Bug juice: harvesting electricity with microorganisms

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Abstract | It is well established that some reduced fermentation products or microbially reduced artificial mediators can abiotically react with electrodes to yield a small electrical current. This type of metabolism does not typically result in an efficient conversion of organic compounds to electricity because only some metabolic end products will react with electrodes, and the microorganisms only incompletely oxidize their organic fuels. A new form of microbial respiration has recently been discovered in which microorganisms conserve energy to support growth by oxidizing organic compounds to carbon dioxide with direct quantitative electron transfer to electrodes. These organisms, termed electricigens, offer the possibility of efficiently converting organic compounds into electricity in self-sustaining systems with long-term stability.

"The disintegration of organic compounds by microorganisms is accompanied by the liberation of electrical energy." M.C. Potter, 1911.

It is well known that microorganisms can produce fuels, such as ethanol, methane and hydrogen, from organic matter. It is less well known that microorganisms can also convert organic matter into electricity in devices known as microbial fuel cells. However, interest in microbial fuel cells is increasing. Microbial fuel cells offer the possibility of harvesting electricity from organic waste and renewable biomass. These are attractive sources of energy because they are 'carbon-neutral'; the oxidation of the organic matter only releases recently fixed carbon back into the atmosphere.

Furthermore, microbial fuel cells could fill a niche that is significantly different from that of the better-known abiotic hydrogen- and methanol-driven fuel cells. For example, abiotic fuel cells require expensive catalysts to promote oxidation of the electron donors[1], whereas naturally occurring microorganisms catalyse the oxidation of the fuels in microbial fuel cells. Abiotic fuel cells often operate at high temperatures[1], but microbial fuel cells can be operated at room temperature and could potentially be designed to function at any temperature at which microbial life is possible. The fuels for abiotic fuel cells are highly explosive or toxic and have to be highly purified to avoid poisoning the catalysts. By contrast, the microorganisms that power microbial fuel cells can oxidize a diverse range of 'dirty' fuels that are often of little perceived value, such as organic waste and the

organic matter in soils and sediments. The ubiquitous and innocuous properties of fuels for microbial fuel cells alleviates the need for the complex and highly regulated distribution systems that are required for hydrogen and methanol. Therefore, microbial fuel cells might be particularly attractive power sources in remote locations and regions of developing countries that are not served by well-developed, centralized power grids.

Microbial fuel cells are also distinct from the better-developed enzymatic fuel cells in which electricity is generated through enzymes or cell extracts rather than whole cells[2]. Enzymatic fuel cells can produce high levels of power for their size and are well suited to applications such as sensors. However, enzymatic fuel cells typically only harvest a small percentage of the electrons available in organic fuels, because incorporating the full complement of enzymes necessary to completely oxidize organic fuels to carbon dioxide is not yet technically feasible. By contrast, as detailed below, microbial fuel cells offer the possibility of extracting over 90% of the electrons from organic compounds, and can be self-sustaining and renewing when populated with microorganisms that conserve energy from electron transfer to electrodes.

One reason that microbial fuel cells are not commonly considered a part of the energy portfolio for the future, is that microbial fuel technology is not yet sufficiently well developed to produce substantial quantities of power in a cost-effective manner. Over the past 40 years it has been suggested that microbial fuel cells might be developed for a wide range of applications, including serving as household electrical generators[3] and powering items

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such as small portable electronic devices[3,4], boats[4], automobiles[3,5], electronics in space[6] and self-feeding robots[7]. There is also interest in developing large-scale microbial fuel cells for the conversion of sewage and other organic waste to electricity[8–10], and the bioremediation of contaminated environments (BOX 1). However, none of these applications is yet practical. The only microbial fuel-cell application with short term potential is to power monitoring devices in remote locations[11].

At present, microbial fuel cells can produce enough current to power small electronic devices for short periods or to trickle-charge capacitors for applications with higher power demands. However, the size of these microbial fuel cells precludes their incorporation into the electronic devices they can power. At the time that this review was completed, the highest power densities reported for the type of microbial fuel cell most likely to be sustainable for long periods of time were approximately 50 Watts per cubic metre of fuel cell volume[12]. Therefore, further optimization is required for most envisioned applications.

Most of the research into optimizing power output from microbial fuel cells has focused on altering their designs in order to overcome electrochemical barriers to electron and proton flow, and to enhance the surface area and reactivity of the anode and cathode (for excellent reviews see REFS 8–10). The literature in this area is extensive, but, for the most part, the actual microbiological processes taking place in the microbial fuel cells were not well defined, and such studies will not be reviewed in detail

here. In addition to further electrochemical engineering, a better understanding of the physiology and ecology of microbial electricity production might be helpful in further optimizing microbial fuel cells. Furthermore, better understanding of the interaction of microorganisms with electrically conductive surfaces will also aid in the development of as-yet-unimagined technologies where small amounts of power or specialized conductive materials are required for micro- or nano-electronic applications.

The purpose of this review is to summarize our present knowledge of the microbiology of electricity production. As detailed below, many microorganisms can contribute to electricity production. However, it is the recent discovery of a new metabolic class of electricity-producing microorganisms that has, for the first time, indicated that a wide diversity of organic compounds can be effectively converted to electricity in self-sustaining microbial fuel cells. These organisms, known as electricigens, can completely oxidize organic compounds to carbon dioxide, with an electrode serving as the sole electron acceptor, and conserve energy to support growth from this electron transfer. The known physiology and ecology of electricigens, their potential mechanisms for electron transfer to electrodes and present concepts for optimizing their performance are reviewed.

What is a microbial fuel cell?

A fuel cell converts chemical energy into electrical energy, without the inefficiencies that arise from combusting fuel

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Box 1 | Microbial fuel cells and bioremediation

One of the main difficulties in the bioremediation of subsurface environments contaminated with organic compounds or metals is the optimum delivery of an electron acceptor or donor to best promote the desired biodegradation. For example, bioremediation of petroleum-contaminated groundwater is often impeded because the introduction of oxygen into the subsurface is technically challenging and expensive. Alternative electron acceptors, such as Fe^{3+} and sulphate, that support anaerobic respiration can also be exploited[78,79], but electrodes represent another, possibly more convenient, electron acceptor. For example, in one study *Geobacter metallireducens* oxidized the prevalent aromatic hydrocarbon contaminant toluene with an electrode serving as the sole electron acceptor[58], and preliminary studies have indicated that electrodes placed in petroleum-contaminated subsurface sediments can accelerate the degradation of aromatic hydrocarbons (R.T. Anderson, personal communication).

