Supplementary

The following information is provided to the article in Energy & Environmental Science on

“Hydrogen production, methanogens inhibition and microbial community structures of psychrophilic single-chamber microbial electrolysis cells”

By

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Analysis and Calculations

The biogases produced by MECs were collected by gas bags (0.25 L capacity; Cali-5-Bond, Calibrated Instruments Inc.), with volumes measured regularly by withdrawing the gases using glass syringes, and composition analyzed by a gas chromatography (Agilent 4890D; J&W Scientific, USA) quipped with a thermal conductivity detector (TCD)\(^1\). The volumes of H\(_2\), CO\(_2\), and CH\(_4\) were calculated by multiplying the total gas production (sum of measured gas and headspace volume) by their specific fractions. Acetate concentrations in the solution before and after MEC experiments were measured by another gas chromatography (Agilent 7890 A; J&W Scientific, USA) equipped with a flame ionization detector (FID), and a polyethylene glycol: 30 m × 0.530 mm × 1.00 μm capillary column (Agilent 19095N-123, J&W Scientific, USA) using a nitrogen carrier gas. Cyclic voltammetry (CV) experiments were conducted at a scan rate of 10 mV s\(^{-1}\), in the potential range between −0.8 V and +0.2 V vs Ag/AgCl electrode using a multichannel potentiostat (WMPG-1000S, WonATech Co., Ltd).

MECs performance was evaluated as previously described\(^2\) in terms of: maximum volumetric hydrogen production rate (\(Q\), m\(^3\) H\(_2\) m\(^{-3}\) reactor day\(^{-1}\)) based on volumetric current density (\(I_V\), A m\(^{-3}\), averaged over 4 h maximum current normalized to the liquid volume of reactor); hydrogen yield (\(Y_{H_2}\), mol H\(_2\) mol\(^{-1}\) acetate), defined as the amount of hydrogen produced from the consumed acetate on a molar basis; Coulombic efficiency (\(C_E\), %) is the conversion efficiency of transforming acetate to electrons by MECs; cathodic hydrogen recovery (\(r_{cat}\), %) as the conversion efficiency of current to actually recovered hydrogen at the cathode; overall hydrogen recovery (\(R_{H_2} = C_E r_{cat}\), %), defined as the
percentage of hydrogen recovered directly from acetate; energy recovery relative to the electrical input \((\eta_{E, \%})\) is the ratio of the combustion energy of hydrogen to the input electrical energy. The moles of hydrogen produced in experiments were calculated based on ideal gas law under one standard atmospheric pressure and respective temperatures.

**Bacterial Community Analysis**

At the end of psychrophilic MECs experiments, the graphite fibers were cut from one of the anodes under each condition and fragmented using sterile scissors. An MEC enriched and operated at 25 °C was sampled as above referred before being switched into psychrophilic environment. The anaerobic granular sludge from a methane-producing EGSB reactor (CH₄ concentration larger than 60%) was used as a control sample in identification of methanogens. Total genomic DNA was extracted from fibers or granular sludge using a PowerSoil DNA Isolation Kit (MoBio Laboratories, Inc., Carlsbad, CA) according to the manufacturer’s instructions. 16S rRNA gene of the extracted DNA was amplified using a pair of universal bacteria primers 8F: 5′-AGAGTTTGATCCTGGCTCAG-3′ \((E. coli\) 16S rRNA positions 8 to 27) and 1541R: 5′-AAGGAGGTGATCCAGCCGCA-3′ \((E. coli\) 16S rRNA positions 1522 to 1541)\(^3\) for bacterial community structures analysis. A pair of universal archaeal 16S rRNA gene primers PRA46F: 5′-(C/T)TAAGCCATGC(G/A)AGT-3′ \((E. coli\) 16S rRNA positions 46 to 60) and PREA1100R: 5′-(T/C)GGGTCTCGCTGGT(G/A)CC-3′ \((E. coli\) 16S rRNA positions 1100 to 1117) was used to detect the possible existence of methanogens.\(^4\) PCR amplification was performed in a Tgradient Thermocycler (Biometra, Germany) as previously described.\(^5\) The presence of PCR products was confirmed by analyzing 5 µl of product on 0.8–1.2% agarose gels and staining with ethidium bromide.
PCR products were purified using a QIAquick Gel Extraction Kit (QIAGEN, Valencia, CA), ligated to vector pCR2.1 and cloned into *Escherichia coli* chemically competent cells using a TOPO TA cloning kit (Invitrogen, Carlsbad, CA) following the manufacturer’s protocol. For bacterial community structures analysis, one hundred plasmids containing positive inserts from each sample were sequenced using an ABI 3730XL sequencer (Applied Biosystems, Foster, CA) with vector-specific primers M13F and M13R. GenBank accession numbers for the sequences are HM124774–HM124851. Thirty positive plasmids were sequenced in archaeal clone libraries used for methanogens identification. The sequences were deposited in GenBank with accession numbers HQ336497-HQ336506.

