# Electromicrobiology

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#### Abstract

Electromicrobiology deals with the interactions between microorganisms and electronic devices and with the novel electrical properties of microorganisms. A diversity of microorganisms can donate electrons to, or accept electrons from, electrodes without the addition of artificial electron shuttles. However, the mechanisms for microbe-electrode electron exchange have been seriously studied in only a few microorganisms. Shewanella oneidensis interacts with electrodes primarily via flavins that function as soluble electron shuttles. Geobacter sulfurreducens makes direct electrical contacts with electrodes via outer-surface, c-type cytochromes. G. sulfurreducens is also capable of long-range electron transport along pili, known as microbial nanowires, that have metallic-like conductivity similar to that previously described in synthetic conducting polymers. Pili networks confer conductivity to G. sulfurreducens biofilms, which function as a conducting polymer, with supercapacitor and transistor functionalities. Conductive microorganisms and/or their nanowires have a number of potential practical applications, but additional basic research will be necessary for rational optimization.

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#### INTRODUCTION

Electromicrobiology is the study of microbial electron exchange with external electronic devices and functionalities of microorganisms that have the potential to contribute to the emerging field of bioelectronics. A wide diversity of microorganisms have the ability to exchange electrons with electrodes, which contribute to a broad range of practical applications (4, 30, 58–60, 62, 63, 101, 102, 108). Furthermore, some microorganisms have surprising electronic characteristics. For example, biofilms of *Geobacter* species have conductivities that rival those of conductive polymers (74), and can function as supercapacitors (71) or transistors (74). The pili of these organisms are capable of long-range (>1 cm) electron transport via a metallic-like conductivity, not previously observed in a biological material (74).

Many of the recent advances in electromicrobiology have arisen from the study of microbial fuel cells, devices initially designed for harvesting electricity from organic matter (58, 59, 101). Difficulties in scaling up microbial fuel cells for extracting energy on an industrial scale have greatly limited their short-term practical use for current production to niche applications, such as harvesting organic matter from aquatic sediments to power electronic monitoring devices (65). Some of the most attractive practical applications of the microbial fuel cell concept are those in which there is no need to harvest current. These include monitoring rates of microbial metabolism in subsurface environments (137) and providing electrodes as an electron acceptor to stimulate the degradation of organic contaminants in sediments (63, 142). Another promising application of the ability of microorganisms to transfer electrons to electrodes is the potential for balancing electron flow within microbial cells by removing excess electrons in order to promote the synthesis of desired products (29).

In a similar manner, new potential applications for electron flow in the reverse direction, i.e., from electrodes to cells, are rapidly emerging (64, 102, 127). Feeding electrons to microorganisms living on electrode surfaces has significant potential to contribute to bioremediation of a diversity of contaminants, including radioactive and toxic metals (38, 124), chlorinated compounds (1, 12, 119, 122, 128), and nitrate (37, 96). Microorganisms have the potential to catalyze the production of hydrogen and methane with electrons derived from electrodes (14, 35, 64, 133, 134). Electrons derived from electrodes can potentially serve as the reductant for effecting microbial reduction of organic compounds to more desirable organic commodities, or for altering fermentation pathways in desired directions (26, 44, 95, 118). Furthermore, with the newly developed process of microbial electrosynthesis (87, 93), it is possible to electrically power the microbial reduction of carbon

#### **Electromicrobiology:**

the study of microbial electron exchange with electronic devices or the investigation of the electronic properties of microorganisms

#### **Bioelectronics:**

technology incorporating electronics in biological applications or developing electronic devices from biological components or electronic components that mimic biological materials

#### Microbial fuel cell:

device for harvesting electricity from organic matter in which microorganisms are the catalyst for oxidizing the organic matter dioxide to liquid transportation fuels and other useful organic commodities (63, 64). When driven with electricity generated from solar technologies, microbial electrosynthesis is functionally an artificial form of photosynthesis with the potential to be much more efficient and environmentally sustainable than biomass-based strategies for fuel and chemical production.

Findings from the study of microbe-electrode exchange have also led to new insights into the functioning of anaerobic ecosystems (63). For over 40 years it has been considered that microorganisms in methanogenic environments exchange electrons primarily via interspecies transfer of hydrogen, with the electron-donating microorganism disposing of electrons by reducing protons to hydrogen and an electron-accepting methanogen oxidizing hydrogen with the reduction of methane (81, 117). However, it is feasible for different species of microorganisms to forge direct electrical connections, similar to those that they establish with electrodes (123), and direct interspecies electron transfer can be the primary mechanism for electron exchange in microbial aggregates converting wastes to methane (86).

These developments demonstrate that a better understanding of the mechanisms by which microorganisms exchange electrons with electrodes could benefit the development of various new technologies as well as provide a better understanding of anaerobic microbial ecology. As more is learned, additional applications will probably emerge. This review summarizes current knowledge on microbe-electrode interactions and the novel electronic materials that some microorganisms can produce.

#### WHY MICROORGANISMS MIGHT INTERACT ELECTRONICALLY WITH ELECTRODES

A key to understanding the mechanisms of microbe-electrode interactions may be to elucidate how this capability evolved. For example, short-term adaptive evolution studies have provided insight into the mechanisms for electron transfer to electrodes and Fe(III) oxides (132, 141). The mere fact that microorganisms are able to exchange electrons with electrodes to produce electric current and can consume current to power their respiration is fascinating, especially when it is considered that electrodes, per se, are not a part of the natural environment. It has been suggested that microbe-electrode exchange is a fortuitous result of the fact that some microorganisms have developed over billions of years of evolution effective strategies for extracellular electron exchange with insoluble minerals and related natural extracellular electron acceptors or donors (59, 64).

However, there is a significant difference between insoluble minerals and electrodes. Electrodes provide a surface with long-term electron-accepting or electron-donating capacity, whereas the ability of individual insoluble minerals to accept or donate electrons is eventually depleted. Hence, the relationship between cells and electrodes is different from that between cells and minerals. This is readily apparent with *Geobacter* species. When actively reducing Fe(III) oxides, *Geobacter* species express flagella and are motile, presumably because they need to continually search for new sources of Fe(III) (15, 63). In contrast, when *Geobacter* species are oxidizing organic compounds with electron transfer to electrodes, they are not planktonic. The cells firmly attach to the electrode surface and form thick (>80  $\mu$ m) metabolically active biofilms (32, 88, 104). The physiological status of these sessile cells packed in biofilms and provided with a constant electron acceptor is expected to be much different from that of planktonic cells actively hunting for minerals on which to dump electrons. Electron transfer to electrodes can be much faster than electron transfer to Fe(III) (4). Is there a better natural analog for electrodes than small individually dispersed minerals?

One option may be graphite deposits. These deposits, which have significant conductivity, can span distances up to 1 km and appear able to transmit electrons between anaerobic and oxic zones

## Microbial electrosynthesis:

strategy for directly converting carbon dioxide to transportation fuels or other organic commodities in which microorganisms accept electrons from an electrode to reduce the carbon dioxide to the desired product, which is excreted from the cell

## Direct interspecies electron transfer:

microbial syntrophy in which microorganisms establish electrical connections for the transfer of electrons from one syntrophic partner to another **Anode:** electrode that accepts electrons

#### **Electron shuttle:**

compound that facilitates electron transfer between microorganisms and electrodes

Long-range electron transport: transport of electrons over multiple cell lengths in the subsurface, producing a geobattery (3). In this initial geobattery concept, electron transfer to the graphite in the anaerobic zone was discussed as abiotic, with reduced chemical species such as ferrous iron donating electrons to the graphite (3). In a similar manner, it was initially proposed that electron transfer to electrodes was abiotic in benthic microbial fuel cells designed to harvest electricity from anaerobic marine sediments (105). However, subsequent studies (5, 41, 125) revealed a specific enrichment of *Geobacteraceae* microorganisms on the surface of the electronaccepting electrodes (the anodes), which could be attributed to the ability of these microorganisms to oxidize organic compounds with direct electron transfer to electrodes. Graphite is a preferred electrode material. The ability of the electron-accepting end of geobattery graphite deposits to serve as a consistent, long-term electron sink would provide an environment highly analogous to the graphite anodes of microbial fuel cells. Therefore, it seems likely that microorganisms that are highly effective in current production in microbial fuel cells may have first perfected this capability as catalysts promoting electron flow in geobatteries. Additional study of these, and possibly other natural analogs for electrodes, could enhance our understanding of microbe-electrode interactions.

