Significance of Biological Hydrogen Oxidation in a Continuous Single-Chamber Microbial Electrolysis Cell

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A single-chamber microbial electrolysis cell (MEC) that used a high density of nonmetal-catalyst carbon fibers as the anode achieved high volumetric current densities from 1470 ± 60 to 1630 ± 50 A/m³ for a hydraulic retention time of 1.6–6.5 h. The high current density was driven by a large anode surface area and corresponded to a volumetric chemical oxygen demand (COD)-removal rate of 27–49 kg COD/m³·d. Observed H₂ harvesting rates were from 2.6 ± 0.10 to 4.3 ± 0.46 m³ H₂/m³·d, but the H₂ production rates computed from the current densities were 16.3–18.2 m³ H₂/m³·d. Tracking all significant electron sinks (residual acetate, H₂, CH₄, biomass, and soluble microbial products (SMP)) in the single-chamber MEC showed that H₂ reoxidation by anode-respiring bacteria recycled H₂ between the cathode and the anode, and this caused the large discrepancy in H₂ production and harvest rates. H₂ recycle accounted for 62–76% of observed current density, and this made the observed Coulombic efficiency 190–310% at steady state. Consequently, the cathodic conversion efficiency was only 16–24%. The current density added by H₂ recycle also increased the applied voltage from ~0.6 V to ~1.5 V for the highest H₂ harvest rate (4.3 m³ H₂/m³·d). CH₄ generation consistently occurred in the continuous single-chamber MEC, and its electrogenesis from consumed acetate was 7–25%. Because of methane formation and biomass/SMP accumulation, the overall H₂ recovery was moderate at 1.8–2.0 mol of H₂/mol of acetate in the MEC. Thus, this study illustrates that a single-chamber MEC with a high anode surface area can generate high volumetric rates for COD removal and H₂ generation, but H₂ recycle and methanogenesis present significant challenges for practical application.

Introduction

A microbial electrolysis cell (MEC) is a new technology that converts the electron equivalents in organic compounds to H₂ gas by combining bacterial metabolism with electrochemical reactions (1, 2). Anode-respiring bacteria (ARB) anaerobically oxidize organic substances and transfer their electrons to an anode. The electrons are circuited to the cathode, where they reduce protons to H₂ gas, which is harvested as it evolves from the water. Some electrical energy must be input to lower the potential of the electrons enough to generate H₂ at the cathode.

The two redox reactions in an MEC have characteristic efficiencies. The oxidation step at the anode is characterized by the Coulombic efficiency (CE = circuited electrons/donor electrons utilized). Strategies capable of achieving a high CE involve suppressing non-ARB reactions (e.g., methanogenesis, O₂ reduction, and biomass synthesis) (3–5). The reduction step at the cathode is defined by the cathodic conversion efficiency (CCE = H₂ electrons captured/circuited electrons). The best strategy to make the CCE high is rapid harvesting of the H₂ gas as soon as it is produced at the cathode (6). The H₂ yield (H₂ electrons/donor electrons utilized) is the product of CE × CCE. To obtain a high H₂ yield, both steps need to succeed, which means that CE and CCE are close to 100%.

Besides high CE and CCE, an MEC should satisfy two additional criteria for success: a high volumetric current density and a low applied voltage to boost the electrons’ energy enough to generate H₂ at the cathode. The volumetric current density relates to the size and capital costs of the MEC, while the applied voltage affects the power cost. A variety of parameters can limit current density in MECs, for example, distance between the anode and cathode, electrolyte resistivity (including the membrane), cathode catalyst, ARB density on the anode, and the specific surface area of the electrodes (7–9). The combination of the two latter options especially can improve the volumetric current density in MECs. For example, using carbon granules (10) or a graphite brush (11, 12) as the anode provided large specific surface areas (18 200 m⁻²) for ARB biofilm formation, and the volumetric current density was improved to 292 A/m³ (3.12 m³ H₂/m³·d), compared to only 62 A/m³ (0.69 m³ H₂/m³·d) with a specific surface area of ~120 m⁻² using a carbon cloth anode (3, 11, 12).

A low applied voltage, another success criterion, can be in opposition to a high current density because the energy losses that develop in an MEC increase with current density. Thus, having a small applied voltage with a high current density will be a key technical challenge for moving bench-scale MECs to commercial applications.