Microbial reduction of the oxidised form of uranium, U^{6+}, with an electrode serving as the electron donor represents a potential strategy for improving the bioremediation of groundwater contaminated with uranium. A simple strategy for preventing the further spread of uranium contamination in groundwater is to add an organic electron donor, such as acetate, to the groundwater[80]. This stimulates the growth of *Geobacter* species, which obtain most of their energy from the oxidation of the acetate with the reduction of the Fe^{3+} oxides that are abundant in most subsurface environments. As U^{6+}-containing groundwater enters the zone of acetate addition, the *Geobacter* species also transfer electrons to the soluble U^{6+} reducing it to U^{4+}, which is highly insoluble. This effectively prevents further migration of the uranium, which is beneficial, but has the drawback that the uranium remains in the subsurface. However, when an electrode serves as an electron donor, the U^{4+} that is produced precipitates on the electrode surface[72]. Therefore, it is possible to envision that by placing electrodes in wells drilled into uranium-contaminated subsurface environments, it will be possible to not only prevent the further mobility of uranium by reducing U^{6+} to U^{4+}, but also to extract the uranium when the electrodes are withdrawn from the wells. The precipitated uranium can easily be extracted from the electrodes with bicarbonate and the electrodes can then be redeployed[72]. Field trials of this concept are underway.

Electrodes could potentially serve as electron donors for the biological removal of other contaminants from contaminated groundwater or waste streams. For example, a diversity of microorganisms, including some Geobacteraceae species[81,82], can remove chlorinated solvents from contaminated groundwater through reductive dechlorination, and a typical strategy to promote dechlorination is to add organic electron donors. However, the electron donors must be added at the right level; adding too little electron donor doesn't adequately promote the process, whereas adding too much stimulates unwanted competitive processes, such as methane production. It might be possible with electrodes to 'dial in' just the right amount of electron donor amendment. Nitrate is a common contaminant with substantial impacts on water quality. Although *Geobacter* species colonizing a nitrate-reducing electrode system in a sediment slurry only reduce nitrate to nitrite[73], other organisms can further reduce nitrite to nitrogen gas[76].

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to produce electricity[13]. In microbial fuel cells the fuel source is generally microbially degradable organic matter. These organic fuel sources cannot be used in currently conceived abiotic fuel cells because, unlike hydrogen, these fuels are not electrochemically active. However, microorganisms can catalyse the release of electrons from organic matter and transfer them to various electron carriers that are electrochemically active.

It is generally regarded that electricity production in microbial cultures was first observed over 90 years ago by Potter[14,15]. Potter's studies embodied most of the principles of modern-day microbial fuel cells. The microbial fuel cell consists of an anode, which accepts electrons from the microbial culture, and a cathode, which transfers electrons to an electron acceptor, typically oxygen for most perceived practical applications. The anode compartment is typically maintained under anoxic conditions, whereas the cathode can be suspended in aerobic solutions or exposed to air. Electrons flow from the anode to the cathode through an external electrical connection that typically includes a resistor, a battery to be charged or some other electrical device. The anode and cathode are often separated by a semi-permeable membrane that restricts oxygen diffusion from the cathode chamber to the anode chamber, while allowing protons that are released from organic matter metabolism, or oxidation of reduced metabolic products, to move from the anode to the cathode. At the cathode, electrons, protons and oxygen combine to form water.

Separating the microorganisms from the source of oxygen in a microbial fuel cell intercepts the flow of electrons to oxygen that microorganisms would catalyse if oxygen were available. Although, as detailed below, some microorganisms can conserve energy from electron transfer to anodes, the electrical connection between the anode and the cathode abiotically completes the final electron transfer steps to oxygen, harvesting some of the energy that the microorganisms would otherwise convert to ATP by oxidative phosphorylation.

Liberating electrons from organic matter

With current microbial fuel-cell technologies, anaerobic metabolism must be promoted at the anode in order to convert organic matter to electricity in an effective manner. Fermentation is a well-known mechanism for anaerobic metabolism of organic matter and, until recently, many microbial fuel-cell studies relied solely on fermentative microorganisms[2,5]. However, fermentation alone cannot be a strategy for efficiently converting organic matter to electricity, because most of the electrons available in the organic fuel remain in fermentation products that do not readily react with electrodes.

Effective anaerobic oxidation of complex assemblages of organic matter, such as those found in most wastes and biomass, requires the fermentation products from the metabolism of sugars, amino acids and related compounds, in addition to other constituents, such as aromatic compounds and long-chain fatty acids, to be oxidized with electron transfer to an electron acceptor. The closest analogues to electrodes for microbial metabolism in natural environments are probably Fe^{3+}
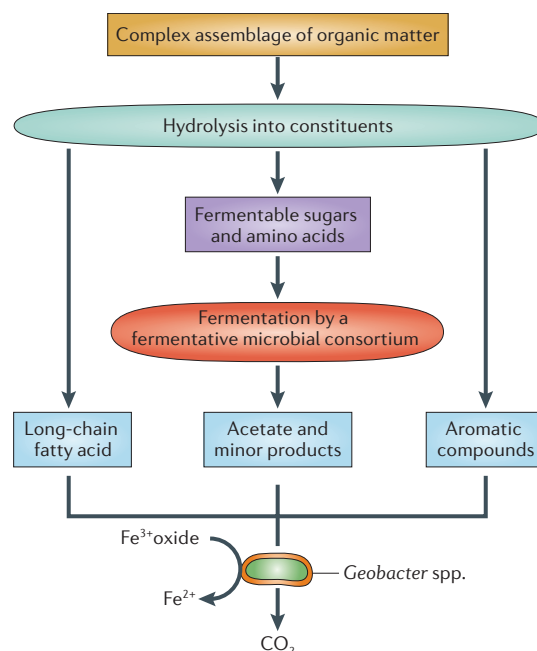

Figure 1 | **Generalized pathway for the anaerobic oxidation of organic matter to carbon dioxide with Fe^{3+} oxide serving as an electron acceptor in temperate, freshwater and sedimentary environments.** The process is mediated by a consortium of fermentative microorganisms and *Geobacter* species.

oxides, because both electrodes and Fe^{3+} oxides are insoluble, extracellular electron acceptors. The oxidation of organic matter, coupled to the reduction of Fe^{3+} oxides in sedimentary environments, requires the cooperation of a consortium of fermentative microorganisms and Fe^{3+}-reducing microorganisms (FIG. 1). Fe^{3+}-reducing microorganisms (most often *Geobacter* species in temperate environments[16] and Fe^{3+}-reducing archaea in hot environments[17]) metabolize the fermentation products and the organic compounds that fermentative microorganisms do not readily metabolize, oxidizing them to carbon dioxide, with Fe^{3+} oxides serving as the electron acceptor. Although there are Fe^{3+} reducers that can completely oxidize fermentable compounds, such as sugars[18] or amino acids[19], to carbon dioxide with the reduction of Fe^{3+}, food chains composed of fermentative microorganisms and Fe^{3+} reducers that oxidize fermentation products are more commonly found, which can be attributed to thermodynamic considerations[20]. It seems likely that, in order to effectively convert organic matter to electricity, similar cooperative consortia and pathways are required, with the exception that an anode serves as the final electron acceptor.