#### MECHANISMS FOR ELECTRON TRANSFER TO ELECTRODES

As previously reviewed in detail (59), many microorganisms can exchange electrons with electrodes when artificial electron shuttles are provided. Shuttles such as methylviologen, neutral red, or thionine can accept electrons from redox-active moieties within cells and transfer the electrons to electrodes. However, the practical benefit of this type of electrical interaction has yet to be proved; electron shuttles are often unstable and toxic, are uneconomical in large-scale processes, and cannot be employed in open environments.

Microorganisms transfer electrons to an electrode without the addition of an artificial electron shuttle in three ways (**Figure 1**): (*a*) electron transfer via microbially produced soluble redox-active molecules, (*b*) short-range direct electron transfer between redox-active molecules on the outer cell surface and the electrode; and (*c*) long-range electron transport through conductive biofilms (62). As detailed below, *Geobacter sulfurreducens*, the microorganism that produces the highest currents in pure culture (92, 141), appears to accomplish this with a combination of long-range electron transport through thick, conductive biofilms and short-range electron transfer between the conductive biofilm and the electrode that is mediated by an extracellular *c*-type cytochrome.

#### **Electron Transfer via Soluble Electron-Shuttling Molecules**

A diversity of both gram-negative and gram-positive microorganisms have the ability to produce electron shuttles to promote electron transfer to electrodes (101). The concept of self-produced electron shuttles facilitating electron transfer to electrodes follows previous studies that demonstrated that some microorganisms produce shuttles that promote electron transfer between cells and insoluble Fe(III) oxides (54, 90, 91, 94). For example, *Geothrix fermentans*, which can reduce Fe(III) oxide enclosed in porous alginate beads via a shuttle (90), also appeared to release an electron shuttle to promote electron transfer to electrodes (7).

*Shewanella* species have a similar ability to reduce Fe(III) with which they are not in direct contact (54, 91); this is attributed to the release of flavin in *S. oneidensis* cultures (135). The finding that cells of *S. oneidensis* were primarily planktonic in microbial fuel cells suggested that an electron shuttle was also involved in electron transfer to electrodes (50). The role of flavins in promoting electron transfer to electrodes with *S. oneidensis* has been well established by electrochemical studies (2, 78). *S. oneidensis* can reduce flavins at the outer cell surface with the *c*-type cytochrome



#### Figure 1

Potential mechanisms for microorganisms to transfer electrons to electrodes. (*a*) Short-range electron transfer by microorganisms in close association with the electrode surface through redox-active proteins, such as *c*-type cytochromes associated with the outer cell surface or in the extracellular matrix. (*b*) Electron transfer via reduction of soluble electron shuttles released by the cell. Oxidized shuttle molecules are reduced at the outer cell surface, and the reduced shuttle molecules donate electrons to the electrode. (*c*) Long-range electron transport through a conductive biofilm via electrically conductive pili, accompanied by short-range electron transfer from the biofilm to the electron mediated by extracellular cytochromes as in panel *a*.

MtrC (18), which is part of a multiprotein complex that transports electrons from the periplasm to the outer surface of the cell (16, 39).

Electrons can hop directly from MtrC to an electrode (2, 55). However, direct electron transfer in intact cells was possible only when anodes were artificially poised at positive potentials significantly higher than those typical of microbial fuel cells, and the rates of electron transfer were much faster in the presence of flavin. These results suggest that electron transfer via a flavin is the preferred route of electron transfer in *S. oneidensis* microbial fuel cells. Furthermore, elegant studies in which direct contact between *S. oneidensis* and electrodes could be prevented with a nonconducting mask with nanohole openings demonstrated that current was produced when the possibility for contact was eliminated as well as when cells established contact (48). The conclusion from these studies was that electron transfer via an electron shuttle was the predominant means of electron transfer even when cells were in contact with the electrode. In a similar manner, it is possible for MtrC to transfer electrons directly to Fe(III) oxides, but the rates of electron transfer are too low to account for observed rates of Fe(III) oxide reduction (109). MtrC serves as flavin reductase (18), and only in the presence of flavin can MtrC transfer electrons to Fe(III) oxide at physiologically relevant rates (109). The maximum current densities produced by microorganisms that rely on electron shuttling to transfer electrons to electrodes are much lower than those for microorganisms capable of long-range electron transport through thick conductive biofilms because the slow diffusive flux of the shuttle is a major limitation (129). Although the shuttling mechanism may be somewhat effective in closed laboratory systems, in open environments this approach suffers from losses of the shuttle from the immediate microbe-electrode interface. For these and other reasons (59), it is not surprising that *Shewanella* species have never been found to be important constituents of anodes harvesting electricity from complex organic matter in open environments (49, 63).

#### Short-Range Direct Electron Transfer via Redox-Active Proteins

Evidence for direct electron transfer to electrodes has been presented for several microorganisms (6, 13, 40, 77, 139, 140). The mechanisms for direct electron transfer to electrodes have been studied most extensively in *G. sulfurreducens*. *G. sulfurreducens* is closely related to the *Geobacter* species that typically predominate on electrodes harvesting current from organic matter, especially when oxygen intrusions are eliminated so that organic substrates are efficiently converted to current, and when the electrode potential is not artificially poised with electronics (49, 66). Early investigations suggested that, just as *Geobacter* species do not use shuttles to reduce Fe(III) oxide (89), shuttles are not involved in electron transfer to electrodes (6). This was subsequently demonstrated more definitively by electrochemical studies (9, 10, 79, 80, 106).

*G. sulfurreducens* has a wide diversity of *c*-type cytochromes (84), many of which are exposed on the outer surface of the cell (20, 45, 53, 83, 99). The outer-surface *c*-type cytochromes that have been purified can reduce known extracellular electron acceptors in vitro (45, 67, 99). Gene deletion studies suggest that these same *c*-type cytochromes transfer electrons to a diversity of extracellular electron acceptors in vivo (51, 52, 83, 112, 136). Numerous studies of current-producing *G. sulfurreducens* biofilms have demonstrated that *c*-type cytochromes are in electrochemical communication with the anode (9, 10, 11, 27, 34, 47, 56, 57, 79, 80, 85, 106, 116, 121). In some instances the cytochromes to the electrode (9) and hence function as the electrochemical gate between cells in contact with the electrode and the electrode surface (21).

Comparison of gene expression in current-producing cells versus expression in cells growing on alternative electron acceptors, as well as gene deletion studies, identified several candidate outer-surface *c*-type cytochromes that might help *G. sulfurreducens* make electrical contacts with electrodes (42, 88). OmcS was implicated in thin biofilms generating low levels of current (42), but OmcZ appears to be the most important cytochrome in biofilms producing high levels of current (88). OmcZ is a hydrophobic protein with a molecular mass of 30 kDa (45). It has eight hemes, which cover a wide range of redox potentials (-420 mV to -60 mV). The midpoint potential is -220 mV. Deletion of *omcZ* greatly inhibited current production (88), as did deletion of another gene that significantly reduced the abundance of OmcZ on the outer cell surface (107). Cyclic voltammetry demonstrated an increased resistance to electron transfer to electrodes in the OmcZdeficient strain. Deleting genes for other outer-surface *c*-type cytochromes did not yield a similar response (106).

Immunogold labeling of current-producing biofilms demonstrated that significant quantities of OmcZ accumulated at the biofilm/anode interface, indicating that it was ideally positioned to facilitate electron transfer to electrodes (45). This accumulation of OmcZ was not observed in biofilms grown on the electrode material, but with fumarate serving as the electron acceptor (45).

Thus, multiple lines of evidence suggest that OmcZ is the key cytochrome for electron transfer between *G. sulfurreducens* biofilms and anodes. Further investigation of the properties that uniquely

suit OmcZ for this purpose is required. Also, although it is possible for *G. sulfurreducens* to overcome deletion of other genes that are highly expressed in current-producing biofilms, such as the gene for the outer-membrane-bound *c*-type cytochrome OmcB (88), this does not mean that electron flow through these components is not important in wild-type cells, because cells may adapt with increased expression of other cell components. A better understanding of the role of other outer-surface components, such as putative multi-copper proteins (43, 82), is also required.

Of the other microorganisms that appear to make direct electrical contact with electrodes, some of the most surprising are the gram-positive species of the genus *Therminocola* (77, 139, 140). The abundant *c*-type cytochromes in *T. potens* were involved in extracellular electron transfer and might be the electrical contacts with electrodes (140). Only cells in direct contact with the electrode appeared to contribute to current production, suggesting that a mechanism for long-range electron transport was absent (140).

#### Long-Range Electron Transport via Conductive Pili

The current production capability of a monolayer of cells in direct contact with an electrode surface is limited by the space available for microorganisms to directly access the electrode surface. Highercurrent densities (current produced per surface area of electrode) are possible from electrically conductive biofilms, which permit multiple layers of cells to contribute to current production (104). As previously reviewed (74), the biofilms of most microorganisms appear to act as insulators rather than conductors and the concept of a conductive biofilm is still rather new and controversial (70).

