

## Microbial battery for efficient energy recovery

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Edited by Harry B. Gray, California Institute of Technology, Pasadena, CA, and approved August 9, 2013 (received for review April 18, 2013)

By harnessing the oxidative power of microorganisms, energy can be recovered from reservoirs of less-concentrated organic matter, such as marine sediment, wastewater, and waste biomass. Left unmanaged, these reservoirs can become eutrophic dead zones and sites of greenhouse gas generation. Here, we introduce a unique means of energy recovery from these reservoirs-a microbial battery (MB) consisting of an anode colonized by microorganisms and a reoxidizable solid-state cathode. The MB has a single-chamber configuration and does not contain ion-exchange membranes. Bench-scale MB prototypes were constructed from commercially available materials using glucose or domestic wastewater as electron donor and silver oxide as a coupled solid-state oxidant electrode. The MB achieved an efficiency of electrical energy conversion of 49% based on the combustion enthalpy of the organic matter consumed or 44% based on the organic matter added. Electrochemical reoxidation of the solid-state electrode decreased net efficiency to about 30%. This net efficiency of energy recovery (unoptimized) is comparable to methane fermentation with combined heat and power.

bioelectrochemical system | microbial fuel cells | exoelectrogens | renewable energy

urrent global energy demand is  $\sim 5.3 \times 10^{20}$  J/y (1). Most of this demand (>80%) is met by extraction and oxidation of the fossil carbon present in concentrated organic reservoirs as oil (32%), coal (27%), and natural gas (21%) (1). About 31% (1.7 ×  $10^{20}$  J/y) is used to produce 7.7 ×  $10^{19}$  J/y of electrical energy with an energy conversion efficiency of ~46% (1). The proven untapped reserves of oil, coal, and natural gas are  $9.1 \times 10^{22}$  J (2). Without carbon sequestration, these supplies would result in significant greenhouse gas emissions (1, 3). Identification of supplies that avoid such releases is a pressing challenge (4). One promising option is the use of less-concentrated reservoirs of organic matter. An untapped resource is the organic matter in marine sediment, estimated to contain  $5.2 \times 10^{22}$  J of stored chemical energy if oxidized with oxygen (5). Other reservoirs of lessconcentrated organic matter are also as yet untapped-in part because they are often viewed as waste, and in part because the technology needed to recover energy from less-concentrated reservoirs is inefficient. If not oxidized, these organics can deplete the oxygen reserves of aquatic ecosystems and stimulate release of methane to the atmosphere. An example is domestic wastewater, a threat to aerobic aquatic ecosystems. The organic matter in domestic wastewater is theoretically sufficient to generate  $\sim 7.4 \times 10^{18}$  J/y: three to four times more energy than is needed to treat the wastewater (6). However, 3% of the electrical load of developed countries is currently required to treat wastewater (7). Another waste example is the biomass produced through photosynthesis, most of which is not used for human needs. Globally,  $4.5 \times 10^{21}$  J/y are stored in biomass generated by photosynthesis (8). About 1% (5.0  $\times$  10<sup>19</sup> J/y) is harvested for human energy needs (9). The remaining biomass can undergo uncontrolled anaerobic biodegradation, with ensuing greenhouse gas emissions.