Mechanisms for electron transfer to electrodes

The key difference in microbial electricity production versus natural biogeochemical processes, such as Fe^{3+} reduction, is that the electrons are transferred to an electrode rather than a natural electron acceptor. Four primary mechanisms for microorganisms to transfer electrons to electrodes have emerged to date.

Indirect electron transfer through the interaction of reduced metabolic products with the anode. The earliest studies on microbial fuel cells documented electricity production with fermentative microorganisms and yeast[4,14,15,21,22], but the mechanisms for power generation were not well understood. It was implied that reduced products of microbial fermentation were abiotically oxidized at the anode surface to provide electrons (FIG. 2a). These products might include hydrogen, alcohols or ammonia[2,13,23–25]. However, to my knowledge there were no studies that actually documented this mechanism or directly quantified which reduced products were oxidized at the anode. Such systems for electricity production are inherently inefficient because many
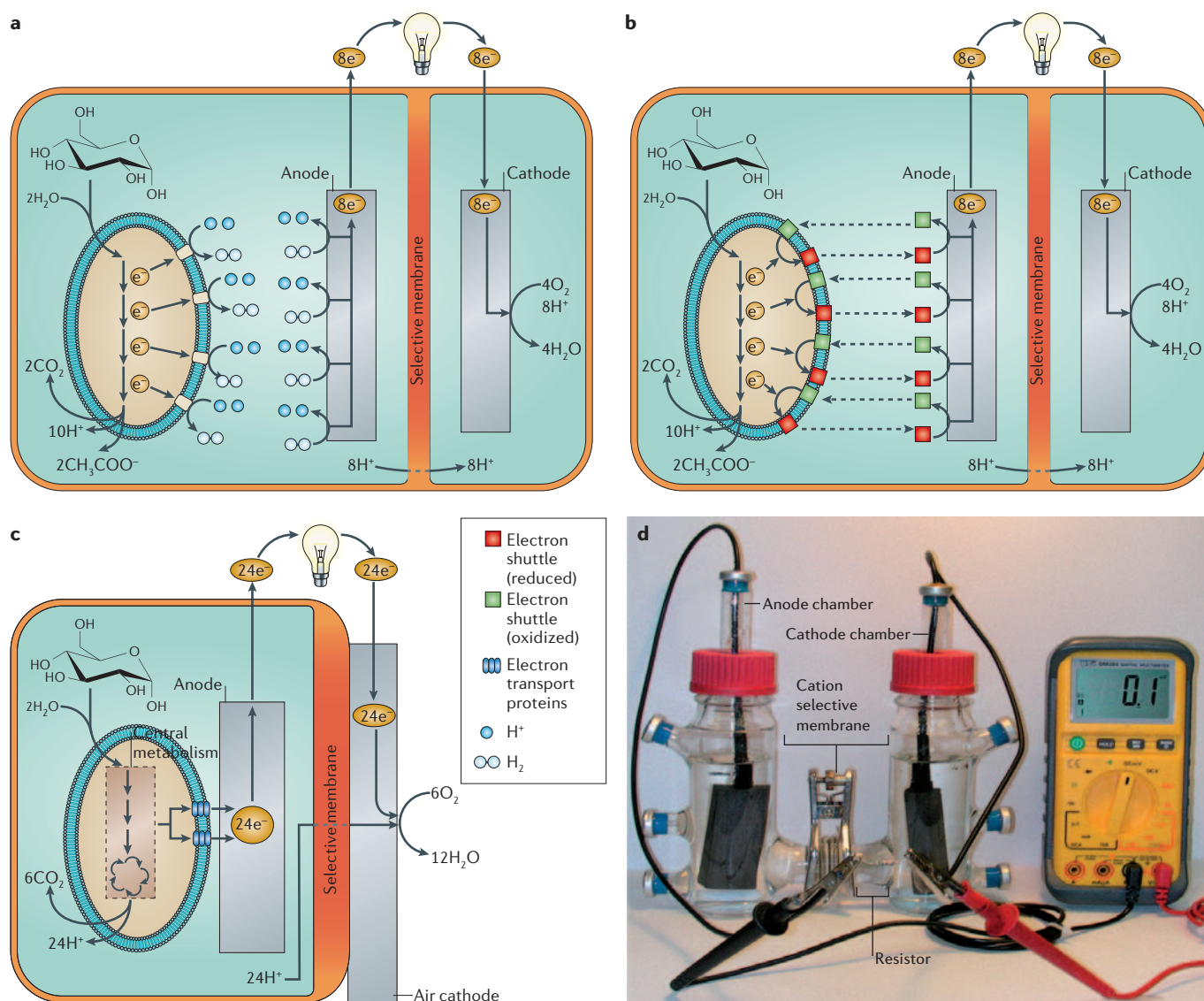

Figure 2 | **Examples of microbial fuel cells producing electricity through different mechanisms of electron transfer to the anode.** Glucose serves as an example fuel. **a** | An indirect microbial fuel cell. A fermentative microorganism converts glucose to an end product, hydrogen, which can abiotically react with the anode to produce electrons and protons. This process only partially recovers the electrons available in the organic fuel as electricity, and results in the accumulation of organic products in the anode chamber. **b** | A mediator-driven microbial fuel cell. An electron-shuttling mediator accepts electrons from reduced cell constituents and abiotically transfers the electrons to the anode. The reoxidized mediator can then undergo repeated cycles of reduction and oxidation. In most instances, the cells that have been used in such fuel cells only incompletely oxidize their organic fuels as shown. **c** | The oxidation of glucose to carbon dioxide with direct electron transfer to the electrode surface. Glucose is taken into the cell and oxidized to carbon dioxide by typical central metabolic pathways, such as the tricarboxylic acid (TCA) cycle. Electrons derived from glucose oxidation are transferred across the inner membrane, periplasm, and outer membrane through electron transport proteins, such as c-type cytochromes. In this example, the system is illustrated with an air cathode rather than a cathode submerged in water. **d** | A two-chambered microbial fuel cell. This system is not optimized for maximum power production but is convenient for microbiological studies.

fermentation products, including organic acids, react very slowly with electrodes, if at all. Although it is possible to modify the composition of anodes to increase their reactivity with some metabolic end products[25], these electrodes tend to foul with oxidation products.