**Conductive pili and biofilms of** *Geobacter sulfurreducens.* The possibility of a conductive biofilm was first proposed in studies on current-producing biofilms of *G. sulfurreducens* (104) and subsequently confirmed by direct measurements (74). Conductive biofilms have been invoked or inferred in other studies (56, 75, 97, 129). However, failure to measure conductivity, as well as highly speculative and unsubstantiated models for conductivity, has led to significant debate about the mechanisms for long-range electron transport through biofilms (70, 73), which can be resolved only by additional direct measurements of conductivity and rigorous experimentation.

The high conductivity of current-producing biofilms of *G. sulfurreducens* (74) allows cells at distances of multiple cell lengths from the anode to contribute to current production (31, 104). The available evidence suggests that the conductivity of the biofilms can be attributed to a dense network of pili with metallic-like conductivity (74).

Initial interest in the type pili of *Geobacter* species came from the observation that *Geobacter metallireducens* expressed pili when growing on insoluble electron acceptors, such as Fe(III) or Mn(IV) oxides, but not when grown with soluble Fe(III) citrate (15), even though Fe(III) citrate is also an extracellular electron acceptor (17). Increased pili production was associated with higher expression of the gene for PilA, the structural protein for type IV pili (15), which are ubiquitous in gram-negative bacteria (19). *G. sulfurreducens* pili have a gross morphology (3–5 nm in width and up to 10–20  $\mu$ m in length) that is similar to that of other type IV pili, but so far only the pili of *G. sulfurreducens* have been shown to be conductive.

Deletion of the gene for PilA inhibited the capacity for Fe(III) oxide reduction, but not the reduction of Fe(III) citrate (103). Addition of anthraquinone-2,6-disulfonate as a soluble electron shuttle alleviated the inhibition of Fe(III) oxide reduction in the *pilA* mutant. These results suggested that the PilA pili were required specifically for Fe(III) oxide reduction, but not for electron transfer to the outer surface of the cell.

Conductivity across the diameter of individual, chemically fixed pili was observed with conducting atomic force microscopy (103). Additional cellular material was often associated with the

#### Microbial nanowires: pili capable of long-range electron transport

pili and acted as insulators for current flow between the conducting tip and the graphite. This observation led to the suggestion that the pili themselves were conductive, rather than the alternative that the pili served as a scaffold for electron-hopping between pilin-associated proteins, and that conduction along the length of the pili permitted *G. sulfurreducens* to greatly extend the potential distance for extracellular electron transfer (103). Thus, the pili were termed microbial nanowires.

Circumstantial evidence that the pili could carry out long-range electron transport came from studies with *G. sulfurreducens* growing on graphite electrodes serving as an electron acceptor. Viability staining indicated that cells at distance from the electrode were viable, and a direct correlation between the extent of current production and biofilm biomass suggested that the cells not in direct contact with the electrode were contributing as much to current production as cells at the electrode surface (104). The simplest explanation for these observations was that long-range electron transport through the biofilm was possible. The finding that a *pilA* mutant did not form thick biofilms on electrodes suggested that a network of microbial nanowires was responsible for the conduction through the biofilm (104). Subsequent observations demonstrated that *pilA* was one of the most highly upregulated genes in current-producing biofilms, providing further circumstantial evidence for the role of pili in current production (88).

Direct measurements of the conductivity of live *G. sulfurreducens* biofilms growing on two gold electrodes that converged across a nonconductive gap demonstrated that the biofilms were conductive, with conductivities rivaling those of synthetic organic conducting polymers (74). Evaluation of different strains of *G. sulfurreducens* revealed significant differences in biofilm conductivity (74); strains that produced more conductive biofilms generated higher current densities in microbial fuel cells (72). There was a strong correlation between conductivity levels and expression of PilA. For example, a strain of *G. sulfurreducens* that was selected specifically for its capacity for high current production and expressed more pili (141) also formed biofilms with the highest conductivity (74).

Surprisingly, the conductivity of the *G. sulfurreducens* biofilms exhibited properties consistent with metallic-like conductivity (74). For example, conductivity initially increased exponentially with a decrease in temperature, a hallmark characteristic of metallic-like conductivity that was previously observed in conducting organic polymers. A similar metallic-like conductivity was observed when pili preparations were spotted on the two-electrode system, forming a network that bridged the nonconducting gap between the electrodes. X-ray diffraction analysis of pili suggested  $\pi$ - $\pi$  stacking, similar to that previously documented in the organic metal polyaniline (74). Thus, a working hypothesis is that aromatic amino acids are aligned along the outer surface of the pili to provide the apparent  $\pi$ - $\pi$  stacking. Another similarity to polyaniline was that the addition of protons to the pili preparations greatly increased their conductivity (74).

The apparent metallic-like conductivity along the pili of *G. sulfurreducens* is in marked contrast to previously described biological electron transfer via electron hopping or tunneling. It is well known that electrons associated with a discrete molecule, such as a cytochrome, can move to another molecule if the two molecules are sufficiently close (<20 Å) (23, 115). However, in metallic-like conductivity the electrons are delocalized. The possibility of delocalized electron transfer in biomolecules has previously been dismissed "due to their lack of periodicity, random fluctuations, and limited conductance values from experiments" (115).

The metallic-like conductive properties of the pili of *G. sulfurreducens* rule out the possibility that electrons are conducted along the length of pili via electron hopping between discrete electron-carrier molecules associated with the pili, such as cytochromes. Furthermore, denaturing cytochromes in pili preparations had no impact on conductivity (74). However, the multiheme *c*-type cytochrome OmcS (100) is specifically associated with the pili of *G. sulfurreducens* (53). Initial observations with immunogold labeling suggested that the OmcS molecules were spaced too far apart for electron hopping between OmcS molecules to account for electron transport along the pili (53), and this finding has subsequently been confirmed by atomic force microscopy (N. Malvankar, unpublished data). OmcS is required for Fe(III) oxide reduction (83). Therefore, it has been proposed that there are barriers to direct electron transfer from pili to Fe(III) oxides and the hypothesized role of OmcS is to facilitate electron transfer from the pili to Fe(III) oxides (63).

In a similar manner, the absolute need for OmcZ, as well as pili, for the production of the highest-current densities in *G. sulfurreducens* biofilms (88, 106), coupled with the localization of OmcZ at the biofilm/anode interface (45), suggests a two-phase electron transport process to electrodes, in which long-range electron transport through the biofilm is along the pili network and OmcZ facilitates the electron transfer from the biofilm to the electrode (63). It is conceivable that with a change in environmental conditions and/or electrode materials that electrochemical gates other than OmcZ may become important. Monitoring electron transfer between specific cytochromes and electrodes as well as cytochrome-to-cytochrome electron transfer may become possible as new tools for simultaneously monitoring the redox status of cytochromes and electron transfer to electrode become available (57).

The present model for long-range electron transport in *Geobacter* biofilms suggests that one avenue to increase current production might be to increase biofilm conductivity. Comparison of direct measurements of biofilm conductivity and the amount of current produced in microbial fuel cells with different strains of *G. sulfurreducens* demonstrated that there was a direct correlation between biofilm conductivity and current production (72). Furthermore, strains with higher biofilm conductivities had lower resistance to electron transfer across the biofilm-anode interface, presumably because electrons were delivered to the interface at a lower potential when resistance to transport through the biofilm was lower.

However, with the best current-producing isolate, strain KN400, the relationship between biofilm conductivity and current production deviated from the strong linear relationship observed with other strains (72). This result suggests that as the current-production capacity of microorganisms is increased, factors other than the maximum potential respiration rate and capacity for long-range electron transport of the organisms begin to limit current production. One possibility is that the protons that must be released from cells during extracellular electron transfer (68) accumulate to levels within the biofilm that inhibit microbial activity (32, 76, 130).

It is not yet known whether the pili of other *Geobacter* species are electronically conductive, but it has been demonstrated that the PilA pili of *G. metallireducens* are required for optimal Fe(III) oxide reduction and current production (131). The metallic-like conductivity of methaneproducing aggregates from a wastewater treatment plant suggested that *Geobacter* species, and possibly other organisms in this mixed natural community, were capable of producing conductive filaments (86).

**Putative conductive filaments in other microorganisms.** Electrically conductive pili greatly benefit *Geobacter* species in their ability to electronically interact with their extracellular environment, and it would be surprising if other microorganisms had not adopted a similar strategy. In fact, preliminary evidence, based on scanning tunneling microscopy, has suggested that a wide diversity of microorganisms produce conductive filaments (36). However, there was significant uncertainty about the filament structure and the mechanisms for conductivity.