Effective energy extraction from less-concentrated organic reservoirs can potentially be achieved with microbial biotechnology. Self-assembled microbial communities have optimized energy extraction systems that allow efficient in situ oxidation of organic matter and other electron donors in diverse environments (4). Because these systems evolved under strong competitive pressures to meet microbial needs for energy (10), hijacking them for human purposes requires understanding of factors that influence microbial competition for energy. The most important factor is the nature of the available oxidants. In reservoirs rich in electron donors, the strongest oxidant ( $O_2$  in aerobic systems) is used first, followed by use of progressively weaker oxidants  $[NO_3^- > Mn$ (IV) minerals > Fe(III) minerals >  $SO_4^{2-}$  >  $CO_2$ ] (10). Carbon dioxide, the least powerful oxidant, is used last and is often the sole remaining oxidant in electron donor-rich anaerobic environments. A significant fraction of the electrons removed  $(\sim 90\%)$  is transferred to methane which can concentrate in the gas phase (11). The high efficiency of electron transfer to methane and the ease of methane recovery from water make methane fermentation a useful benchmark for energy recovery via microbial biotechnology. However, use of methane fermentation for energy generation has limitations: efficient digestion of biomass requires hydrolysis and fermentation at warm temperatures (>20 °C) (6, 11); inefficient capture of methane and losses during transport lead to energy losses, greenhouse gas emissions, and explosion hazards; and the collected biogas often requires clean-up to remove contaminants (hydrogen sulfide and siloxanes), limiting applications to large-scale digesters (12).

Microbial fuel cells (MFCs) offer an option for direct electricity generation from electron donors oxidized by microorganisms (13) and have been used to recover electricity from domestic wastewater and marine sediment (6, 14–16). Like chemical fuel cells, oxidation occurs at an anode, and electrons pass through an external circuit to a cathode where  $O_2$  is reduced. At the anode, however, chemical catalysts are replaced by exoelectrogens—microorganisms that oxidize the electron donors and transfer the electrons to an electrode (17). For MFCs, energy recovery is limited by a voltage loss when  $O_2$  is reduced at the cathode. This loss is exacerbated by MFC operating conditions atmospheric pressure, ambient temperature, and an aqueous

## Significance

This work introduces a microbial battery for recovery of energy from reservoirs of organic matter, such as wastewater. Microorganisms at an anode oxidize dissolved organic substances, releasing electrons to an external circuit, where power can be extracted. The electrons then enter a solid-state electrode that remains solid as electrons accumulate within it. The solid-state electrode is periodically removed from the battery, oxidized, and reinstalled for sustained power production. Molecular oxygen is not introduced into the battery, and ion-exchange membranes are avoided, enabling high efficiencies of energy recovery.

Author contributions: X.X., C.S.C., and Y.C. designed research; X.X., M.Y., P.-C.H., and N.L. performed research; X.X., M.Y., C.S.C., and Y.C. analyzed data; and X.X., M.Y., P.-C.H., N.L., C.S.C., and Y.C. wrote the paper.

The authors declare no conflict of interest.

This article is a PNAS Direct Submission.

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This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10. 1073/pnas.1307327110/-/DCSupplemental.



Fig. 1. Schematic of two-step energy generation process using microbial batteries (MBs). In step 1, electron donors are oxidized by microorganisms, releasing electrons to an intermediate solid-state electrode; in step 2, the solid-state electrode is regenerated by supplying a terminal electron acceptor. The key components are a microbial anode and a solid-state oxidant cathode. Organics are oxidized at the anode by the enzymatic activity of exoelectrogens. At the cathode, a solid-state oxidant is reduced.

electrolyte at near-neutral pH. Diffusion of dissolved  $O_2$  into the anode compartment is also a problem, allowing formation of aerobic biomass and oxidation of organic matter without energy production. Finally, methane production is sometimes reported in the anode compartment (18). This represents yet another energy loss and signals that methanogens are outcompeting exoelectrogens for the available electrons. Building on previous MFC studies, this work introduces a previously undescribed microbial electrochemical device for energy recovery where the key difference is the use of a solid-state cathode to replace the oxygen gas cathode of a MFC. Operation of the anode is like that of a MFC anode, but operation of the cathode is like that of a rechargeable battery. We therefore refer to this device as a microbial battery (MB).