The one reduced product of anaerobic metabolism that will readily react with electrodes is hydrogen sulphide, and power has been generated in fuel cells using sulphate reducers[2]. However, the primary oxidation product is likely to be insoluble sulphur (S^0) (REFS 13,26). Reduction of sulphate to sulphide requires eight electrons, but oxidation of sulphide to S^0 releases only two of these eight electrons at the electrode surface. Therefore, this is not an efficient method for transferring electrons to an anode. Furthermore, the *Desulfovibrio* species typically used in such systems only incompletely oxidize their organic electron donors to acetate, which will not react with electrodes, further limiting efficiency.

Enhanced electron transfer with artificial mediators. Artificial mediators, sometimes referred to as electron shuttles, offer the possibility for microorganisms to generate reduced products that are more electrochemically active than most fermentation products (FIG. 2b). These electron shuttles are typically capable of crossing cell membranes, accepting electrons from one or more electron carriers within the cell, exiting the cell in the reduced form and then transferring electrons onto the electrode surface[2,5]. Mediators are important in microbial fuel cells that use microorganisms such as *Escherichia coli*, *Pseudomonas*, *Proteus*, and *Bacillus* species that are unable to effectively transfer electrons derived from central metabolism to the outside of the cell[2]. Common electron shuttles include thionine, benzylviologen, 2,6-dichlorophenolindophenol, 2-hydroxy-1,4-naphthoquinone and various phenazines, phenothiazines, phenoxoazines, iron chelates and neutral red[2].

In many instances, the reduced constituents within the cell that interact with artificial mediators were not determined, but there are many mechanisms by which mediators might intercept electrons[27]. Detailed investigations on the action of neutral red, which seems to be one of the most effective mediators[28,29], have demonstrated that it can accept electrons from NADH and can be enzymatically reduced by a hydrogenase, and possibly formate dehydrogenase[27].

As previously noted[28], the stoichiometries of conversion of organic electron donors to electricity in the presence of mediators have typically not been determined. It is likely that, in most instances, fermentation products continue to be produced, reducing the efficiency of conversion of fuel to electricity[27,28]. However, cell suspensions of *Proteus vulgaris* were able to completely oxidize sucrose to carbon dioxide, with quantitative electron transfer to an electrode, in a system in which thionine served as the mediator[30].

In addition to incomplete oxidation of most fuels, microbial fuel cells using artificial electron shuttles have several other significant drawbacks. For example, the ability of a microorganism to conserve energy to support growth by electron shuttling to an electrode with an artificial mediator has yet to be demonstrated. This is an important consideration because, for long-term operations, the microorganisms catalysing the oxidation of the organic fuels require some energy for maintenance and possibly growth. A major objection to the use of artificial electron shuttles in the development of practical microbial fuel cells is that most applications will probably require the processing of the organic fuel in open systems, in which there is a continuous or semi-continuous input of the fuel source. This will require continual addition of the electron shuttle, which adds expense. Furthermore, many of the electron shuttles used in previous studies are toxic to humans and could not be responsibly released into the environment.

Microorganisms that produce their own mediators. In some instances, microorganisms might produce their own mediators to promote extracellular electron transfer. This was first proposed as a mechanism to facilitate electron transfer to Fe^{3+} in *Shewanella oneidensis*[31]. Although the conclusions from that initial study were questioned[32], studies in which *Shewanella* species were shown to reduce Fe^{3+} oxides at substantial distances from the cell surface have confirmed the concept of electron shuttles[33–35]. Other organisms, such as *Geothrix fermentans*[36] and *Pseudomonas* species[37,38] also produce electron shuttles. However, some Fe^{3+} reducers, such as *Geobacter* species do not[39], and have instead evolved strategies to seek out[40] and establish contact with Fe^{3+} oxides for direct electron transfer[41]. Biosynthesizing an electron shuttle is energetically expensive[42] and therefore an electron shuttle must be recycled many times in order to recoup this energy investment. For this reason, microorganisms that produce electron shuttles are expected to be at a competitive disadvantage in open environments in which the shuttle will rapidly be lost from the site of release[33]. This might explain why species from the Geobacteraceae predominate over other species under Fe^{3+}-reducing conditions in many sedimentary environments[16].

The same considerations are likely to apply to microbial fuel cells. Electron shuttles were produced in a microbial fuel cell that was sequentially fed glucose over time, but without substantial medium replacement[43]. *Pseudomonas* species isolated from this fuel cell, and *Pseudomonas aeruginosa,* produce phenazine electron shuttles that could aid in electron transfer to electrodes[38,43]. Furthermore, some 16S rRNA gene sequences in the fuel cell were closely related to other organisms, such as *Lactobacillus* and *Enterococcus* species, that might also release electrochemically active compounds[43]. Releasing an electron shuttle might be an adaptive strategy under these conditions because the shuttle is not lost from the system. However, microbially produced electron shuttles are not likely to play an important role in the open flow-through systems necessary for waste treatment[44,45]. In pure culture studies, a one-time replacement of the medium from a *Geothrix* fuel cell decreased power production by 50% (REF. 46). If an electron shuttle was continually being flushed from the system, this would result in a net energetic loss to the

organism. The possible effect of this can be seen in the sediment microbial fuel cells, discussed below, in which *Geobacter* species that do not produce an electron shuttle outcompete *Geothrix* species that do[47].

Another significant factor limiting the effectiveness of electricity production by several of the microorganisms that produce an electron shuttle is that they only incompletely oxidize their organic fuels. For example, the electron-shuttling *Psuedomonas* species recovered from the closed glucose-oxidizing microbial fuel cell could not account for the highly efficient conversion of glucose to electricity in that system, because the isolates had a fermentative metabolism, leaving most of the electrons initially available in glucose in fermentation products[38,43].