The studies focused primarily on filaments of *S. oneidensis*. The diameter of the filaments (50 to >150 nm) was much too broad for the filaments to be type IV pili. Furthermore, direct examination of a role of pili in extracellular electron transfer suggested they are not important in extracellular electron transfer in *S. oneidensis*, as strains that could not produce pili filaments continued to produce electrical current better than wild-type strains (8).

It was suggested that cytochromes associated with *S. oneidensis* filaments conferred conductivity because conductive filaments could not be detected in a mutant strain that did not produce two outer-surface *c*-type cytochromes or a mutant strain deficient in a type II secretion system required for cytochrome export (36). Cytochrome-based conductivity was also inferred from studies using conducting tip atomic force microscopy (24). However, no direct evidence for the association of cytochromes with the filaments has ever been reported and it is generally regarded that the cytochromes in question are associated with the outer surface of the cell body rather than filaments (113). Furthermore, it seems unlikely that cytochromes could be packed tightly enough along pili to confer conductivity via cytochrome-to-cytochrome electron hopping.

As noted above, there is substantial evidence that much of the extracellular electron transfer in *S. oneidensis* is likely to proceed via soluble electron shuttles. The fact that *S. oneidensis* cannot form thick biofilms on electrodes under strict anaerobic conditions (50, 74) further suggests that it is not capable of long-range electron transfer via conductive pili. Therefore, even though conductance could be measured along the length of a filament of *S. oneidensis* (25), the available evidence suggests that it is unlikely that long-range electron transport along conductive filaments is a significant process in *S. oneidensis*.

Scanning tunneling microscopy also suggested that a strain of *Synechocystis*, a phototrophic cyanobacterium, produced conductive filaments and that the thermophilic fermentative bacterium *Pelotomaculum thermopropionicum* produced conductive filaments that established connections with the methanogen *Methanothermobacter thermautotrophicus* (36). A physiological role for the filaments of *Synechocystis* has yet to be determined. Subsequent studies with the *P. thermopropionicum*—*M. thermautotrophicus* coculture identified the filament spanning between the two organisms as a flagellum (114), suggesting that the role of the filament is to establish contact between the two microorganisms, not to mediate electron transfer (81). As previously discussed in detail (61), long-range electron transport via microbial nanowires should not be invoked without evidence for conduction along the length of the proposed nanowires and a demonstration that the filaments are required for the reduction of the proposed electron acceptor.

#### FEEDING ELECTRONS TO MICROBES

As limitations in producing electrical current with microbial fuel cells have become apparent, there has been a significant shift in focus toward the development of practical applications in which electrons flow from electrodes to microorganisms (64, 65, 102). Feeding electrons to microbes typically involves an input of energy that can help alleviate many of the limitations that arise when trying to extract energy with microbial fuel cells.

As previously reviewed (127), electrons can be supplied indirectly to microorganisms via artificial electron shuttles, but this approach has the same limitations for practical applications that were discussed above for current production. It is also possible to electrochemically reduce protons to hydrogen gas, but the low solubility and explosive nature of hydrogen gas limit the usefulness of this approach for most applications (127). Furthermore, efficient production of hydrogen gas typically requires expensive metallic catalysts or substantial inputs of energy to overcome sluggishness in proton reduction at electrode surfaces. Therefore, direct electron transfer from electrodes to microorganisms is expected to be the best choice for most applications as long as sufficiently high rates of electron transfer can be established (127).

The possibility that direct electron transfer from electrodes to microbes could drive microbial respiration was first noted in *Geobacter* species that have the capacity to reduce fumarate (37), nitrate (37), uranium (38), and chlorinated compounds (119, 122) with an electrode as the sole electron donor. A series of control studies demonstrated that hydrogen gas was not an

intermediate in electron transfer between the electrode and the cells, and all the available evidence suggested that the *Geobacter* species were accepting electrons directly from the electrode (37). Gene expression patterns in biofilms of *G. sulfurreducens* reducing fumarate with an electrode as the electron donor were significantly different from those in *G. sulfurreducens* biofilms producing current (120). Deletion of genes, such as *omcZ* and *pilA*, that are essential for current production, had no impact on current consumption, whereas deletion of a cytochrome gene that is essential for current consumption had no impact on electrodes into *Geobacter* species is different from that for electron transfer from electrodes into *Geobacter* species is different from that for electron transfer in the opposite direction, a conclusion that is also supported by electrochemical studies (22). In contrast, electrons provided to *S. oneidensis* for fumarate reduction appear to enter via the same Mtr pathway that is responsible for electron flow to the outer cell surface (110).

Mechanisms for energy conservation in cells receiving electrons from electrodes are poorly understood (64, 108). A potential source of energy conservation is the proton gradient across the inner membrane that should be generated when protons are consumed to reduce electron acceptors in the cytoplasm (64). Biofilms of current-consuming *Geobacter* species are much thinner than current-producing biofilms, suggesting that energy conservation is poorer for current consumption than for current generation.

Only a few pure cultures other than *Geobacter* and *Shewanella* species have been shown to carry out anaerobic respiration on cathodes. It was suggested that the methanogen *Methanobacterium palustre* (14) was capable of accepting electrons directly from electrodes, but there was the possibility of significant hydrogen production under the conditions employed (64, 134). *Anaeromyxobacter debalogenans* could reduce fumarate and reductively dehalogenated 2-chlorophenol to phenol with an electrode serving as the sole electron donor (119). Proof-of-concept studies for microbial electrosynthesis, the process in which microorganisms use electrons derived from electrodes for the reduction of carbon dioxide to organic products, demonstrated that a number of acetogenic microorganisms accepted electrons for the reduction of carbon dioxide to acetate at potentials too high for hydrogen to serve as the intermediate for electron transfer (87, 93). Deleting the gene for the hydrogen-uptake hydrogenase in one of these acetogens, *Clostridium ljungdablii*, had no impact on current consumption, further suggesting that electrons are transferred from the electrode directly to the cells (T. Ueki & K.P. Nevin, unpublished data).

#### BIOELECTRONICS

Electrically active microorganisms have the potential to make significant contributions to the emerging field of bioelectronics (138). For example, the ability of microorganisms to sense a wide diversity of chemicals and environmental conditions, coupled with the possibility of translating a response into an electrical signal, suggests many possibilities for the development of biological sensors and biocomputing (126). Furthermore, the discovery of unexpected electronic properties of conductive biofilms and the prospect of long-range electron transport through networks of conductive pili have opened the possibility that these materials may serve as models for the development of new synthetic electronic materials or may even be used directly in novel "living" electronic devices.

Electronics grown or constructed from living materials have the potential benefits that they can be produced from inexpensive feedstocks with little waste generation and avoid the use of toxic compounds. If the living microorganisms and their components are part of the electronic application, they can have the capacity for self-repair and replication. Charge can be transmitted and stored underwater. Furthermore, the demonstrated ability of some microorganisms and/or their extracellular components to make electrical contacts with electrodes suggests that they may be

**Cathode:** electrode that donates electrons

ideal tools for establishing electrical connections between abiological and biological components in medical devices and sensors.

In addition to their high conductivity, biofilms of *G. sulfurreducens* can function as supercapacitors (71). The abundant *c*-type cytochromes in the biofilms (28, 57, 61, 111) provide a capacitance comparable to that of synthetic supercapacitors with low self-discharge rates (71). The likelihood of manipulating both conductivity and capacitance with genetic engineering (71, 74), as well as improving the cohesiveness and other beneficial properties of the biofilms (C. Leang, unpublished data), demonstrates the potential for further developing these materials for practical applications. *G. sulfurreducens* biofilms can function as transistors, offering the possibility of developing field-effect transistors and other logic devices based on microbial nanowires (74, 98).

The metallic-like conductivity of pili offers the possibility of mass-producing novel wires for electronics. As the mechanisms for conductivity are elucidated, it should be feasible to modify the properties of the wires for specific applications. If the concept that cytochromes associated with pili facilitate electrical connections between pili and external electron acceptors/donors is correct, then it can be envisioned that it may be possible to genetically modify the structure of the electrical connection or to introduce new connectors to provide new functionalities.

#### **FUTURE DIRECTIONS**

There are many promising future research avenues in electromicrobiology. Our understanding of how microorganisms donate electrons to electrodes is still rather superficial and even less is known about electron transfer from electrodes to cells. Furthermore, previous study on this topic has been limited to a few microbes. It is remarkable that the organisms that have been studied in detail, *T. potens, S. oneidensis*, and *G. sulfurreducens*, have significantly different approaches for transferring electrons to electrodes. The intensive study of the electrophysiology of *Shewanella* and *Geobacter* species has been possible only because of substantial earlier investments that facilitated systemsscale investigations (33, 69). Similar in-depth investigations of other organisms are warranted. Elucidation of the mechanisms for metallic-like conductivity along pili and further investigation into the diversity of microorganisms that possess conductive filaments, the function of those filaments, and their mechanisms for conduction are needed.