## **Results and Discussion**

Sustained operation of the MB system requires two steps. In step 1, microorganisms at the anode oxidize electron donors

(e.g., carbohydrates and methane), releasing electrons that pass through an external circuit to a cathode comprising (or containing) a solid-state oxidant, such as silver oxide (Fig. 1, step 1). The cathode material becomes reduced. In the case of a silveroxide electrode, silver metal is produced. Step 1 is an energy extraction process like that of the discharge process in batteries. In step 2, the cathode is removed and oxidized by exposure to a terminal electron acceptor, such as oxygen, water, or carbon dioxide, under conditions favorable for its oxidation (Fig. 1, step 2). The oxidized electrode is then reinstalled—a step that differs only slightly from the normal process used to recharge batteries—and the process is repeated. The major drawbacks of MFCs—voltage losses at oxygen cathodes and diffusion of oxygen into the anode compartment—are avoided by single-chamber operation without the introduction of oxygen.

A design challenge for the MB is identification of a stable solid-state electrode with a suitable electrochemical potential.



**Fig. 2.** Carbon cloth-supported silver-oxide cathode (*A* and *B*) and carbon cloth microbial anode (*C* and *D*). (*A*) Scanning electron microscope (SEM) images of a silver-oxide electrode. The particles are less than 50 nm. (*B*) Reduction potential of a silver-oxide electrode in a phosphate buffer solution (PBS, 200 mM, pH 7) at current densities ranging from 0.01 to 1 mA/cm<sup>2</sup>. Current density values are labeled above the potential curve. The dashed line indicates the standard reduction potential. (*C*) SEM images of a colonized carbon cloth electrode. Zoom-in image (*Inset*) shows a cluster of exoelectrogens connected by microbial nanowires. (*D*) Cyclic voltammograms (CVs) of a colonized carbon cloth electrode and a plain carbon cloth electrode in a glucose electrolyte (~1 g/L). Scan rate, 1 mV/s.



**Fig. 3.** Performance of glucose-fed MBs and regeneration of the solid-state  $Ag_2O/Ag$  electrode. (*A*) Potentials of the microbial anode and the  $Ag_2O/Ag$  electrode during oxidation of a glucose solution (~1 g/L) at a fixed current density of 0.1 mA/cm<sup>2</sup>. The red arrows indicate regeneration of the  $Ag_2O/Ag$  electrode. (*B*) Polarization curves show the applied voltage and potentials of both electrodes during regeneration of the  $Ag_2O/Ag$  electrode with  $O_2$  reduction catalyzed by a commercially available carbon cloth supported platinum (Pt) electrode. The electrolyte was a sodium hydroxide (NaOH, 1 M, pH 14) solution bubbled with air (~100 mL/min). After changing the current density, potentials were recorded until equilibrium was reached (at least 10 min). (*C* and *D*) Voltage profiles of plate-shaped MBs with different external loadings (*C*) and in three consecutive cycles with a 2,000  $\Omega$  loading. The MBs were equipped with precolonized carbon cloth anodes and silver-oxide cathodes. The feeding was a glucose solution (~1 g/L). The red arrows in *D* indicate the replacement of fresh glucose feeding and the regeneration of the  $Ag_2O/Ag$  electrode.

One candidate couple is silver oxide/silver (Ag<sub>2</sub>O/Ag), a couple that has been used for decades in batteries (19). This couple offers several advantages: (*i*) both silver and silver oxide remain in the solid state in water and are stable under conditions favorable for microbial growth, i.e., ambient pressure and temperature and near-neutral pH; (*ii*) the standard reduction potential at pH 7 is 0.76 V vs. standard hydrogen electrode (SHE)—a value that is higher than the value for other electron acceptors commonly present in anaerobic environments (such as  $SO_4^{2-}$  and  $CO_2$ ), but lower than oxygen evolution (0.82 V vs. SHE at

pH 7); (*iii*) the potential difference between the anode and the silver oxide electrode is large enough to support energy extraction for human needs and to also sustain exoelectrogen growth and competition for electron donors (11, 17); (*iv*) hydroxyl alkalinity generated by reduction of silver oxide to silver at the cathode neutralizes acidity produced at the anode; and (*v*) silver has well-documented antimicrobial properties, preventing growth of microorganisms on the silver-oxide electrode and enabling operation within a single chamber without an ion-exchange membrane.