A means to lower the applied potential is to eliminate the membrane to create a single-chamber MEC (3, 6, 10–12). Eliminating the membrane attenuated pH energy loss and ohmic energy loss (6, 10), which were significant for a dual-chamber MEC (13). Cathode overpotential becomes significant at a high current density, while anode overpotential reaches ~0.2 V for saturated current density (14). Addressing the cathode overpotential, Selimbo et al. (15) and Call et al. (11) reported that nickel or stainless steel cathodes showed performances similar to or better than that of platinum for H₂ production in single-chamber MECs. They reported applied voltages in the range of 0.6–0.9 V for volumetric current density of 188–222 A/m³ (1.5–1.7 m³ H₂/m³-d), suggesting that the cathode catalyst mainly would affect the energy loss of an MEC.

Although membrane elimination can help reduce energy losses, single-chamber MECs can introduce other risks. If H₂ harvesting from the cathode is not rapid enough, hydrogenotrophic methanogens or ARB can oxidize the H₂ generated at the cathode. Hydrogenotrophic methanogenesis reduces the H₂ yield (mol of H₂/mol of acetate utilized) by routing H₂ electrons into CH₄, which evolves from the water and diverts electron equivalents out of the MEC. In principle, acetoclastic methanogens also can decrease H₂ yield by...
diverting acetate electrons to CH₄ (16), but a body of literature (4, 6, 17) is showing that CH₄ produced in a microbial fuel cell (MFC) fed by a fermentable substrate comes solely from H₂-utilizing methanogens, not acetoclastic methanogens, which are out- competed by acetate-oxidizing ARB. In contrast to ARB, hydrogenotrophic methanogens do not need an anode as the terminal electron acceptor for their respiration since they use bicarbonate dissolved in water as their final electron acceptor and H₂ as their electron donor; thus, hydrogenotrophic methanogens do not need to attach on the anode. This metabolic feature suggests that a very short hydraulic retention time (HRT) may prevent H₂ loss by hydrogenotrophic methanogenesis in a continuous single-chamber MEC, given that the methanogens are able to grow suspended in the water.

In contrast to H₂-oxidizing methanogens, H₂-oxidizing ARB must resipre electrons to the anode. When the H₂ oxidized by the ARB comes from the cathode, it creates an H₂-recycle effect that generates current beyond that attributable to organic-substrate oxidation. Lee et al. (6) recently proved that H₂ generated at a cathode of a single-chamber MEC was oxidized by ARB on an anode in a non-steady-state manner during batch operation. The extra current by the ARB’s H₂ oxidation can increase energy losses and the applied voltage. Then, the energy efficiency of the single-chamber MEC deteriorates because the extra current increases the energy losses and applied voltage without increasing H₂ production.

A few studies have reported or claimed that H₂ can be recycled by ARB in single-chamber MECs (6, 16, 18), but did not comment on its impact on the applied voltage. The main reason for overlooking the impact was that the H₂ production rate was small (less than dozens of milliliters of H₂ per day) and reactors were run for short periods with batch mode (11, 12, 15). However, a continuous single-chamber MEC generating large H₂ volume could create a niche for H₂-utilizing ARB to accumulate on the anode. Then the current by H₂ recycle can be significant, and the energy efficiency of the MEC can drop considerably. This H₂ recycle may be a serious problem with a large-scale single-chamber MEC because of the large volume of H₂ generation. No study has evaluated the effect of the H₂-recycled current in continuous steady-state single-chamber MECs generating high currents.

Here, we explore the impacts of achieving high volumetric rates of H₂ production by providing large anode surface area and the effects of H₂ oxidation by ARB and methanogenesis in a continuously fed single-chamber MEC. To increase the H₂ volumetric production rate and COD-removal loading rate, we minimized the HRT in an MEC having a very high specific surface area for the anode. We also evaluated the possibility of improving the CE by suppressing CH₄ formation by HRT control. We assessed H₂ oxidation by ARB for steady-state conditions in the single-chamber MEC by monitoring CE, CCE, and acetate concentrations for a range of short HRTs. We quantified all energy losses so that we could precisely assess attenuation of ohmic energy loss and PH energy loss by membrane elimination and limitation of metal-free carbon cathode. Finally, we evaluated the effects of H₂ recycle on the energy efficiency of the single-chamber MEC.