Electromicrobiology has the potential to alleviate pressing societal needs. Although the justification for many of the early studies in electromicrobiology was further optimization of microbial fuel cells for energy harvesting, many more promising concepts for applications for microbeelectrode interactions have recently emerged and undoubtedly more will be envisioned. For example, as noted in the Introduction, the ability to favorably alter microbial fermentation with a supply of electrons from electrodes has been demonstrated, but only with the addition of soluble mediators. Developing a system for direct electron transfer might make this technology practical at a large scale. The rationale development of any of these technologies will depend on continued study of basic mechanisms of electromicrobiology.

#### SUMMARY POINTS

- 1. Electromicrobiology is a rapidly emerging field of microbiology.
- 2. Some microorganisms have the ability to either donate electrons to, or accept electrons from, electrodes.
- 3. Some microorganisms exchange electrons with electrodes via soluble molecules that facilitate electron transfer between the cells and the electrodes.

- 4. Other microorganisms can directly exchange electrons with electrodes via outer-surface, redox-active proteins, such as *c*-type cytochromes.
- 5. *Geobacter* species can produce thick, conductive biofilms with supercapacitor and transistor properties and conductivities that rival those of synthetic conductive polymers.
- 6. The pili of *G. sulfurreducens* can function as electrical wires, transporting electrons with metallic-like conductivity, a property not previously observed in biological materials.
- 7. Microbe-electrode exchange offers a number of potential practical applications in bioenergy, sensing, and bioremediation and serves as a model for important natural phenomena, such as interspecies electron transfer in anaerobic environments.
- The electronic materials that microorganisms can produce have the potential to be incorporated into novel electronic devices or serve as models for the production of new synthetic electronic materials.

#### **DISCLOSURE STATEMENT**

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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#### LITERATURE CITED

- Aulenta F, Canosa A, Reale P, Rossetti S, Panero S, Majone M. 2009. Microbial reductive dechlorination of trichloroethene to ethene with electrodes serving as electron donors without the external addition of redox mediators. *Biotechnol. Bioeng.* 101:85–91
- Baron DB, LaBelle E, Coursolle D, Gralnick JA, Bond DR. 2009. Electrochemical measurements of electron transfer kinetics by *Shewanella oneidensis* MR-1. *J. Biol. Chem.* 284:28865–73
- Bigalke J, Grabner EW. 1997. The geobattery model: a contribution to large scale electrochemistry. *Electrochim. Acta* 42:3443–52
- Bond DR. 2010. Electrodes as electron acceptors and the bacteria who love them. In *Geomicrobiology: Molecular and Environmental Perspectives*, ed. L Barton, M Mandl, A Loy, pp. 385–99. New York: Springer
- Bond DR, Holmes DE, Tender LM, Lovley DR. 2002. Electrode-reducing microorganisms that harvest energy from marine sediments. *Science* 295:483–85
- Bond DR, Lovley DR. 2003. Electricity production by *Geobacter sulfurreducens* attached to electrodes. *Appl. Environ. Microbiol.* 69:1548–55
- 7. Bond DR, Lovley DR. 2005. Evidence for involvement of an electron shuttle in electricity generation by *Geothrix fermentans. Appl. Environ. Microbiol.* 71:2186–89
- 8. Bouhenni RA, Vora GJ, Biffinger JC, Shirodkar S, Brockman K, et al. 2010. The role of *Shewanella oneidensis* MR-1 outer surface structures in extracellular electron transfer. *Electroanalysis* 22:856–64

5. First demonstration that microorganisms could oxidize organic compounds to carbon dioxide with direct electron transfer to electrodes.

8. Illustrates a genetic approach to directly test electron transfer concepts. 10. Good example of sophisticated approaches that are becoming available for studying microbe-electrode interactions.

16. Provides important insight into how *Sbewanella oneidensis* transports electrons to the outer cell surface (see also Reference 39).

- Busalmen JP, Esteve-Nunez A, Berna A, Feliu JM. 2008. C-type cytochromes wire electricity-producing bacteria to electrodes. Agnew. Chem. Int. Ed. 47:4874–77
- Busalmen JP, Esteve-Nunez A, Berna A, Feliu JM. 2010. ATR-SEIRAs characterization of surface redox processes in G. sulfurreducens. Bioelectrochemistry 78:25–29
- Busalmen JP, Esteve-Nunez A, Feliu JM. 2008. Whole cell electrochemistry of electricity-producing microorganisms evidence an adaptation for optimal exocellular electron transport. *Envrion. Sci. Technol.* 42:2445–50
- 12. Butler C, Clauwaert P, Green SJ, Verstraete W, Nerenberg R. 2010. Bioelectrochemical perchlorate reduction in a microbial fuel cell. *Environ. Sci. Technol.* 44:4685–91
- Chaudhuri SK, Lovley DR. 2003. Electricity generation by direct oxidation of glucose in mediatorless microbial fuel cells. *Nat. Biotechnol.* 21:1229–32
- Cheng S, Xing D, Call DF, Logan BE. 2009. Direct biological conversion of electrical current into methane by electromethanogenesis. *Environ. Sci. Technol.* 43:3953–58
- Childers SE, Ciufo S, Lovley DR. 2002. Geobacter metallireducens accesses insoluble Fe(III) oxide by chemotaxis. Nature 416:767–69
- 16. Clarke TA, Edwards MJ, Gates AJ, Hall A, White GF, et al. 2011. Structure of a cell surface decaheme electron conduit. *Proc. Natl. Acad. Sci. USA* 108:9384–9
- Coppi MV, O'Neil RA, Leang C, Kaufmann F, Methé BA, et al. 2007. Involvement of *Geobacter sulfurre*ducens SfrAB in acetate metabolism rather than intracellular Fe(III) reduction. *Microbiology* 153:3572–85
- Coursolle D, Baron DB, Bond DR, Gralnick JA. 2010. The Mtr respiratory pathway is essential for reducing flavins and electrodes in *Shewanella oneidensis*. *J. Bacteriol.* 192:467–74
- Craig L, Piquie ME, Tainer JA. 2004. Type IV pilus structure and bacterial pathogenicity. Nat. Rev. Microbiol. 2:363–78
- Ding YHR, Hixson KK, Giometti CS, Stanley A, Esteve-Nunez A, et al. 2006. The proteome of dissimilatory metal-reducing microorganism *Geobacter sulfurreducens* under various growth conditions. *Biochim. Biophys. Acta* 1764:1198–206
- 21. Dumas C, Basseguy R, Bergel A. 2008. Electrochemical activity of *Geobacter sulfurreducens* biofilms on stainless steel anodes. *Electrochim. Acta* 53:5235–41
- Dumas C, Basseguy R, Bergel A. 2008. Microbial electrocatalysis with *Geobacter sulfurreducens* biofilm on stainless steel cathodes. *Electrochm. Acta* 53:2494–500
- 23. Edwards PP, Gray HB, Lodge MTJ, Williams RJP. 2008. Electron transfer and electronic conduction through an intervening medium. *Angew. Chem. Int. Ed.* 47:6758–65
- 24. El-Naggar MY, Gorby YA, Xia W, Nealson KH. 2008. The molecular density states in bacterial nanowires. *Biophys. J.* 95:L10–12
- El-Naggar MY, Wanger G, Leung KM, Yuzvinsky TD, Southam G, et al. 2010. Electrical transport along bacterial nanowires from *Shewanella oneidensis*. Proc. Natl. Acad. Sci. USA 107:18127–31
- Emde R, Schink B. 1990. Enhanced propionate formation by Propionibacterium freudenreichii subsp. freudenreichii in a three-electrode amperometric culture system. Appl. Environ. Microbiol. 56:2771–76
- Esteve-Nunez A, Busalmen JP, Berna A, Gutierrez-Garran C, Feliu JM. 2011. Opportunities behind the unusual ability of *Geobacter sulfurreducens* for exocellular respiration and electricity production. *Energy Environ. Sci.* 4:2066–69
- Esteve-Nunez A, Sosnik J, Visconti P, Lovley DR. 2008. Fluorescent properties of c-type cytochromes reveal their potential role as an extracytoplasmic electron sink in *Geobacter sulfurreducens. Environ. Microbiol.* 10:497–505
- Flynn JM, Ross DE, Hunt KA, Bond DR, Gralnick JA. 2010. Enabling unbalanced fermentations by using engineered electrode-interfaced bacteria. *mBio* 1:e00190–10
- 30. Franks AE, Nevin KP. 2010. Microbial fuel cells, a current review. Energies 3:899-919
- Franks AE, Nevin KP, Glaven RH, Lovley DR. 2010. Microtoming coupled to microarray analysis to evaluate the spatial metabolic status of *Geobacter sulfurreducens* biofilms. *ISME J*. 4:509–19
- 32. Franks AE, Nevin KP, Jia H, Izallalen M, Woodard TL, Lovley DR. 2009. Novel strategy for threedimensional real-time imaging of microbial fuel cell communities: monitoring the inhibitory effects of proton accumulation within the anode biofilm. *Energy Environ. Sci.* 2:113–19