As noted above, *Shewanella* species, which produce electricity with lactate as an electron donor[48–50], also produce electron shuttles. The role of shuttles in electricity production by *Shewanella* species has yet to be fully evaluated. However, metabolic constraints also limit the effectiveness of *Shewanella* species in producing electricity. These organisms can only incompletely oxidize a limited number of organic acids such as lactate and pyruvate to acetate under anaerobic conditions[51], even when an electrode serves as the electron acceptor[48,50]. This limits the efficiency of electricity production. A direct coupling between lactate consumption and electricity production was not observed with *Shewanella* because power production declined as consumption of the lactate electron donor continued[48]. In a subsequent study[49], it was suggested that contaminants in the system might be contributing to lactate consumption. This might account for the calculation[52] that in some instances less than 0.03% and never more than 9% of the electrons derived from the conversion of lactate to acetate were recovered as electricity.

Direct electron transfer to electrodes. It was first proposed that microorganisms might be able to transfer electrons to an electrode surface when it was discovered that cultures of *Shewanella putrefaciens* produced electricity while metabolizing lactate[48]. However, this was prior to the discovery, discussed above, that *Shewanella* species produce an electron shuttle, which could account for the electron transfer to the electrode. Cell growth in the presence of an electrode was documented as an increase in optical density[48], suggesting that a high percentage of the cells were planktonic rather than in direct contact with the electrode and, as noted above, growth was not linked to electricity production. Although some cells were observed on the anode, coverage was sparse. It was proposed that electrons might be directly transferred from the cell to the electrode through outer-membrane c-type cytochromes[48], but no direct evidence for this was provided. Furthermore, it is now recognized that outer-membrane cytochromes are important in electron shuttle reduction in *Shewanella*[35].

Other organisms believed to directly transfer electrons to the electrode included a strain of *Aeromonas hydrophila* that produced electricity with yeast extract as the fuel[53]. No studies were done on electron recovery, which make it impossible to determine the efficiency

of conversion of organic matter to electricity. However, the finding that *A. hydrophila* could not use acetate as an electron donor for Fe^{3+} reduction[53] indicates that it was not able to effectively oxidize organic compounds under anaerobic conditions. Current was also produced in a fuel cell inoculated with a *Clostridium* species[54], but current production was not linked to glucose metabolism or growth of the organism, and it was subsequently calculated that less than 0.04% of the electrons available in glucose were recovered as electricity[52].

In summary, the studies described in this section demonstrate the potential for microbial cultures to produce electricity and have greatly advanced understanding in this field. However, none of the types of metabolism documented in this section affords the possibility for complete oxidation of a wide variety of organic compounds coupled with electron transfer to an electrode that, as outlined above, is considered to be necessary for effective conversion of organic matter to electricity. Furthermore, microbial growth that is fuelled by the generation of energy derived from electron transfer to electrodes has not been shown, which is an important consideration in the long-term sustainability of microbial fuel cells.

Oxidation of organic matter with electricigens

The microbial fuel cell that is probably closest to practical application is the sediment microbial fuel cell, also known as a Benthic Unattended Generator or BUG. BUGs (FIG. 3) produce current from the organic matter stored in aquatic sediments[11,55]. Their potential application is to power electronic devices, such as monitoring equipment, at the bottom of the ocean and in other aquatic environments[11,56,57]. An electrode, typically a plate of graphite, is embedded in anoxic sediments and serves as the anode. When the anode is connected to another graphite electrode in the overlying aerobic water, which serves as the cathode, there is a flow of electrons.

It was initially proposed that BUGs operate by principles similar to those outlined above for previously described microbial fuel cells with reduced end products of microbial metabolism, such as sulphide, reacting with the anode, and/or by electron shuttling between microbes and electrodes with naturally occurring electron shuttles, such as humic substances[55]. However, analysis of the microbial community colonizing the surface of the anodes, by characterization of 16S rRNA genes, revealed that there was an enrichment of microorganisms in the family Geobacteraceae on anodes harvesting electricity from sediments[11,47,58]. This Geobacteraceae enrichment has been observed on anodes harvesting electricity from a diversity of marine and freshwater sediments[47], and on anodes harvesting electricity from organic waste matter, such as swine waste[59]. Typically Geobacteraceae account for over half of the microorganisms on the energy-harvesting anodes, whereas they generally constitute less than 5% of the community on control electrodes not connected to a cathode. In marine environments the predominant Geobacteraceae are *Desulfuromonas* species, which prefer marine salinities, whereas in freshwater environments *Geobacter* species predominate.

The hallmark physiological characteristic of microorganisms in the Geobacteraceae is the ability to oxidize organic compounds with electron transfer to insoluble Fe^{3+} oxides[16,60]. Other extracellular electron acceptors, such as humic substances[61] and Mn^{4+} oxides[62], are also reduced. As noted above, Geobacteraceae are often the predominant Fe^{3+}-reducing microorganisms in sedimentary environments in which organic matter oxidation coupled to Fe^{3+} oxide reduction is an important process[16], and the available evidence indicates that Geobacteraceae do not produce electron shuttles, but need to be in direct contact with Fe^{3+} oxides in order to reduce them[16,39].

Pure cultures of Geobacteraceae were found to oxidize their typical electron donors with electron transfer to electrodes, and they conserve energy to support growth from this newly recognised form of respiration[52,58,63]. Acetate is probably the most important electron donor because of its central role in the degradation of organic matter by anaerobic microbial consortia (FIG. 1), but other organic acids, ethanol and aromatic compounds are also used, as is hydrogen. The organic compounds are oxidized to carbon dioxide, with nearly full recovery of the electrons derived from organic-matter oxidation as electricity. Species of Geobacteraceae shown to be capable of this form of respiration include *Geobacter sulfurreducens*, *Geobacter metallireducens*, *Geobacter psychrophilus*, *Desulfuromonas acetoxidans* and *Geopsychrobacter electrodiphilus*. Many of the studies on electron transfer to electrodes in Geobacteraceae have focused on *G. sulfurreducens* because the genome sequence[64] and a genetic system[65] are available, making it the species of choice for physiological studies. Once power production was established with *G. sulfurreducens*, the medium in the anode chamber could be replaced without affecting power production[52]. This demonstrated that cells attached to the anode were responsible for the power production, consistent with the observation that there were few planktonic cells present. These results also showed that a soluble electron shuttle was not involved in electron transfer to the electrode, because the electron shuttle would have been removed when the medium was exchanged, (FIG. 2c).

