STANDARDIZED MICROBIAL FUEL CELL ANODES OF SILICA-IMMOBILIZED SHEWANELLA ONEIDENSIS (POSTPRINT)

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**Standardized Microbial Fuel Cell Anodes of Silica-Immobilized Shewanella oneidensis (POSTPRINT)**

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- **Sponsoring/Monitoring Agency**: ^Air Force Research Laboratory

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- **ABSTRACT**: Populations of metabolically active bacteria were associated at an electrode surface via vapor-deposition of silica to facilitate in situ characterization of bacterial physiology and bioelectrocatalytic activity in microbial fuel cells.

- **SUBJECT TERMS**: microbial fuel cells, MFC, Shewanella oneidensis, S. oneidensis, biofilms, silica coated bacteria cells
Standardized microbial fuel cell anodes of silica-immobilized
*Shewanella oneidensis*

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Populations of metabolically active bacteria were associated at
an electrode surface via vapor-deposition of silica to facilitate
in situ characterization of bacterial physiology and bio-
electrocatalytic activity in microbial fuel cells.

Microbial fuel cells (MFC) convert chemical energy to electrical
energy by capitalizing on the metabolic and respiratory
processes of particular microbial species.1 Dissimilatory
metal-reducing bacteria such as *Shewanella oneidensis* will
transfer electrons from reduced electron donors (e.g. lactate)
into insoluble electron acceptors (e.g. iron and manganese
oxides).2 In MFC, the electrode surface will act as a respiratory
sink for the available electrons and when combined with a
suitable cathode will yield electricity.3 The association between
bacteria and electrodes, however, is inconsistent due to
inherent variations in bacterial growth due to changes in
physiological conditions (e.g., electron donor concentrations,
diffusion limitations, pH, growth phase, etc.), that are difficult
to control in the MFC reactor, especially after extended
culture periods.4,5 In addition, in order to most effectively
use insoluble electron acceptors, the metal-reducing bacteria
form a complex biofilm.6 Biofilms generally benefit MFC
catalyst power output as the bacterial cells become tightly associated
with the electron acceptor (i.e., the electrode in MFC). Bio-
film, however, require significant time to become established
and as such can lead to irreproducible power density.3

In order to address the design optimization of MFC, specific
variables and limitations of the system must be defined. In
order to evaluate modifications at the cathode, for example,
MFC require a standardized anode in which the number,
activity and status of the bacterial population are known
and controlled. Here, we demonstrate a method to associate
cellular bacteria in a silica matrix by using *S. oneidensis* as a
model system to produce standardized anodes with defined
bacterial physiology and electrochemical activity. Silica
sol-gel materials have served well for diverse applications in
electrochemistry but the process can often lead to cellular
lysis.7,8 A one-step vapor deposition of silica provides an alternative
to aqueous sol-gel formations that retains the activity of
biomolecules and preserves integrity of whole cells.9,10
*S. oneidensis* DSP-10 cultures were harvested, washed and
resuspended to a defined cell density (1 × 10^9 cfu mL^-1).† The
cells were physisorbed onto porous graphite felt (GF) and
exposed to tetramethylorthosilicate (TMOS) in vapor phase
which undergoes rapid and complete hydrolysis in aqueous
solvents. Further condensation and cross-linking of the hydro-
lyzed silica monomers occurs with high salt concentrations in
the reaction and leads to the sol-gel and particulate silica
formation. The resulting matrix of silica particles helps
immobilize the bacterial cells directly on the GF (Si/cells–GF).
The vapor-phase process eliminates the use of co-solvents or
catalysts which are commonly used in sol-gel synthesis.7 The
effective immobilization of cells was likely due to combination
of hydrogen bonding and electrostatic interactions between
the cell membrane and silica particles.10 Control electrodes
were physisorbed to GF with no subsequent TMOS exposure
(cells–GF).