Fig. 4. MB energy recovery with a 2,000  $\Omega$  loading and a Ag<sub>2</sub>O/Ag solid-state electrode with coupled electrochemical regeneration of the Ag<sub>2</sub>O/Ag electrode. The heights of the boxes indicate the energy percentages.

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Electrode samples were prepared by embedding silver nanoparticles on a carbon cloth substrate followed by electrochemical oxidation (SI Appendix, Fig. S1). X-ray diffraction (XRD) verified the formation of silver oxide (SI Appendix, Fig. S2). Scanning electron microscope (SEM) images of the electrode surface are shown in Fig. 24. The silver-oxide electrodes were tested in a phosphate buffer solution (PBS, 200 mM, pH 7) to simulate operational conditions in MBs. The reduction potential under different current densities was recorded (Fig. 2B). Electrode potentials were constant during reduction, indicating that solidphase reduction of silver oxide to silver was stable. With reducing currents that varied from 0.01 to 1 mA/cm<sup>2</sup> (a typical operating current density range for microbial anodes), the reduction potentials of the electrodes remained stable at 0.53-0.49 V vs. SHE (Fig. 2B and SI Appendix, Fig. S3). These values were much higher than those of oxygen reduction (from 0.43 to -0.07 V vs. SHE) catalyzed by a commercially available platinum (Pt) electrode (Pt nanoparticles on a carbon cloth substrate) under the same conditions (SI Appendix, Fig. S3). Higher voltage outputs are attributed to lower overpotential losses (0.23-0.27 V for Ag<sub>2</sub>O vs. 0.39-0.89 V for O<sub>2</sub>), and the superior electrochemical properties of the Ag<sub>2</sub>O/Ag couple. When the same Ag<sub>2</sub>O/Ag electrode was operated in a sodium hydroxide electrolyte (NaOH, 1 M, pH 14), a typical condition in Ag<sub>2</sub>O/Ag batteries, the overpotential loss was less than 0.05 V at a current density of 1 mA/cm<sup>2</sup> (SI Appendix, Fig. S4). The greater losses observed in the PBS electrolyte were probably due to surface changes in the Ag<sub>2</sub>O/Ag electrode at neutral pH or local increase in pH near the Ag<sub>2</sub>O/Ag electrode surface during reduction.

The anode materials for MBs can be carbon cloth (20), graphite brush (21), or carbon nanotube/graphene-coated macroporous substrate, such as sponge (22, 23). Fig. 2C shows SEM images of a commercially available carbon cloth electrode colonized by exoelectrogens. The exoelectrogens remove electrons from electron donors in the electrolyte and transfer the electrons to the electrode by direct surface contact, diffusion of electron shuttles, and/or conduction through microbial nanowires (17, 24, 25). Electrochemical impedance spectroscopy (SI Appendix, Fig. S5) and cyclic voltammetry (Fig. 2D) established that exoelectrogens were actively oxidizing organics: In electrolyte amended with glucose (~1 g/L, an electron-equivalent concentration similar to that of typical domestic wastewater), a strong signal for electron transfer was only seen with carbon cloth colonized by microorganisms (SI Appendix, Fig. S5), and a positive current peak resulted from the oxidation of glucose (Fig. 2D).

Single-chamber MBs were built by placing the precolonized microbial anodes and silver-oxide cathodes into a bottle containing 100 mL glucose electrolyte ( $\sim 1$  g/L). The potentials of the microbial anode and the Ag<sub>2</sub>O/Ag electrode were recorded for a fixed current density of  $0.1 \text{ mA/cm}^2$ . As shown in Fig. 3A, the anode potential was stable at -0.12 V vs. SHE. This indicates that anode microbial activity was not affected by colocation of the Ag<sub>2</sub>O/Ag electrode in the same chamber. Because the volume of the system was large and the current relatively small, the decrease in glucose levels was not large enough to change the anode potential. The operating potential of the Ag<sub>2</sub>O/Ag electrode was about 0.52 V. The potential dropped quickly when the Ag<sub>2</sub>O/Ag electrode reached its capacity for storage of electrons, and most of the silver oxide was reduced to silver. At this point, the Ag<sub>2</sub>O/Ag electrode requires removal and regeneration with a terminal electron acceptor, such as oxygen (Fig. 1, step 2).

