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Abstract

Geobacter sulfurreducens can form electrically conductive biofilms, but the potential for conductivity through mixed species biofilms has not been examined. A current-producing biofilm grown from a wastewater sludge inoculum was highly conductive with low charge transfer resistance even though microorganisms other than *Geobacteraceae* accounted for nearly half the microbial community.

The discovery of long-range electron transport through electronically conductive biofilms offers new possibilities in microbe-electrode interactions and bioelectronics (10, 11, 16, 22) and has revealed the potential for microorganisms to make direct electrical connections for interspecies electron transfer (8, 19, 27). Most biofilms that have been studied are insulating (1, 2, 16, 20). The possibility of electrically conductive biofilms was first suggested based on the findings that: 1) *Geobacter sulfurreducens* produced thick (40-50 μ m) biofilms when growing on anode surfaces; 2) biofilm cells not in contact with the anode contributed to current production as much as cells in direct contact; and 3) the production of thick current-producing biofilms was dependent on the presence of conductive pili (25). Subsequent studies modeling current production in biofilms in which *Geobacter* species predominated found that it was necessary to include an empirically fitted conductivity value in the model in order to accurately predict observed current densities (18, 29).

Direct measurements of conductivity in current-producing biofilms of *Geobacter sulfurreducens* revealed high conductivities, rivaling those of synthetic conducting polymers (16). Multiple lines of evidence indicated that, as previously proposed (25), conductivity could be attributed to a network of pili (16). Multiple lines of evidence suggested that, surprisingly, the

pili have metallic-like conductivity (16). Metallic-like conductivity is a new paradigm for longrange electron transport in biological systems (12, 22) and it has been suggested that electron hopping between *c*-type cytochromes in biofilms, a more traditional mechanism of electron transfer, might account for electron transport through *G. sulfurreducens* biofilms (26). However, many experimental findings refute the electron-hopping hypothesis (12, 15).

Conductivity through biofilms is essential for high current densities in microbial fuel cells because it permits microorganisms not in direct contact with the anode to contribute to current production (10, 14, 25, 28). Conductive networks may also make it possible for microorganisms to directly exchange electrons in syntrophic partnerships (19, 27), which may be a more efficient mode of syntrophic interaction than interspecies hydrogen transfer (8).

Electrical conductivity of mixed-species current-producing biofilms.

The anodes of microbial fuel cells generating current from wastewater or organic matter in aquatic sediments can be colonized by a diversity of microorganisms (6, 9). In order to evaluate the conductivity of a mixed-species current-producing biofilm, an inoculum of anaerobic digester sludge from the Pittsfield, Massachusetts wastewater treatment plant was prepared as described earlier (21) and immediately inoculated into previously described (16) 'ministack' microbial fuel cells that contained two gold anodes (total 6.45 cm² geometric area) separated by a 50 μ m non-conducting gap. Anodes were connected by a 560 Ω load to a carboncloth cathode which was immersed in a 50 mM FeCN solution. External potential was not applied to the anode for the MFC operation, ensuring true fuel cell mode.10 mM acetate served as the electron donor and the incubation temperature was 37 °C. All results were confirmed by repeated measurements on multiple biofilms. The production of current in the microbial fuel cells (Fig. 1a) was associated with the growth of a biofilm that covered the two anodes and converged, bridging the non-conducting gap (Fig. 1b and Fig. 3a). When electrical conductance across the gap was measured as previously described (16), there was significant biofilm conductance (Fig. 1c). Biofilm conductivity (Fig. 1d), calculated with conformal mapping as previously described (16), was comparable to that previously reported for current-producing biofilms of strain KN400 (16) (See Supplemental material for details). As previously described, the effluent from the anode chamber was passed to another chamber which was identical with the exception that the two gold electrodes were not connected to the cathode (16). No biofilm grew in the control chamber and conductance between the two electrodes was low (Fig. 1c). The demonstrated high electrical conductivity of mixed-species derived biofilms provides an explanation for their capacity for high-current densities, $(0.9 \pm 0.45 \text{ A/m}^2)$, comparable to those obtained with *G. sulfurreducens* biofilms grown in the same type of 'ministack' microbial fuel cells, (0.7 A/m^2) , under similar conditions (16, 21).

