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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.

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 ×*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.

2.4. MEC calculations

Methane generation rate was used as the metric to assess MEC performance instead of total methane generated per cycle, as total methane produced was affected by the timing of media changes. Methane generation rate was calculated between each weekly headspace measurement. The overall methane generation rate for each cycle was calculated by averaging weekly rates, starting from the date methane was first measured in the headspace, and therefore excluding the start of the stationary phase (<10% methane increase) (details in the Supporting information, SI).

Coulombic recoveries (*CR*s) were used to assess how much of the charge transferred through the circuit was converted into methane. The *CR*s were calculated as:

$$CR = \frac{8\ F\ V_m}{Q} \quad (1)$$

where F is the faraday constant, 8 is the number of electrons transferred during the reduction of CO_2 to methane, V_m is the volume of methane measured in the cathode headspace at STP, and Q is the total coulombs transferred through the circuit. Coulombs transferred was calculated as the integral current over time for the cycle.

2.5. Microbial community analysis

GAC samples were removed from the acclimation reactors and MECs at the end of reactor operation, with tests conducted using 0.25 g of GAC from each reactor. Carbon brush fibers were cut from MEC cathodes for DNA extraction, with fibers removed from the side of the brush facing the membrane. DNA extraction was performed on all of the brush fibers removed (approx. 0.25 g). A Mo Bio PowerSoil DNA extraction kit was used to isolate DNA from GAC and fiber samples, with the following modifications. Glass beads (0.1 mm, Mo Bio) were used to aid in cell lysis instead of the garnet beads included with the kit, and 750 μL of bead solution was added to the bead beating tube and glass beads. Cell lysis was performed in a bead mill (Bead Ruptor 12 Homogenizer, Kennesaw, GA) for 45 s on the medium setting, instead of vortexing the sample. Centrifuge time was increased from 0.5 to 1 min. Incubation times at 4 °C were increased from 5 to 10 min.

To target both bacteria and archaea, primer set 515F/805R was used for PCR amplification of DNA. Primer sequences for 515F and 805R are modified with the Illumina adaptors, and are listed as 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGGTGYCAGCMGCCGCGGTAA-3′ and 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC-3′, respectively. The underlined regions of the primer sequences target the 16S rRNA gene. Illumina MiSeq was used for next-generation sequencing of 16S rRNA gene amplicons. Amplicon sequences were initially sorted by the KAUST Bioinformatics Team based on a Phred score of >30. The primers, barcodes and adaptors were then trimmed off and remaining sequences < 280 nt were removed. The remaining amplicons which passed the quality screening as were classified using the Ribosomal Database Project (RDP) at a 95% confidence interval. Bray-Curtis similarities were calculated from square-root transformed relative abundance data. Multidimensional scaling (MDS) plots were generated from bootstrapped Bray-Curtis similarities using Primer-E software, version 7 [19]. All high-throughput sequencing data were deposited in the European Nucleotide Archive (ENA) under study accession number PRJEB14995.

3. Results and discussion

3.1. Acclimation with GAC

Hydrogen-fed acclimation reactors (H) produced the greatest volume of methane at 17 ± 15 mL per cycle (80% of theoretical methane production, SI Table S2), while methanol-fed acclimation reactors (M) most rapidly promoted stable methane generation at 7.4 ± 1.2 mL per cycle (90% of theoretical production) (Fig. 1). The large standard deviation with hydrogen gas as the feed reflects the large changes in methane production over successive cycles compared to more consistent production rates for the other substrates. The VFA mix, consisting of methanol, acetate, and propionate (MAP), produced only 1.9 ± 0.6 mL methane per cycle (18% of theoretical production). Methane production increased with successive cycles for the M, H, and MAP acclimation reactors, but acetate-fed (A) and wastewater-fed (W) acclimation reactors ceased to produce methane after two cycles.

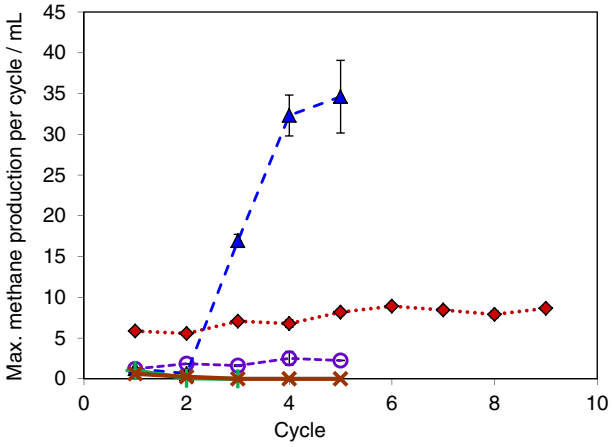

Fig. 1. Maximum methane production per cycle for fed-batch acclimation reactors with the following substrates: methanol (M, ···◆···); a methanol, acetate, and propionate mix (MAP, –○–); hydrogen (H, –▲–); acetate (A, –+–); and wastewater (W, –×–). Error bars indicate the range for duplicates.