All sequences were checked for chimeras using the CHECK_CHIMERA program (http://rdp8.cme.msu.edu/cgi/chimera.cgi?su=SSU) at RDP II and BELLEROPHON (http://foo.maths.uq.edu.au/~huber/bellerophon.pl). Operational taxonomic units (OTUs) from clone libraries were defined with DOTUR 1.53 program at a cutoff value of 0.03. 16S rRNA gene sequences were analyzed in the GenBank database (http://www.ncbi.nlm.nih.gov) and Ribosomal Database Project II (http://rdp.cme.msu.edu). Neighbor-joining phylogenetic trees were constructed with the Molecular Evolutionary Genetics Analysis package (MEGA 4.1) using the Jukes-Cantor algorithm and bootstrap test (1000 repetitions).
Table S1 Characterization of archaeal 16S rRNA gene clone library and derived from the anode biofilm of an MEC operated at 25 ºC and a control sample of methane-producing anaerobic granular sludge.

<table>
<thead>
<tr>
<th>Name of OUT (NCBI Accession)</th>
<th>Percentage clones (%)</th>
<th>Closest relative (NCBI Accession)</th>
<th>Homology (%)</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEC_CH4_1 (HQ336497)</td>
<td>100.0</td>
<td>Methanobrevibacter arboriphilus DC (AY196664)</td>
<td>99</td>
<td>MEC operated at 25 ºC</td>
</tr>
<tr>
<td>AGS_1 (HQ336498)</td>
<td>6.7</td>
<td>Uncultured archaeon clone (AB447835)</td>
<td>95</td>
<td>Anaerobic granular sludge</td>
</tr>
<tr>
<td>AGS_2 (HQ336499)</td>
<td>56.7</td>
<td>Methanoseta concilii Opfikon (NR_028242)</td>
<td>99</td>
<td></td>
</tr>
<tr>
<td>AGS_3 (HQ336500)</td>
<td>3.3</td>
<td>Uncultured archaeon clone (AB447835)</td>
<td>94</td>
<td></td>
</tr>
<tr>
<td>AGS_4 (HQ336501)</td>
<td>6.7</td>
<td>Uncultured Methanoseta sp. clone A11 (EU888815)</td>
<td>95</td>
<td></td>
</tr>
<tr>
<td>AGS_5 (HQ336502)</td>
<td>3.3</td>
<td>Uncultured Methanoseta sp. clone A11 (EU888815)</td>
<td>97</td>
<td></td>
</tr>
<tr>
<td>AGS_6 (HQ336503)</td>
<td>3.3</td>
<td>Uncultured Methanolinae sp. clone (AB479405)</td>
<td>93</td>
<td></td>
</tr>
<tr>
<td>AGS_7 (HQ336504)</td>
<td>6.7</td>
<td>Uncultured Methanoseta sp. clone A11 (EU888815)</td>
<td>92</td>
<td></td>
</tr>
<tr>
<td>AGS_8 (HQ336505)</td>
<td>10</td>
<td>Uncultured crenarchaeote clone F31 (EU910616)</td>
<td>98</td>
<td></td>
</tr>
<tr>
<td>AGS_9 (HQ336506)</td>
<td>3.3</td>
<td>Methanosarcina barkeri (AY196682)</td>
<td>93</td>
<td></td>
</tr>
</tbody>
</table>

Table S2 Comparison of diversity parameters of three MECs communities enriched and operated at 4 ºC, 9 ºC and 25 ºC.