The ability of Geobacteraceae to oxidize acetate and other organic compounds to carbon dioxide with an anode serving as an electron acceptor, and their heavy colonization of the anodes of sediment microbial fuel cells, provides a likely explanation for much of the power production with sediment microbial fuel cells. As noted above, complex organic matter in anaerobic sediments is typically degraded by a consortium of fermentative microorganisms and Geobacteraceae when Fe^{3+} is available as an electron acceptor (FIG. 1). During energy harvesting with sediment microbial fuel cells, the Geobacteraceae divert electron flow to the anode rather than reducing Fe^{3+} (FIG. 3a).

Furthermore, *Geobacter* species can serve as the catalyst in more compact fuel cells that are fed various fuels. In flow-through systems with an air cathode, *G. sulfurreducens* generates 350 milliWatts per metre squared of electrode surface and 60 Watts per cubic metre of reactor volume (K.P. Nevin and D.R.L., unpublished observations), which is comparable to the power output of mixed communities in state-of-the-art microbial fuel cells designed as prototypes for extracting electricity from large volumes of organic waste[12,66]. These *G. sulfurreducens* fuel cells are likely to produce power as long as they are provided with fuel. They have been maintained in our laboratory with periodic replenishment of acetate for over a year with no loss in power production.

Other electricigens

The ability of *Geobacter* species to completely oxidize organic compounds with an electrode serving as the electron acceptor, and to conserve energy to support
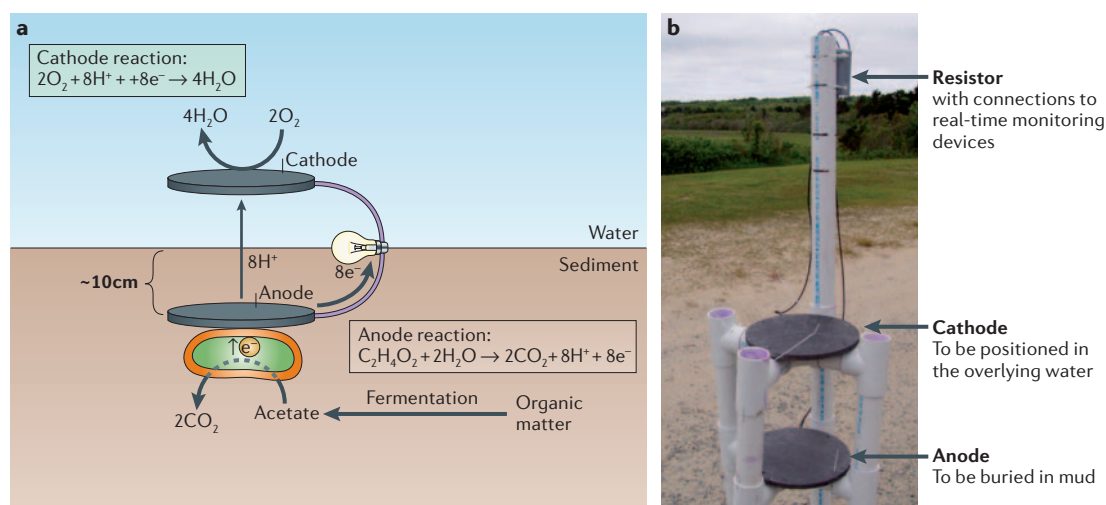

Figure 3 | **A sediment microbial fuel cell. a** | A schematic of a sediment microbial fuel cell. Organisms in the family Geobacteraceae can oxidize acetate and other fermentation products, and transfer the electrons to graphite electrodes in the sediment. These electrons flow to the cathode in the overlying aerobic water where they react with oxygen. **b** | An actual sediment fuel cell before deployment.

growth from this metabolism, represents a novel form of microbial respiration. The oxidation of electron donors, such as acetate, which are key constituents of carbon and electron flow during anaerobic degradation of organic matter, demonstrates that it is possible to effectively convert complex organic matter to electricity with a combination of *Geobacter* species and the appropriate fermentative microorganisms. The capacity to conserve energy from this metabolism is essential for the long-term sustainability of a microbial fuel cell.

In order to facilitate discussion, one-word terms are generally coined to describe various forms of microbial respiration. For example, microorganisms that conserve energy to support growth from methane production are commonly referred to as methanogens. Therefore, it is suggested that microorganisms that conserve energy from electron transfer to an electrode be referred to as electricigens. Previously, the terms electrodophile and anodophile have been used to describe microorganisms associated with anodes[29,50]. However, these terms have also been associated with microorganisms that have not been shown to conserve energy to support growth from electron transfer to electrodes. The term anodophile is also not specific enough to describe the respiration of electricigens because many microorganisms might attach to the surface of an anode, but not necessarily contribute to electricity production. Furthermore, it has not been demonstrated that any microorganism is actually anodophilic, with a specific affinity for electrodes.

Several electricigens outside the Geobacteraceae have been described. *Rhodoferax ferrireducens*, which was isolated from subsurface sediments as an Fe^{3+} reducer[67], oxidizes sugars, such as glucose, fructose, sucrose, lactose and xylose, to carbon dioxide with over 80% recovery of the electrons derived from sugar oxidation as electricity[18]. This organism is of special interest because of the long history of attempts to convert sugars to electricity in microbial fuel cells. As was observed with *G. sulfurreducens*, power production by *R. ferrireducens* could be attributed to the cells attached to the electrode surface and power production was sustained for long periods of time[18]. When the electrical connection in the *R. ferrireducens* fuel cell was disconnected for 36 hours, leaving *R. ferrireducens* with no means of energy generation, power production resumed as soon as the connection was restored. The capacity for storage under idle conditions without deterioration in performance is a desirable characteristic for a microbial fuel cell and further demonstrated the long-term survival abilities of *R. ferrireducens*. *R. ferrrireducens* has not yet been identified on sediment microbial fuel cells. This is analogous to the finding, summarized above, that communities of fermentative microorganisms and acetate-oxidizing Fe^{3+}-reducers outcompete Fe^{3+}-reducing microorganisms that can completely oxidize sugars in sediments. However, a microorganism with a metabolism like that of *Rhodoferax* might be an ideal candidate for a pure culture system for converting sugars to electricity (FIG. 2c).