3.2. MEC operation

When the GAC from reactors M, H and MAP was transferred to the MECs, there was no significant difference in methane generation rates due to the different substrates used in pre-acclimation (ANOVA, $P > 0.05$, cycles 4–7). These pre-acclimated reactors generated 24 ± 10 nmol cm^{-3} d^{-1} for cycles 4–7. Methane production rate appeared to increase with cycle number (Fig. 2), but the change was not significant (linear regression, $P > 0.30$), with average production rates of 25 ± 10 nmol cm^{-3} d^{-1} (M), 22 ± 11 nmol cm^{-3} d^{-1} (MAP), and 22 ± 9 nmol cm^{-3} d^{-1} (H). However, methane production rates in these three MECs were significantly higher (*t*-test, $P < 0.04$) than the control MEC inoculated with bog sediment and fresh (non-acclimated) GAC (GAC + bog), which produced 13 ± 4 nmol cm^{-3} d^{-1} of methane. There was a significant increase in methane production by the GAC + bog MEC over time (linear regression, $P = 0.05$). Even if production rate would have continued to increase to equal that in the pre-acclimated reactors, the GAC + bog MEC reactor acclimation time was five times longer (441 days) than the hydrogen (81 days) or MAP pre-acclimated (60 days) reactors. Such a long acclimation time would not be useful compared to the other methods. Methane production rate for the control MEC inoculated without any GAC (bog-only) ceased methane production after the second cycle, perhaps due to low abundance of methanogens in the bog sediment inoculum, as discussed below.

*CR*s based on recovery of methane for the measured current showed similar trends to methane production rate, with similar *CR*s for reactors M, MAP, and H, and lower *CR*s for GAC + bog (Fig. 3). The high initial *CR* for the MECs without GAC (bog) MECs was likely due to a combination of very low current and methane production from organics in the inoculum. Methane was only routinely measured in the cathode chamber, but gas measurements from the anode chamber showed low concentrations of methane. Therefore, total methane production was

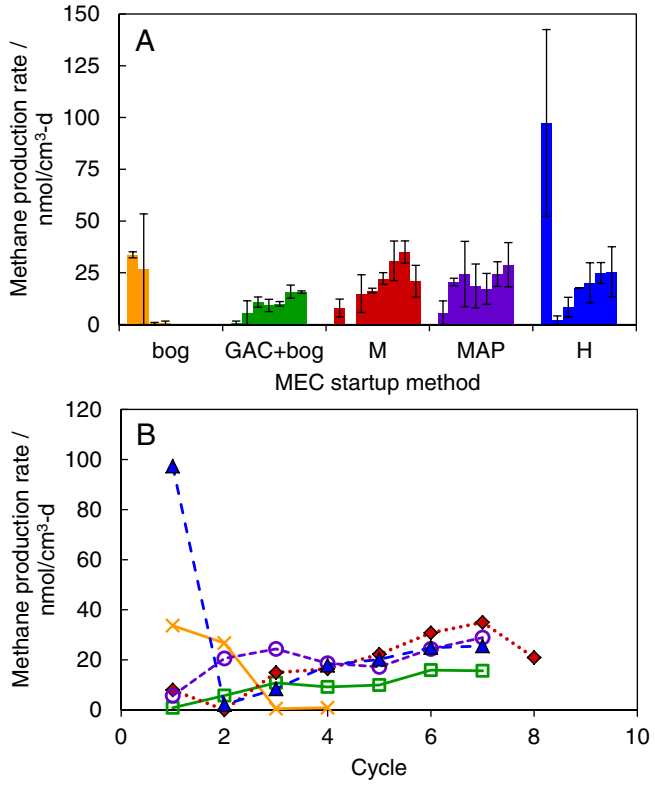

Fig. 2. Both (A) and (B) show methane production rate across successive cycles in MECs (error bars show duplicate range). The MEC inoculation methods shown are: bog inoculum only (bog, –×–), bog inoculum with fresh GAC (GAC + bog, –□–), methanol-acclimated GAC (M, ···◆···), methanol/acetate/propionate acclimated GAC (MAP, –○–), and hydrogen-acclimated GAC (H, –▲–).

likely underestimated due to loss of some methane into the anode chamber. A high *CR* was obtained for bog-only MECs because of low charge transfer.

