Microbes previously found in methanogenic MECs, or previously shown to be capable of exocellular electron transfer, were enriched on the GAC. Pre-acclimation using GAC is therefore a simple approach to enrich electroactive communities, improve methane generation rates, and decrease startup times in MECs.

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1. Introduction

Microbial electrolysis cells (MECs) use microbes grown on one or both electrodes to produce gaseous fuels with the addition of external electrical input [1]. Exoelectrogenic microorganisms on the anode can produce electrical current from the oxidation of organic matter. At the cathode, hydrogen (H_2) can be produced abiotically, or certain microorganisms can be used to reduce electrode overpotentials and produce different chemical species, including methane and acetic acid [2–4]. Methane can be generated by microbes on the cathode through carbon dioxide (CO_2) fixation and oxidation of H_2 or through direct uptake of electrons. The formation of H_2 under standard conditions requires cathode potentials more negative than -410 mV vs a standard hydrogen electrode (SHE). However, if methane is generated by direct uptake of electrons from the cathode, minimum potentials can be more positive (-240 mV vs SHE) [3]. The exact mechanism for methane production is controversial, with direct electron transfer indicated in some studies [2,3], while others have concluded hydrogen gas or formate formation are primary routes for methane production [5,6].

Higher current densities are needed to increase the economic viability of methane production from MECs. In microbial fuel cells (MFCs) and other bioelectrochemical systems, the generation of high power densities is associated with the predominance of anode communities by various Geobacter species, such as Geobacter sulfurreducens and Geobacter anodireducens [7,8]. The specific microbes needed on the cathode to enhance MEC performance for biocathodic methane production are not known, but likely they require a predominance of Methanobacterium. In most MEC studies with cathodes that are poor catalysts for hydrogen gas evolution, where methane production predominates over other terminal products, the predominant archaea are hydrogenotrophic Methanobacterium [2,9–11]. Hydrogenotrophic microorganisms are almost always more abundant than acetoclastic methanogens, even in acetate-fed MECs [9].

Methods to enrich electrotrophic biocathode communities have not been well studied, but materials and acclimation procedures are important. When several different electrode materials were compared in two-chamber MECs, Methanobrevibacter predominated on a platinum-coated cathode, but Methanobacterium predominated on almost all others (graphite blocks; graphite blocks coated with carbon black, or carbon black with stainless steel, nickel, ferrihydrite, magnetite, iron sulfide, molybdenum disulfide; and carbon fiber brushes) [10]. The only exception was a duplicate reactor with a carbon brush cathode, where Methanosaeta was predominant, and that had poor performance for methane production compared to the other carbon brush reactor duplicate and all other materials. Inocula obtained from a natural bog sediment with high quantities of hydrogenotrophic methanogens showed

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higher methane generation in MECs than reactors inoculated with anaerobic digester sludge with mostly acetoclastic methanogens [9].

It has recently been shown that methane generation rates are increased in anaerobic digesters containing granular activated carbon (GAC), possibly due to direct electron exchange between syntrophic microbial communities of bacteria and methanogenic archaea [12]. Direct electron transfer was found to occur in co-culture studies of *Geobacter metallireducens* and *G. sulfurreducens*, and *G. metallireducens* and *Methanosarcina barkeri* [12]. In addition, rates of methanogenesis have been increased for methanogenic sludge by addition of magnetite [13–15], and direct electron transfer has been observed between *G. sulfurreducens* and *Thiobacillus denitrificans* using GAC and electrically conductive magnetite [16]. This suggested that the development of methanogenic communities on GAC, in the absence of an electrode, might be an effective method to enrich microbial communities for subsequent use in methanogenic MECs. However, anaerobic digester studies using GAC have focused on co-cultures involving acetoclastic methanogens and have found acetoclastic methanogens to dominate in mixed-culture studies [12], while hydrogenotrophic methanogens have dominated in MEC studies [9,10].

The impact of microbial community development on GAC was examined here using an inorganic (hydrogen gas) or different organic substrates that included methanol, acetate, and propionate. Following enrichment of methanogenic communities on GAC with these different substrates, the GAC was added to two-chamber MECs. The rate of methane production of the pre-acclimated MECs was compared to controls lacking GAC, or containing GAC with no pre-acclimation. The subsequent performance of the MECs was examined in terms of startup time for methane production, and methane generation rates.

2. Materials and methods

2.1. Inoculum and culture medium

An ammonium chloride and bicarbonate medium, including vitamins and minerals, was used to support microbial growth [9]. Bog sediment was chosen as an inoculum source for its microbial diversity and higher methane production rates compared to anaerobic digester inocula in MECs [9]. Sediment was obtained from the Black Moshannon bog (40°54′20.6″N, 78°03′11.1″W) and maintained under anaerobic conditions by flushing with nitrogen gas before storage. The sample was sieved to remove fibers in an anaerobic chamber (Coy Lab Products, Grass Lake, MI) that contained an atmosphere of hydrogen (2%) and nitrogen (98%). The sample was then centrifuged for 5 min at $7650 \times g$ (Sorvall Evolution RC Centrifuge), decanted, and mixed with bicarbonate medium to create a 50/50 (v/v) slurry. The slurry was stored at 4 °C and used to inoculate acclimation reactors and MECs at different times.