Materials and Methods

Inoculation and Start-up. We collected effluent from a batch MEC operated for over 9 months with an acetate feed and with current density of ~7 A/m², and we used the supernatant as inoculum to the upflow single-chamber MEC reactor described in the next section. After sparging the single-chamber MEC with N₂ gas (99.999%) for 5 min, we operated the MEC with a feed medium (25 mM acetate, 100 mM phosphate buffer, and other nutrients (5)) in batch mode for ~3 months. We switched from batch mode to continuous operation after the currents exceeded 100 mA (800 A/m²) since 100 mA was close to the maximum current achieved in preliminary experiments.

Reactor Configuration. Figure S1 illustrates the upflow single-chamber MEC used in our experiments. The total volume of the MEC was 145 mL, and the working volume was 125 mL. We report volumetric current density or volumetric H₂ production rate based on the working volume of 125 mL. We used three bundles of graphite fiber (24K Carbon Tow, Fibre Glast, Brookville, OH) as the anode and one bundle as the cathode, all lacking metal catalysts. The specific surface area of the fibers was 571 400 m²/m³ (fiber’s diameter, 7 µm; its length, 20 cm). One bundle consisted of 24 000 fibers, and its geometric surface area per bundle was 1060 cm². The geometric surface area of the anodes per the MEC volume was 2530 m²/m³ of working volume, which can allow high ARB biofilm density in the MEC. Before using the fibers as electrodes in the MEC, we cleaned them with chemicals for 3 days; 1 N nitric acid for 1 day, 1 N acetone for 1 day, and 1 N ethanol for 1 day in series. We cleaned the fibers with 18 MQ deionized water before using them in the MEC, and surrounded the cathode with a nonconductive mat made up of polyethylene to prevent short-circuiting. The distance between the anode bundles and the cathode bundle was less than 1 mm. We tied the graphite fibers with a graphite rod (GraphiteStore.com, Inc., Buffalo Grove, IL) for electrical connection to a potentiostat (VMP3, Bio-Logic, Knoxville, TN) that provided the applied voltage for the MEC. We mixed the MEC by circulating liquid between the bottom and the middle of the MEC with a peristaltic pump (Masterflex L/S, Cole-Parmer, Vernon Hills, IL). The effluent recycle rate at 42 mL/min was for mixing the MEC, and the cathodic circulation rate at 14 mL/min was for releasing H₂ gas to the gas phase (Figure S1). We placed an Ag/AgCl reference electrode (MF-2052, Bioanalytical Systems, Inc., West Lafayette, IN) less than 1 cm away from the cathodes at the top of the MEC. Gas was released from the top of the MEC, and we measured its volume using a Milligas counter (Calibrated Instruments, Inc., Hawthorne, NY).

Operation. Acetate was the sole added electron donor and organic-carbon source to the MEC in all experiments. We fed the MEC using a peristaltic pump (Masterflex L/S, Cole-Parmer), varying the HRT from 6.5 to 1.6 h for the continuous MEC, while the influent acetate concentration was fixed at 17 mM. The influent COD loading rate ranged from 32 to 133 kg COD/m³·d as the HRT went from high to low. We fixed the anode potential at −0.126 V (vs standard hydrogen electrode, SHE) for all experiments, while the cathode potential varied in proportion to the overpotentials.

Besides 17 mM acetate, the feed medium contained 100 mM K₃PO₄/NaH₂PO₄, and trace minerals, as reported in Lee et al. (5). The influent pH was 7.3–7.4, and the effluent pH remained in the same range because of self-neutralization. We operated the single-chamber MEC at 30–32 °C and recorded current, anode potential, cathode potential, and applied voltage every 120 s using EC lab software (Version 9.5, Bio-Logic, Knoxvville, TN).