29. Demonstrates a promising biotechnological application for microbe-electrode electron exchange.

- Fredrickson JK, Romine MF, Beliaev AS, Auchtung JM, Driscoll ME, et al. 2008. Towards environmental systems biology of *Shewanella*. Nat. Rev. Microbiol. 6:592–603
- Fricke K, Harnisch F, Schroder U. 2008. On the use of cyclic voltammetry for the study of anodic electron transfer in microbial fuel cells. *Energy Environ. Sci.* 1:144–47
- Geelhoed JS, Hamelers HVM, Stams AJM. 2010. Electricity-mediated biological hydrogen production. Curr. Opin. Microbiol. 13:307–15
- Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, et al. 2006. Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proc. Natl. Acad. Sci. USA* 103:11358–63
- Gregory KB, Bond DR, Lovley DR. 2004. Graphite electrodes as electron donors for anaerobic respiration. *Environ. Microbiol.* 6:596–604
- Gregory KB, Lovley DR. 2005. Remediation and recovery of uranium from contaminated subsurface environments with electrodes. *Environ. Sci. Technol.* 39:8943–47
- Hartshorne RS, Reardon CL, Ross D, Nuester J, Clarke TA, et al. 2009. Characterization of an electron conduit between bacteria and the extracellular environment. *Proc. Natl. Acad. Sci. USA* 106:22169–74
- Holmes DE, Bond DR, Lovley DR. 2004. Electron transfer by *Desulfobulbus propionicus* to Fe(III) and graphite electrodes. *Appl. Environ. Microbiol.* 70:1234–37
- Holmes DE, Bond DR, O'Neil RA, Reimers CE, Tender LR, Lovley DR. 2004. Microbial communities associated with electrodes harvesting electricity from a variety of aquatic sediments. *Microb. Ecol.* 48:178– 90
- Holmes DE, Chaudhuri SK, Nevin KP, Mehta T, Methe BA, et al. 2006. Microarray and genetic analysis of electron transfer to electrodes in *Geobacter sulfurreducens*. Environ. Microbiol. 8:1805–15
- 43. Holmes DE, Mester T, O'Neil RA, Larrahondo MJ, Adams LA, et al. 2008. Genes for two multicopper proteins required for Fe(III) oxide reduction in *Geobacter sulfurreducens* have different expression patterns both in the subsurface and on energy-harvesting electrodes. *Microbiology* 145:1422–35
- Hongo M, Iwahara M. 1979. Application of electro-energizing method to L-glutamic acid fermentation. Agric. Biol. Chem. 10:2075–81
- Inoue K, Leang C, Franks AE, Woodard TL, Nevin KP, Lovley DR. 2011. Specific localization of the *c*-type cytochrome OmcZ at the anode surface in current-producing biofilms of *Geobacter sulfurreducens*. *Environ. Microbiol. Rep.* 3:211–17
- 46. Inoue K, Qian X, Morgado L, Kim BC, Mester T, et al. 2010. Purification and characterization of OmcZ, an outer-surface, octaheme *c*-type cytochrome essential for optimal current production by *Geobacter sulfurreducens*. Appl. Environ. Microbiol. 76:3999–4007
- Jain A, Gazzola G, Panzera A, Zanoni M, Marsili E. 2011. Visible spectroelectrochemical characterization of *Geobacter sulfurreducens* biofilms on optically transparent indium tin oxide electrode. *Electrochim. Acta* 47:12530–32
- 48. Jiang X, Hu J, Fitzgerald LA, Biffinger JC, Xie P, et al. 2010. Probing electron transfer mechanisms in *Shewanella oneidensis* MR-1 using a nanoelectrode platform and single-cell imaging. *Proc. Natl. Acad. Sci. USA* 107:16806–10
- Kiely PD, Regan JM, Logan BE. 2011. The electric picnic: synergistic requirements for exoelectrogenic microbial communities. *Curr. Opin. Biotechnol.* 22:378–85
- Lanthier M, Gregory KB, Lovley DR. 2008. Growth with high planktonic biomass in *Shewanella oneidensis* fuel cells. *FEMS Microbiol. Lett.* 278:29–35
- Leang C, Adams LA, Chin K-J, Nevin KP, Methé BA, et al. 2005. Adaption to disruption of electron transfer pathway for Fe(III) reduction in *Geobacter sulfurreducens*. *J. Bacteriol*. 187:5918–26
- Leang C, Coppi MV, Lovley DR. 2003. OmcB, a c-type polyheme cytochrome, involved in Fe(III) reduction in *Geobacter sulfurreducens*. J. Bacteriol. 185:2096–103
- Leang C, Qian X, Mester T, Lovley DR. 2010. Alignment of the *c*-type cytochrome OmcS along pili of Geobacter sulfurreducens. Appl. Environ. Microbiol. 76:4080–84
- Lies DP, Hernandez ME, Kappler A, Mielke RE, Gralnick JA, Newman DK. 2005. Shewanella oneidensis MR-1 uses overlapping pathways for iron reduction at a distance and by direct contact under conditions relevant for biofilms. *Appl. Environ. Microbiol.* 71:4414–26

48. Innovative investigation that helped resolve the mechanisms for electron transfer to electrodes in *S. oneidensis*.

- Liu H, Newton GJ, Nakamura R, Hashimoto K, Nakanishi. 2010. Electrochemical characterization of a single electricity-producing bacterial cell of *Shewanella* using optical tweezers. *Angew. Chem. Int. Ed.* 49:6596–99
- Liu Y, Kim H, Franklin R, Bond DR. 2010. Gold line array electrodes increase substrate affinity and current density of electricity-producing *G. sulfurreducens* biofilms. *Energy Environ. Sci.* 3:1782–88
- Liu Y, Kim H, Franklin RR, Bond DR. 2011. Linking spectral and electrochemical analysis to monitor *c*-type cytochrome redox status in living *Geobacter sulfurreducens* biofilms. *ChemPhysChem* 12:2235–41
- 58. Logan BE. 2009. Exoelectrogenic bacteria that power microbial fuel cells. Nat. Rev. Microbiol. 7:375-81
- 59. Lovley DR. 2006. Bug juice: harvesting electricity with microorganisms. Nat. Rev. Microbiol. 4:497-508
- Lovley DR. 2006. Microbial fuel cells: novel microbial physiologies and engineering approaches. Curr. Opin. Biotechnol. 17:327–32
- Lovley DR. 2008. Extracellular electron transfer: wires, capacitors, iron lungs, and more. *Geobiology* 6:225–31
- Lovley DR. 2008. The microbe electric: conversion of organic matter to electricity. Curr. Opin. Biotechnol. 19:564–71
- Lovley DR. 2011. Live wires: direct extracellular electron exchange for bioenergy and the bioremediation of energy-related contamination. *Energy Environ. Sci.* 4:4896–906
- Lovley DR. 2011. Powering microbes with electricity: direct electron transfer from electrodes to microbes. *Environ. Microbiol. Rep.* 3:27–35
- Lovley DR, Nevin KP. 2011. A shift in the current: new applications and concepts for microbe-electrode electron exchange. *Curr. Opin. Biotechnol.* 22:441–48
- Lovley DR, Ueki T, Zhang T, Malvankar NS, Shrestha PM, et al. 2012. Geobacter: the microbe electric's physiology, ecology, and practical applications. Adv. Microb. Physiol. 59:1–100
- Magnuson TS, Isoyama N, Hodges-Myerson AL, Davidson G, Maroney MJ, et al. 2001. Isolation, characterization and gene sequence analysis of a membrane-associated 89 kDa Fe(III) reducing cytochrome *c* from *Geobacter sulfurreducens*. *Biochem.* 7. 359:147–52
- Mahadevan R, Bond DR, Butler JE, Esteve-Nunez A, Coppi MV, et al. 2006. Characterization of metabolism in the Fe(III)-reducing organism *Geobacter sulfurreducens* by constraint-based modeling. *Appl. Environ. Microbiol.* 72:1558–68
- Mahadevan R, Palsson BO, Lovley DR. 2011. In situ to in silico and back: elucidating the physiology and ecology of *Geobacter* spp. using genome-scale modelling. *Nat. Rev. Microbiol.* 9:39–50
- Malvankar NS, Lovley DR. 2012. Microbial nanowires: a new paradigm for biological electron transfer and bioelectronics. *ChemSusChem* 5:1039–46
- Malvankar NS, Mester T, Tuominen MT, Lovley DR. 2012. Supercapacitors based on *c*-type cytochromes using conductive nanostructured networks of living bacteria. *ChemPhysChem* 13:463–68
- Malvankar NS, Tuominen MT, Lovley DR. 2012. Biofilm conductivity as a decisive variable for the high-current-density *Geobacter sulfurreducens* microbial fuel cells. *Energy Environ. Sci.* 5:5790–97
- Malvankar NS, Tuominen MT, Lovley DR. 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 Environ. Sci.* 5:6247–49
- Malvankar NS, Vargas M, Nevin KP, Franks AE, Leang C, et al. 2011. Tunable metallic-like conductivity in nanostructured biofilms comprised of microbial nanowires. *Nat. Nanotechnol.* 6:573–79
- Marcus AK, Torres CI, Rittmann BE. 2007. Conduction-based modeling of the biofilm anode of a microbial fuel cell. *Biotechnol. Bioeng.* 98:1171–82
- Marcus AK, Torres CI, Rittmann BE. 2011. Analysis of microbial electrochemical cell using the proton condition in a biofilm (PCBIOFILM) model. *Bioresour. Technol.* 102:253–62
- Marshall C, May H. 2009. Electrochemical evidence of direct electrode reduction by a thermophilic gram-positive bacterium *Thermincola ferriacetica*. *Energy Environ. Sci.* 2:699–705
- Marsili E, Baron DB, Shikhare I, Coursolle D, Gralnick JA, Bond DR. 2008. Shewanella secretes flavins that mediate extracellular electron transfer. Proc. Natl. Acad. Sci. USA 105:3968–73