Reoxidation of silver by oxygen to silver oxide is thermodynamically favorable ( $\Delta G = -22.6 \text{ kJ/mol}$  at 25 °C and 1 atm O<sub>2</sub>) and is therefore expected to occur spontaneously (26). A thin silver-oxide layer is in fact observed when a clean silver surface is exposed to oxygen at normal pressures (27, 28). Complete oxidation occurs at 300 °C and 2 MPa O<sub>2</sub> (29). Simply bubbling oxygen through an aqueous solution of silver nanoparticles results in partial oxidation (30). Three different regeneration methods were investigated: direct exposure to air at room temperature (~20 °C), exposure to air at 90 °C, and immersion in aerated water bubbled with air. In all cases, the open circuit potential of the Ag<sub>2</sub>O/Ag electrode returned to about 0.48 V vs. SHE after regeneration, but the operating potential dropped significantly when a reducing current was applied (SI Appendix, Fig. S6A). The oxidation capacity-defined as the amount of charge delivered to the cathode before the operating potential of the Ag<sub>2</sub>O/Ag electrode fell to values less than 0.3 V vs. SHEincreased with the regeneration time (SI Appendix, Fig. S6B). After a 24-h regeneration period, the oxidation capacity could last for a few minutes when the reducing current was 0.1 mA/cm<sup>2</sup> (SI Appendix, Fig. S6A). Direct oxidation through such means could avoid use of noble metal catalysts for electrochemical reduction of O<sub>2</sub>. Electrochemical reoxidation is also an option for regeneration. Because this step occurs outside the MB, temperature, pressure, and electrolyte composition can be optimized. As proof-of-concept, we used a sodium hydroxide solution (NaOH, 1 M, pH 14) as the electrolyte and a commercially available Pt electrode for oxygen reduction. As shown by the polarization curves in Fig. 3B, a voltage of 0.20-0.35 V was needed at a regeneration current of  $0.01-1 \text{ mA/cm}^2$ . After regeneration, the Ag<sub>2</sub>O/Ag electrode was reinstalled in the MB with resumption of energy recovery (Fig. 3A). Because the Ag<sub>2</sub>O/Ag redox couple is reversible, the charge-discharge of Ag<sub>2</sub>O/Ag electrode in a NaOH electrolyte had a coulombic efficiency >97% over 200 cycles. A 20% drop in charge storage capacity occurred over the first 30 cycles—probably due to some loss of Ag<sub>2</sub>O/Ag, but  $\sim$ 70% of the capacity remained after 200 cycles (SI Appendix, Fig. S7).

Plate-shaped MBs with compact design  $(3 \times 3 \times 0.3 \text{ cm}^3)$  were constructed with precolonized microbial anodes  $(3 \times 3 \text{ cm}^2)$ and silver-oxide cathodes  $(3 \times 3 \text{ cm}^2)$  to estimate the efficiencies of MBs (*SI Appendix*, Fig. S8). The chamber was filled with 2 mL of glucose solution with an initial chemical oxygen demand (COD) of 1,170 mg/L. In this case, the oxidation capacity of the Ag<sub>2</sub>O/Ag electrode was sufficient to maintain a stable operating potential for one cycle, whereas potential of the microbial anode changed as glucose concentration became limiting. Output voltage of the cell decreased. The open circuit voltage was 0.78 V. With a 2,000-Ω external loading, the maximum output voltage was 0.69 V (Fig. 3C). When the voltage dropped to 0.4 V, solution COD decreased to 130 mg/L, a removal efficiency of 89%. The total charge, calculated by integrating the generated current over time, established that ~92% of the organics removed were



