The role of homoacetogenic bacteria as efficient hydrogen scavengers in microbial electrochemical cells (MXCs)

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ABSTRACT

We evaluated the consumption of hydrogen gas at the anode of a microbial electrolysis cell (MEC) and characterized the significance of new interactions between anode respiring bacteria (ARB) and homo-acetogens. We demonstrated the significance of biofilm limitation for direct consumption of H₂ over acetate by ARB, using the deep biofilm model. Selective inhibition of the major competing hydrogen sink at the biofilm anode, methanogenesis, resulted in significant increase in electron recovery as electric current (~10–12 A/m²). The presence of acetate at high concentration in the anode compartment and detection of formate, a known intermediate of the acetyl-CoA pathway, provide evidence towards the role of homoacetogenic bacteria. We also assessed the activity of homoacetogens with reverse transcription quantitative PCR targeting formyltetrahydrofolate synthetase (FTHFS) transcripts, and observed a comparable decrease in the FTHFS transcript numbers with current density and acetate concentrations as we decreased the HRT below 4.5 h. The biofilm anode community was predominated by Deltaproteobacteria (70% of total readouts) along with a fraction of the homoacetogenic genus, Acetobacterium (4% of total readouts), established by pyrosequencing targeting the V6 region of the 16S rRNA. Homoacetogens seem to play a major role as syntrophic members of the biofilm anode community when electron recovery is high.

Key words | deep biofilm, formyl tetrahydrofolate synthetase (FTHFS), homoacetogens

INTRODUCTION

Microbial electrochemical cells (MXCs) are an increasingly well understood discipline, and they have promise for practical applications in bioenergy. The biofilm anode and its coulombic efficiency are common for microbial fuel cells and electrolysis cells. Hydrogen consumption is the key link for achieving higher coulombic efficiencies in MXCs fed with fermentable substrates, and it has been demonstrated that hydrogen-oxidizing methanogens outcompete other hydrogen sinks in the biofilm anode (Freguia et al. 2008; Parameswaran et al. 2009; Wang et al. 2009). While acetate is readily consumed by several known anode respiring bacteria (ARB) belonging to various genera such as Geobacteraceae, Rhodopseudomonas, and Shewanella (Nevin et al. 2008; Xing et al. 2008), H₂ is not readily used by ARB in mixed culture, except in pure culture studies and in acetate-fed biofilms (Torres et al. 2007; Rozendal et al. 2008; Lee et al. 2009). Deep biofilm anode limitation for H₂ consumption fundamentally limits high current densities, as explained below.

Deep biofilm flux analysis for acetate and H₂ into a biofilm anode

Apart from the microbial limitation for H₂ consumption, biofilm kinetics also dictates the maximum current densities. A deep biofilm gives the maximum substrate flux for a given substrate concentration, because the substrate concentration is driven very close to zero within the biofilm (Rittmann & McCarty 2001). Substrate flux into a
deep biofilm ($J_d$) can be calculated by the following equation (Rittmann & McCarty 2001)

$$J_d = \left[2q_{\text{max}}X_fD_f\left(S_s + K_s \ln\left(\frac{K}{K + S_s}\right)\right)\right]^{1/2}$$

(1)

where $J_d$ is the substrate flux in terms of g COD/m²·day, $S_s$ is the measured liquid phase concentration of electron donor (g COD/m³), $K_s$ is the half maximum rate concentration for ARB and corresponding electron donor (g COD/m³), $q_{\text{max}}$ is the maximum specific substrate utilization rate for ARB and a specific electron donor (g COD/g VS·day), and $X_f$ is the biomass concentration in the biofilm anode (assumed as 50,000 g/m³).

For acetate, the following values can be used for the equation:

$$D_{\text{IAc}} = 7.52 \times 10^{-5} \text{ m}^2/\text{day}, \quad q_{\text{max}} = 22.6 \text{ g COD/g VS·day}, \quad K_a = 22.6 \text{ g COD/m}^3,$$

as reported in Lee et al. (2009), for G. sulfurreducens consuming acetate in mixed culture.

