ABSTRACT
A microbial fuel cell with a microfabricated electrode and exoelectrogenic bacteria has been demonstrated. The superior compatibility of the organism's microbiology makes this approach more attractive and practical over previously micromachined microbial fuel cell designs. Both electrical performance and longevity of the device are greatly improved with continuous power output for over a week and enhanced performance over time. Using a 1mm² anode, the fuel cell reaches and maintains 619 mV of open circuit voltage and delivers 0.12 μW maximum power using potassium ferricyanide at the cathode after 10 days of operation.

INTRODUCTION
The surge of oil prices along with concern for global warming has motivated the search for both short and long term alternatives to current energy technologies. Consequently, efficient and sustainable energy conversion is the focus of many research programs. Common areas of investigation in this field include improving the production of biofuels for combustion, solar cells, and hydrogen fuel cells. Conversely, a less studied biomimetic approach involves the direct conversion of simple sugars and alcohols into electricity using microorganisms. Termed microbial fuel cells (MFCs), these electrochemical devices utilize the enzymatic redox reactions of living microorganisms' metabolism to efficiently break down organic fuels and produce electric current. This is an attractive technology for it remedies both environmental and energy endeavors. The use of photosynthetic end products (biomass) as fuel, closes the carbon cycle, and the extraction of electricity directly through an electrochemical cell removes the Carnot efficiency limitation of thermal energy conversion. In addition, the use of inexpensive and self-renewable bacteria as the catalyst frees the demand of precious metals that are also a limited natural resource. In principle, potential applications of microbial fuel cells range from off-grid and portable electronics to industrial wastewater treatment, but the ultimate cost of materials and fabrication will dictate target markets. As we will show, MFCs are well suited for MEMS applications requiring steady, low level power sources with minimal maintenance.

Earlier MEMS-based MFCs have shown low energy conversion efficiency, low capacity, and short life time. Previously, we used Saccharomyces cerevisiae (baker’s yeast) as the catalyst, and immobilized Thylakoids, to convert biomass into electrical energy [1-3]. Electron shuttles were required in these systems to carry electrons from living cells or enzymes to the inorganic electrodes. This results in low efficiency as current density is lost through the long diffusion process, and mediators compromise the microorganisms' viability. Here, we tackle these bottlenecks by adopting exoelectrogenic bacteria that break down various organic fuels into electrons and protons. These bacteria transport the separated charge extracellularly, eliminating the need for toxic exogenous compounds. In addition, the bacteria produce “organic nanowire” appendages used as electrical connections to transfer electrons directly to the electrode for enhanced efficiency [4-7].

PRINCIPLES OF OPERATION
We have adopted Geobacter sulfurreducens as our preferred organism because of its ability to thrive in highly anoxic (low oxygen) environments and transport electrons extracellularly through its intrinsic metabolic processes. In addition, these bacteria are capable of fully oxidizing compounds such as acetate, ethanol, and pyruvate to carbon dioxide with insoluble irons or electrodes as the electron acceptor for respiration, much like aerobic cells use oxygen. As illustrated in Fig. 1, electrical energy is harvested extracellularly from the bacteria through an electrochemical cell by inoculating an anode with the microorganisms that then colonize the electrode to form bacterial films that can reach over 40 µm in thickness [5].

![Exoelectrogenic bacteria membrane schematic (inset) illustrating electron externalization model and the pilins, “organic nanowires”, of individual cells that form a network between cells and electrode. Here, bacteria break down acetate into electrons and protons to provide electricity.](image-url)
In the case of acetate consumption, charged species (protons and electrons) and CO₂ are the metabolic byproducts, as shown in Equation 1. Electrons are transferred to the electrode via appendage nanowires that serve as electrical conduits or through direct electrode-cytochrome (membrane bound protein involved in charge transfer) contact between individual cells and the inorganic electrode [6-7].

\[
\text{CH}_3\text{COO}^- + 2\text{OH}^- \rightarrow 2\text{CO}_2 + 8\text{e}^- + 5\text{H}^+ \quad (1)
\]

To complete the circuit, protons released from the bacteria to regulate internal acidity travel through a proton exchange membrane (PEM) towards the cathode. At the cathode, protons combine with an oxidant as shown in Figure 2.

DEFINITION AND FABRICATION

The prototype fuel cell anode geometry was designed to conform to the bacteria and biofilm dimensions. The anode consisted of a micro-patterned gold electrode that was 2 µm in width, the length of a single bacterium for single cell contact, arrayed at a 100 µm pitch to promote biofilm separation through the spacing of electrodes, as shown in Fig. 3a. The total anode surface area was 1 mm². The fabrication process started with the growth of a 500 nm oxide layer on a silicon wafer through a wet-furnace process, as shown in Fig. 3b. Next, photoresist was applied and patterned to define the electrodes using a lift-off process.

The electrochemical cell consisted of a two chamber flow-through configuration as shown in Fig. 4. The anodic chamber housed the bacteria in a 350 µL volume. The cathode consisted of a coiled gold wire with 100 mm² surface area immersed in 200 µL of catholyte. The two chambers were separated by a 50 mm² Nafion 212 membrane. A miniature reference Ag/AgCl electrode was incorporated in the anolyte. Lastly, because the anodic catalysis is of primary interest to this study, the cathodic reaction losses were mitigated by using potassium ferricyanide (K₃[Fe(CN)₆]) as an electron sink. The ferricyanide reduction to ferrocyanide proceeds as follows,

\[
[\text{Fe(CN)}_6]^{3-} + \text{e}^- \rightarrow [\text{Fe(CN)}_6]^{4-} \quad (2)
\]

EXPERIMENTAL PROCEDURE

The bacteria, *Geobacter sulfurreducens*, was initially cultured using anaerobic media in a PIPES buffer using 20 mM acetate and 80 mM fumarate as electron acceptor at 30°C. The bacteria were then washed and added to the anolyte to colonize the electrode. Prior to taking all measurements, anaerobic media and acetate (5mM) were added to the anodic chamber. Quantitative measurements were acquired with a Gamry (Warminster, PA) Reference 600 potentiostat.