Charge transfer resistance.

Charge transfer resistance represents an energy barrier at the electrode interface (14, 28). In addition to promoting long-range electron transport through biofilms, high biofilm conductivity can lower the charge transfer resistance (R_{cl}) at biofilm/anode interface because electrons reaching the biofilm/anode interface after traveling through a biofilm with higher conductivity will have greater energy than electrons transported through biofilms of lower conductivity (14, 28). This higher energy will reduce the energy barrier at the biofilm/anode interface that will lower the charge transfer resistance. This possibility was evaluated by measuring the charge transfer resistance using electrochemical impedance spectroscopy (14, 23).

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In this configuration, both the sides of the split-anode were connected to each other and used as the working electrode. The reference electrode was Ag/AgCl, placed in the anode chamber and the counter electrode was a carbon cloth, placed in the cathode chamber. The anode was disconnected from the cathode and all of the impedance measurements were performed at the open circuit potential of the anode (-550 mV vs. Ag/AgCl) (13, 14). For all comparisons between mixed-species and G. sulfurreducens biofilms, the amplitude excitation was 0.1V ac (4, 5). Linearity of the ac signal was ensured by measuring impedance over the amplitude range of 0.001 V - 0.1 V at the open circuit potential (Supplementary Fig. 2). The charge transfer resistance was evaluated from the measured impedance spectra by fitting (Supplementary Table 1) the previously described (14, 17, 23) equivalent circuit model (Supplementary Fig. 3). As expected, the charge transfer resistance of the mixed-species biofilms was much lower than in uninoculated controls (Fig. 2a, Supplementary Fig. 4). Charge transfer resistance of the mixedspecies biofilms declined with increased maturity, presumably reflecting enhanced electrical contact with the anode (Fig. 2b). In comparison to other measurements of charge transfer resistance made under similar conditions of open circuit potential, the charge transfer resistance of the mature mixed-species biofilms grown on gold electrodes $(1.45 \pm 0.32 \text{ K}\Omega \cdot \text{cm}^2)$ was higher than the 0.48 K Ω cm² previously reported for another mixed species biofilm grown on carbon electrodes (24), but comparable with biofilms of G. sulfurreducens strain KN400 (1.1 $K\Omega \cdot cm^2$), and much lower than the 204 $K\Omega \cdot cm^2$ reported for biofilms of Shewanella oneidensis (17).

Community analysis.

Current-producing mixed-species biofilms had two distinct layers (Fig. 3a)– a top, outer brown layer that was loosely attached to the anode (Fig. 3b) and a bottom, inner pink layer that was strongly attached to the anode (Fig. 3c). In order to identify the microorganisms which were conferring conductivity to mixed-species biofilms, clone libraries of 16S ribosomal RNA genes were constructed from the initial inoculum, as well as for inner pinkish biofilm, which was closely attached to the electrode, and for outer brownish biofilm, which was loosely attached to the electrode. At day 54 the outer and inner layers of the biofilm were individually sampled with a micropipette for community analysis. As previously described (3, 19), genomic DNA was extracted, 16S rRNA gene sequences were amplified with PCR, cloned, and sequenced. Detailed experimental procedure for community analysis is provided in supplemental material and the results are presented in Fig. 3c. The initial inoculum had 8% of *Geobacteraceae* whereas the proportion of *Geobacteraceae* in the inner and the outer biofilm zones was ca. 50 and 10%, respectively.

Implications.