For H₂, we normalized all reported concentrations to values in terms of g COD/m³ by first converting reported H₂ partial pressures into concentration with Henry’s law

$$C_{\text{H₂}} = K_{\text{H₂}}P,$$

where $C_{\text{H₂}}$ = equilibrium liquid phase concentration of H₂ (moles/L), $K_{\text{H₂}}$ = Henry’s constant for H₂ gas at 30°C and atmospheric pressure (8.1 × 10⁻⁴ moles/L·atm), $P$ = partial pressure of H₂ in headspace (atm). We then converted the resulting mole H₂/L to g COD/m³ by $1 \text{ g COD of } H₂/m^3 = (1 \text{ mole } H₂/L) \cdot (2 \text{ e }^- H₂/mole H₂) \cdot (8 \text{ g COD/e }^- \text{eq}) \cdot (1 \text{ L/}10^{-3} \text{ m}^3$.

Correspondingly, we used the following values for H₂ in the deep-biofilm equation: $D_{\text{I(H₂)}} = 3.89 \times 10^{-4} \text{ m}²/\text{day}$ and $K_s = 0.39 \text{ g COD/m}^3$ for G. sulfurreducens grown with H₂ as electron donor and Fe(III) as electron acceptor (Brown et al. 2005). $q_{\text{max}}$ is not yet reported for H₂ consumption by G. sulfurreducens; hence, we used the same value as for acetate consumption (22.6 g COD/g VS·day), as G. sulfurreducens consumes acetate and H₂. The resulting $J_d$ values were converted to current densities using the relationship $1 \text{ A} = 0.14 \text{ g COD/day}$ (Lee et al. 2009).

As shown in Figure 1, current density values are two times lower for maximum practical H₂ concentration (1 atm $H₂ = 13 \text{ g COD/m}^3$) than for a modest acetate concentration commonly observed in organic waste streams (5 mmol/L acetate = 320 g COD/m³).

H₂ concentrations actually observed in anaerobic systems are in the range of 100 nmol/L to a few μmol/L (Yang & McCarty 1998), and the corresponding current densities according to the model are ≤0.5 A/m². Acetate concentrations in natural anaerobic ecosystems (1–10 mmol/L) produce current densities of 5–50 A/m² according to the deep biofilm model (Shigematsu et al. 2004).

These results demonstrate that current densities from acetate consumption by ARB should be much higher than H₂ in biofilm anodes for practical concentrations of both substrates.

H₂ consumption in MXCs achieves even more significance during the consumption of fermentable substrates which get converted to acetate and H₂. Hydrogenotrophic methanogenesis outcompetes direct H₂ consumption by ARB, resulting in low coulombic efficiency (Parameswaran et al. 2009). Selective inhibition of methanogenesis favored homoacetogens as hydrogen scavengers, resulting in higher coulombic efficiency (Parameswaran et al. 2009).

Homoacetogenic bacteria grow chemolithoautotrophically on H₂ and CO₂ at higher H₂ thresholds (Drake 1993). Although the role of homoacetogens in microbial electrochemical systems has not been evaluated so far, we have documented initial evidence for the presence of homoacetogens in biofilm anodes fed with fermentable substrates (Parameswaran et al. 2010). The goal of this study is to investigate if homoacetogens play a significant role in improving electron recovery from a H₂-fed anode, and the goal is achieved with a combination of chemical and molecular microbial tools.

**MATERIALS AND METHODS**

**Microbial electrolysis cell (MEC) operation**

We fed a H-type MEC with 80% $H₂ : 20% CO₂$ as the electron donor and carbon source, with media conditions and operating parameters as described in Parameswaran et al. (2011).
We monitored current density with a potentiostat (Princeton Applied Research, Model VMP3, Oak Ridge, TN), while soluble products were monitored with a HPLC using an Aminex HPX/87H column kept at 50 °C. We monitored the gas percentages of H₂, CO₂, and CH₄ (if any) in the head-space of the MEC in samples taken with a gas-tight syringe (SGE 500 μL, Switzerland) using a gas chromatograph (GC 2010, Shimadzu) equipped with a thermal conductivity detector and a packed column (ShinCarbon ST 100/120 mesh, Restek Corporation, Bellefonte, PA) for separating sample gases.

**Nucleic acids extraction – DNA and RNA**

At the end of each batch run following continuous-flow steady state, we centrifuged the entire volume of the anode compartment to obtain a pellet for DNA extraction. We used 0.25 g of the pellet for extracting DNA following the recommendations for the MOBIO® Powersoil DNA extraction kit.

