Fate of \( \text{H}_2 \) in an Upflow Single-Chamber Microbial Electrolysis Cell Using a Metal-Catalyst-Free Cathode

HYUNG-SOOL LEE,∗ CÉSAR I. TORRES, PRATHAP PARAMESWARAN, AND BRUCE E. RITTMANN

Center for Environmental Biotechnology, The Biodesign Institute at Arizona State University, 1001 S. McAllister Ave. Tempe, Arizona 85287-5701

Received January 21, 2009. Revised manuscript received March 19, 2009. Accepted March 20, 2009.

With the goal of maximizing the \( \text{H}_2 \)-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 \( \text{H}_2 \) 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 \( \text{H}_2 \) was 98 ± 2%, the Coulombic efficiency (CE) was 60 ± 1%, the \( \text{H}_2 \) 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 \( \text{H}_2 \) loss due to \( \text{CH}_4 \) accumulation: up to 14 ± 1% and 16 ± 2% of the biogas at 30 and 80 mM of acetate, respectively. Quantitative PCR proved that no aceticlastic methanogens were present, but that hydrogenotrophic methanogens (i.e., \text{Methanobacteriales}) were present on both electrodes. The hydrogenotrophic PCR proved that no acetoclastic methanogens were present, but bacterial consortia involving fermenters and ARB (7, 8). Second, nonfermentable substrates can be completely oxidized to \( \text{CO}_2 \), 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., \( \text{O}_2 \)) from the cathode, which forces electrons to be donated to \( \text{H}^+ \) ions (or \( \text{H}_2\text{O} \)), thereby producing \( \text{H}_2 \) at the cathode. The second change is to provide a certain amount of electrical energy to make the cathode potential negative enough to generate \( \text{H}_2 \). The standard electrical potentials at pH 7 and a temperature of 25 °C for reduction half-reactions for acetate (\( E^\circ \)), for protons to \( \text{H}_2 \) (\( E^\circ \)) are −0.284 and −0.421 V, respectively. Thus, the \( E^\circ \) value is −0.137 V for the redox reaction of acetate oxidation to form \( \text{H}_2 \) (\( \text{CH}_3\text{COO}^- + 2\text{H}_2\text{O} = \text{CO}_2 + 2\text{H}_2 + 4\text{e}^- \)).

The first requirement for a high \( \text{H}_2 \) yield is that the cathode potential should be high, and this demands that \( e^- \) equiv of the donor substrate not get lost before they are transferred to the cathode. Possible \( e^- \) sinks decreasing CE under anaerobic conditions can be biomass synthesis, soluble microbial products (SMP), or \( \text{CH}_4 \) gas; SMP can be produced and accumulate despite complete oxidation of electron donor in bacterial catabolism (9). \( \text{H}_2\text{O} \) can be the significant electron sink if \( \text{O}_2 \) 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 \( \text{O}_2 \) leakage.

The second requirement for a high \( \text{H}_2 \) yield is that the cathode potential should be high. While some studies showed steady CCE values up to 100% in a dual-chamber MEC (10), \( \text{H}_2 \) loss by diffusion into the anode chamber was large in some cases (11, 12), decreasing the CCE down to 6–33%. Loss of \( \text{H}_2 \) by diffusion can seriously limit MEC applications, since \( \text{H}_2 \) yield can be small even though the CE is high. Thus, an MEC must be designed to prevent \( \text{H}_2 \) loss, as well as have a high CE, if the \( \text{H}_2 \) 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 \( \text{H}_2 \) loss. In the dual-chamber MEC, the membrane

* Corresponding author phone: +1-480-727-0849; fax: +1-480-727-0889; e-mail: hyungsool@asu.edu.
is a barrier to movement of H₂ from the cathode to the anode, but it adds expense and complexity. If we can collect H₂ 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 5–98% with 0.3–1.15 V applied voltage (13–16). The most likely sink for H₂ in a single-chamber MEC would seem to be hydrogenotrophic methanogens that consume the H₂ produced at the cathode before it can be recovered (8, 14). Up to now, no studies proved H₂ consumption by hydrogenotrophic methanogens in single-chamber MECs, although CH₄ gas was observed (13, 14). Another H₂ sink can be its oxidation by ARB, if they are able to utilize H₂ as an electron donor (17, 18). Unlike methane generation, H₂ oxidation by ARB might not be a significant H₂ loss in a single-chamber MEC, since current produced by H₂ oxidation produces H₂ gas on the cathode again, while the biomass yield of ARB is very small (8, 13, 18, 19). However, H₂ recycle between the cathode and the anode can harm overall performance (net H₂ recovery related to applied voltage), because current generated by H₂ 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 H₂-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 archaean is 0.76 days (22), if the methanogens are suspended in the flowing liquid.