3.3. Microbial communities in acclimation reactors

GAC acclimation to the different substrates enriched the inoculum communities in archaea, *Geobacter*, and sulfate-reducing bacteria (SRB). The bog sediment inoculum contained low archaeal abundance, and therefore also low methanogen abundance. Bog sediment used for inoculating the MAP and H reactors only contained 3.0 ± 0.02% archaea, which increased to 19 ± 12% archaea in acclimation reactors M, MAP,

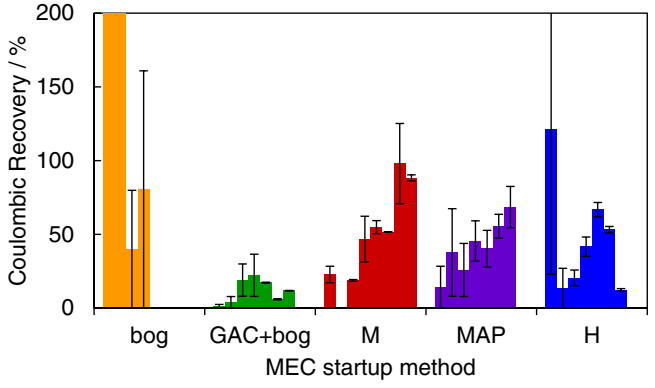

Fig. 3. Coulombic recoveries across successive cycles for different GAC acclimation methods. Labeling is identical to that in Fig. 2. Bog cycle 1 (2307%, 3635%) and bog cycle 2 (499%, 499%) are out of the range shown.

and H. *Geobacter* was the only bacterial group that was classified to the genus level in the bog inoculum, and only accounted for 3.0 ± 0.05% of DNA reads (Fig. 4). In the acclimation reactor samples, abundance of *Geobacter* increased to 42 ± 22%. SRB, as measured by the most highly abundant groups in the acclimation reactor samples (*Desulfomonile*, *Desulfovibrionales*, *Desulfovibrionaceae*, *Desulfovibrio*, *Desulfuromonadales*, and *Desulfobulbus*) increased from 0.7 ± 0.1% in the inocula to 19 ± 12% in the acclimation reactors.

Microbial communities in the M and MAP acclimation reactors showed a high degree of similarity, averaging 74 ± 8% similarity among all M and MAP duplicates (Table S7). Based on this high similarity and the low methane production in MAP acclimation reactors, it is likely that only methanol was consumed in the MAP mix of substrates, which may be due to carbon catabolite repression of the microorganisms present in the inoculum. GAC communities from M and MAP acclimation reactors were enriched with SRB and *Geobacter*, which have both been associated with exocellular electron transfer in microbial electrochemical technologies [20]. GAC may therefore have been a favorable environment for these bacteria due to its high conductivity, which could enable electron transfer between microorganisms [12]. SRB composed 21 ± 4% of M reactor DNA reads and 31 ± 2% of MAP reactor reads (Fig. 4B). In contrast, H reactors were only 5 ± 3% SRB. *Geobacter* comprised 50 ± 17% of M reactor DNA reads and 59 ± 3% of MAP reactor reads, while only comprising 16 ± 7% of H reads. *Geobacter* was the most abundant bacterial genus in a single-chamber mixed-culture MEC fed with acetate [9], but not in a two-chamber mixed-culture MEC without organic substrate addition [10]. In addition to transferring electrons to an electrode, *Geobacter* has been shown to be capable of direct interspecies electron transfer (DIET) with other methanogens [12,21] in studies that focused on acetoclastic methanogens. *Methanomethylovorans*, a member of the aceto- and methylotrophic family *Methanosarcinaceae* [22], were found in acclimation reactors M and MAP (3.7 ± 2.2%). They are able to consume methanol in the substrate, and are more closely related to the acetoclastic methanogens used in DIET studies [21], implying a possible similar interaction between *Geobacter* and *Methanomethylovorans*.