Analyses. We quantified the acetate concentration and other possible liquid products using high-performance liquid chromatography (HPLC; Model LC-20AT, Shimadzu, Pleasanton, CA) equipped with an Aminex 87H column (Bio-Rad Laboratories, Inc., Hercules, CA). Detailed information on HPLC operating conditions is in Lee et al. (19). The compounds detected by the HPLC were formate, acetate, lactate, pyruvate, fumarate, succinate, n-butylate, isobutyrate, propionate, valerate, acetone, ethanol, butanol, and propanol. We established calibration curves for each compound for each set of analytical runs and measured samples in triplicate.
To measure ohmic resistances in the single-chamber MEC, we performed electrochemical impedance spectroscopy (EIS) using the potentiostat. Detailed description for the EIS tests is in the Supporting Information. We used an electrical circuit (Figure S2) consisting of an ohmic-resistance component (solution resistance) and a constant-phase element in parallel with a combined resistance for charge transfer and diffusion. To accurately estimate ohmic resistances, we fit the EIS data with a Levenberg–Marquardt nonlinear least-squares algorithm in EC lab software (Version 9.5).

We quantified gas percentages of H2, O2, CH4, and CO2 by sampling the gas with a gas-tight syringe (Hamilton Company, Reno, NV) and analyzing it using a gas chromatograph (GC 2010, Shimadzu, Pleasanton, CA) with a thermal conductivity detector and a packed column (ShinCarbon ST 100/120 mesh, Restek Corporation, Bellefonte, PA) for gas separation. We established new calibration curves for each gas using analytical grade H2, O2, CH4, and CO2 gases (Matheson Tri-gas, USA) each time we measured biogas composition. The GC operating conditions are described in Lee et al. (19).

We measured dissolved H2 concentration in the steady-state continuous MEC with a trace analytical gas chromatograph (ta 3000, Ametek) using a reduction gas detector. We measured dissolved H2 concentration of the MEC in duplicate for each HRT. Detailed information on sampling and operation of the GC can be found in the Supporting Information.

Calculations. We computed the H2 yield using eq 1.

\[
\text{H2 yield} = \frac{\text{mol of H2}}{\text{mol of acetate}_{\text{ox}}} = \frac{\frac{\text{mol of H2}}{\text{Coulombs}}}{\frac{\text{mol of acetate}_{\text{ox}}}{\text{Coulombs}}} = \frac{\text{mol of H2} \times \text{mol of acetate}_{\text{ox}}}{\text{Coulombs} \times \text{Coulombs}} = \frac{\text{mol of H2}}{\text{mol of acetate}_{\text{ox}}} = \frac{8e^– \text{equiv of acetate}_{\text{ox}}}{2e^– \text{equiv of acetate}_{\text{ox}}} = \frac{e^– \text{equiv of H2}}{2e^– \text{equiv of H2}} = \frac{4 \times \text{CE} \times \text{CCE} \times \text{mol of H2}}{\text{mol of acetate}_{\text{ox}}} (1)
\]

where acetateox is the moles of acetate oxidized in a given time, Coulombs are electrons transferred to an anode in acetate oxidation, the constant 4 is the conversion from moles of acetate to moles of H2, CE is the observed Coulombic efficiency for acetate (the cumulative Coulombs normalized by the acetate oxidized in a given time, e– equiv of Coulombs/e– equiv of acetate oxidized), and CCE is cathodic conversion efficiency (cumulative H2 gas volume normalized by the cumulative Coulombs for a given time, e– equiv of H2/e– equiv of Coulombs). We converted the H2 volume into e– equiv using the ideal gas law.

We determined the cumulative H2 volume with eq 2:

\[
\text{H2 volume}_{\text{cum}} = \Delta \text{gas}_{\text{cum}} \times \left(\frac{H_{2,1} + H_{2, t-1}}{2}\right) (2)
\]

where H2 volumecum is the cumulative H2 volume in a reaction time (mL), Δgas cum is the cumulative gas volume in a given time (mL), H2,1 is the H2 percentage of gas measured by the GC in initial sampling time, and H2, t-1 is H2 percentage of gas measured by the GC in terminal sampling time. We measured H2 volume over at least 5 h of reaction time. The reported H2 volume was the average of four measurements over the reaction time.