The most direct way to stop H₂ loss in a single-chamber MEC is to recover the H₂ gas upon its release from the cathode so efficiently that H₂ recovery out-competes methanogens, ARB, or any other sinks. Since the solubility of the H₂ molecule is extremely low (K₀(T) = 7.65 × 10⁻⁴ mol/L-atm at one atmosphere and a temperature of 30°C) (23), rapid recovery of H₂ 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 H₂ 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 H₂ in the single-chamber MEC to quantify any sinks. Finally, we determined which types of methanogens are responsible for CH₄ 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 H₂ 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 N₂ 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: H₂ 68 ± 3%, CO₂ 21 ± 5%, and CH₄ <0.5%), we shifted into batch mode for accurately quantifying the H₂ 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 7.5–7.4. 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 CH₄ 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 H₂ oxidation at the anode of the B-MEC. We fed this MEC with the same medium, but lacking acetate, and we provided H₂ gas (99.99% H₂) 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/m² for tests using B-MEC, while current density is presented as A/m² for the upflow single-chamber MEC.

Analyses. We quantified gas percentages of H₂, CH₄, and CO₂ with a gas chromatograph (GC 2010, Shimadzu) equipped with a thermal conductivity detector and a packed column (ShinCarbon ST 100/120 mesh, Restek 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.
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 \( \text{Methanosarcinaceae} \), \( \text{Methanobacteriales} \), and \( \text{Methanomicrobiales} \). 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 \( \text{H}_2 \) yield in the Supporting Information. We quantified cumulative \( \text{H}_2 \) volume by analyzing \( \text{H}_2 \) percentage and volume change in headspace in the MEC.

Results

\( \text{H}_2 \) Recovery and \( \text{CH}_4 \) Formation in the B-MEC. We tested \( \text{H}_2 \) 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 \( \text{CH}_4 \) formation was detected (5% in the biogas). For a reaction time of 25 h, the CCE decreased to 48%, with 9% \( \text{CH}_4 \) in the biogas. The \( \text{CH}_4 \) percentage increased up to 26% by 100 h, and the CCE dropped to 22%. These results indicate that \( \text{H}_2 \) 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/m³ for 6–22 h. The cumulative CE and CCE for 32 h were 60 ± 2% and 98 ± 2%, respectively, but their discrete values at different time intervals varied. The average CCE was 98 ± 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 ± 1% (\( n = 3 \)), and the \( \text{H}_2 \) yield was 59 ± 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/m³, respectively, and the acetate concentration declined from 0.74 ± 0.00 mM to 0.43 ± 0.08 mM. The computed CE was 161 ± 25% (\( n = 3 \)) for the period of 27–32 h, in which coulombs generated by acetate consumption were small. Due to the high CE, \( \text{H}_2 \) yield was 138 ± 21% for this time (100% = 4 mol \( \text{H}_2 \)/mol acetate). For the baseline condition, the upflow, single-chamber MEC efficiently prevented \( \text{H}_2 \) loss to methanogenesis, since \( \text{CH}_4 \) peaks (detected only at the end of the test) were too small to be quantified (<0.5%), which means that the \( \text{e}^- \)-equiv of cumulative \( \text{CH}_4 \) was 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/m³), which equals a volumetric \( \text{H}_2 \) production rate of 0.57 ± 0.02 m³ \( \text{H}_2 /\text{m}^3-\text{d} \) of MEC working volume. This production rate is significant, since we were able to double the \( \text{H}_2 \)-producing rate (with an applied voltage 1.06 V) over the rate obtained (0.3 m³ \( \text{H}_2 /\text{m}^3-\text{d} \)) with a dual-chamber MEC using platinum catalyst at the cathode and applied voltage 1 V (\( \text{II} \)), 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/m³ (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 ± 9%, cumulative CE was stable at 60 ± 2%, the cumulative \( \text{H}_2 \) yield was 53.4%, and only a small, unquantifiable \( \text{CH}_4 \) 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 \( \text{H}_2 \) yield declined a small amount.

\( \text{H}_2 \) Oxidation by ARB. To test for \( \text{H}_2 \) 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/m³, and its maximum was 76.5 A/m³ (0.85 m³ \( \text{H}_2 /\text{m}^3-\text{d} \)). At 34 h, acetate was no longer detected, but we observed a stable current density of 5.4 to 10.1 A/m³ (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 \( \text{H}_2 \) was the electron donor once acetate was not detectable, since \( \text{H}_2 \) 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 \( \text{H}_2 \) reoxidation.