Another electricigen discovered from molecular analysis of the anode surfaces of sediment microbial fuel cells is *Desulfobulbus propionicus*. Electrodes harvesting electricity from sediments with high concentrations of
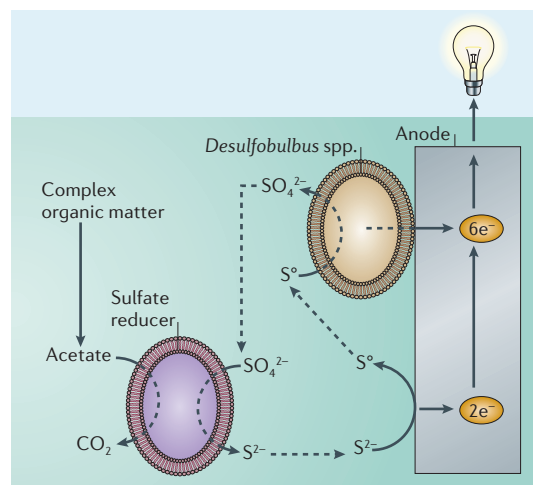

Figure 4 | **Mechanisms by which reduced sulphur compounds can contribute to electricity production in sediment microbial fuel cells in sulphide-rich sediments.** Sulphate (SO_4^{2-}) reducers produce sulphide (S^{2-}) which can abiotically react with the anode, yielding two electrons and sulphur (S^0). *Desulfobulbus* species that colonize the anode can oxidize S^0 extracting six additional electrons and recycling the sulphate.

sulphide (S^{2-}) were colonized by microorganisms in the family *Desulfobulbaceae*[47]. Studies with *D. propionicus*, a pure culture representative of this family, revealed that it oxidized S^0 to sulphate (SO_4^{2-}) with an electrode serving as the sole electron acceptor[26]. This might be an important reaction at the anode surface in sediments with high concentrations of sulphide because, as noted above, the sulphide produced might abiotically react with electrodes producing S^0. This abiotic reaction only harvests two of the eight electrons potentially available from sulphide oxidation (FIG. 4). Oxidation of S^0 to sulphate extracts six more electrons and regenerates sulphate as an electron acceptor for further microbial reduction.

It seems likely that there is wide diversity of electricigens yet to be discovered. For example, molecular analysis of the microbial communities associated with the degradation of acetate in a flow-through system[44], or glucose in a fed-batch system[43], indicated that there were many phylogenetically distinct constituents in each of these communities. This is in contrast to the less diverse communities enriched on sediment microbial fuel cells, and there is a strong need to further evaluate the microbiology of such systems.

Mechanisms of electron transfer to electrodes

As noted in the introduction, much of the focus in improving the output of microbial fuel cells has been on improving the electrochemical design of the devices. However, if there was a better understanding of how electricigens transfer electrons to anodes, this information might be useful in designing better anode materials to interact with the appropriate electron transfer proteins[29]. It is important to recognize that although microorganisms have had billions of years to optimize electron transfer to natural extracellular electron

acceptors, such as Fe^{3+} oxides, it is unlikely that there has been substantial evolutionary pressure to select for the most effective strategies for electricity production. This indicates that it might be possible to enhance electricity production through adaptive evolution or genetic engineering.

In the absence of any evidence that microbial electron transfer to graphite is an important natural phenomenon, it is assumed that the ability of electricigens to produce electricity is related to their capacity to transfer electrons onto natural extracellular electron acceptors, such as Fe^{3+} and Mn^{4+} oxides and humic substances. Insights into electricity production might come from the understanding of electron transfer to these natural electron acceptors.

It is clear that one of the most formidable barriers to microorganisms transferring electrons onto Fe^{3+} or electrodes is the non-conducting lipid-membrane system that serves as an insulator, separating the cytoplasm,

where electrons are extracted from organic matter during central metabolism, from the outside of the cell where the final electron transfer must take place. A potential strategy for *G. sulfurreducens* to transfer electrons that are derived from the central metabolism onto extracellular Fe^{3+} oxides is beginning to emerge (FIG. 5). Current evidence suggests that a series of c-type cytochromes associated with the inner membrane, the periplasm, and the outer membrane might interact to transfer electrons to the outer membrane surface[16]. However, growth on Fe^{3+} oxides also requires the presence of specialized pili that are localized to one side of the cell[41]. Fe^{3+} oxides specifically associate with these pili, which are electrically conductive[41]. This suggests that pili are the electrical conduit between the cell and Fe^{3+} oxides.

Initial studies indicated that *G. sulfurreducens* formed little more than a monolayer on the surface of electrodes, suggesting that close contact between the cells and the anode was required[52]. Under these conditions, current
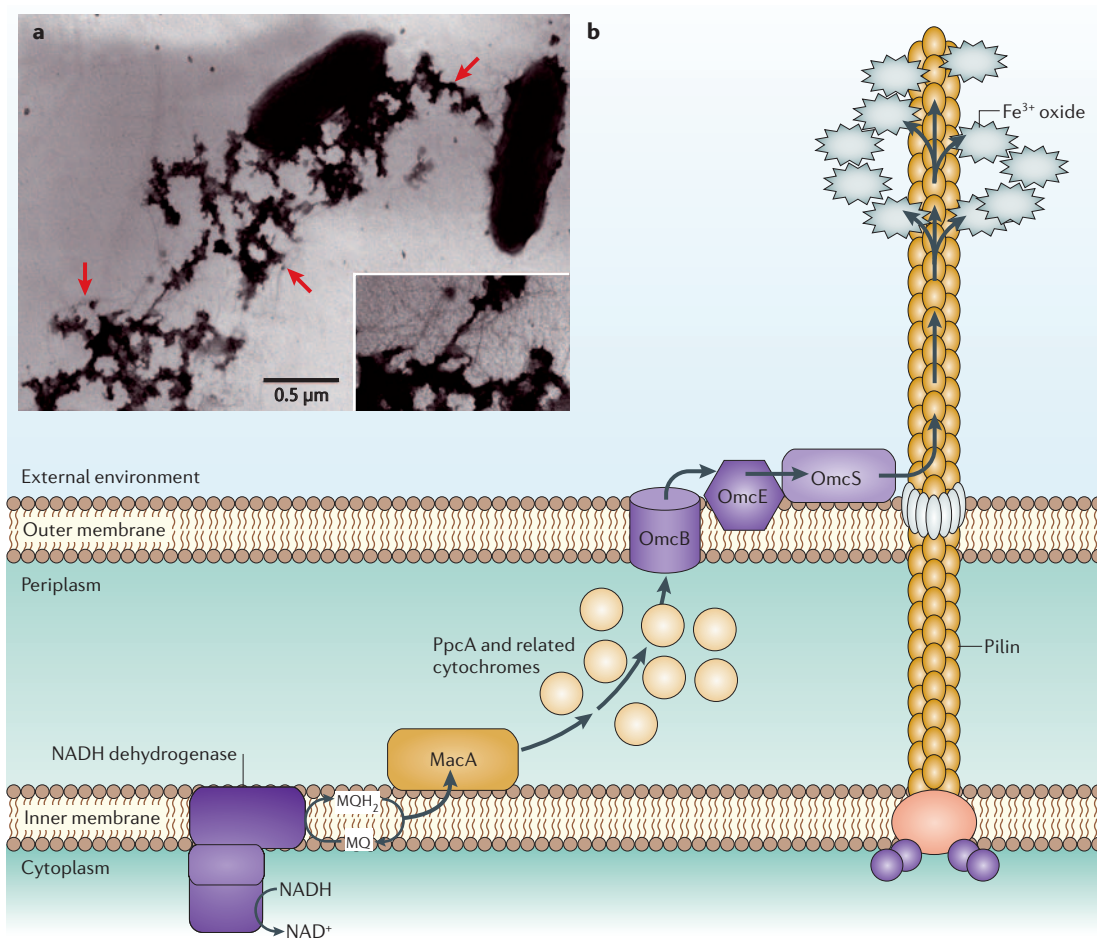