2.2. Pre-acclimation of microorganisms with GAC

Microbial communities were acclimated with GAC in 120 mL glass serum bottles (in duplicate) operated under fed batch mode, where the end of a cycle occurred when methane production plateaued. These acclimation reactors were prepared inside an anaerobic chamber, with each bottle containing 40 mL bicarbonate medium, 10 mL bog sediment slurry, and 3.4 g GAC (dry weight, DARCO MRX, 10×30 mesh, Norit Activated Carbon). Substrates were added to the bottles by injection at the beginning of each cycle: methanol (M), a VFA mix (MAP), acetate (A), hydrogen (H), and wastewater (W) as substrates. M reactors were fed with 20 μL of methanol, MAP reactors with 0.5 mL of a VFA mix (17 mL/L methanol, 37 g/L sodium acetate, and 10 g/L sodium propionate) [17,18], and A reactors with 0.5 mL of a 100 g/L sodium acetate solution. H reactors were flushed with H_2/CO_2 (20% CO_2, 80% H_2) for 15 min. After cycle one, the headspace was then filled to 200 kPa with H_2/CO_2. For W reactors, 40 mL of primary clarifier effluent (Penn State Wastewater Treatment Plant, 490 ± 90 mg COD/L) was bubbled with nitrogen gas and added to the reactor instead of bicarbonate medium.

Controls for methane production in the absence of substrate were run in duplicate and operated identically. For each set of controls, the medium was changed at the same time as their associated test duplicate. Methane generation by the controls was subtracted from maximum methane generation for the test reactors for each cycle.

Reactors were incubated with shaking at 31 °C (80 RPM, MaxQ400, ThermoScientific, MA). Methane production was measured every 1–4 days by gas chromatography (SRI 310C, SRI Instruments, Torrance, CA). At the end of a cycle, the medium was changed by removing 40 mL of the liquid, including inoculum, in the anaerobic chamber, and replacing with fresh bicarbonate medium (40 mL). Bottles were flushed with N_2/CO_2 (20% CO_2, 80% N_2) for 15 min to remove hydrogen from the headspace. Substrate was then added using a syringe.

2.3. MEC setup and operation

Two-chamber MECs were assembled from two glass bottles with side arms, with the tops sealed with butyl rubber stoppers and a cap, with the side arms separated by a Nafion membrane (Nafion 117, Fuel Cell Store, Boulder, CO, U.S.A.) and an O-ring [3]. Each half of the reactor held 100 mL of liquid and had 55 mL of headspace. A syringe was inserted into the rubber stopper of the cathode chamber to release gas pressure.

To prepare the electrodes, titanium wires (0.032 gauge, 12 cm in length) were cut and cleaned with coarse sand paper. For the anode, the wire was inserted through the butyl rubber stopper and secured to a piece of ruthenium mixed metal oxide electrode (2.0 ± 0.3 mm thick; Magneto, The Netherlands). For the cathode, the wire was inserted through the stopper and attached to carbon brushes (23/311629, Millrose, Mentor, OH) by wrapping the wires around the brush stem. Brushes were baked at 450 °C for 1 h before use [3]. A 4 mm hole was drilled through the stopper for the Ag/AgCl reference electrode (-200 ± 5 mV vs SHE; model RE-5B, BASi, West Lafayette, IN) filled with 3 M NaCl saturated with AgCl. Electrodes were refurbished by replacing the solution and frit, and a refurbished electrode was inserted at the start of each cycle.

GAC from M, H, and MAP acclimation reactors (serum bottles) were used as the inoculum for M, H, and MAP MECs. Two MEC controls were used, one with fresh GAC and bog sediment (GAC + bog), and the other with only bog sediment (bog) and no GAC. Reactors were assembled in the anaerobic chamber, with 100 mL bicarbonate medium in the anode. Pre-acclimated reactors were assembled with 93 mL bicarbonate medium and 7 mL (10 g wet weight) of GAC from the serum bottles in the cathode chamber. Fresh GAC + bog controls were made by adding 83 mL of bicarbonate medium, 7 mL GAC wetted with bicarbonate medium, and 10 mL bog sediment slurry to the cathode. For the bog-only controls, 90 mL of bicarbonate medium and 10 mL bog sediment slurry were added to the cathode. The headspace in the reactors was flushed with N_2/CO_2 (80% N_2 and 20% CO_2) for 15 min after assembly or medium change. During the first three cycles, carbon brushes were not in contact with the lower GAC. During the third, the carbon brushes were lowered into the GAC to make a direct contact.

Reactors were operated at a set cathode potential of -600 mV vs SHE. Methane volume in the headspace of the cathode was measured weekly by gas chromatography. A cycle was ended when one of the duplicate reactors showed less than a 10% increase in headspace methane volume over a week [3], regardless of the performance of the other duplicate. To start a new cycle, 80 mL of liquid was removed from both the anolyte and catholyte with a wide mouth pipette, and 80 mL of fresh bicarbonate medium was added to each in an anaerobic chamber. Catholyte with GAC or bog sediment was stirred manually before removing liquid.