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Fate of H_2 in an Upflow Single-Chamber Microbial Electrolysis Cell Using a Metal-Catalyst-Free Cathode

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With the goal of maximizing the H_2-harvesting efficiency, we designed an upflow single-chamber microbial electrolysis cell (MEC) by placing the cathode on the top of the MEC and carried out a program to track the fate of H_2 and electron equivalents in batch experiments. When the initial acetate concentration was 10 mM in batch-evaluation experiments lasting 32 h, the cathodic conversion efficiency (CCE) from coulombs (i.e., electron equivalents in current from the anode to the cathode) to H_2 was 98 ± 2%, the Coulombic efficiency (CE) was 60 ± 1%, the H_2 yield was 59 ± 2%, and methane production was negligible. However, longer batch reaction time (∼ 7 days) associated with higher initial acetate concentrations (30 or 80 mM) led to significant H_2 loss due to CH_4 accumulation: up to 14 ± 1% and 16 ± 2% of the biogas at 30 and 80 mM of acetate, respectively. Quantitative PCR proved that no acetoclastic methanogens were present, but that hydrogenotrophic methanogens (i.e., *Methanobacteriales*) were present on both electrodes. The hydrogenotrophic methanogens decreased the CCE by diverting H_2 generated at the cathode to CH_4 in the upflow single-chamber MEC. In some experiments, the CE was greater than 100%. The cause was anode-respiring bacteria oxidizing H_2 and producing current, which recycled H_2 between the cathode and the anodes, increasing CE to over 100%, but with a concomitant decline in CCE, despite negligible CH_4 formation.

Introduction

Biological production methods have the potential to provide renewable H_2, but major technical bottlenecks need to be overcome. Photobiological H_2 production is far too slow (0.038−0.086 m^3 H_2/m^3-d) (*1*), is limited by climate conditions (i.e., sustained sunlight), and normally produces O_2 that must be separated from H_2. The rate of dark-fermentative bioH_2 can be more substantial, up to 64.5 m^3 H_2/m^3-d (*2*), but the H_2 yield (e^- equiv H_2/e^- equiv of electron donor removed) is small, typically ∼17% (*2*−*4*). For practical application of biological H_2 production, H_2 yields and production rates must be increased simultaneously.

Liu et al. (*5*) introduced a new concept of capturing H_2 out of organic fuels by converting the microbial fuel cell (MFC) into a microbial electrolysis cell (MEC). An MEC is similar to an MFC in that it uses specific bacteria, which we refer to as anode-respiring bacteria (ARB), that can transfer electrons extracted from organic donors to the anode in the MEC. The electrons transported to the anode pass through a circuit and reach the cathode where the electrons react with H^+ ions (or H_2O) to produce H_2. MECs have two main advantages over other biohydrogen processes. First, a variety of organic donor substrates can be used as fuel (*6*) by using bacterial consortia involving fermenters and ARB (*7*, *8*). Second, nonfermentable substrates can be completely oxidized to CO_2, resulting in high conversion yields of 67−91% (*6*).

The conversion from an MFC to an MEC requires two changes. The first change is to exclude oxidants (e.g., O_2) from the cathode, which forces electrons to be donated to H^+ ions (or H_2O), thereby producing H_2 at the cathode. The second change is to provide a certain amount of electrical energy to make the cathode potential negative enough to generate H_2. The standard electrical potentials at pH 7 and a temperature of 25 °C for reduction half-reactions for acetate ($E_{acetate}^{\circ\prime}$) and for protons to H_2 ($E_{H_2}^{\circ\prime}$) are −0.284 and −0.421 V, respectively. Thus, the $E^{\circ\prime}$ value is −0.137 V for the redox reaction of acetate oxidation to form H_2 ($CH_3COO^- + 3H_2O = CO_2 + HCO_3^- + 4H_2$); thus, we need to provide applied voltage of at least 0.137 V to produce H_2 in the MEC using acetate as the electron donor.

An MEC involves two redox steps, each of which has a characteristic efficiency of electron capture. The first redox step is oxidation of an electron donor by the ARB, with electrons transferred to the anode. The Coulombic efficiency (CE) is the ratio of electron equivalents (e^- equiv) converted to electrical flow (i.e., coulombs) normalized to the number of e^- equiv consumed from the organic donor. The second redox step is a reduction in which the electrons transferred through the circuit react with H^+ (or H_2O) at the cathode and produce H_2. The cathodic conversion efficiency (CCE) is the ratio of e^- equiv donated to H_2 normalized to the e^- equiv transferred in the circuit from the anode to the cathode. A CCE less than 100% means that H_2 produced on the cathode gets lost to other reactions (e.g., diffusion to an anode compartment, leaking from an MEC, or biological oxidation).