The finding that mixed-species biofilms can possess electrical conductivity comparable to pure culture biofilms of *G. sulfurreducens* with low charge transfer resistance provides an explanation for the capacity of mixed-species biofilms to produce the thick biofilms necessary for high current densities. Modeling studies have previously demonstrated that invoking a highly conductive biofilm could explain the effective function of high-current-density multi-species biofilms in which *Geobacter* species predominated (18, 29, 30). The conductivity of the mixed-species biofilms was an order of magnitude higher than that of multi-species methanogenic

aggregates derived from wastewater digesters (19) and two orders of magnitude higher than that of dual-species *Geobacter* aggregates (27). There may be stronger selection for higher conductivity in current-producing biofilms than in the previously described conductive aggregates because electrons released from microorganisms near the outer surface of currentproducing biofilms need to be transported much farther than in cell aggregates, in which electrons only need to be transported to nearby cells.

It is not possible to determine from the data available whether microorganisms other than *Geobacter* species contributed the conductivity of the mixed-species biofilms. Biofilms of *Escherichia coli* and *Pseudomonas aeruginosa* grown on the two-electrode device described here were not conductive (16) and other microbial biofilms were also found to have poor conductivity (1, 2, 20). Other current-producing microorganisms such as *Shewanella oneidensis* (7) and *Thermincola* strain JR (31) do not form thick biofilms when producing current, suggesting that they are incapable of forming highly conductive biofilms. Thus, these results indicate that biofilms containing high proportions of organisms other than *Geobacter* species may be conductive, but whether the other organisms contribute to biofilm conductivity warrants further investigation.

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REFERENCS

- Dheilly, A., I. Linossier, A. Darchen, D. Hadjiev, C. Corbel, and V. Alonso. 2008. Monitoring of microbial adhesion and biofilm growth using electrochemical impedancemetry. Applied Microbiology and Biotechnology 79(1):157-64.
- Herbert-Guillou, D., B. Tribollet, D. Festy, and L. Kiéné. 1999. In situ detection and characterization of biofilm in water by electrochemical methods. Electrochimica Acta 45:1067-1075.
- 3. Holmes, D. E., K. P. Nevin, T. L. Woodard, A. D. Peacock, and D. R. Lovley. 2007. Prolixibacter bellariivorans gen. nov., sp. nov., a sugar-fermenting, psychrotolerant anaerobe of the phylum Bacteroidetes, isolated from a marine-sediment fuel cell. International Journal of Systematic and Evolutionary Microbiology 57:701-707.
- Hong, J., D. S. Yoon, S. K. Kim, T. S. Kim, S. Kim, E. Y. Pak, and K. No. 2005. AC frequency characteristics of coplanar impedance sensors as design parameters. *Lab Chip* 5:270-279.
- Hong, J., D. S. Yoon, M. I. Park, J. Choi, T. S. Kim, G. Im, S. Kim, Y. E. Pak, and K. No. 2004. A dielectric biosensor using the capacitance change with AC frequency integarated on glass substrates. Jpn. J. Appl. Phys 43:5639-5645.
- Kiely, P. D., J. M. Regan, and B. E. Logan. 2011. The electric picnic: synergistic requirements for exoelectrogenic microbial communities. Current Opinion in Biotechnology 22:378-385.

- Lanthier, M., K. B. Gregory, and D. R. Lovley. 2008. Growth with high planktonic biomass in *Shewanella oneidensis* fuel cells. FEMS Microbiology Letters 278:29-35.
- Lovley, D. 2011. Reach out and touch someone: potential impact of DIET (direct interspecies energy transfer) on anaerobic biogeochemistry, bioremediation, and bioenergy. Reviews in Environmental Science and Biotechnology 10:101-105.
- Lovley, D. R. 2006. Bug juice: harvesting electricity with microorganisms. Nature Reviews: Microbiology 4:497-508.
- Lovley, D. R. 2011. Live wires: direct extracellular electron exchange for bioenergy and the bioremediation of energy-related contamination. Energy & Environmental Science 4:4896-4906.
- Lovley, D. R. 2011. Powering microbes with electricity: direct electron transfer from electrodes to microbes. Environmental Microbiology Reports 3:27-35.
- Malvankar, N. S., and D. R. Lovley. 2012. Microbial Nanowires: A new paradigm for biological electron transfer and bioelectronics. ChemSusChem DOI:10.1002/cssc.201100733.
- Malvankar, N. S., T. Mester, M. T. Tuominen, and D. R. Lovley. 2012. Supercapacitors based on c-type cytochromes using conductive nanostructured networks of living bacteria. ChemPhysChem 13:463 - 468.