We performed RNA extraction only during the step HRT (hydraulic retention time) experiments in Run 2, described earlier. We collected about 50 mL of effluent from the anode compartments after steady state was achieved at each HRT of the continuous-flow experiments in Run 2. We obtained about 0.5 g of pellets in each case, for a total of 5 HRT conditions (17–1 h). We extracted RNA from the samples as per the RNeasy Mini Kit, and recommendations for the optimum Lysozyme concentration. We stored the extracted RNA at −80 °C. We quantified DNA and RNA using a NanoDrop® spectrophotometer.

**Reverse transcription and quantitative PCR**

We performed reverse transcription of the RNA extracted from step HRT (hydraulic retention time) experiments in Run 2, with a final goal of assessing the formyltetrahydrofolate synthetase (FTHFS) genes. We removed any DNA that was co-extracted with the RNA by performing DNase treatment. The resulting RNA was subjected to two-step reverse transcriptase PCR with an Omniscript RT–PCR kit (Qiagen, CA) and random hexamers (Promega, PA) to produce cDNA in the first step. The resulting cDNA was normalized to a concentration of 10 ng/μL before we quantified the target transcripts with quantitative PCR (qPCR).

We targeted the FTHFS gene transcripts and 16S rRNA of general *Bacteria* in the cDNA samples as per the conditions of Xu *et al.* (2009) and Ritalahti *et al.* (2006). We performed SYBR Green I assays for the three targets, with a Realplex 4S quantitative PCR unit for 10 μL total reaction volumes. Each reaction consisted of: 0.5 μL nuclease free H₂O, 4.5 μL of SYBR Realmastermix (5 Prime, CA), 0.5 μL each of forward and reverse primers for a final concentration of 0.5 μmol/L, and 4 μL of template cDNA. We performed the assay in triplicate with a six-fold dilution of standard for developing a calibration curve, along with the samples.

**Pyrosequencing analysis targeting the V6 region of 16S rRNA**

We used bacterial primers 967f and 1046r to amplify the V6 region of the 16S rRNA gene, as described by Sogin *et al.* (2006) and Zhang *et al.* (2009), on biofilm and suspension at the end of steady-state operation for the 9-h HRT. We performed PCR as follows: 94 °C for 2 min, 25 cycles of denaturation at 94 °C for 30 s each, 57 °C annealing for 45 s, 72 °C for 1 min extension, and a final extension at 72 °C for 2 min. Excess primer dimers and dNTPs were removed with QiaQuick spin columns (Qiagen). Amplicon pyrosequencing was performed using a 454/Roche and sequenced on 454/GSFLX sequencers as per manufacturer’s specifications. We used the RDP Pyrosequencing pipeline project along with MOTHUR software to trim and analyze the readouts. We obtained around 19,285 and 15,867 sample readouts for H₂ MEC suspension and biofilm anode samples, respectively.

**RESULTS AND DISCUSSION**

**Current density and acetate production**

We performed batch MEC experiments with 1 mL each of anaerobic digested sludge and return activated sludge as the inoculum, repeating the experiments twice (Runs 1 and 2). Both runs showed a concomitant increase in current production and acetate formation during the batch operation phase, as elucidated in Parameswaran *et al.* (2011). Acetate accumulation during both runs indicated that acetate production was faster than acetate consumption by anode respiration. Moreover, the current approach is novel for startup of an MEC fed with H₂ alone, in that acetate was not used in the anode to establish a biofilm anode, as shown in Table 1. We selected for the community that could consume H₂, either directly or indirectly. High current densities (10–12 A/m²) were observed in H₂-fed biofilm anodes during this study (Parameswaran *et al.* 2011). During
continuous operation in both runs (HRT = 9 h), acetate levels at constant current densities were around 6 mmol/L, and the maximum current densities were comparable between the two runs.