The first requirement for a high H_2 yield is that the CE should be high, and this demands that e^- equiv of the donor substrate do not get lost before they are transferred to the anode. Possible e^- sinks decreasing CE under anaerobic conditions can be biomass synthesis, soluble microbial products (SMP), or CH_4 gas; SMP can be produced and accumulate despite complete oxidation of electron donor in bacterial catabolism (*9*). H_2O can be the significant electron sink if O_2 leaks into the anode compartment. Lee et al. (*8*) showed that biomass (15−26%) and SMP-like organics (11−18%) were the largest nonelectricity sinks in an MFC that had no O_2 leakage.

The second requirement for a high H_2 yield is that the CCE also is high. While some studies showed steady CCE values up to 100% in a dual-chamber MEC (*10*, *11*), H_2 loss by diffusion into the anode chamber was large in some cases (*11*, *12*), decreasing the CCE down to 6−33%. Loss of H_2 by diffusion can seriously limit MEC applications, since H_2 yield can be small even though the CE is high. Thus, an MEC must be designed to prevent H_2 loss, as well as have a high CE, if the H_2 yield is to be maximized.

A single-chamber MEC has its anode and cathode placed in the same compartment, and it may be one of the solutions to reduce H_2 loss. In the dual-chamber MEC, the membrane

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is a barrier to movement of H_2 from the cathode to the anode, but it adds expense and complexity. If we can collect H_2 gas produced from the cathode much more rapidly than it can diffuse to the anode or be consumed by a reaction, we may not need a membrane to separate the two chambers. The single-chamber system makes an MEC system more compact and cost-effective because of the absence of the membrane; the single-chamber MEC also can minimize the ohmic overpotential due to ion transport through the membrane (*13*−*15*) and the concentration overpotential due to a large pH gradient between the two chambers (*11*).

Several studies have tested single-chamber MECs, but they showed unstable CCEs in the range of 5−96% with 0.3−1.15 V applied voltage (*13*−*16*). The most likely sink for H_2 in a single-chamber MEC would seem to be hydrogenotrophic methanogens that consume the H_2 produced at the cathode before it can be recovered (*8, 14*). Up to now, no studies proved H_2 consumption by hydrogenotrophic methanogens in single-chamber MECs, although CH_4 gas was observed (*13, 14*). Another H_2 sink can be its oxidation by ARB, if they are able to utilize H_2 as an electron donor (*17, 18*). Unlike methane generation, H_2 oxidation by ARB might not be a significant H_2 loss in a single-chamber MEC, since current produced by H_2 oxidation produces H_2 gas on the cathode again, while the biomass yield of ARB is very small (*8, 13, 18, 19*). However, H_2 recycle between the cathode and the anode can harm overall performance (net H_2 recovery related to applied voltage), because current generated by H_2 oxidation increases overpotentials (i.e., energy losses) and the applied voltage.

To ensure a high CCE when hydrogenotrophic methanogens are a risk, we can try to inhibit H_2-utilizing methanogenesis with a specific inhibitor (e.g., BES), intermittent exposure to air, an acidic pH, or a short solids retention time (SRT). Using inhibitors is not practical for field applications, due to their expense, toxicity potential, or difficult handling. Exposure to air also is not practical because it adds an alternative electron sink that will reduce the CE significantly. Hu et al. (*14*) attempted to use an acidic pH for preventing the methanogens' growth, but it was not effective. In addition, an acidic pH could lower the current, since substrate-utilization rates are normally inhibited in acidic pH (*20, 21*), though some ARB may acclimate to acidic conditions (*14*). Short SRT can be efficient for suppressing the methanogens' activity, since the absolute minimum SRT of the archaea is 0.76 days (*22*), if the methanogens are suspended in the flowing liquid.

The most direct way to stop H_2 loss in a single-chamber MEC is to recover the H_2 gas upon its release from the cathode so efficiently that H_2 recovery out-competes methanogens, ARB, or any other sinks. Since the solubility of the H_2 molecule is extremely low ($K_H = 7.65 \times 10^{-4}$ mol/L-atm at one atmosphere and a temperature of 30 °C) (*23*), rapid recovery of H_2 gas should be feasible if the reactor configuration is optimized for this purpose.

