Exoelectrogenic Biofilm as a Template for Sustainable Formation of a Catalytic Mesoporous Structure

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ABSTRACT: Mesoporous structures can increase catalytic activity by maximizing the ratio of surface area to volume, but current synthesis techniques utilize expensive polymers and toxic chemicals. A Geobacter sulfurreducens biofilm was used as a sustainable template to form mesoporous Pd structures while eliminating the need for synthetic chemicals. The bulk of the biofilm material was removed by thermal treatments after nanoparticle formation, producing a catalytic Pd mesoporous (pore size 9.7 ± 0.1 nm) structure attached to the graphite electrode with a 1.5–2 μm thick backbone composed of nanoparticles (~200 nm). A control electrode electrochemically plated with Pd in the absence of a biofilm exhibited a variable planar Pd base (~0.5–3 μm thick) with sporadic Pd extrusions (~2 μm across, 1–5 μm tall) from the surface. The biotemplated mesoporous structure produced 15–20% higher stable current densities during H2 oxidation tests than the electrochemically plated control electrode, even though 30% less Pd was present in the biotemplated catalyst. These results indicate that electroactive biofilms can be used as a sustainable base material to produce nanoporous structures without the need for synthetic polymers.

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Nanoporous structures with high pore volumes can increase the catalytic activity of surfaces by maximizing the ratio of surface area to volume. Generating catalytic mesoporous structures (2–50 nm pore size; Fendler, 1996) from stabilized nanoparticle suspensions and functionalized polymers in block copolymer assemblies (Li and El-Sayed, 2001; Mackay et al., 2006; Warren et al., 2008) minimizes effective surface area reduction due to agglomeration of nanoparticles during deposition directly onto supports (Wang et al., 2013). Catalytic mesoporous structures are formed by removing the copolymers and can subsequently be bound to an electrode and used in catalysis, such as H2 oxidation (Orilall and Wiesner, 2011; Warren et al., 2008). The usual synthesis methods for mesoporous structures are often environmentally unfriendly and costly because they require potentially hazardous strong reductants to precipitate soluble metals and synthetic polymers to form an ordered structure and bind it to an electrode. Other methods require electrodeposition onto supports and a subsequent dealloying procedure in an acidic environment to obtain porosity (Tominaka et al., 2010). An alternative method to create a catalytic surface was developed here using biofilms of Geobacter sulfurreducens, eliminating the need for these synthetic chemicals and/or acidic conditions.

Dissimilatory metal reducing bacteria (DMRB), such as G. sulfurreducens, have been shown to sustainably reduce soluble metals from waste streams (Mabbert et al., 2005) to nanoparticles using naturally produced polymers as stabilizers and capping agents (De Windt et al., 2006; Lovley, 1993; Yates et al., 2013; Yong et al., 2002). Geobacter sulfurreducens produces electrically conductive pili (Malvankar et al., 2012), which increase the reduction rate of soluble metals (Cologgi et al., 2011) and enable number agreement attachment and extracellular electron transfer to insoluble electron acceptors at the highest known rates among microorganisms (Bond et al., 2012). Nanostructure formation using biogenic templates has received increased interest recently as the development of green synthesis techniques become more desirable (Chen et al., 2010; Li et al., 2012). Palladium catalysts formed by Desulfovibrio desulfuricans (Yong et al., 2007) and Escherichia coli (Orozco et al., 2010) have been previously studied for hydrogen oxidation. However, these studies use cell suspensions under a hydrogen atmosphere to form nanoparticles. The palladized cells are then carbonized and applied to an electrode using an expensive Nafton binder. Formation of mesoporous structures with a G. sulfurreducens biofilm allows direct synthesis on
an electrode without the need for strong reductants or synthetic polymers and binders. These attributes make *G. sulfurreducens* an attractive, sustainable alternative for the formation of catalytic mesoporous structures.

This communication reports the in situ formation of palladium nanoparticles by a *G. sulfurreducens* biofilm attached to a polarized graphite electrode. The resulting nanoparticle-impregnated biofilm was then pyrolyzed and oxidized to remove the bulk of the cell material and expose a catalytic mesoporous Pd structure. The resulting structure was characterized electrochemically for H₂ oxidation and compared to control graphite electrodes electroplated with Pd or coated with a Pd black/Nafion suspension.