**Continuous flow experiments**

When we decreased the HRT successively at the end of run 2, we observed that the acetate concentrations decreased simultaneously with the current density (Figure 2(a)), as published previously by us (Parameswaran et al. 2011). While current density increased from an HRT of 17–10 h, it started to decrease from a HRT of 4.5 h, along with a decrease in acetate. Current production stabilized at 3 A/m² at a HRT of 1 h. We observed a corresponding decrease in the number of FTHFS transcripts starting from a HRT of 4 h (Figure 2(b)), and they remained at low levels at lower HRTs. Unlike the FTHFS transcripts, general *Bacteria* 16S rRNA concentrations first increased with decreasing HRT, possibly due to proliferation of other dominant groups or due to biofilm detachment at higher flow rates (lower HRT). This finding suggests preferential homoacetogen washout from the suspension. Previous research had ruled out the possibility of homoacetogens in an anode chamber fed with H₂ and at an HRT of 1.5 h (Freguia et al. 2008). Our findings indicate that homoacetogens may not be completely washed out at HRTs below 1.5 h, indicating that either they possess rapid metabolic rates or they are able to reside in the reactor as part of the biofilm. This finding has significant implications for effective competition of homoacetogens against hydrogen-oxidizing methanogens, which are severely limited at lower retention times <2 h (Lee et al. 2009; Wang et al. 2009).

**Pyrosequencing analysis of biofilm anode and suspension microbial communities**

Microbial community analysis by pyrosequencing revealed a clear difference between biofilm anode and suspension microbial communities at the 9-h HRT (Figure 3). Homoacetogens represented by the genus *Acetobacterium*, belonging to the *Eubacteriaceae* family accounted for 46% of the total reads, while *Desulfovibrio* genus represented

<p>| Summary of startup and operating conditions along with results obtained from research on H₂ consumption by ARB |
|-----------------|-----------------|-----------------|-----------------|</p>
<table>
<thead>
<tr>
<th><strong>Startup condition</strong></th>
<th><strong>Current density (A/m²)</strong></th>
<th><strong>Dominant ARB</strong></th>
<th><strong>Reference</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>10 mmol/L acetate with 99% H₂</td>
<td>1.5–2</td>
<td>Uncharacterized</td>
<td>Rozendal et al. (2008)</td>
</tr>
<tr>
<td>Acetate + 100% H₂</td>
<td>0.3</td>
<td><em>Geobacter sulfurreducens</em> (pure culture)</td>
<td>Bond &amp; Lovley (2003)</td>
</tr>
<tr>
<td>10% H₂ : 90% N₂</td>
<td>0.13</td>
<td>Mixed culture (<em>Geobacteraceae</em> dominant genus)</td>
<td>Torres et al. (2007)</td>
</tr>
<tr>
<td>80% H₂ : 20% CO₂ (biofilm grown with acetate)</td>
<td>1–1.2</td>
<td>Mixed culture (<em>Geobacter sulfurreducens</em>)</td>
<td>Call et al. (2009)</td>
</tr>
<tr>
<td>80% H₂ : 20% CO₂</td>
<td>10</td>
<td>Mixed culture (<em>Geobacteraceae</em>)</td>
<td>Current study</td>
</tr>
</tbody>
</table>

![Figure 2](https://iwaponline.com/wst/article-pdf/65/1/1/443494/1.pdf)
8% of the total reads. Pseudomonas, belonging to the subphylum Gammaproteobacteria, accounted for 7.5% of the total reads. Pseudomonas has been implicated to be an ARB that performs electron transfer through soluble electron shuttles (Pham et al. 2007).

On the other hand, the biofilm anode community was dominated by the phylum Deltaproteobacteria (accounting for 70% of the total reads), to which the known ARB genus Geobacteraceae belongs. Dysgonomonas, which belongs to the phylum Bacteroidetes, accounted for 5% of the total reads; 4% of the total sequence reads belonged to the homoacetogenic genus, Acetobacterium. The results from pyrosequencing are in close accord with a clone-library analysis previously reported by Parameswaran et al. (2010). The ability of homoacetogens to thrive in the biofilm anode and current density observed at the low HRT of 1 h reinforce the value of promoting homoacetogen ARB partnership in the biofilm anode.

CONCLUSIONS

High current density could be obtained from H2-fed anodes of MXCs when homoacetogens were the predominant H2 scavengers. Accumulation of acetate, detection of formate, sustained current generation, and acetate accumulation at a very low HRT of 1 h provide multiple lines of evidence that homoacetogens were not washed out and could effectively compete with hydrogenotrophic methanogens.

REFERENCES


First received 21 October 2010; accepted in revised form 10 January 2011.