- Malvankar, N. S., M. T. Tuominen, and D. R. Lovley. 2012. Biofilm conductivity as a decisive variable for high-current-density microbial fuel cells. Energy & Environmental Science 5:5790-5797.
- Malvankar, N. S., M. T. Tuominen, and D. R. Lovley. 2012. Comment on "On electrical conductivity of microbial nanowires and biofilms" by S. M. Strycharz-Glaven, R. M. Snider, A. Guiseppi-Elie and L. M. Tender, Energy Environ. Sci., 2011, 4, 4366 Energy & Environmental Science 5:6247-6249.
- Malvankar, N. S., M. Vargas, K. P. Nevin, A. E. Franks, C. Leang, B.-C. Kim, K. Inoue, T. Mester, S. F. Covalla, J. P. Johnson, V. M. Rotello, M. T. Tuominen, and D. R. Lovley. 2011. Tunable metallic-like conductivity in microbial nanowire networks. Nature Nanotechnology 6:573-579.
- Manohar, A. K., O. Bretschger, K. H. Nealson, and F. Mansfeld. 2008. The use of electrochemical impedance spectroscopy (EIS) in the evaluation of the electrochemical properties of a microbial fuel cell. Bioelectrochemistry 72:149-154.
- Marcus, A. K., C. I. Torres, and B. E. Rittmann. 2007. Conduction-based modeling of the biofilm anode of a microbial fuel cell. Biotechnology and Bioengineering 98:1171-1182.
- Morita, M., N. S. Malvankar, A. E. Franks, Z. M. Summers, L. Giloteaux, A. E. Rotaru, C. Rotaru, and D. R. Lovley. 2011. Potential for Direct Interspecies Electron Transfer in Methanogenic Wastewater Digester Aggregates. mBio 2:e00159-11.

- Muñoz-Berbel, X., F. J. Muñoz, N. Vigués, and J. Mas. 2006. On-chip impedance measurements to monitor biofilm formation in the drinking water distribution network. Sensors & Actuators: B. Chemical 118:129-134.
- Nevin, K. P., H. Richter, S. F. Covalla, J. P. Johnson, T. L. Woodard, A. L. Orloff, H. Jia, M. Zhang, and D. R. Lovley. 2008. Power output and coulombic efficiencies from biofilms of *Geobacter sulfurreducens* comparable to mixed community microbial fuel cells. Envionmental Microbiology 10:2505-2514.
- Qian, F., and Y. Li. 2011. Biomaterials: A natural source of nanowires. Nature Nanotechnology 6:538-539.
- Rabaey, K., L. Angenent, U. Schröder, and J. Keller. 2009. Bioelectrochemical Systems: From Extracellular Electron Transfer to Biotechnological Application. Intl Water Assn.
- Ramasamy, R. P., Z. Ren, M. M. Mench, and J. M. Regan. 2008. Impact of initial biofilm growth on the anode impedance of microbial fuel cells. Biotechnology and bioengineering 101:101-108.
- Reguera, G., K. P. Nevin, J. S. Nicoll, S. F. Covalla, T. L. Woodard, and D. R. Lovley. 2006. Biofilm and nanowire production leads to increased current in *Geobacter sulfurreducens* fuel cells. Applied and Environmental Microbiology 72:7345-7348.