A *G. sulfurreducens* biofilm grown on a polished, poised (−0.15 V vs. SHE) graphite electrode (duplicates) was an effective template for the formation of a mesoporous structure with increased catalytic activity. The mesoporous Pd structure was formed by pyrolysis (450°C in Ar, 5°C/min ramp followed by immediate cooling) and oxidation (450°C in air, 2 h) of the *G. sulfurreducens* template after in situ reduction of Pd(II) to Pd(0) nanoparticles (Fig. 1). Some Pd(0) was likely converted to palladium oxide during the oxidation step of the process (Chen and Ruckenstein, 1981). In H₂ oxidation tests, the biotemplated Pd structures produced higher catalytic current densities at 0.4 V during linear sweep voltammetry (LSV) scans (1 mV/s) in 0.1 M H₂SO₄ compared to the electroplated electrodes (Fig. 2) at rotation rates of 2,000 rpm (3.5 vs. 2.7 mA/cm²) and 1,000 rpm (2.3 vs. 2.0 mA/cm²) with continuous H₂ sparging. Current densities are compared at 0.4 V to account only for the catalytic current due to hydrogen oxidation and not include current from the large, non-catalytic oxidation peak. Catalytic current densities were 9–23% larger for biotemplated electrodes above 0.25 V during rotation and 75–100% larger above 0.08 V without rotation. Duplicate electrodes generally exhibited minimal variations in current density profiles (Fig. S1). In one case (biotemplated electrode at 0 rpm), the maximum current peak was three times larger than its duplicate, but it still exhibited a similar stable current density between 0.08 and 0.4 V. Voltammograms exhibited a current peak at ∼0.15 V during H₂ sparging tests likely due to corrosion of the carbon support (Roen et al., 2004) or pyrolyzed carbonaceous cell material remaining in the structure. A polymeric binder was not needed for attachment of the biotemplated structure to the graphite electrode because carbonaceous cell material likely bonded the Pd structure to the graphite surface. It is possible that some of this carbonaceous material could have blocked active Pd sites, decreasing maximum attainable catalytic current by the biotemplated catalyst. The control electrode coated with a Pd black and Nafion suspension produced less current at 1,000 rpm than both electrochemically formed electrodes (1.1 mA/cm²), likely due to the diffusion resistance of H₂.
through the Nafion binder. Biotemplated electrodes that were pyrolyzed, but not oxidized, produced approximately half the current density (1.2 mA/cm²) at 0.4 V and 1,000 rpm (Fig. S2) as electrodes that were both pyrolyzed and oxidized, likely because much of the palladium structure was encased in carbonized cell material. The graphite electrode control lacking a catalyst or binder produced negligible current (0.16 mA/cm², 1,000 rpm). The current density of the biotemplated Pd structure (0.35 mA/cm²) at 0.4 V obtained by LSVs without rotation was higher than the electrochemically plated graphite electrode (0.2 mA/cm²) and a bulk Pd flag electrode (0.1 mA/cm²). Electrical connectivity between the palladium layer and the graphite support was confirmed by current generation through hydrogen oxidation in a two-chambered fuel cell without potentiostatic control (Fig. S3).

Voltammograms generated with N₂ sparging of the solutions gave similar current densities in RDE tests at 1,000 and 2,000 rpm (0.43 ± 0.10 mA/cm²; Fig. 2). A carbon corrosion peak was observed under N₂ sparging conditions, but it was shifted to a more negative potential of ~100 mV to 0.05 V, compared to H₂ sparging conditions. The presence and similar magnitude of peaks with both N₂ and H₂ sparging implies the peak is a result of a non-catalytic oxidation current, likely due to the corrosion of the graphite support (Fig. S4) or carbonized cell material that remained in the structure due to incomplete oxidation (Yasuda et al., 2006).

Mesoporous structures generated using a *G. sulfurreducens* biofilm as a template for Pd reduction had higher porosities than the electrochemically coated graphite electrode control. Interconnected Pd nanoparticles (~100–300 nm) produced by *G. sulfurreducens* formed the backbone (Figs. 3 and S5) of the structure, which resulted in increased void space after oxidation of the cell material. Sintering of Pd nanoparticles during oxidation at 450°C likely influenced the final shape of the porous structure as it can decrease (Chen and Ruckenstein, 1981) the total active surface area. However, removal of carbonized cell material at this temperature increased the catalytic current density and surface area (detailed below) versus the un-oxidized electrode by removing the bulk of the carbonized cell material to expose

**Figure 3.** ESEM images of (A–B) a biotemplated mesoporous structure created by *G. sulfurreducens* and (C–D) an electrochemically formed structure under high (~10,000) and low magnification (~1,300).
catalytically active sites within the porous Pd backbone. The electrode surfaces were composed of ~97% Pd (by weight) after pyrolysis and oxidation compared to 50% carbon and 50% Pd after pyrolysis alone, for areas analyzed with EDS (Fig. S6), highlighting the importance of the oxidation step. The mesoporous palladium layer formed by *G. sulfurreducens* was ~3 µm thick after the cell material was removed (Fig. 3). Electrochemically plated Pd control electrodes exhibited a variable planar base layer (~0.5–3 µm thick) with sporadic extrusions (~2 µm diameter, ~1–5 µm tall) from the surface (Fig. 3) after undergoing similar thermal treatments. The structural difference between the two methods highlights the advantage of using a *G. sulfurreducens* biofilm as a template for the formation of a mesoporous structure composed of Pd nanoparticles with increased available catalytic surface area without the use of any synthetic chemicals.