The catholyte consisted of a solution of 50mM phosphate buffer (pH 7) and 50 mM K₃[Fe(CN)₆] with a redox potential of 436 mV (vs. SHE) at neutral pH. The reaction is quasi-reversible and the oxidized form can be renewed with aeration, but the set up used was anoxic and required continuous replenishment of the catholyte.
RESULTS AND DISCUSSION

In contrast to our previous work, this system does not require an electron mediator. The Geobacter electrochemical cell generates electrical power, as shown in Fig. 2, by harnessing electrons from the bacterial metabolic breakdown of acetate. The bacteria retrieve energy from the fuel for biological maintenance functions or cell division, then release electrons as metabolic waste to be collected by an electrode as useful electrical energy. The mechanism is analogous to a standard fuel cell but Geobacter acts as the catalyst and acetate as the fuel, while the “organic nanowires” act as interfaces to the inorganic electrode, eliminating the need for an electron mediator.

Performance

Figure 5 shows the MFC polarization over time as the bacteria colonize the electrode surface. With increasing time and cell count on the electrode, the overpotential or connectivity losses are mitigated, and the open circuit voltage and current density increase. The maximum current obtained after 10 days of continuous operation, using acetate as fuel and potassium ferricyanide as the catholyte, was 1.4 µA/1 mm². SEM images of the anode indicated that only a monolayer of bacteria covered the electrode during the attainment of these measurements. The maximum current could be increased by using a catholyte with a more oxidizing redox potential (such as pure oxygen) and allowing the bacteria to cover the anode surface with a fully developed biofilm. Figure 6 depicts the power obtained as a function of current after ten days of bacterial respiration. In this case, a maximum power of 0.12 µW occurs at 0.61 µA.

The bacteria can produce a high potential upon inoculation of the anode due to a “pre-charging” of the membrane that occurs during test tube incubation, assuming that fuel has been metabolized without an electron acceptor. Prior studies estimate the redox potential of the electrons from the bacteria at -0.2 V (vs. SHE) [4], making the maximum open circuit voltage, $V_{oc}$, possible from the cell here presented roughly 630 mV. Figure 7 displays open circuit voltages under various operating conditions. Upon bacterial addition to the anode, a $V_{oc} \approx 630$ mV is obtained. Here, the cells were incubated in a test tube for two weeks prior to anolyte inoculation. Next, after an initial “discharging” of the bacteria, the $V_{oc}$ reduces to 100 mV but increases over time as is illustrated by Fig. 5. After 10 days of respiration on the anode, $V_{oc}$ values ranged from 550-600 mV and maintained a low standard deviation (roughly 1 mV) over 10 minute intervals. The maximum steady state $V_{oc}$ was 619 mV or 98% of the maximum expected. Lastly, the control readings, taken with media and acetate but without bacteria, demonstrate negligible potential across the electrodes.

Figure 8 shows the MFC time response to different loads. These measurements were recorded 6 days after inoculation of the bacteria at a point where $V_{oc} \approx 0.2$ V. In each case, 90% of the $V_{oc}$ was regained immediately upon load removal. In addition, the power output (voltage and current) was stable for all the loadings over time.
Bacteria Viability

In addition to performance, the viability of the bacterial catalysts during respiration onto the electrode is also of interest. SEM images in Fig. 9 illustrate bacterial growth patterns on gold electrodes and on insulating oxide. After six days of respiration in the anodic chamber, bacteria more densely populate the electrode, as expected. Proximity to the conductive surface facilitates the release of electrons from charged enzymes used in metabolism and allows them to repeat the process efficiently. However, as is shown in Fig. 9b, cell division is common on the insulating surface making the current density contribution and connectivity means of these cells uncertain.

Figure 10 depicts the intricate rooting of bacteria on an electrode. Bacteria grow multiple appendages that form interconnects to the electrode and other bacteria [7].

CONCLUSIONS

Demonstrated here is a micro-fabricated MFC that uses Geobacter sulfurreducens as living catalyst and acetate as fuel. The system polarization (and power density) increases over time as the bacteria colonize the electrode surface. With a 1 mm² anode area, the fuel cell delivered \( V_{oc} = 619 \text{ mV} \) and \( P_{max} = 0.12 \mu \text{W} \) using potassium ferricyanide as catholyte. We hypothesize that further performance improvements are possible by allowing the bacterial biofilm to fully cover the anode, increasing the electrode surface area, and enhancing the connectivity of the bacteria to the electrode.

In addition to promising performance, the presented approach of using exoelectrogentic catalysts offers a number of advantages over previous methods. Because the bacteria externalize electrons through their natural metabolic processes, no oxidizing electron shuttle is needed and viability is maintained. The system presented harvested energy for over a week. In addition, Geobacter appears to have the capability of transferring high energy electrons directly to the electrode via membrane contact or organic pilins.

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