- Strycharz-Glaven, S. M., R. M. Snider, A. Guiseppi-Elie, and L. M. Tender. 2011. On the electrical conductivity of microbial nanowires and biofilms. Energy & Environmental Science 4:4366-4379.
- Summers, Z. M., H. E. Fogarty, C. Leang, A. E. Franks, N. S. Malvankar, and D. R. Lovley. 2010. Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. Science 330:1413-1415.
- Torres, C. I., A. K. Marcus, H. S. Lee, P. Parameswaran, R. Krajmalnik-Brown, and B. E. Rittmann. 2009. A kinetic perspective on extracellular electron transfer by anode-respiring bacteria. FEMS Microbiology Reviews 34:3-17.
- 29. Torres, C. I., A. K. Marcus, P. Parameswaran, and B. E. Rittmann. 2008. Kinetic experiments for evaluating the Nernst-Monod model for anode-respiring bacteria (ARB) in a biofilm anode. Environmental Science & Technology 42:6593-6597.
- Torres, C. s. I., R. Krajmalnik-Brown, P. Parameswaran, A. K. Marcus, G. Wanger,
 Y. A. Gorby, and B. E. Rittmann. 2009. Selecting Anode-Respiring Bacteria Based on
 Anode Potential: Phylogenetic, Electrochemical, and Microscopic Characterization.
 Environmental Science & Technology 43:9519-9524.
- Wrighton, K. C., P. Agbo, F. Warnecke, K. A. Weber, E. L. Brodie, T. Z. DeSantis,
 P. Hugenholtz, G. L. Andersen, and J. D. Coates. 2008. A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. Isme J 2:1146-1156.

FIGURES LEGENDS

FIG. 1. (a) Current production and **(c)** conductance data over days for mixed-species biofilm in split-anode microbial fuel cell and corresponding control. Error bars: Standard deviation. The fuel cell was switched to flow through mode at day 10 that removes planktonic cells (21).

(b) Confocal laser scanning microscopy image (3D-reconstruction) of mixed-species biofilm showing that the biofilm bridged the non-conductive gap. Gap is designated by arrows. Scale bar, 250 μ m. Biofilm thickness is 50.28 ± 8.13 μ m. Biofilms were stained with the LIVE/DEAD BacLight Bacterial Viability Kit. (d) The conductivity of mixed-species biofilm and corresponding control which lacked biofilm at day 54. Error bars: Standard deviation.

FIG. 2 (a) Charge-transfer resistance for the mixed-species biofilm as a function of fuel cell current. Error bars: Standard deviation. **(b)** Comparison of charge transfer resistance of mixed-species biofilm and corresponding control. Error bars: Standard deviation.

FIG. 3 (a) Schematic of mixed-species biofilm formation. **(b)** Image of outer, top-layered brownish biofilm that is loosely attached to the anode. Scale bar 1 cm. **(c)** Image of inner, bottom-layered pinkish biofilm that is strongly attached to the anode. Scale bar 1 cm. **(d)** Community analysis of wastewater inoculum as well as in the inner and outer biofilms. Left hand side charts show the division based on the phylums and right hand side charts show the division based on species.



FIGURES

FIG. 1. (a) Current production and (c) conductance data over days for mixed-species biofilm in split-anode microbial fuel cell and corresponding control. Error bars: Standard deviation. The fuel cell was switched to flow through mode at day 10 that removes planktonic cells (21). (b) Confocal laser scanning microscopy image (3D-reconstruction) of mixed-species biofilm showing that the biofilm bridged the non-conductive gap. Gap is designated by arrows. Scale bar, 250 μ m. Biofilm thickness is 50.28 ± 8.13 μ m. Biofilms were stained with the LIVE/DEAD BacLight Bacterial Viability Kit. (d) The conductivity of mixed-species biofilm at day 54 and corresponding control which lacked biofilm. Error bars: Standard deviation.



FIG. 2 (a) Comparison of charge transfer resistance of mature mixed-species biofilm and corresponding control. Error bars: Standard deviation. **(b)** Charge-transfer resistance for the mixed-species biofilm as a function of fuel cell current. Error bars: Standard deviation.



FIG. 3 (a) Schematic of mixed-species biofilm formation. (b) Image of outer, top-layered brownish biofilm that is loosely attached to the anode. Scale bar 1 cm. (c) Image of inner, bottom-layered pinkish biofilm that is strongly attached to the anode. Scale bar 1 cm. (d) Community analysis of wastewater inoculum as well as in the inner and outer biofilms. Left hand side charts show the division based on the phylums and right hand side charts show the division based on families.