The surface area of the electrodes formed over a biotemplate or by electrochemical plating was quantified from N₂ adsorption/desorption isotherms using the Brunauer–Emmett–Teller (BET) gas adsorption method (Fig. S7). The average BET surface area of the blank graphite electrodes before treatment was 0.65 ± 0.05 m²/g (Table I). The Pd structure formed over the *G. sulfurreducens* template exhibited an increased BET surface area (569 ± 75 m²/g) and pore volume (1.40 ± 0.17 cm³/g) compared to the electrochemically plated Pd electrode (330 ± 45 m²/g and 0.90 ± 0.14 cm³/g). The average pore size of the biotemplated structure (9.7 ± 0.1 nm) was similar to the electroplated structure (11.1 ± 0.3 nm), and both structures are classified as mesoporous materials (2–50 nm pore size). Although an ordered mesoporous structure was not included as a control in this study, the surface area of the electroplated catalyst was similar to other chemically formed mesoporous palladium and carbon electrodes (345 m²/g, Wan et al., 2009; 241 m²/g, Yang et al., 2012). The increase in surface area is likely due to the surface roughness of the carbon support, which could provide an irregular pattern and more facets for palladium growth compared to a smooth surface, such as a gold electrode.

The mass of Pd (normalized by electrode surface area) was determined by carefully removing the Pd film from the electrode surface and weighing. The biotemplated structure contained 33% less Pd (0.23 ± 0.01 mg Pd/cm²) than the electroplated electrode (0.35 ± 0.02 mg/cm²). Normalizing the current density of the catalysts by the surface area and the mass of palladium in the catalyst layer results in a 47% higher current density for the biotemplated catalyst (14.8 mA/cm² mg Pd) compared to the electroplated catalyst (7.8 mA/cm² mg Pd) at 0.4 V and 2,000 rpm.

These results show that exoelectrogenic biofilms of *G. sulfurreducens* served as a facile, sustainable biogenic template for the formation of catalytic mesoporous structures by producing a porous interconnected nanoparticle framework in situ. The mesoporous structure had an increased surface area and pore volume compared to an electrochemically coated Pd structure, resulting in higher stable current densities during H₂ oxidation tests despite having 33% less Pd in the catalyst layer. Although the process was not optimized here, the results indicate that *G. sulfurreducens* can successfully and sustainably form a mesoporous catalytic structure without addition of any chemical reductants or synthetic polymers. The ability for *G. sulfurreducens* to form a porous structure from soluble metals using only natural processes has implications for utilizing biofilms to recover and disperse metal as nanoparticles conductive surfaces to sustainably form porous catalysts tailored to different applications.

## Materials and Methods

Two-chamber reactors (duplicates) with a Nafion 117 proton exchange membrane to separate the anode and cathode chambers were used for all tests. Graphite working electrodes (1 cm × 2 cm) were sanded with 400 and 1,500 grit sandpaper, cleaned with 1 M HCl and de-ionized water and connected to a titanium wire. Electrodes had a contact resistance <0.7 Ω. Working electrodes for rotating disk electrode (RDE) tests were prepared identically, but were cylindrical in shape (1.4 cm diameter) to fit onto the RDE apparatus. Counter electrodes were Pt wires (15 cm length × 0.25 cm diameter). Reference electrodes (Ag/AgCl, 3 M NaCl, +0.2 V vs. SHE) were inserted into the working electrode chamber.

*Geobacter sulfurreducens* PCA was obtained from stocks frozen at −80°C. Stocks were cultured in ATCC medium 1957 with 30 mM acetate. Cultures were incubated at 30°C. The anode chambers of reactors were inoculated with 1:10 ratio of *G. sulfurreducens* culture to ATCC 1957 medium, excluding fumarate. Electrochemically plated graphite plates were run under the same conditions without inoculation of *G. sulfurreducens*. Anode potentials were set to −0.15 V (vs. SHE) with a potentiostat (Biologic, Knoxville, TN) in a 30°C temperature controlled chamber. Anode chambers were stirred at ~200 rpm with a magnetic stir bar to help reduce diffusion limitations.