Methanobacterium dominated in H acclimation reactors, with 71 ± 0.1% of the total number of DNA reads for both bacteria and archaea (Fig. 2). These hydrogenotrophic methanogens were the dominant archaeal genus found previously in many mixed-culture methane-generating MECs [2,9–11]. A high abundance of methanogens in the inoculum of an MEC was previously found to correlate with a higher methane generation rates [9]. The H acclimation reactors also showed the presence of *Methanobrevibacter*, another hydrogenotrophic methanogen which is in the same family as *Methanobacterium* [22]. *Methanobrevibacter* was previously reported to be present in MECs using a platinum coated graphite cathode [10]. The high abundance of hydrogenotrophic methanogens in H acclimation reactors is likely to have accounted for a higher methane production compared to M and MAP acclimation reactors.

3.4. Microbial communities in MECs

For MECs, community similarity was higher between GAC samples (65 ± 9%, Table S8, GAC), than between brush samples (59 ± 10%, Table S8, Brush) or among all samples (59 ± 10%). Many of the genera of microbes that were more highly abundant in GAC samples, as compared with carbon fiber brush samples, have previously been reported to be associated with methanogenic MECs or they have been shown to be capable of exocellular electron transfer (Fig. 5). Cathodic archaeal communities were dominated by *Methanobacterium*, which has also been found to predominate on the cathode in previous methanogenic MEC studies [2,9–11]. While *Methanomethylovorans* was the predominant methanogen in M and MAP acclimation reactors, *Methanobacterium* dominated the methanogen population in the corresponding MECs. For all

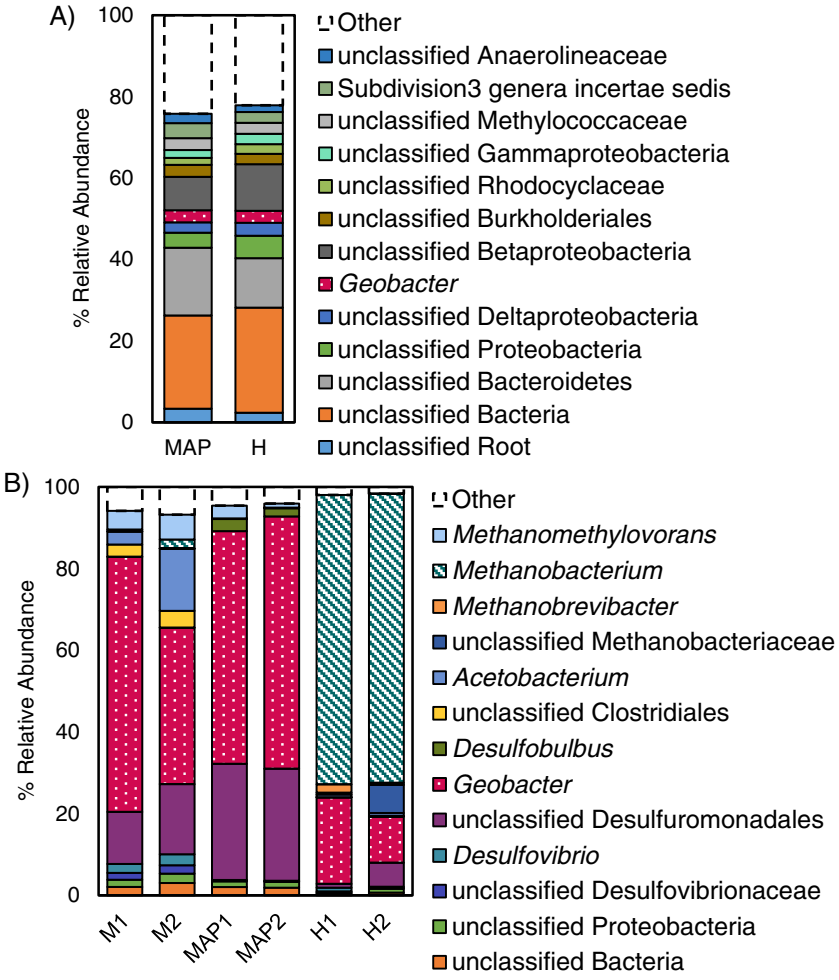

Fig. 4. Phylogenetic affiliations of microbial populations with >2% relative abundance in (A) the bog sediment used to inoculate MAP and H acclimation reactors, and (B) GAC from M, MAP, and H acclimation reactors.

