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

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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 bioelectrocatalytic activity in microbial fuel cells.

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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) to 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 power output as the bacterial cells become tightly associated with the electron acceptor (*i.e.*, the electrode in MFC). Biofilms, 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 bacterial cells 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 hydrolyzed 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

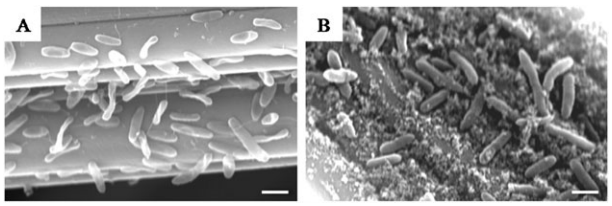

Fig. 1 SEM of bacterial cells on GF electrodes in the absence (A) and presence (B) of a silica matrix. Scale bars are 1 μm.

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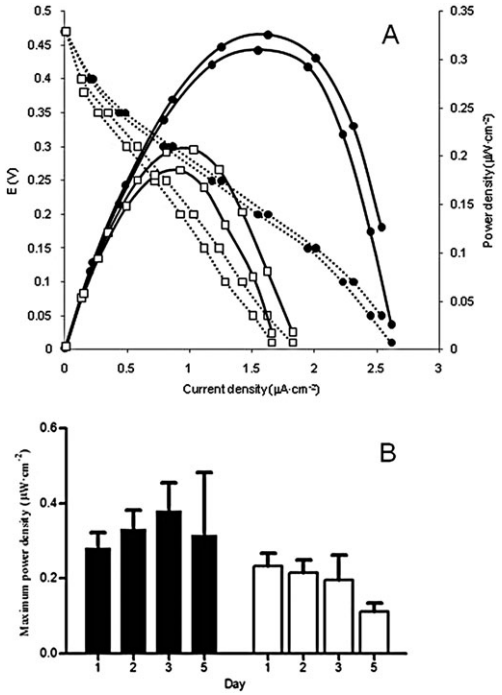

Fig. 2 (A) Power (solid lines) and polarization (dashed lines) curves for duplicate MFC at 24 hours; Si/cells–GF (●), cells–GF (□). (B) Maximum power density over 5 days; Si/cells–GF (black bars), cells–GF (white bars).

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 ± 0.03 μW cm^{-2}) was higher than for cells–GF (0.236 ± 0.03 μW 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 (~3.45 mg protein cm^{-2} after 5 days) than for cells–GF (~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 loss of cells due to flow-through elution was confirmed by varying the flow rate through the MFC. Under continuous flow, Si/cells–GF produced higher OCV, less variability in output and significantly higher voltage under load (330 Ω) than cells–GF (Table 1). The difference in output voltage under load was less significant at higher flow rates (1 mL min^{-1}) but the maximum voltage sustained was higher for Si/cells–GF than for cells–GF.

Silica-encapsulation of *S. oneidensis* clearly provides reproducible loading on GF that can be used to standardize anodes for further MFC studies. As a proof of concept, we investigated the electrocatalytic activity of *S. oneidensis* in response to a range of potential electron donors (Table 2). The influence of electrolyte was determined in the same manner by substituting buffer for growth medium (Table 2). Glucose (under anaerobic conditions) was a comparatively poor substrate for power generation relative to lactate, in agreement with previous studies.[11] Acetate and pyruvate demonstrated comparable power output to lactate, but were dependent upon substrate concentration and electrolyte (*i.e.* growth medium or buffer). There was no significant variation in protein density for Si/cells–GF electrodes incubated with growth media or buffer (296 ± 42 and 306 ± 42 μg mL^{-1} protein ($n = 6$) with 20 mM lactate in media and PBS respectively).

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

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

			Potential under 330 Ω load/mV		
	Flow rate/mL min^{-1}	OCV/mV	Min.	Max.	Mean ± SD
Cells–GF	0.5	92 ± 31	0.012	0.11	0.05 ± 0.24
Si/cells–GF	0.5	162 ± 4	0.256	0.47	0.35 ± 0.05
Cells–GF	1.0	146 ± 13	0.073	0.32	0.14 ± 0.04
Si/cells–GF	1.0	189 ± 6	0.036	0.71	0.14 ± 0.08

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

	OCV in media/mV		OCV in buffer/mV		
Electron donor	5 mM	20 mM	5 mM	20 mM	Max. power[a]/μW cm^{-2}
None	174 ± 83		188 ± 19		0.008
Glucose	196	314	ND[b]	268 ± 52	0.018
Acetate	245	219	ND	395 ± 50	0.035
Pyruvate	304 ± 74	367 ± 75	385 ± 39	446 ± 14	0.220
Lactate	398 ± 107	474 ± 25	431 ± 79	386 ± 33	0.129

[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^{-1}) at 30 °C, with gentle agitation (100 rpm). Cells were harvested at late stationary phase ($OD_{600} \approx 4$–5) by centrifugation, washed (3×) and resuspended in phosphate buffered saline (PBS) to a final OD_{600} of 5. Corresponding colony forming units (cfu mL^{-1}) 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″, Morgan AM&T, Inc. Greenville, SC) electrodes allowing ~10 cm for connections. The material was washed with PBS and then sterilized by autoclaving before use. The sterilized GF was placed in a glass Petri dish (100 × 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 Biffinger 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[12] 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 Ohms law and normalized, based on a geometric surface area of 21 cm^2. 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).

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