The biofilm was grown until current production began to decrease from its maximum (~5 days), when the medium was exchanged for fresh ATCC medium 1957, excluding

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**Table I.** Pore volume analysis of Pd structures formed with a biotemplate or electrochemically.

<table>
<thead>
<tr>
<th>Structure</th>
<th>Average surface area (m²/g)</th>
<th>Average pore volume (cm³/g)</th>
<th>Average pore size (nm)</th>
<th>Mass of Pd (mg/cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biotemplated</td>
<td>569 ± 75</td>
<td>1.40 ± 0.17</td>
<td>9.7 ± 0.1</td>
<td>0.23 ± 0.01</td>
</tr>
<tr>
<td>Abiotic plated</td>
<td>330 ± 45</td>
<td>0.90 ± 0.14</td>
<td>11.1 ± 0.3</td>
<td>0.35 ± 0.02</td>
</tr>
<tr>
<td>Graphite block</td>
<td>0.65 ± 0.05</td>
<td>0.001</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

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fumarate, to remove cells in suspension. Sodium tetrachloropalladate (170 mg/L final concentration; Na2PdCl4, Sigma–Aldrich, St. Louis, MO) and sodium acetate (10 mM) were added to the working electrode chamber while the working electrode was set to −0.15 V (vs. SHE). Electrodes were disconnected from the potentiostat after 1 h of incubation, removed from the reactor, and stored in anaerobic sodium bicarbonate medium at 4°C until pyrolysis (<2 h). Control electrodes were subjected to identical potentiostatic control, but were not inoculated with *G. sulfurreducens*.

Electrodes were pyrolyzed in a Lindberg tube furnace (Thermo Scientific, Waltham, MA) under an argon atmosphere. The furnace temperature was ramped at 5°C/min to 450°C and immediately allowed to cool to ambient temperature to carbonize cell material. The carbon was then oxidized away at 450°C in air for 2 h.

Catalytic activity of electrodes for H2 oxidation was evaluated using LSV in H2 saturated 0.1 M H2SO4. The electrolyte was prepared by sparging 0.1 M H2SO4 with pure H2 gas for at least 30 min. Electrochemical performance was tested using an RDE to decrease the effect of diffusion resistance. Tests were run with continuous H2 or N2 sparging to differentiate the catalytic currents gained by oxidizing H2 from the background currents. Potentials were cycled between −0.1 to 0.4 V (vs. SHE) at 1 mV/s during LSVs with an electrode rotation rate of 2,000 or 1,000 rpm to evaluate electrode performance. Current densities were normalized to the geometric surface area of the electrode.

Data were recorded on a Solartron potentiostat (Solarton, Farnborough, Hampshire, UK). Pt wire counter electrodes and Ag/AgCl (3 M NaCl) reference electrodes were used in all tests. Bare graphite electrodes and a Pd flag (Sigma–Aldrich) connected to a titanium wire were also characterized electrochemically as controls. Electrical connectivity between the palladium layer and the graphite support was tested using prepared electrodes as hydrogen fuel cell anodes in two-chamber reactors with identical setup as described above for *G. sulfurreducens* growth.

The electrode surface structure was examined using environmental scanning electron microscopy (E-SEM, FEI Quanta 200) equipped with electron dispersive X-ray spectrometry (EDS). Elemental spectra of the electrode surfaces were obtained after pyrolysis and after oxidation using EDS. Particle sizes that composed the mesoporous structure were obtained with a field emission scanning electron microscope (FE-SEM, FEI NanoSEM 630) at high magnification (up to 100,000×). The height and evenness of the Pd layers was determined by measuring profile images along the edge of the structure with ImageJ software measuring tools. Thin sections (70 nm) of biofilms embedded in Eponate resin, after staining with osmium tetroxide and uranyl acetate and dehydrating in an ethanol and acetone series, were analyzed by TEM (JEOL JEM 1200 EXII) to show that the biofilm reduced the palladium to palladium nanoparticles in the extracellular space (Fig. S4).

Surface area, pore volume, and average pore size of the structures formed on the electrodes were obtained from N2 adsorption/desorption isotherms with a Micromeritics ASAP 2000 (Micromeritics, Norcross, GA) analyzer using the BET gas adsorption method. The weight of palladium on the electrodes was determined gravimetrically by gently scraping the palladium layer off the graphite support with a clean scalpel and weighing it with a Mettler-Toledo UMT2 microbalance (Mettler-Toledo, Columbus, OH).

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**References**


**Supporting Information**

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