We established e– equiv balances for the single-chamber MEC using eq 3.

\[
\Delta e_{\text{acetate}} = e_{\text{plp}} + e_{\text{H2}} + e_{\text{CH4}} + e_{\text{SMPs}} + e_{\text{biomass}} (3)
\]

where Δe acetate is the average amount of acetate oxidized for a given time (e– equiv), e plp is a possible liquid product from acetate, eH2 is the average cumulative H2 for the given time (e– equiv), eCH4 is the average cumulative CH4 for the given time (e– equiv), eSMP is the accumulation of soluble microbial products (SMPs) during the given time (e– equiv), and ebiomass is the biomass synthesis during the given time (e– equiv). On the basis of the measurements from the literature (5), we assumed that SMP and biomass synthesis comprised 11% and 15% of Δe acetate, respectively.

Results and Discussion

Current Density and COD Removal. Figure 1 shows that the volumetric current density increased as HRT decreased. The average volumetric current densities were 1470 ± 60, 1590 ± 70, and 1630 ± 50 A/m2, respectively, for HRT of 6.5, 3.1, and 1.6 h. Volumetric current densities were averaged with 1 month of data at steady state. Applied voltage increased with decreasing HRT: 1.49 ± 0.03, 1.47 ± 0.05, and 1.43 ± 0.04 V, respectively, for HRTs of 6.5, 3.1, and 1.6 h. Because the substrate-utilization rate is proportional to current density in an MEC (20, 21), low acetate concentration at longer HRT probably limited the substrate-utilization rate and the current density in the MEC. Effluent COD concentrations were 188, 429, and 687 mg COD/L for HRT 6.5, 3.1, and 1.6 h, respectively, and these correspond to volumetric COD-removal rates of 27–49 kg COD/m3·d for COD loading rates of 32–133 kg COD/m3·d. Previous studies reported COD-removal rates from 0.25 to 2.3 kg COD/m3·d for COD loading rates of 1.4–4.1 kg COD/m3·d in MFC/MECs (22–24). The volumetric removal rates in our work are comparable to anaerobic digestion (10–45 kg COD/m3·d) (25, 26) and favor MEC applicability for high-strength wastewater streams, where the effluent COD concentrations observed here are acceptable. However, an input organic donor other than acetate might cause the COD-removal loading rates to be lower in an MFC/MEC.

Gas Composition and CH4 Formation. The off gas was composed of 49–53% H2, 19–31% CO2, and 1.8–6.9% CH4. Gas-percentage values for each HRT are reported in Figure S3 in the Supporting Information. The short HRT’s kept the CH4 gas percentages lower in the continuous reactor, compared to those from batch mode (3, 6, 15). However, the small, but consistent accumulation of CH4 despite an HRT as low as 1.6 h suggests that methanogens (presumably hydrogenotrophs (6)) were attached to the electrodes, not suspended, since suspended methanogens should have been washed out. This result is consistent with our previous finding (6) that hydrogenotrophic methanogens (mostly Methanobacteriales) were found in biofilms on the anode and cathode in a single-chamber MEC. Steady CH4 accumulation, even in short HRT, is one of the bottlenecks for the single-chamber MEC configuration, and it appears that methanogenesis cannot be suppressed solely by HRT control in continuous single-chamber MECs.

H2 Yield and Electron-Equivalent Balance. The H2 yield was stable over the HRT range 1.81 ± 0.19 to 2.03 ± 0.07 mol of H2/mol of acetate (Table 1), which correspond to 45–51% H2-capture efficiency from the e– equiv of acetate utilized (Δe acetate); H2 yields determined by eq 1 were the same as those measured by observed H2 per acetate oxidized. Approximately half of the donor electrons ended up in other electron sinks—CH4, SMP, and biomass; we did not detect any liquid products from acetate with HPLC analyses. Figure 2 shows distributions of electron equiv (or COD) by HRT; in these balances, we assumed that SMP and biomass (ARB + methanogens) synthesis comprised 11% and 15% of Δe acetate. (5) The mass-balance closures on electron equiv-
lents were typical of carefully controlled continuous studies, from \(-2.3\%\) to \(+20.1\%\). The second-largest non-H\(_2\) sink was CH\(_4\) in most cases, from 7 to 25 \((1.8-6.9\%)\) of \(\Delta e\) acetate, despite its small percentage \((1.8-6.9\%)\) in the gas. Since 1 mol of CH\(_4\) contains 8 \(e^-\) equiv versus 2 \(e^-\) equiv per mole of H\(_2\), CH\(_4\) had an impact on H\(_2\) yield larger than its gas percentage; for instance, 10\% CH\(_4\) percentage of gas in headspace is equivalent to 40\% H\(_2\) in \(e^-\) equiv.