<table>
<thead>
<tr>
<th>Sample</th>
<th>No.of clones</th>
<th>No.of OTUs&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Estimators (95% confidence interval)</th>
<th>Coverage&lt;sup&gt;b&lt;/sup&gt; (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>H&lt;sub&gt;Shannon&lt;/sub&gt;</td>
<td>S&lt;sub&gt;Chao1&lt;/sub&gt;</td>
</tr>
<tr>
<td>4 ºC</td>
<td>100</td>
<td>24</td>
<td>2.39 (2.14-2.64)</td>
<td>46 (31-99)</td>
</tr>
<tr>
<td>9 ºC</td>
<td>100</td>
<td>30</td>
<td>2.64 (2.38-2.90)</td>
<td>61 (39-129)</td>
</tr>
<tr>
<td>25 ºC</td>
<td>100</td>
<td>24</td>
<td>1.62 (1.27-1.97)</td>
<td>51 (32-114)</td>
</tr>
</tbody>
</table>

<sup>a</sup> The OTUs are defined at a DNA distance of 0.03.

<sup>b</sup> Coverage estimate is calculated as [1-(n/N)] ×100, where n is the number of singleton clones, and N is the total number of clones.
Figure S1. Single-chamber MEC shown with gas collection tube (top).

Figure S2. CV (10 mV s$^{-1}$) analysis of biological anodes in MFCs started up at 4 °C, 9 °C and 25 °C before being transferred to MECs under turn-over conditions.
Figure S3. Agarose gel analyses of PCR amplified products with the archaeal primers. The communities from five different conditions were investigated: MEC operated at 25 °C, MEC operated at 25 °C and then switched to 9 °C and 4 °C operation, MEC operated at 4 °C and 9 °C, and methane-producing anaerobic granular sludge (AGS). Marker, DNA size marker; NC, negative control.
**Figure S4.** Collector’s curves of observed and estimated OTUs derived by the DOTUR program from 16S rRNA gene clone libraries. Phylotypes were defined at DNA distance of 0.03 (97% OTU cutoff). (A) and (B) represent MECs enriched and operated at 4 °C and 9 °C, respectively; (C) represent MEC enriched and initially operated at 25 °C and then switched to 4 °C and 9 °C. Curves include observed OTUs (solid line), Chao1 (short dash), and abundance-based coverage estimator (ACE) (long dash).
**Figure S5.** Phylogenetic tree of α-Proteobacteria, β-Proteobacteria, γ-Proteobacteria, Spirochaetes, and Acidobacteria groups (A) and δ-Proteobacteria, ε-Proteobacteria, Fusobacteria, Firmicutes, and Bacteroidetes groups (B) illustrating the phylogenetic distances between 16S rRNA gene recovered from the MEC anodes and their closest relatives (an identified species has the closest sequence with MEC clone) in the GenBank and RDP databases. Red rectangle highlights the phylogenetic affiliations of the majority of psychrophilic clones with the main psychrotolerant exoelectrogens found in psychrophilic MECs. A total of 100 clones were sequenced for each sample representing 24 OTUs (enriched at 4 ºC, ○), 30 OTUs (9 ºC, ◆) and 24 OTUs (25 ºC, △), respectively, with the total number of clone sequences for each OTU in parentheses (bold). The sequences accession number in the GenBank database also in parentheses. Bar indicates 5% divergence.

**References**