Fig. 5. Voltage profile of a plate-shaped MB oxidizing municipal wastewater organic matter with a 2,000  $\Omega$  loading. The MBs were equipped with precolonized carbon cloth anodes and silver-oxide cathodes. The feed was domestic wastewater with an initial COD of 390 ± 38 mg/L.

oxidized and contributed to current generation. No microbial growth was observed on the Ag<sub>2</sub>O/Ag cathode. Operation at two other loadings (1,000 Ω and 500 Ω) was also investigated (Fig. 3C) with similar results. COD removal efficiencies were 91 and 92% for 1,000 Ω and 500 Ω loadings, respectively, and, in both cases, coulombic efficiency was 91%. Fig. 3D shows the voltage profiles of plate-shaped MBs in three consecutive cycles with a 2,000-Ω loading. A higher voltage cutoff of 0.5 V was applied to maintain microbial activity over multiple cycles. The performance of the later two cycles was similar. The maximum output voltages (~0.62 V) were not as high as the first cycle, probably because there was insufficient time for recovery of microbial activity before glucose concentration again became limiting. COD removal efficiencies were 85 (cycle 1), 84 (cycle 2), and 82% (cycle 3), and coulombic efficiencies were 91–92% in all cases.

Fig. 4 summarizes the energy flow of the MB with a  $2,000-\Omega$ load (Fig. 3C) followed by electrochemical regeneration of the solid-state (Ag<sub>2</sub>O/Ag) electrode (SI Appendix, for calculation details, and Figs. S9 and S10). For this analysis, we consider all of the added glucose and assume that the total chemical energy is the combustion energy  $(2.8 \times 10^3 \text{ kJ/mol for glucose})$  (26). This assumption enables comparison with energy conversion by methane fermentation with combined heat and power (CHP). The energy balance is as follows: about 11% of the energy in the added glucose is retained in the electrolyte as residual electron donors; of the 89% removed, ~7% is used for biomass synthesis, leaving ~82% for microbial energy production; ~12% is used for microbial metabolism; overpotential at the solid-state electrode resulted in another 16% energy loss; and 10% energy loss resulted from losses due to internal resistance and diffusion limitations. The gross efficiency of electrical energy production was  $\sim$ 44%. This number increases to  $\sim$ 49% if the energy recovered is divided by the energy of the organic matter consumed. The energy required for electrochemical reoxidation ranged from ~14–24%, depending upon the current density  $(0.01-1 \text{ mA/cm}^2)$ applied. This step involves a tradeoff: reoxidization at higher current densities consumes more energy but is more rapid, so less electrode material is required for continuous electricity generation. The net energy conversion efficiency for energy production and electrode reoxidation was ~20-30% based on the organic matter added and ~21-33% based on the organic matter consumed. These values are comparable to methane fermentation with CHP (7).

The MB offers an alternative for energy recovery from soluble electron donors. Compared with methane production with CHP, it is likely to have fewer safety concerns and reduced environmental impact because methane production is avoided. The single-chamber design without ion-exchange membranes should be adaptable to different scales of operation. An obvious application is removal of biodegradable organics from domestic and industrial wastewaters. The same plate-shaped MB was tested with real domestic wastewater collected from a sewer at Escondido Village at Stanford University. Fig. 5 illustrates the voltage profile for oxidation of this wastewater-fed MB with a  $2,000-\Omega$ external loading. The voltage profile decreased gradually from 0.69 to 0.4 V during operation. This differed from the voltage profile of the glucose-fed MB, where a stable voltage plateau was followed by a decrease as glucose levels became limiting. This difference was likely due to differences in the biodegradability of

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municipal wastewater organics, with initial oxidation of readily biodegradable substances followed by oxidation of less-biodegradable substances. The COD removal efficiency was 53% from 390 to 170 mg/L, and the coulombic efficiency was 84%. The efficiency of electrical energy conversion was 38% based on the combustion enthalpy of COD removed or 22% based on total COD in the wastewater. The results demonstrate that the MB concept can be generalized for treatment of complex organic mixtures. For larger-scale applications, however, the high capital cost of the silver-based electrode would be prohibitive. More costeffective materials are needed for fabrication of rechargeable solid-state electrodes.