We designed an upflow-type single-chamber MEC, positioning the cathode near the top of the MEC, which can allow us to capture H_2 gas efficiently. We evaluated the CE and CCE of the new MEC configuration over a range of operating conditions, showing that it was possible to have simultaneously high CE and CCE. Most important is that we comprehensively tracked the fates of electrons and H_2 in the single-chamber MEC to quantify any sinks. Finally, we determined which types of methanogens are responsible for CH_4 formation in the single-chamber MEC when methanogenesis was not suppressed.

Materials and Methods

Inoculation and Reactor Configuration. We provide details of inoculation, design, and operation of the upflow, single-chamber MEC in the Supporting Information. The most critical aspect of the design is that the cathode (a carbon felt) was placed above the anode (a bed of graphite spheres) and just below the liquid surface so that H_2 gas could bubble out and be harvested rapidly.

Experiments. Acetate was the sole electron donor and organic-carbon source in all experiments. We acclimated the MEC in the continuous mode with an internal recirculation rate of 20 mL/min using a peristaltic pump (Masterflex L/S, Cole-Parmer, U.S.) and a feed rate at 0.88 mL/min; we sparged the MEC with N_2 gas (99.9999%) for 5 min before initiating continuous experiments. The hydraulic retention time (HRT) based on empty-bed volume was 2.3 h during continuous operation. When the CE reached a steady-state value of 64 ± 2% (gas composition: H_2 68 ± 3%, CO_2 21 ± 5%, and CH_4 <0.5%), we shifted into batch mode for accurately quantifying the H_2 yield and electron flows.

We evaluated two operating parameters: acetate concentration (10−80 mM) and internal recirculation flow rate (7 and 40 mL/min). Other parameters were constant when we varied one parameter per experiment. The baseline condition was an initial acetate concentration of 10 mM and an internal recycle rate 7 mL/min. We used the same composition of the mineral medium as in Lee et al. (*8*); key were the 100 mM phosphate buffer and 1.7 S/m medium conductivity; the relatively high buffer concentration and conductivity are higher than in typical domestic wastewater, but they minimized the chances that proton transport limited current generation in our experiments (*20*). We operated the MEC at 30 ± 2 °C, and the medium pH was 7.3−7.4. We replaced the medium in the MEC by operating the cell in continuous mode for 5 HRTs between each experiment each time we varied a parameter.

We fixed the anode potential at −0.2 V vs Ag/AgCl, to minimize the chance that the anode potential influenced the current density (*24*−*27*); the anode potential was +0.07 V vs the standard hydrogen electrode for our medium solution. We provided power to the MEC using a potentiostat (VMP3, Applied Princeton Research, TN) at the fixed anode potential, which allowed us to determine what applied voltage corresponded to the maximum current density; the cathode potential varied with current, and we measured applied voltage as the current changed. We recorded current, anode potential, cathode potential, and applied voltage (cathode potential - anode potential) every 120 s using an EC laboratory software (Bio-Logic, TN).

We investigated the effect of cathode position on CCE and CH_4 formation in a single-chamber MEC by placing the cathode alongside the anode, instead of above it; we call this a bottle-type single-chamber MEC (B-MEC). We used the same inoculum and medium. We provide details for the reactor configuration and its operating conditions in the Supporting Information.

We tested the possibility of direct current generation by ARB doing H_2 oxidation at the anode of the B-MEC. We fed this MEC with the same medium, but lacking acetate, and we provided H_2 gas (99.99% H_2) as the sole electron donor for 2 h at a flow rate of 150 mL/min while monitoring current; we express current density as A/m^2 for tests using B-MEC, while current density is presented as A/m^3 for the upflow single-chamber MEC.

Analyses. We quantified gas percentages of H_2, CH_4, and CO_2 with a gas chromatograph (GC 2010, Shimadzu) equipped with a thermal conductivity detector and a packed column (ShinCarbon ST 100/120 mesh, Resteck Corporation) in the GC. We measured concentrations of organic acids and alcohols using high performance liquid chromatography (HPLC; model LC-20AT, Shimadzu). We provided details for sampling and operating procedures for the GC and the HPLC in the Supporting Information.