Silica-coated bacterial cells were visible on the fibers of GF
by scanning electron microscopy (SEM) (Fig. 1). Energy
dispersive spectroscopy (EDS) conducted during SEM
analysis confirmed that the particulate structure consisted of
silicon and oxygen (data not shown). Samples of Si/cells–GF
were subcultured to fresh growth medium and grew to pure
culture, confirming that silica encapsulation did not hinder the
viability of the cells.

Although physisorbed cells were retained on the carbon fibers
(Fig. 1), the silica immobilization is expected to enhance
reproducibility and association of cells at the electrode surface.
Electrodes were assembled as anodes in a simple flow-through
MFC with unmodified GF as cathode.‡ The stabilized open
circuit voltage (OCV) was 430 ± 29 mV (n = 11) for
Si/cells–GF and 385 ± 117 mV (n = 9) for cells–GF with lactate
as electron donor, in agreement with previous studies.4 The
Si/cells–GF electrodes retained stable OCV for 48 hours
(445 ± 22 mV, n = 6) whereas the cells–GF electrode decreased

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† Electronic supplementary information (ESI) available: Schematic of
MFC apparatus. See DOI: 10.1039/c0ec01255f

Fig. 1 SEM of bacterial cells on GF electrodes in the absence (A) and
presence (B) of a silica matrix. Scale bars are 1 μm.
over 48 hours (340 ± 11 mV, \(n = 5\)). The standard deviation for cells–GF was significantly greater than for Si/cells–GF, confirming that the silica-encapsulation provides a reproducible means for fabrication of MFC anodes. The maximum power density for Si/cells–GF (0.358 \(\pm 0.03\) mW cm\(^{-2}\)) was higher than for cells–GF (0.236 \(\pm 0.03\) mW cm\(^{-2}\)). In addition, Si/cells–GF anodes retained a higher maximum power density with no loss of activity after 5 days of operation (Fig. 2).

The increased power density for Si/cells–GF was attributed to a greater number of active cells on the electrode surface. Protein determination assays confirmed that more cells were associated with Si/cells–GF (\(\sim 3.45\) mg protein cm\(^{-2}\) after 5 days) than for cells–GF (\(\sim 2.05\) mg protein cm\(^{-2}\)). The loss of protein is due to cells being flushed from the MFC during the addition of medium, which results in a loss of power density and long term stability. Increased power output in Si/cells–GF may also be attributed to more efficient passage of electrons as the cells are tightly associated with the electrode. The vapor-deposition of silica creates a particulate matrix that enhances bacterial association with surfaces and eliminates the solvent toxicity of conventional aqueous sol–gel techniques. The resulting silica matrix helps provide a stable and defined microbial community. In MFC, the ability to stabilize and

![Fig. 2](image-url)  
(A) Power (solid lines) and polarization (dashed lines) curves for duplicate MFC at 24 hours; Si/cells–GF (●), cells–GF (□). (B) Maximum power density over 5 days; Si/cells–GF (black bars), cells–GF (white bars).

Table 1  Output potentials for MFC with *S. oneidensis* anodes under continuous flow

<table>
<thead>
<tr>
<th>Flow rate/mL min(^{-1})</th>
<th>OCV/mV</th>
<th>Potential under 330 (\Omega) load/mV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Min.</td>
<td>Max.</td>
</tr>
<tr>
<td>Cells–GF</td>
<td>0.5</td>
<td>92 ± 31</td>
</tr>
<tr>
<td>Si/cells–GF</td>
<td>0.5</td>
<td>162 ± 4</td>
</tr>
<tr>
<td>Cells–GF</td>
<td>1.0</td>
<td>146 ± 13</td>
</tr>
<tr>
<td>Si/cells–GF</td>
<td>1.0</td>
<td>189 ± 6</td>
</tr>
</tbody>
</table>

Table 2  Open circuit voltage and maximum power density for MFC with Si/cells–GF anodes and various electron donors