To further prove \( \text{H}_2 \) oxidation by ARB on anode biofilm, we ran the B-MEC fed with \( \text{H}_2 \) gas as the only added electron donor. Figure 2b shows the results. Endogenous decay
currents were consistent at 0.24 \( \pm \) 0.01 \( \text{A/m}^2 \) for 10 h before \( \text{H}_2 \) was applied. With \( \text{H}_2 \) gas applied, the current density immediately increased, reached a peak at 3.34 \( \text{A/m}^2 \), 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 (10). Significant current generation with \( \text{H}_2 \) as the sole electron donor supports that \( \text{H}_2 \)-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 \( \text{CH}_4 \) 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 \( \text{H}_2 \) conversion to current either through direct oxidation by ARB or indirectly after acetogenesis at nonsteady state. The independent evidence for \( \text{H}_2 \)-oxidizing ARB suggests that they were mainly responsible for fluctuation in CE and CCE in our experiments.

**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\% \( \text{H}_2 \) yield in the baseline experiment (10 mM acetate), which produced negligible \( \text{CH}_4 \). Methanogenesis became significant for the higher acetate concentrations and the longer reaction times needed to consume the acetate. Electron fractions for \( \text{CH}_4 \) were 32 and 37\% for 30 and 80 mM of acetate concentrations, respectively, and the \( \text{H}_2 \) yields dropped to 28–31\%. The significant declines in \( \text{H}_2 \) 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 \( \text{H}_2 \) yield for the high-acetate concentrations supports that hydrogenotrophic methanogens oxidized \( \text{H}_2 \) 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, Methanosetaceae, Methanosarcinaceae, Methanobacteriales, Methanomicrobiales, and Methanobacteriales. Geobacteraceae were 41\% of total bacteria in the anode’s biofilm, whereas they were only 7\% in cathode’s biofilm.
Methanosetaeae and Methanosaetaeae were not amplified for the anode and cathode biofilms, which indicates that acetoclastic methanogens were negligible in the upflow single-chamber MEC. Although Methanomicriobiales were not amplified in either biofilm sample, H₂-oxidizing Methano bacteriales 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 CH₄ 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 H₂ 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 H₂-production rate for the upflow single-chamber MEC was 0.57 ± 0.02 m³ H₂/m³-d at an applied voltage of ~1V, even though the cathode had no metal catalyst. This H₂ 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 H₂ production rates (0.48–3.12 m³ H₂/m³-d) at applied voltages of 0.7–1.3 V (10, 13). While platinum-catalyzed cathodes typically increase H₂ production rates and lower applied voltages, using platinum may not be practical in field application due to cost. Rozendoal 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 CH₄ 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 H₂-yield dropped to 28–31%, and significant CH₄ accumulated (32–37% of electron equiv of acetate utilized). Other investigators also reported CH₄ accumulation (10, 12, 13). It was not clear if anode potential (varied by different applied voltage) or reaction time mainly caused CH₄ 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 CH₄ 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 H₂ yield was due to a lower CCE (48–53%), which supports that hydrogenotrophic methanogenesis was mainly responsible for CH₄ production. This suggests that the acetate-oxidizing ARB out-competed acetoclastic methanogens for acetate. Acetogenic methanogens typically have half-maximum-rate concentration (Kᵥ) values of 180–430 mg COD/L (22, 30, 31) and maximum specific substrate-utilization rate (qᵥmax) values of 7.6 mg COD/mg VSS-d (17). In comparison, Geobacter sulfurreducens, a known ARB, has a relatively low Kᵥ (0.64 mg COD/L) and a relatively high qᵥmax (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, Methano bacteriales, 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 H₂ produced at the cathode or diffusing back from the gas phase made the cathode an ideal ecological niche for the H₂-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 H₂-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 CH₄ 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-channel 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.

H₂ Oxidation by ARB. H₂ is universal electron donor for anaerobic microorganisms (22), and three studies showed current generation by ARB H₂ oxidation (12, 17, 18). In our study, significant current generation with no acetate present and the rapid current increase when only H₂ was supplied (Figure 2b) strongly support that we had H₂ oxidation by ARB in our upflow single-chamber MEC. Although acetogenesis might be involved in H₂ recyle, we never observed a build up of acetate during current generation, which suggest negligible effect of homoacetogens in our system. Thus, H₂ oxidation by ARB can be a sink for H₂ if the rate of H₂ transfer to gas phase is too slow to harvest the H₂ 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 CH₄ formation was negligible. Although H₂ recycle from its oxidation by ARB (either directly or indirectly via homoacetogenesis) would not affect the overall H₂ yield significantly by itself, these reactions can increase the current density or decrease the measured H₂ production rate. In this case, the observed H₂ gas production rate is not necessarily equivalent to the volume computed from the current because H₂ produced at the cathode is consumed at the anode. Evidence for H₂ oxidation by ARB supports that the non-steady-state oxidation of H₂ was mainly responsible for an apparent CE up to 161%, a CCE down to 86%, and a H₂ 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 H₂ 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 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.

**Acknowledgments**

This research was funded by OpenCEL, LLC and by the Biohydrogen Initiative of Arizona State University.

**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 evolution 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.

**Literature Cited**


ES900204J