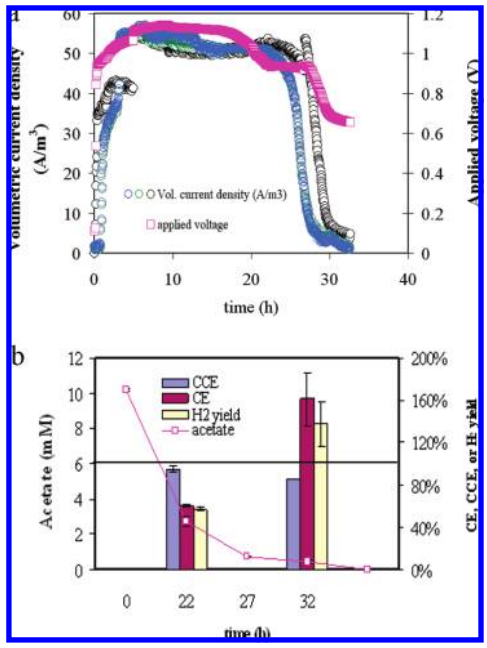

FIGURE 1. The evolution of parameters with time in the upflow single-chamber MEC run at the baseline condition (influent acetate 10 mM and recirculation rate 7 mL/min). (a) volumetric current density and applied voltage, and (b) Coulombic efficiency (CE), cathodic conversion efficiency (CCE), and acetate concentration. The CE and CCE values at 22 h are averages for reaction time from 0 to 22 h. The CE and CCE values at 32 h are averages for reaction time from 27 to 32 h.

We observed biofilm formation on the cathode using scanning electron microscopy (SEM) at the end of the upflow MEC experiments. We extracted DNA from the anode bed and the cathode from the upflow single-chamber MEC at the end of the tests. We performed quantitative real-time PCR targeting the 16S rRNA genes for *Geobacteraceae*, general bacteria, acetoclastic methanogens (*Methanosaetaceae* and *Methanosarcinaceae*), and hydrogenotrophic methanogens (*Methanomicrobiales* and *Methanobacteriales*). We offer detailed information on SEM, DNA extraction, and the real-time PCR in the Supporting Information.

Calculations. We provide equations for calculating CE, CCE, and H_2 yield in the Supporting Information. We quantified cumulative H_2 volume by analyzing H_2 percentage and volume change in headspace in the MEC.

Results

H_2 Recovery and CH_4 Formation in the B-MEC. We tested H_2 losses in the B-MEC having its cathode alongside the anodes. The results are shown in the Supporting Information. The CCE was 72% over a reaction time of 12 h, and significant CH_4 formation was detected (5% in the biogas). For a reaction time of 25 h, the CCE decreased to 48%, with 9% CH_4 in the biogas. The CH_4 percentage increased up to 26% by 100 h, and the CCE dropped to 22%. These results indicate that H_2 produced on the cathode was easily accessible for hydrogenotrophic methanogens before its release to gas phase when the cathode was alongside the anode.

Performance for the Baseline Condition in the Upflow MEC. Figure 1a shows the applied voltage and volumetric current density with time in the MEC batch experiment run at the baseline condition (in triplicate tests). The average volumetric current density was 51.4 ± 1.6 A/m^3 for 6−22 h. The cumulative CE and CCE for 32 h were $60 \pm 2\%$ and $98 \pm 2\%$, respectively, but their discrete values at different time intervals varied. The average CCE was $98 \pm 2\%$ for the first 22 h ($n = 3$), during which time the acetate concentration declined to 2.77 ± 0.30 mM, the CE was $60 \pm 1\%$ ($n = 3$), and the H_2 yield was $59 \pm 2\%$ (Figure 1b); n is the number of measurements. From 27 to 32 h, the CCE and current density decreased to 86% and 4.85 A/m^3, respectively, and the acetate concentration declined from 0.74 ± 0.00 mM to 0.43 ± 0.08 mM. The computed CE was $161 \pm 25\%$ ($n = 3$) for the period of 27−32 h, in which coulombs generated by acetate consumption were small. Due to the high CE, H_2 yield was $138 \pm 21\%$ for this time (100% = 4 mol H_2/mol acetate). For the baseline condition, the upflow, single-chamber MEC efficiently prevented H_2 loss to methanogenesis, since CH_4 peaks (detected only at the end of the test) were too small to be quantified (<0.5%), which means that the e^- equiv of cumulative CH_4 were less than 1.6% of the electron donor consumed.

The applied voltage was 1.06 ± 0.08 V for the average maximum volumetric current density (51.4 ± 1.6 A/m^3), which equals a volumetric H_2 production rate of 0.57 ± 0.02 m^3 H_2/m^3-d of MEC working volume. This production rate is significant, since we were able to double the H_2-producing rate (with an applied voltage 1.06 V) over the rate obtained (0.3 m^3 H_2/m^3-d) with a dual-chamber MEC using platinum catalyst at the cathode and applied voltage 1 V (*11*), even though we had no metal catalyst. The high phosphate buffer (100 mM) and the lack of a membrane should attenuate pH and ohmic overpotentials in our MEC, which means that overpotentials were at the anode and cathode. We observed that the cathode developed a white coating, which was biofilm confirmed by an SEM image (Supporting Information Figure S5). While it is possible that the biofilm allowed the cathode to function as a biocathode (*12*) and attenuated applied voltage, the applied voltage of ~1 V was consistent from the beginning to the end of experiments, which implies negligible biocathode activity.