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>OCV in media/mV</th>
<th>OCV in buffer/mV</th>
<th>Max. power(^a)/(\mu W) cm(^{-2})</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>5 mM</td>
<td>20 mM</td>
<td>5 mM</td>
</tr>
<tr>
<td>None</td>
<td>174 ± 83</td>
<td>188 ± 19</td>
<td>0.008</td>
</tr>
<tr>
<td>Glucose</td>
<td>196</td>
<td>268 ± 52</td>
<td>0.018</td>
</tr>
<tr>
<td>Acetate</td>
<td>245</td>
<td>395 ± 50</td>
<td>0.035</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>304 ± 74</td>
<td>385 ± 39</td>
<td>0.220</td>
</tr>
<tr>
<td>Lactate</td>
<td>398 ± 107</td>
<td>431 ± 79</td>
<td>0.129</td>
</tr>
</tbody>
</table>

\(^a\) Max power in buffer (Note: power output in growth medium with lactate is given in the text). \(^b\) ND = not determined.
standardize populations of *S. oneidensis* with respect to electrocatalytic performance provides a platform for a range of characterization studies. Although the proof of concept demonstrated in this communication has an emphasis on MFC research, the methodology described can be readily applied to immobilizing other microorganisms for industrial applications. The silica matrix, in essence, mimics the exopolysaccharide ‘glue’ that binds cells in a natural biofilm. This synthetic process, however, eliminates the significant cultivation times and variability typically associated with natural biofilm formation, thereby facilitating electrochemical studies on a standardized platform.

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Notes and references

‡ *S. oneidensis* DSP-10 was cultured in Luria Bertani broth containing rifampicin (5 μg mL<sup>−1</sup>) at 30 °C, with gentle agitation (100 rpm). Cells were harvested at late stationary phase (OD<sub>600</sub> ≈ 4–5) by centrifugation, washed (3×) and resuspended in phosphate buffered saline (PBS) to a final OD<sub>600</sub> of 5. Corresponding colony forming units (cfu mL<sup>−1</sup>) were determined by serial dilution and plate counts. Titanium wire (~20 cm length (0.25 mm dia, Goodfellow, Oakdale, PA)) was woven through the GF (5 cm, 1/8″ length (0.25 mm dia, Goodfellow, Oakdale, PA)) was woven through the GF electrode. 5 mL of the harvest and washed culture was applied to the top of the graphite felt and TMOS (1 mL, Sigma Aldrich, St. Louis, MO) was deposited in the anodes containing cells in the base of a plastic powder funnel (10 mm) that was modified with a central glass well designed to hold the GF electrode. 5 mL of the harvested and washed culture was applied to the top of the graphite felt and TMOS (1 mL, Sigma Aldrich, St. Louis, MO) was deposited in the outer ring. Glass beads were added to the outer well to increase the surface area for evaporation. The dish was covered and incubated for 30 min at 37 °C. The flow through MFC were prepared by first placing the anodes containing cells in the base of a plastic powder funnel (55 mm dia), overlaying the anode with a polycarbonate membrane (0.2 μm) as separator, and then placing the GF cathode on the separator (Personal Communication, Justin Bifflinger and Brad Ringeisen, Naval Research Laboratory, USA). A photograph and schematic diagram of the MFC apparatus are shown in ESI.‡ All experiments were maintained at room temperature (~22 °C) unless otherwise stated. Sterile defined media<sup>3</sup> with lactate (20 mM) was used as MFC feed and electrolyte unless described otherwise. Open circuit electrode voltage (OCV) was measured using a Personal Daq/54 (IOtech, Cleveland, OH). Polarization potentials were measured on a VersaSTAT 3 potentiostat/galvanostat (Princeton Applied Research, Oak Ridge, TN) by varying the potential and recording steady state current values. Power was calculated using Ohm’s law and normalized, based on a geometric surface area of 21 cm<sup>2</sup>. Samples were cut from the electrodes (in triplicate) and incubated in NaOH (0.1 N, 90 °C, 30 minutes) to cause cellular lysis and release immobilized protein. Total protein content of cells was determined using the bicinchoninic acid protein (BCA) assay according to the manufacturer’s instructions (Pierce Biotechnology, Rockford, IL).