74. Demonstrates metallic-like conductivity for transport along pili and through biofilms offers an unprecedented mechanism for long-range electron transport in biology.

- Marsili E, Rollefson JB, Baron DB, Hozalski RM, Bond DR. 2008. Microbial biofilm voltammetry: direct electrochemical characterization of catalytic electrode-attached biofilms. *Appl. Environ. Microbiol.* 74:7329–37
- Marsili E, Sun J, Bond DR. 2010. Voltammetry and growth physiology of *Geobacter sulfurreducens* biofilms as a function of growth stage and imposed potential. *Electroanalysis* 22:865–74
- McInerney MJ, Sieber JR, Gunsalus RP. 2009. Syntrophy in anaerobic global carbon cycles. Curr. Opin. Biotechnol. 20:623–32
- Mehta T, Childers SE, Glaven R, Lovley DR, Mester T. 2006. A putative multicopper protein secreted by an atypical type II secretion system involved in the reduction of insoluble electron acceptors in *Geobacter* sulfurreducens. Microbiology 152:2257–64
- Mehta T, Coppi MV, Childers SE, Lovley DR. 2005. Outer membrane c-type cytochromes required for Fe(III) and Mn(IV) oxide reduction in *Geobacter sulfurreducens*. Appl. Environ. Microbiol. 71:8634–41
- Methé BA, Nelson KE, Eisen JA, Paulsen IT, Nelson W, et al. 2003. The genome of *Geobacter sulfurre*ducens: insights into metal reduction in subsurface environments. Science 302:1967–69
- Millo D, Harnisch F, Patil SA, Ly HK, Schröder U, Hildebrandt P. 2011. In situ spectroelectrochemical investigation of electrocatalytic microbial biofilms by surface-enhanced resonance Raman spectroscopy. *Angew. Chem. Int. Ed.* 50:2625–27
- Morita M, Malvankar NS, Franks AE, Summers ZM, Giloteaux L, et al. 2011. Potential for direct interspecies electron transfer in methanogenic wastewater digester aggregates. *mBio* 2:e00159–11
- Nevin KP, Hensley SA, Franks AE, Summers ZM, Ou J, et al. 2011. Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. *Appl. Environ. Microbiol.* 77:2882–86
- Nevin KP, Kim BC, Glaven RH, Johnson JP, Woodard TL, et al. 2009. Anode biofilm transcriptomics reveals outer surface components essential for high density current production in *Geobacter sulfurreducens* fuel cells. *PLoS One* 4:e5628
- Nevin KP, Lovley DR. 2000. Lack of production of electron-shuttling compounds or solubilization of Fe(III) during reduction of insoluble Fe(III) oxide by *Geobacter metallireducens*. *Appl. Environ. Microbiol.* 66:2248–51
- Nevin KP, Lovley DR. 2002. Mechanisms for accessing insoluble Fe(III) oxide during dissimilatory Fe(III) reduction by *Geothrix fermentans. Appl. Environ. Microbiol.* 68:2294–99
- Nevin KP, Lovley DR. 2002. Mechanisms for Fe(III) oxide reduction in sedimentary environments. Geomicrobiol. J. 19:141–59
- Nevin KP, Richter H, Covalla SF, Johnson JP, Woodard TL, et al. 2008. Power output and columbic efficiencies from biofilms of *Geobacter sulfurreducens* comparable to mixed community microbial fuel cells. *Environ. Microbiol.* 10:2505–14
- Nevin KP, Woodard TL, Franks AE, Summers ZM, Lovley DR. 2010. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. *mBio* 1:e00103–10
- Newman DK, Kolter R. 2000. A role for excreted quinones in extracellular electron transfer. *Nature* 405:93–97
- Park DH, Laivenieks M, Guettler MV, Jain MK, Zeikus JG. 1999. Microbial utilization of electrically reduced neutral red as the sole electron donor for growth and metabolite production. *Appl. Environ. Microbiol.* 65:2912–17
- Park HI, Kim DK, Choi Y-J, Pak D. 2005. Nitrate reduction using an electrode as direct electron donor in a biofilm-electrode reactor. *Process Biochem.* 40:3383–88
- Picioreanu C, Head IM, Katuri KP, van Loosdrecht MCM, Scott K. 2007. A computational model for biofilm-based microbial fuel cells. *Water Res.* 41:2921–40
- 98. Qian F, Li Y. 2011. A natural source of nanowires. Nat. Nanotechnol. 6:538-9
- Qian X, Reguera G, Mester T, Lovley DR. 2007. Evidence that OmcB and OmpB of Geobacter sulfurreducens are outer membrane surface proteins. FEMS Microbiol. Lett. 277:21–27
- 100. Qian XL, Mester T, Morgado L, Arakawa T, Sharma ML, et al. 2011. Biochemical characterization of purified OmcS, a *c*-type cytochrome required for insoluble Fe(III) reduction in *Geobacter sulfurreducens*. *Biochim. Biophys. Acta* 1807:404–12

93. Proof of concept for an artificial form of photosynthesis with many potential advantages over biomass-based strategies for the production of transportation fuels and other organic commodities.