Effects of Internal Recycle Rate. The higher circulation rate of 40 mL/min (from the baseline of 7 mL/min) improved the volumetric current density so that its maximum value rose to 68.1 ± 1.2 A/m^3 (a 32% increase over the control) at an applied voltage of 1.15 ± 0.03 V. Acetate was undetected after 32 h, the cumulative CCE averaged $89 \pm 9\%$, cumulative CE was stable at $60 \pm 2\%$, the cumulative H_2 yield was 53.4%, and only a small, unquantifiable CH_4 peak (<0.5%) was observed at the end of the test. These results show that improved mass-transport could increase the acetate utilization rate and current generation, although the CCE and H_2 yield declined a small amount.

H_2 Oxidation by ARB. To test for H_2 oxidation by ARB in the upflow single-chamber MEC, we monitored current density until and after the acetate concentration became undetectable (<0.0625 mM of acetate). Figure 2a shows the results for the baseline starting concentration of 10 mM acetate for an MEC that had been operated for 6 months prior to the batch experiment. With a well-developed ARB biofilm on the anode, the volumetric current density immediately reached 62.2 A/m^3, and its maximum was 76.5 A/m^3 (0.85 m^3 H_2/m^3-d). At 34 h, acetate was no longer detected, but we observed a stable current density of 5.4 to 10.1 A/m^3 (0.76 to 1.41 mA) for the final 9 h of the experiment. The applied voltage for the maximum current density was 1.09 V, and it decreased to 0.69−0.72 V for the time with no acetate. These results support that H_2 was the electron donor once acetate was not detectable, since H_2 was continually being produced at the cathode, while the acetate concentration was undetectable (<0.0625 mM). Although the oxidation of storage products within the bacteria might allow current generation with no acetate (*28*), the stable current for 9 h is more consistent with H_2 reoxidation.

To further prove H_2 oxidation by ARB on anode biofilm, we ran the B-MEC fed with H_2 gas as the only added electron donor. Figure 2b shows the results. Endogenous decay

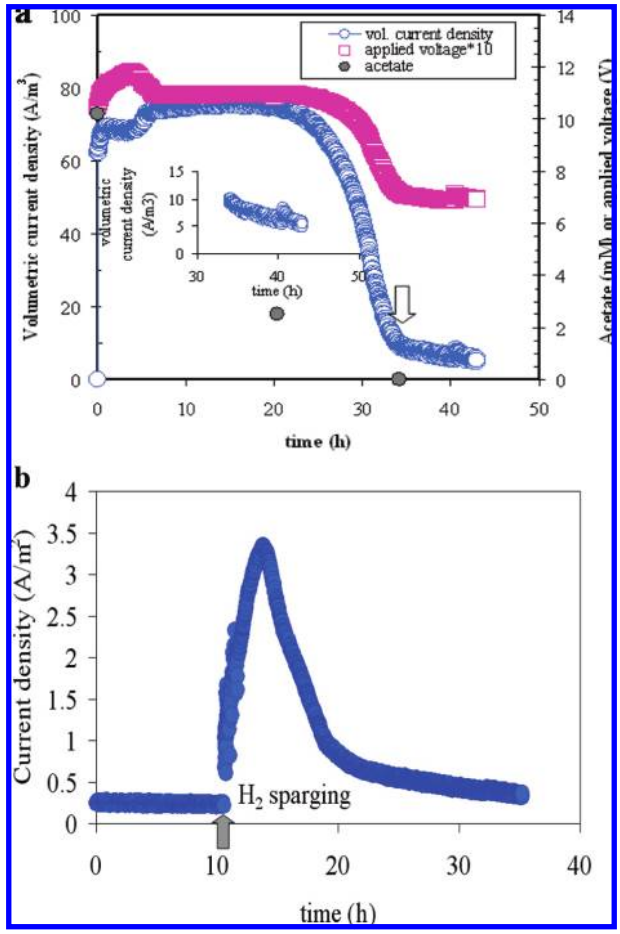

FIGURE 2. Evidence for direct current generation by ARB carrying out H_2 oxidation in single-chamber MECs. (a) the evolution of volumetric current density, applied voltage, and acetate concentration at an initial acetate concentration of 10 mM of acetate in the upflow single-chamber MEC. The block arrow indicates current generation in the absence of detectable acetate (<0.0625 mM). (b) current generation by H_2 oxidation of ARB in a B-MEC having H_2 as sole electron donor. Block arrows indicate the starting time for H_2 sparging.