**H\(_2\) Production Rates.** If all the measured current density was captured as H\(_2\) gas (i.e., CCE = 100\%), the volumetric H\(_2\) production rates would be 16.3–18.2 m\(^3\) of H\(_2\)/m\(^3\)-d. However, the observed average H\(_2\) production rates were 2.64 ± 0.10, 3.70 ± 0.03, and 4.32 ± 0.46 m\(^3\) of H\(_2\)/m\(^3\)-d, respectively, for HRT 6.5, 3.1, and 1.6 h. These large differences between the computed and observed H\(_2\) production rates indicate either significant H\(_2\) loss (perhaps by a gas leak, O\(_2\) reduction, H\(_2\) loss in effluents, or CH\(_4\) accumulation) or supplemental current generated by electron donors other than acetate. A gas leak and O\(_2\) reduction were not possible because the MEC was well-sealed, and we never detected O\(_2\) in off-gas analyses. The average dissolved-H\(_2\) concentration was consistent at 1.37 ± 0.12 µM in all experiments. Thus, H\(_2\) loss in effluent discharged from the MEC corresponded to only \(\sim 64\%\) of observed H\(_2\) production \((0.33-0.54\ L of H_2/d)\). CH\(_4\) electron equiv was 7–25\% of \(\Delta e\) acetate in the HRT-variation tests; although significant, this amount was too small to cause the substantial differences in H\(_2\) production rates. Thus, current generation by ARB oxidizing substrates other than acetate had to have been the reason for the large discrepancy between the observed and computed H\(_2\) production rates in the single-chamber MEC.

**H\(_2\) Recycle.** At steady state in the single-chamber MEC, the observed CE was consistently higher than 100\%, ranging from 190 ± 7 to 310 ± 3\%, as shown in Table 1. Thus, the consistently observed CE over 100\% confirms that ARB utilized electron donors other than acetate for producing current in the single-chamber MEC.

**Figure 3** shows the possible fates of acetate electrons in the single-chamber MEC. Acetate electrons could be diverted to cell synthesis, CH\(_4\) (by acetoclastic methanogens), SMP, and circuited electrons (transformed into H\(_2\) at the cathode). ARB might utilize the SMP, CH\(_4\), and H\(_2\) as electron donors for current generation, and the ARB’s endogenous respiration can add current. The electron fraction of acetate electrons in SMP typically is around 11\%, which is not significant compared to other electron sinks (5); this amount cannot cause the CE to exceed 100\%. In addition, SMPs do not seem usable for high-rate current generation because residual soluble COD remained stable and did not give a current.

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**Table 1.** Influent/Effluent Acetate Concentration, Coulombic Efficiency, Cathodic Conversion Efficiency, and H\(_2\) Yield to Different HRTs in the Single MEC

<table>
<thead>
<tr>
<th>HRT (h)*</th>
<th>acetate (mM)</th>
<th>observed CE (%)</th>
<th>CCE (%)</th>
<th>H(_2) yield (mol of H(_2)/mol of acetate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.5</td>
<td>17.0 ± 0.11</td>
<td>310 ± 3</td>
<td>16 ± 0</td>
<td>2.03 ± 0.07</td>
</tr>
<tr>
<td>3.1</td>
<td>17.0 ± 0.05</td>
<td>230</td>
<td>21</td>
<td>1.88</td>
</tr>
<tr>
<td>1.6</td>
<td>17.0 ± 0.01</td>
<td>190 ± 7</td>
<td>24 ± 2</td>
<td>1.81 ± 0.19</td>
</tr>
</tbody>
</table>

* Anode potential was fixed at \(-0.126\ V vs SHE\). Observed CE is \(e^-\) equiv of Coulombs normalized by \(e^-\) equiv of acetate oxidized; CCE is \(e^-\) equiv of H\(_2\) volume relative to \(e^-\) equiv of Coulombs. H\(_2\) yield was computed by eq 1.
increase in studies with MFC/MECs (5, 27). If ARB could oxidize methane, it could be another source of current; however, no studies have reported direct CH4 oxidation by ARB or microbial consortia involving anaerobic methanotrophs and ARB. Good electron closure (over 90%) in MEC/MFCs having significant CH4 accumulation supports that CH4 would not be used as electron donor for ARB to generate current (4–6, 23). We produced the decay current with acetate-free medium for 1 day, and the decay current was less than 1% of observed currents in our study. Therefore, H2 oxidation by ARB is the only sink that could be large enough to account for the consistent observed CE over 100% at steady state in the single-chamber MEC.