Figure 5 | **A mechanism for extracellular electron transfer by *Geobacter sulfurreducens*. a** | A transmission electron micrograph showing the association of Fe^{3+} oxide (indicated by arrows) with pilin expressed by *Geobacter sulfurreducens*. The inset shows pili that are intertwined with Fe^{3+} oxides. **b** | Potential route for electron transfer to Fe^{3+} oxides by *Geobacter sulfurreducens*. This model is based on a previous model[16] and subsequent findings[41,69]. MacA, PpcA, OmcB, OmcE and OmcS are c-type cytochromes which genetic studies have indicated are required for optimal Fe^{3+} reduction. The proposed electron flow between the cytochromes is based on their reported location within the bacterial cell. It is important to note that the genome of *G. sulfurreducens* contains genes that encode approximately 100 c-type cytochromes[64], some of which might also participate in this electron transfer process. MQH_2, menaquinol; MQ, menaquinone. Panel (**a**) is reproduced with permission from *Nature* REF. 41 © (2005) Macmillan Publishers Ltd.

can be produced in the absence of pili providing the cell retains the ability to produce the outer-membrane cytochrome, OmcS[68]. OmcS, which is also essential for Fe^{3+} oxide reduction, is displayed on the outer surface of the cell[69]. It might be that OmcS can make electrical contact with the relatively flat surface of electrodes, alleviating the need for the conductive pili that seem to be required for effective contact with heterogeneously dispersed Fe^{3+} oxides. However, current production in those initial studies was low. In improved systems with a tenfold or greater power output, thick, visually apparent, biofilms formed on the anode. The conductive pili are essential for the development of these thicker biofilms and higher levels of current production[70]. This suggests that the pili are involved in electron transfer to the anode for cells that are not in direct contact with the anode surface. Further understanding of the mechanisms of electron transfer to electrodes is likely to accelerate now that techniques are available for monitoring gene expression during growth on electrodes[68,71].

The firm attachment of *Geobacter* and *Rhodoferax* species to electrodes contrasts with the current model for the behaviour of dissimilatory metal-reducing microorganisms in sedimentary environments[16], in which permanent attachment to the Fe^{3+} oxide surface is unlikely to be beneficial because Fe^{3+} reducers must have the mobility to search for new sources of Fe^{3+} once the Fe^{3+} oxide in one location is depleted. By contrast, electrodes represent a more enduring electron sink and therefore more permanent attachment to electrode surfaces might be advantageous. How *Geobacter* or other organisms make this distinction in the quality of the electron acceptors is unknown.

Electrodes as an electrode donor

In addition to using electrodes as an electron acceptor, *Geobacter* species can accept electrons from an electrode poised at low redox potentials[72,73]. In this instance, the microorganisms are interacting with the cathode, which provides electrons for anaerobic respiration. The ability of *Geobacter* species to 'run backwards' in this manner is analogous to processes seen in sediments. With organic compounds serving as the electron donor *Geobacter* species reduce Fe^{3+} oxides to Fe^{2+} and transfer electrons onto the quinone moieties of extracellular

humic substances[61]. However, when nitrate is available as an electron acceptor, the same *Geobacter* species can accept electrons from solid-phase Fe(II)-bearing minerals[74] and reduced humic substances[75] with the reduction of nitrate. Electron acceptors that *Geobacter* species can reduce with an electrode serving as the electron donor, include nitrate and fumarate[73] and the oxidized form of uranium, U^{6+} (REF. 72). Other as-yet-unidentified microorganisms might be involved in electrode-driven nitrate reduction in other environments[76].

Specific enrichments of microorganisms have been observed on the cathodes of sediment microbial fuel cells[47] and therefore microorganisms might be involved in, or influenced by, electron transfer to oxygen at the cathode surface. A new microbiological approach to improve cathode performance is to use iron or manganese as a mediator or component of the electrode, with renewal of the oxidized form of the metals catalysed by metal-oxidizing microorganisms[7,57,77].

Conclusions

This is an exciting time in microbial fuel-cell research. The field is in its infancy, but the discovery of pure cultures that can serve as models for complete oxidation of organic compounds with direct electron transfer to electrodes provides an important tool for understanding the ecology and physiology of fuel cells that can harvest energy from complex wastes. The availability of complete genome sequences and genetic systems for a number of these organisms means that detailed mechanistic investigations into extracellular electron transfer to electrodes and the possibilities for accelerating this process are feasible. Furthermore, it seems likely that there are many microorganisms yet to be discovered that might be beneficial for electricity production. The development of the myriad of potential practical applications of microbial fuel cells will require the well-coordinated efforts of electrochemists, materials scientists, engineers and microbiologists. Even if the generation of high levels of electricity from microbial fuel cells is a long way off, an understanding of the coupling of organic matter oxidation to electron transfer to electrodes is likely to yield important insights into the diversity of microbial respiratory capabilities and might lead to as-yet-unforeseen applications in nano-electronics.

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DATABASES

The following terms in this article are linked online to:
Entrez Genome Project: http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
Desulfuromonas acetoxidans | *Escherichia coli* | *Geobacter metallireducens* | *Geobacter sulfurreducens* | *Geothrix ferementans* | *Pseudomonas aeruginosa* | *Rhodoferax ferrireducens* | *Shewanella oneidensis* | *Shewanella putrefaciens*

FURTHER INFORMATION

Derek R. Lovley's homepage: http://www.geobacter.org
Benthic Unattended Generator (BUG) homepage: http://www.nrl.navy.mil/code6900/bug
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