currents were consistent at 0.24 ± 0.01 A/m^2 for 10 h before H_2 was applied. With H_2 gas applied, the current density immediately increased, reached a peak at 3.34 A/m^2, and it decreased slowly with time (Figure 2b). This is the typical pattern of the current-versus-time profile in the presence of electron donor in MFCs/MECs (*18*). Significant current generation with H_2 as the sole electron donor supports that H_2-oxidizing ARB were active in the single-chamber MEC.

Effects of Acetate Concentration and Reaction Time. The CCE was 92% for starting acetate concentrations of 30 and 80 mM during the first 24 h of each batch experiment ($n = 2$), but this conversion efficiency dropped to $53 \pm 8\%$ and $48 \pm 19\%$ for 30 and 80 mM acetate, respectively, when the CCE was averaged for 7 days ($n = 3$). At the end of the tests on day 7, the CH_4 percentage of the biogas increased up to $14 \pm 1\%$ and $16 \pm 2\%$ at 30 and 80 mM acetate, respectively ($n = 3$) (see Supporting Information Figure S6).

The cumulative CE was $87 \pm 38\%$ for the highest acetate concentration, although it fluctuated in the range of 52−151% for individual time intervals throughout the batch experiment. Although the fluctuations in CE could have been caused to a small degree by errors in measurements of acetate consumed for individual time intervals, the values much greater than 100% indicate H_2 conversion to current either through direct oxidation by ARB or indirectly after acetogenesis at nonsteady state. The independent evidence for H_2-oxidizing ARB suggests that they were mainly responsible for fluctuation in CE and CCE in our experiments.

TABLE 1. Average Percent Distributions of Electron Equivalents at the End of the Batch Experiments for the Three Initial Acetate Concentrations

acetate	coulombs	H_2	CH_4	$H_2 + CH_4$	SMP	unaccounted
10 mM	60 ± 1	59 ± 0.5	2	61	13 ± 2	26 ± 1
30 mM	58	31	32	63	15	22
80 mM	58 ± 3	28 ± 2.1	37	65	17 ± 4	18 ± 3

$n = 2$ for data without standard deviation. CEs over 100% was not counted for the averages. Except for acetate, no liquid end products were detected by the HPLC. The unaccounted electron fraction is the sum of biomass and unidentified electron sinks.

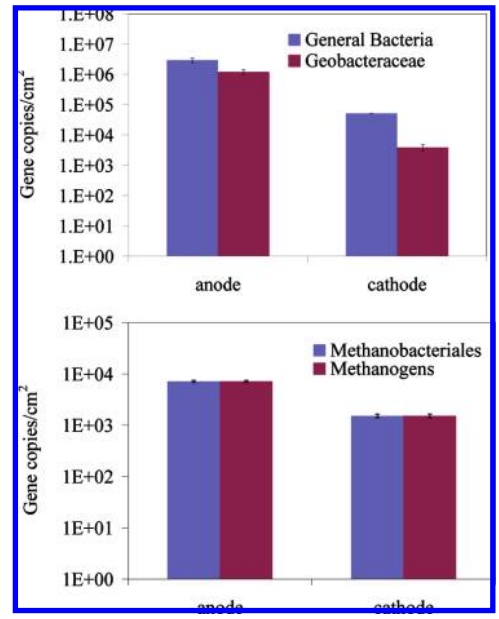

FIGURE 3. Distributions of *Geobacteraceae* and methanogens on anodes and a cathode in the upflow single-chamber MEC. Total methanogens are the sum of *Methanobacteriales, Methanomicrobiales, Methanosaetaceae*, and *Methanosarcinaceae*.

Electron Equivalent Balances. Table 1 summarizes electron-equivalent balances at each acetate concentration at the end of the batch experiments. The moderate CE and high CCE led to a 59% H_2 yield in the baseline experiment (10 mM acetate), which produced negligible CH_4. Methanogenesis became significant for the higher acetate concentrations and the longer reaction times needed to consume the acetate. Electron fractions for CH_4 were 32 and 37% for 30 and 80 mM of acetate concentrations, respectively, and the H_2 yields dropped to 28−31%. The significant declines in H_2 yield occurred despite stable CE values of 58−60% for all acetate concentrations (CE over 100% was not counted for the averages). Having a high CE, but a low H_2 yield for the high-acetate concentrations supports that hydrogenotrophic methanogens oxidized H_2 and lowered the CCE. Thus, the modestly long batch reaction time, about a week, allowed hydrogenotrophic methanogens to accumulate in the single-chamber MEC, even though the cathode was located above the anode.