Equation 4 describes how observed CE can be above 100% in a steady-state single-chamber MEC,

$$\text{observed CE} = \frac{C_{\text{obs}}}{C_{\text{acetate}} + \frac{\Delta C_{\text{H}_2}}{\Delta \text{acetate}}} + \frac{C_{\text{SMPs}} + C_{\text{decay}} + C_{\text{CH}_4}}{\Delta \text{acetate}}$$

where $C_{\text{obs}}$ is the observed cumulative electron equivalents circuited in a given time (e- equiv), $C_{\text{acetate}}$ is the electron equivalents of acetate utilized in a given time (e- equiv), $C_{\text{H}_2}$ is the cumulative electrons circuited from H2 oxidation in a given time (e- equiv), $C_{\text{SMPs}}$ is the cumulative electron equivalents circuited from SMP utilization in a given time (e- equiv), $C_{\text{decay}}$ is the cumulative electron equivalents circuited from endogenous decay of ARB, and $C_{\text{CH}_4}$ is the cumulative electron equivalents circuited by ARB’s CH4 oxidation. On the basis of the discussion earlier in this section, only $C_{\text{acetate}}$ and $C_{\text{H}_2}$ are significant.

We estimated current densities from acetate and H2 oxidations by assuming a realistic value for the true CE for acetate (CEacetate): 74% with the assumption that SMP and biomass (ARB + methanogens) synthesis comprised 11% and 15% of $\Delta \text{acetate}$, respectively. We multiplied 0.74 times the e- equiv of acetate consumed in a given time for computing electron equivalents circuited from acetate. Then we subtracted that value from the observed total electrons circuited to estimate electron equivalents circuited from H2. Figure 4 reveals that the current density from acetate was only 24–38% of the total, which means that 62–76% of observed current density was from H2 oxidation. Our previous study showed that H2 oxidation by ARB became more important at low acetate concentration (6). This study also supports the H2-current density increasing (from 1060 to 1140 A/m3) with decreasing acetate concentration. The Supporting Information also shows results for CEacetate = 90%, which is close to the highest CE reported in the literature (28). Current density generated from acetate was still below half of the observed current density when we used CEacetate = 90% (see Figure S4).

Significant H2 recycle by ARB led to a low CCE, only 16–24%. We hoped to release H2 readily to the headspace by generating intensive advection flow in the single-chamber MEC (40 mL/min of effluent recycle and 14 mL/min of cathodic recycle). However, these hydrodynamic conditions were not sufficient for causing H2 transport to the gas phase before it could be utilized by ARB or methanogens. This supports the rate of H2 transfer to the gas phase needing to be very rapid to keep the CCE high.

**Energy Loss Characterization.** We computed and compared all energy losses in the single-chamber MEC by HRT;
all values are summarized in the Supporting Information. Ohmic resistance was very low at 0.49 Ω in the single-chamber MEC (Figure S5), one of the merits of single-chamber MECs (6, 10), and ohmic energy losses only were 0.09–0.1 V even at highest volumetric current density 1470–1630 A/m³. pH energy loss also was negligible because of pH self-neutralization in the single-chamber MEC (pH was constant at 7.3–7.4). These results clearly indicate that ohmic energy loss and pH energy loss can be as small as 0.1 V, even for such high current density in single-chamber MECs (Table S1).