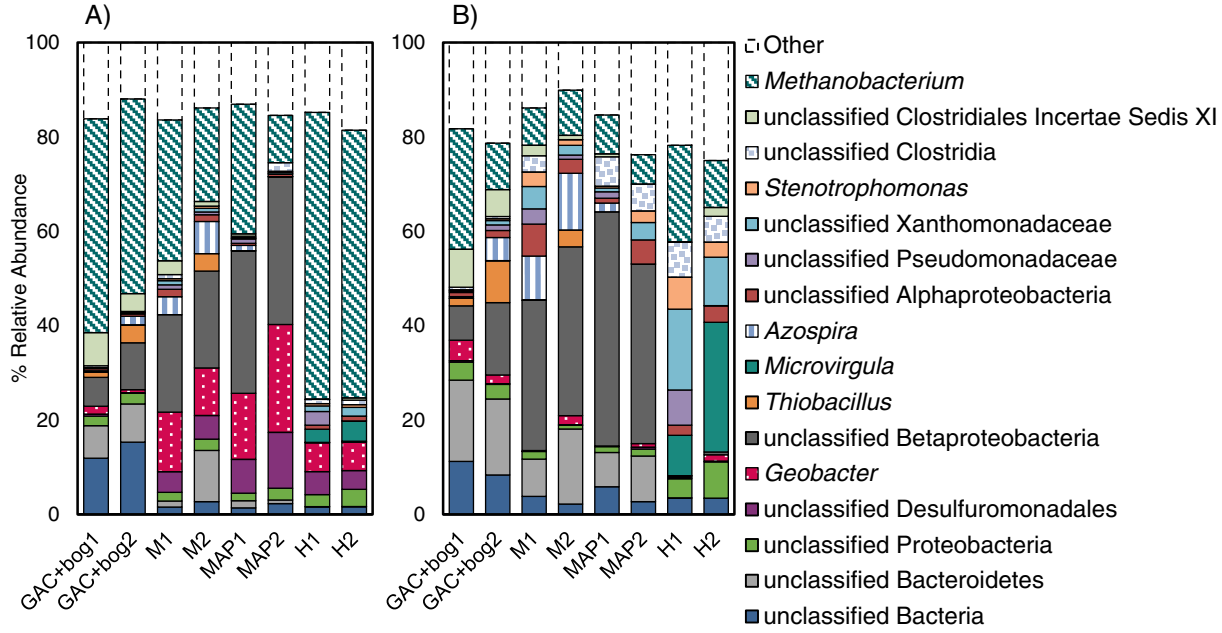

Fig. 5. Phylogenetic affiliations of microbial populations with >5% relative abundance for MECs. Samples were taken from A) GAC and B) carbon brush cathode fibers taken from GAC + bog, M, MAP, and H reactors.

pre-acclimated MECs, GAC samples showed greater relative abundance of *Methanobacterium* (34 ± 20%) compared to fiber samples (10 ± 5%). GAC samples from pre-acclimated reactors also showed greater relative abundance of *Geobacter* (12 ± 6%) compared to fiber samples (0.8 ± 0.8%), and a greater abundance of *Desulfuromonadales* (6 ± 3%) compared to fiber samples (0.1 ± 0.1%). *Desulfuromonas*, within the order *Desulfuromonadales*, has been found to exist at high relative abundances on various cathode types in methanogenic MECs, including carbon brush cathodes [10]. The reason GAC selected for *Geobacter*, *Desulfuromonadales*, and *Methanobacterium* but carbon brushes did not, is not clear. Adsorption of natural organic matter onto the GAC may allow greater access to adsorbed substrate, or surface charge and electron transfer properties may select for certain microbes. The role of direct electron uptake in methanogenesis in the MECs could not be concluded, as the experiment did not include targeted mechanistic studies.

4. Conclusions

Hydrogen-fed, pre-acclimated GAC showed higher relative abundance of *Methanobacterium* (71 ± 0.05%) than methanol-fed and VFA-fed acclimation reactors (0.7 ± 1%). However, all pre-acclimated MECs showed similar methane generation rates and startup times (24 ± 10 nmol cm^{-3} d^{-1} for cycles 4–7). Pre-acclimation of GAC to a fed chemical substrate showed improved methane generation rate as compared to MEC startup with inoculum and fresh GAC (13 ± 4 nmol cm^{-3} d^{-1}), as well as decreased startup time. The presence of GAC in MECs using bog sediment inoculum showed improved charge transfer and more consistent methane generation than MECs with only bog inoculum. GAC may be an effective growth support for enriching microbes associated with methanogenic MECs and exocellular electron transfer, as GAC had greater relative abundances of *Geobacter*, *Desulfuromonadales*, and *Methanobacterium* on the GAC (52 ± 14%) as compared to the carbon fiber brush (11 ± 5%).

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bioelechem.2016.08.003.

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