## **Materials and Methods**

Silver-oxide electrodes were prepared using a slurry-coating process followed by electrochemical oxidation. Silver nanoparticles (20 nm, SkySpring Nanomaterials, Inc., 85% wt) were mixed with conductive carbon black (Super-P, TIMCAL, 8% wt) and PVDF (Sigma-Aldrich, 7% wt) in NMP (Sigma-Aldrich). The mixture was stirred overnight and successively coated onto a carbon cloth (Fuel Cell Earth, LLC). The electrodes were dried in vacuum, resulting in a mass loading of ~20 mg/cm<sup>2</sup>. The silver electrode was then oxidized electrochemically in a sodium hydroxide solution (NaOH, 1 M, pH 14) at a current density of 1 mA/cm<sup>2</sup>. The process was stopped after the potential increased to 0.3 V vs. Ag/AgCl to avoid formation of silver peroxide (AgO). A carbon cloth-supported platinum (Pt) electrode (Pt loading 0.5 mg/cm<sup>2</sup>, Fuel Cell Earth, LLC) was used for oxygen reduction. Carbon cloth microbial anodes were used. These anodes were colonized in traditional H-shaped MFCs for more than 1 mo until a stable current was obtained (22). Titanium wires were used to connect the electrodes to the external circuits.

Plate-shaped MBs were manufactured at the Varian Physics Machine Shop at Stanford. Two pieces of Plexiglas, with a groove of  $3 \times 3 \times 0.15$  cm on each piece, were screwed together to form a chamber of  $3 \times 3 \times 0.3$  cm. A precolonized carbon cloth microbial anode ( $3 \times 3$  cm) and a carbon cloth-supported silver-oxide cathode ( $3 \times 3$  cm) were installed on each side with a separation distance of about 0.2 cm. Different resistors (500–2,000  $\Omega$ ) were applied as external loading, and the output voltages were recorded. The MBs were first filled with a phosphate buffer (PBS, 200 mM, pH 7). Oxidation of residual organic matter was accompanied by a decline in output voltage to 0.4 V. The electrolyte volume (2 mL) was replaced with fresh PBS buffer containing ~1 g/L glucose, and the current generation cycle was repeated. After the output voltage returned to 0.4 V, operation was stopped and the electrolyte analyzed.

Electrochemical characterization was achieved with a three-electrode setup using a BioLogic VMP3 potentiostat-galvanostat equipped with an electrochemical impedance spectroscopy (EIS) board. A double-junction Ag|AgCl|KCl (3.5 M) reference electrode (RE) was used for the measurement, with a Pt counter electrode (CE) used as necessary. EIS was conducted at the open circuit voltage (OCV) in the frequency range of 105-0.1 Hz with a 10 mV peak-to-peak sinusoidal potential perturbation. Cyclic voltammetry tests were performed on the microbial anodes over the potential range, -0.5 to -0.2 V vs. RE at a sweep rate of 1 mV/s. Polarization curves were obtained by changing the current density from 0.01 to 1 mA/cm<sup>2</sup> and monitoring potential until equilibrium was observed (at least 10 min). Oxygen reduction measurements were performed while the electrolyte was bubbled with air (~100 mL/min). XRD measurements were carried out with a PANalytical X'Pert (Ni-filtered Cu K $\alpha$  radiation), and scanning electron microscope (SEM) images were taken by FEI Nova NanoSEM. For SEM, the anode sample was pretreated with a fixing and critical point-drying process (22). The COD of the electrolyte was determined using a HACH COD analysis kit (HACH, Co.).

ACKNOWLEDGMENTS. X.X. acknowledges the support from the Stanford Interdisciplinary Graduate Fellowship.

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