Anode overpotential was 0.154 V, on the basis of the standard potential at pH 7 for the reducing half reaction, \(1/2\text{CH}_3\text{COO}^- + 1/2\text{HCO}_3^- + \text{H}^+ + e^- = \text{CH}_4 + 1/2\text{H}_2\text{O}\) (canonic potential, −0.126 V; standard potential for acetate reaction, −0.28 V); concentration and temperature correction had a small impact on potential value (data not shown), and we used the standard potential for overpotential characterization. Cathode overpotentials were 1.15–1.21 V, which were 82% of the total energy loss. The high cathode overpotential caused the applied voltage to be high at 1.43–1.49 V (see Table S1). This high applied voltage did not lead to water electrolysis (i.e., \(\text{H}_2\text{O}\) oxidation to \(\text{O}_2\)) on the anode because we fixed anode potential at −0.126 V (vs SHE); thus, our results are not associated with water electrolysis. The large overpotentials for the cathode with no metal catalyst mean that a metal-free carbon cathode is not practical when the MEC produces high volumetric current density of 1470–1630 A/m³, although the carbon cathode may be useful for current density in ranges of dozens to hundreds A/m² (6, 10). The characterization of all energy losses at a high current density emphasizes the significance of cathode overpotential in large-scale MECs, which indicates the demands for good, low-cost catalysts, such as nickel or stainless steel (14).

**Implication of H₂ Recycle in Single-Chamber MECs.** Our study proved that removing the membrane from the MECs can attenuate pH and ohmic energy loss to less than 0.1 V even at 1470–1630 A/m³. However, these advantages can be counteracted if the \(\text{H}_2\) is oxidized by ARB at the anode. Although \(\text{H}_2\) oxidation by ARB did not affect the \(\text{H}_2\) yield, it seriously harmed values of CCE and energy loss. These deleterious effects occurred because \(\text{H}_2\) recycle increased the current density without increasing the net yield of \(\text{H}_2\) gas; that is, the CCE declined. Supporting Information (Figures S6 and S7) provides a quantitative analysis of why preventing \(\text{H}_2\) recycle is critically important for maintaining acceptable CCE and energy loss in single-chamber MECs. In brief, the required applied voltage is only 0.6 V to generate 4.3 m³ of \(\text{H}_2\) m³/d (≈400 A/m³) at CCE = 100%, but it increases to ∼1.5 V in our single-chamber MEC when \(\text{H}_2\) recycle causes the current density to be 3.8 times higher (1100 A/m³ from \(\text{H}_2\) oxidation). Thus, \(\text{H}_2\) recycle increases the applied voltage by 0.9 V for the MEC using metal-free carbon cathode, but the net \(\text{H}_2\) harvesting rate remains the same, 4.3 m³ of \(\text{H}_2\) m³/d.

Using a good metal catalyst on the cathode (perhaps platinum, nickel, or stainless steel) could alleviate some of the extra energy loss by \(\text{H}_2\) recycle. However, extra current still will be deleterious to electrode overpotentials and does not contribute to the \(\text{H}_2\) yield. A traditional ion-exchange membrane to separate the anode from a cathode compartment can produce more pure \(\text{H}_2\) and improve \(\text{H}_2\) yield because of the absence of hydrogenotrophic methanogens. However, membrane separation may not be effective if \(\text{H}_2\) diffusion from the cathode to the anode were significant (13). Also, it will foster high pH energy loss and ohmic energy loss (13). For these reasons, we must develop new configurations or materials that can readily release \(\text{H}_2\) gas from MECs, prevent \(\text{H}_2\) transport to the anode, and still permit rapid ion transport between electrodes. We may accelerate \(\text{H}_2\) harvesting, such as by vacuum collection, to help decrease \(\text{H}_2\) recycle in single-chamber MECs. If we cannot stop considerable \(\text{H}_2\) recycle in single-chamber MECs, we need to use a dual-chamber MEC having a separator that permits ion transport, but not gas transport, as has been used for chemical batteries (e.g., Celgard LLC).

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**Supporting Information Available**

A schematic diagram of the upflow single-chamber MEC, an equivalent circuit, sampling and analysis of dissolved \(\text{H}_2\) in liquid, gas compositions in the single-chamber MEC, characterization of current density from acetate and \(\text{H}_2\), quantification of ohmic resistance, characterization of anode and cathode overpotential, and estimation of \(\text{H}_2\) production rate at no \(\text{H}_2\) recycle and methanogenesis. This material is available free of charge via the Internet at http://pubs.acs.org.

**Literature Cited**


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(26) Van Lier, J. *Current and future trends in anaerobic digestion: diversifying from waste (water) treatment to resource oriented conversion techniques,* 11th World Congress on Anaerobic Digestion, Brisbane, Australia, September 27, 2007; IWA: Brisbane, Australia, 2007.


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