- Rabaey K, Rodriguez J, Blackall LL, Keller J, Gross P, et al. 2007. Microbial ecology meets electrochemistry: electricity-driven and driving communities. *ISME J*. 1:9–18
- Rabaey K, Rozendal RA. 2010. Microbial electrosynthesis—revisiting the electrical route for microbial production. Nat. Rev. Microbiol. 8:706–16
- Reguera G, McCarthy KD, Mehta T, Nicoll JS, Tuominen MT, Lovley DR. 2005. Extracellular electron transfer via microbial nanowires. *Nature* 435:1098–101
- Reguera G, Nevin KP, Nicoll JS, Covalla SF, Woodard TL, Lovley DR. 2006. Biofilm and nanowire production leads to increased current in *Geobacter sulfurreducens* fuel cells. *Appl. Environ. Microbiol.* 72:7345– 48
- Reimers CE, Tender LM, Fertig S, Wang W. 2001. Harvesting energy from the marine sediment-water interface. *Environ. Sci. Technol.* 35:192–95
- 106. Richter H, Nevin KP, Jia H, Lowy DA, Lovley DR, Tender LM. 2009. Cyclic voltammetry of biofilms of wild type and mutant *Geobacter sulfurreducens* on fuel cell anodes indicates possible roles of OmcB, OmcZ, type IV pili, and protons in extracellular electron transfer. *Energ. Environ. Sci.* 2:506–16
- Rollefson JB, Stephen CS, Tien M, Bond DR. 2011. Identification of an extracellular polysaccharide network essential for cytochrome anchoring and biofilm formation in *Geobacter sulfurreducens*. J. Bacteriol. 193:1023–33
- Rosenbaum M, Aulenta F, Villano M, Angenent LT. 2011. Cathodes as electron donors for microbial metabolism: Which extracellular electron transfer mechanisms are involved? *Biores. Technol.* 102:324–33
- Ross DE, Brantley SL, Tien M. 2009. Kinetic characterization of OmcA and MtrC, terminal reductases involved in respiratory electron transfer for dissimilatory iron reduction in *Shewanella oneidensis* MR-1. *Appl. Environ. Microbiol.* 75:5218–26
- 110. Ross DE, Flynn JM, Baron DB, Gralnick JA, Bond DR. 2011. Towards electrosynthesis in Sbewanella: energetics of reversing the Mtr pathway for reductive metabolism. PLoS One 6:e16649
- Schrott GD, Bonnani PS, Robuschi L, Esteve-Núñez A, Busalmen JP. 2011. Electrochemical insight into the mechanism of electron transport in biofilms of *Geobacter sulfurreducens*. Electrochim. Acta 56:10791–95
- 112. Shelobolina ES, Coppi MV, Korenevsky AA, DiDonato LN, Sullivan SA, et al. 2007. Importance of *c*-type cytochromes for U(VI) reduction by *Geobacter sulfurreducens*. BMC Microbiol. 7:16
- Shi L, Squier TC, Zachara JM, Fredrickson JK. 2007. Respiration of metal (hydr)oxides by Shewanella and Geobacter: a key role for multihaem c-type cytochromes. Mol. Microbiol. 65:12–20
- 114. Shimoyama T, Kato S, Ishii S, Watanabe K. 2009. Flagellum mediates symbiosis. Science 323:1574
- Shinwari MW, Deen MJ, Starikov EB, Cuniberti G. 2010. Electrical conductance in biological molecules. Adv. Funct. Mater. 20:1865–83
- Srikanth S, Marsili E, Flickinger MC, Bond DR. 2008. Electrochemical characterization of *Geobacter sulfurreducens* cells immobilized on graphite paper anodes. *Biotechnol. Bioeng*. 99:1065–73
- Stams AJ, Plugge CM. 2009. Electron transfer in syntrophic communities of anaerobic bacteria and archaea. Nat. Rev. Microbiol. 7:568–77
- Steinbusch KJJ, Hamelers HVM, Schaap JD, Kampman C, Buisman CJN. 2010. Bioelectrochemical ethanol production through mediated acetate reduction by mixed cultures. *Environ. Sci. Technol.* 44:513– 17
- Strycharz SM, Gannon SM, Boles AR, Nevin KP, Franks AE, Lovley DR. 2010. Anaeromyxobacter debalogenans interacts with a poised graphite electrode for reductive dechlorination of 2-chlorophenol. Environ. Microbiol. Rep. 2:289–294
- 120. Strycharz SM, Glaven RH, Coppi MV, Gannon SM, Perpetua LA, et al. 2011. Gene expression and deletion analysis of mechanisms for electron transfer from electrodes to *Geobacter sulfurreducens*. *Bioelectrochemistry* 80:142–50
- 121. Strycharz SM, Malanoski AP, Snider RM, Yi H, Lovley DR, Tender LM. 2011. Application of cyclic voltammetry to investigate enhanced catalytic current generation by biofilm-modified anodes of *Geobacter sulfurreducens* strain DL1 versus variant strain KN400. *Energy Environ. Sci.* 4:896–913
- 122. Strycharz SM, Woodward TL, Johnson JP, Nevin KP, Sanford RA, et al. 2008. Graphite electrode as a sole electron donor for reductive dechlorination of tetrachlorethene by *Geobacter lovleyi. Appl. Environ. Microbiol.* 74:5943–47

- 123. Summers ZM, Fogarty HE, Leang C, Franks AE, Malvankar NS, Lovley DR. 2010. Direct exchange of electrons within aggregates of an evolved syntrophic coculture of anaerobic bacteria. *Science* 330:1413–15
- 124. Tandukar M, Huber SJ, Onodera T, Pavlostathis SG. 2009. Biological chromium(VI) reduction in the cathode of a microbial fuel cell. *Environ. Sci. Technol.* 43:8159–65
- 125. Tender LM, Reimers CE, Stecher HA, Holmes DE, Bond DR, et al. 2002. Harnessing microbially generated power on the seafloor. *Nat. Biotechnol.* 20:821–25
- 126. TerAvest MA, Li Z, Angenent LT. 2011. Bacteria-based biocomputing with cellular computing circuits to sense, decide, signal, and act. *Energy Environ. Sci.* 4:4907–16
- 127. Thrash JC, Coates JD. 2008. Review: direct and indirect electrical stimulation of microbial metabolism. *Environ. Sci. Technol.* 42:3921–31
- 128. Thrash JC, Van Trump IV, Weber KA, Miller E, Achenbach LA, Coates JD. 2007. Electrochemical stimulation of microbial perchlorate reduction. *Environ. Sci. Technol.* 41:1740–46
- 129. Torres CI, Marcus AK, Lee H-S, Parameswaran P, Krajmalnik-Brown R, Rittmann BE. 2010. A kinetic perspective on extracellular electron transfer by anode-respiring bacteria. *FEMS Microbiol. Rev.* 34:3–17
- 130. Torres CI, Marcus AK, Rittmann BE. 2008. Proton transport inside the biofilm limits electrical current generation by anode-respiring bacteria. *Biotechnol. Bioeng.* 100:872–81
- 131. Tremblay P-L, Aklujkar M, Leang C, Lovley DR. 2011. A genetic system for *Geobacter metallireducens*: role of flagella and pili in extracellular electron transfer. *Environ. Microbiol. Rep.* 4:82–88
- 132. Tremblay P-L, Summers ZM, Glaven RH, Nevin KP, Zengler K, et al. 2011. A *c*-type cytochrome and a transcriptional regulator responsible for enhanced extracellular electron transfer in *Geobacter sulfurreducens* uncovered by adaptive evolution. *Environ. Microbiol.* 13:13–23
- Van Eerten-Jansen MCAA, Heijne AT, Buisman CJN, Hamelers HVM. 2012. Microbial electrolysis cells for production of methane from CO<sub>2</sub>: long-term performance and perspectives. *Int. J. Energy Res.* 36:809–19
- Villano M, Aulenta F, Ciucci C, Ferri T, Giuliano A, Majone M. 2010. Bioelectrochemical reduction of CO<sub>2</sub> to CH<sub>4</sub> via direct and indirect extracellular electron transfer by a hydrogenophilic methanogenic culture. *Biores. Technol.* 101:3085–90
- 135. von Canstein H, Ogawa J, Shimizu S, Lloyd JR. 2008. Secretion of flavins by *Sbewanella* species and their role in extracellular electron transfer. *Appl. Environ. Microbiol.* 74:615–23
- 136. Voordeckers JW, Izallalen M, Kim B-C, Lovley DR. 2010. Role of *Geobacter sulfurreducens* outer surface *c*-type cytochromes in the reduction of soil humic acid and the humics analog anthraquinone-2,6-disulfonate. *Appl. Environ. Microbiol.* 76:2371–75
- 137. Williams KN, Nevin KP, Franks AE, Englert A, Long PE, Lovley DR. 2010. Electrode-based approach for monitoring in situ microbial activity during subsurface bioremediation. *Environ. Sci. Technol.* 44:47–54
- 138. Willner I, Katz E, eds. 2005. Bioelectronics: From Theory to Applications. Weinheim: Wiley-VCH
- Wrighton KC, Agbo P, Warnecke F, Weber KA, Brodie EL, et al. 2008. A novel ecological role of the *Firmicutes* identified in thermophilic microbial fuel cells. *ISME J*. 2:1146–56
- 140. Wrighton KC, Thrash JC, Melnyk RA, Bigi JP, Byrne-Bailey KG, et al. 2011. Evidence for direct electron transfer by a gram-positive bacterium isolated from a microbial fuel cell. *Appl. Environ. Microbiol.* 77:7633–39
- 141. Yi H, Nevin KP, Kim B-C, Franks AE, Klimes A, et al. 2009. Selection of a variant of *Geobacter sul-furreducens* with enhanced capacity for current production in microbial fuel cells. *Biosens. Bioelectron*. 24:3498–503
- 142. Zhang T, Gannon SM, Nevin KP, Franks AE, Lovley DR. 2010. Stimulating the anaerobic degradation of aromatic hydrocarbons in contaminated sediments by providing an electrode as the electron acceptor. *Environ. Microbiol.* 12:1011–20

#### RELATED RESOURCES

Geobacter Project. http://www.geobacter.org/ www.electrofuels.org 126. Forward-looking discussion of exciting possibilities in electromicrobiology.

129. Excellent discussion of the role of biofilm conductivity in generating high-current densities in microbial fuel cells.

130. First study emphasizing the importance of proton accumulation as a factor limiting current production in anode biofilms.

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