Microbial Community in the Anodes and the Cathode. Figure 3 shows quantitative real-time PCR results targeting *Geobacteraceae*, general bacteria, *Methanosaetaceae*, *Methanosarcinaceae*, *Methanobacteriales*, and *Methanomicrobiales*. *Geobacteraceae* were 41% of total bacteria in the anode's biofilm, whereas they were only 7% in cathode's biofilm.

Methanosaetaceae and *Methanosarcinaceae* were not amplified for the anode and cathode biofilms, which indicates that acetoclastic methanogens were negligible in the upflow single-chamber MEC. Although *Methanomicrobiales* were not amplified in either biofilm sample, H_2-oxidizing *Methanobacteriales* were present in the anode and the cathode biofilms.

Discussion

The upflow single-chamber MEC, constructed by placing the cathode above the anode bed, achieved the goal of a high CCE, up to 98% (at 32 h of reaction time), along with negligible CH_4 production when evaluated in the batch mode with a starting acetate concentration of 10 mM (baseline). The CE was stable at 60%, and consequently the H_2 yield was 59% in the upflow single-chamber MEC. In comparison, the CCE was only 48% (at 25 h of reaction time) in the B-MEC having its cathode alongside the anode, and the CCE dropped to 26% for 100 h of reaction time.

The H_2-production rate for the upflow single-chamber MEC was 0.57 ± 0.02 m^3 H_2/m^3-d at an applied voltage of ~1V, even though the cathode had no metal catalyst. This H_2 production rate is about 2-fold higher than that in an MEC using a platinum-coated cathode (*11*) at similar applied voltage. More recent studies using platinum catalysts showed higher H_2 production rates (0.48−3.12 m^3 H_2/m^3-d) at applied voltages of 0.7−1.3 V (*10, 13*). While platinum-catalyzed cathodes typically increase H_2 production rates and lower applied voltages, using platinum may not be practical in field application due to cost. Rozendal et al. (*12*) suggested using a biocathode as the alternative to platinum. In comparison, our study suggests that a cathode with no metal catalyst may have applicability for field application of MECs.

The high CCE and small CH_4 production for this baseline condition indicate that methanogenesis (reactions 3 and 3′ in Supporting Information Figure S1) were negligible in these experiments. The relatively short reaction time, below 34 h in the batch experiment, precluded significant growth and accumulation of acetoclastic or hydrogenotrophic methanogens anywhere in the MEC.

For higher acetate concentrations, for which the reaction time was ~7 days to deplete the acetate, the H_2-yield dropped to 28−31%, and significant CH_4 accumulated (32−37% of electron equiv of acetate utilized). Other investigators also reported CH_4 accumulation (*10, 12, 13*). It was not clear if anode potential (varied by different applied voltage) or reaction time mainly caused CH_4 formation in the other studies, since a smaller applied voltage elongated reaction times, but also decreased the anode potential (*13*). A more negative anode potential can influence the competition between ARB and methanogens by lowering the energy gain for ARB (*29*). Our study showed the significance of reaction time for CH_4 formation, since anode potential was fixed at +0.07 V (vs SHE), where the acetate oxidation rate by ARB should not be limited by anode potential (*20*).

Because the CE did not decline much (~58%; not counting CEs over 100%) with higher acetate concentrations, the loss of H_2 yield was due to a lower CCE (48−53%), which supports that hydrogenotrophic methanogenesis was mainly responsible for CH_4 production. This suggests that the acetate-oxidizing ARB out-competed acetoclastic methanogens for acetate. Acetogenic methanogens typically have half-maximum-rate concentration (K_s) values of 180−430 mg COD/L (*22, 30, 31*) and maximum specific substrate-utilization rate (q_{max}) values of 7.6 mg COD/mg VSS-d (*17*). In comparison, *Geobacter sulfurreducens*, a known ARB, has a relatively low K_s (0.64 mg COD/L) and a relatively high q_{max} (22.7 mg COD/mg VSS-d) for acetate (*19*), both of which should help them outcompete acetoclastic methanogens for acetate. Quantitative PCR showed that *Geobacteraceae* were 41% of the bacteria in the anode's biofilm.

Quantitative PCR targeting four major groups of methanogens detected only hydrogenotrophic methanogens, *Methanobacteriales*, in the upflow single-chamber MEC. We observed a white coating on the black cathode felt at the end of acetate experiments. SEM images on the cathode clearly showed biofilm formation, and quantitative PCR proved that hydrogenotrophic methanogens accumulated on the cathode. The immediate access to H_2 produced at the cathode or diffusing back from the gas phase made the cathode an ideal ecological *niche* for the H_2-oxidizing methanogens, as compared to the anode biofilm; the methanogens have no significant ecological benefit for growing in an anode biofilm of acetate-fed MECs, where they must compete with ARB that can respire to the anode. However, we found *Methanobacteriales* in the anode's biofilm at a level 5-fold higher than in the cathode biofilm (Figure 3), probably due to denser biofilm formation on the anodes than on the cathode. Finding H_2-oxidizing methanogens in the anode's biofilm suggests an interaction between ARB and hydrogenotrophic methanogens that is not explained with electron-donor competition alone. The accumulation of hydrogenotrophic methanogens in an anode biofilm could decrease the CCE despite a short HRT in a continuous MEC, as the anode biofilm has long SRT (~7 days) compared to the HRT.

The trends in CE, CCE, and CH_4 show that the batch reaction time was critical for allowing methanogenesis. Based on our batch experiments, HRT less than 34 h may efficiently wash out methanogens in the bulk liquid of a continuous-flow, single-chamber MEC, but it may not be effective for methanogens in biofilms on the electrodes. On the other hand, a short HRT may be a challenge for direct utilization of complex organic fuels, which may need to be pretreated by hydrolysis and fermentation prior to the MECs to avoid kinetic roadblocks from hydrolysis and fermentation in an MEC with a short HRT.

H_2 Oxidation by ARB. H_2 is universal electron donor for anaerobic microorganisms (*22*), and three studies showed current generation by ARB H_2 oxidation (*12, 17, 18*). In our study, significant current generation with no acetate present and the rapid current increase when only H_2 was supplied (Figure 2b) strongly support that we had H_2 oxidation by ARB in our upflow single-chamber MEC. Although acetogenesis might be involved in H_2 recycle, we never observed a build up of acetate during current generation, which suggest negligible effect of homoacetogens in our system. Thus, H_2 oxidation by ARB can be a sink for H_2 if the rate of H_2 transfer to gas phase is too slow to harvest the H_2 as fast as it is produced at the cathode.

Non-Steady State Effects on the CE and the CCE. In some cases, we observed an apparent CE over 100% and a significant drop in the CCE for individual time intervals even though CH_4 formation was negligible. Although H_2 recycle from its oxidation by ARB (either directly or indirectly via homoacetogenesis) would not affect the overall H_2 yield significantly by itself, these reactions can increase the current density or decrease the measured H_2 production rate. In this case, the observed H_2 gas production rate is not necessarily equivalent to the volume computed from the current because H_2 produced at the cathode is consumed at the anode. Evidence for H_2 oxidation by ARB supports that the non-steady-state oxidation of H_2 was mainly responsible for an apparent CE up to 161%, a CCE down to 86%, and a H_2 yield of 138%. The CCE drop and CE increase became most significant when the current from acetate oxidation was small, such as at the end of batch tests; then, the fraction of current from H_2 oxidation by ARB was a large portion of the total observed current.

Although the upflow single-chamber MEC was able to harvest H_2 rapidly enough to give a CCE of almost 100% at first, the longer-term development of H_2-oxidation by ARB and of hydrogenotrophic methanogens means that H_2 transport to gas phase was not able to out-compete those reactions once they became well established. These results indicate that H_2 transport process from the liquid phase to the gas phase was limited, despite low solubility of H_2 and placing the cathode very near the gas−liquid interface. Other works (*32, 33*) also have shown the limitations of the H_2 transport rate.

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Supporting Information Available

Description of possible electron flows in a single-chamber MEC, inoculation, reactor configuration, and its operation of an upflow single-chamber MEC and a bottle-type single-chamber MEC, sampling, operating conditions, and analyses in the GC and the HPLC, computation of Coulombic efficiency, cathodic conversion efficiency, and H_2 yield, measurement of H_2 gas volume, DNA extraction, and quantitative real-time PCR, field emission scanning electron microscopy, SEM image on the cathode biofilm, and evolutions of gas compositions in the upflow single-chamber MEC at acetate 30 and 80 mM. This material is available free of charge via the Internet at http://pubs.acs.org.

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