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Live wires: direct extracellular electron exchange for bioenergy and the bioremediation of energy-related contamination

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PERSPECTIVE

Live wires: direct extracellular electron exchange for bioenergy and the bioremediation of energy-related contamination

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Microorganisms that can form direct electrical connections with insoluble minerals, electrodes, or other microorganisms can play an important role in some traditional as well as novel bioenergy strategies and can be helpful in the remediation of environmental contamination resulting from the use of more traditional energy sources. The surprising discovery that microorganisms in the genus *Geobacter* are capable of forming highly conductive networks of filaments that transfer electrons along their length with organic metallic-like conductivity, rather than traditional molecule to molecule electron exchange, provides an explanation for the ability of *Geobacter* species to grow in subsurface environments with insoluble Fe(III) oxides as the electron acceptor, and effectively remediate groundwater contaminated with hydrocarbon fuels or uranium and similar contaminants associated with the mining and processing of nuclear fuel. A similar organic metallic-like conductivity may be an important mechanism for microorganisms to exchange electrons in syntrophic associations, such as those responsible for the conversion of organic wastes to methane in anaerobic digesters, a proven bioenergy technology. Biofilms with conductivities rivaling those of synthetic polymers help *Geobacter* species generate the high current densities in microbial fuel cells producing electric current from organic compounds. Electron transfer in the reverse direction, *i.e.* from electrodes to microbes, is the basis for microbial electrosynthesis, in which microorganisms reduce carbon dioxide to fuels and other useful organic compounds with solar energy in a form of artificial photosynthesis that is more efficient and avoids many of the environmental sustainability concerns associated with biomass-based bioenergy strategies. The ability of *Geobacter* species to produce highly conductive electronic networks that function in water opens new possibilities in the emerging field of bioelectronics.

Introduction

Direct extracellular electron transfer (DEET), one of the most recently discovered forms of microbial respiration, offers the possibility of novel bioenergy strategies as well as cost-effective, sustainable approaches for the remediation of environments contaminated in the extraction, transportation, or

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use of fossil and nuclear fuels. In DEET, microorganisms form direct electrical connections with insoluble materials that can either accept or donate electrons. Common examples are Fe(III) oxides, electrodes, and even other microorganisms.

It is well known that microorganisms capable of DEET have a major impact on the natural cycling of carbon, metals, and nutrients[1,2] and this topic will not be reviewed here. Rather, the focus is on surprising new information on how DEET is possible and new concepts for the application of DEET to

Broader context

Novel biological processes are a potential source of solutions for the need for new, sustainable energy strategies and the necessity of dealing with the legacy of environmental contamination associated with more traditional energy sources. The genomes of the microbial world encode a vast metabolic potential, which for the most part is poorly understood, but may provide some help for energy needs. This perspective gives a quick primer on the basic principles of direct extracellular electron transfer, a relatively recently discovered form of microbial respiration, and summarizes how continuing developments in the study of this one form of microbial respiration has led to a number of new concepts for bioenergy and the restoration of environments contaminated as the result of energy-related activities.

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bioenergy and the restoration of environments contaminated as the result of energy practices.

Distinguishing DEET from other forms of microbial respiration

DEET requires unique physiological adaptations, which make it feasible for microorganisms to establish direct electrical connections with extracellular electron acceptors. DEET is fundamentally different than most other commonly considered forms of microbial respiration, such as reduction of oxygen, nitrate, sulfate, or carbon dioxide, in which the electron acceptors enter the cell and are reduced internally. DEET, by definition, requires that microorganisms transfer electrons outside the cell in order to reduce them.

An equally important distinction, is the significant difference between microorganisms that transfer electrons onto insoluble extracellular electron acceptors with DEET and microorganisms that produce soluble electron shuttles to transfer electrons to those electron acceptors. For example, *Shewanella* species are the most intensively microorganisms capable of reducing insoluble Fe(III) oxides or transferring electrons onto electrodes.[3,4] However, *Shewanella* species release soluble molecules that shuttle electrons between the outer cell surface and Fe(III) oxides[5–7] or electrodes.[8–10] Although it has been suggested that *Shewanella oniedensis* can also make direct electrical contact with extracellular electron acceptors *via* conductive filaments,[11,12] multiple lines of evidence have indicated that this is unlikely, as detailed below. Elegant studies have demonstrated how *S. oniedensis* can transfer electrons to the outer surface of the cell to the multi-heme cytochrome MtrC,[13,14] but MtrC is a flavin reductase[15] and requires flavin to reduce insoluble Fe(III) oxide at physiologically relevant rates.[16]

The difference between DEET and reduction *via* an electron shuttle has important ecological consequences. It is frequently stated that *Shewanella* species play an important role in Fe(III) reduction and bioremediation in soils and sediments, but, to my knowledge there is not a single example where this has actually been shown to be the case. Molecular studies that avoid culture bias have repeatedly demonstrated that *Shewanella* species are not abundant in environments in which Fe(III) reduction is important and *Shewanella* species are not abundant when Fe(III) reducing microorganisms are stimulated to promote the reduction of organic or metal contaminants.[2,17,18] Although many factors may influence which Fe(III) reducers predominate in soils and sediments,[19] the necessity to produce an electron shuttle that may be lost from the cell may be a metabolic burden that lowers competitiveness in open environments.[6,20] In a similar manner, *Shewanella* species are rarely detected on anodes in which a complex community serves as the inoculum for harvesting electricity.[21] The elegance of studies on *Shewanella* extracellular electron transport are without question. However, DEET and electron transfer *via* a shuttle are fundamentally different mechanisms and investigations whose ultimate purpose is to understand the mechanisms of metal reduction in subsurface environments or current production from organic wastes might more productively focus on other organisms.

It is likely that there are many microorganisms that are capable of DEET. However, to date, *Geobacter* species are the only microbes clearly demonstrated to rely on DEET to conserve energy for anaerobic growth that are also genetically tractable. *Geobacter* species are frequently numerically abundant organisms in soils and sediments and on the surface of electrodes where DEET is important,[18,21] which suggests that they are environmentally significant agents of DEET. Unfortunately, *Geobacter* species are studied in few laboratories. Thus, this review of DEET and its energy and environmental impacts is strongly weighted with references from the author's laboratory.

Insoluble electron acceptors

Fe(III) is the most abundant electron acceptor available for DEET in soils and sediments. When soils and sediments become waterlogged, oxygen is rapidly depleted and Fe(III) is typically the most abundant potential electron acceptor for microbial respiration.[1,22] Fe(III) is very poorly soluble at circumneutral pH, and is found as various Fe(III) oxides and other minerals, such as clays.[23]

Mn(IV) oxides are second to Fe(III) in abundance as extracellular electron acceptors in many soils and sediments and may provide about 10% of the electron-accepting capacity of Fe(III) in these environments. The total electron flow to Fe(III) and Mn(IV) is based not only on the amount of Fe(III) and Mn(IV) in the environment, but also the extent to which the Fe(II) and Mn(II) produced from Fe(III) and Mn(IV) reduction are recycled back to Fe(III) and Mn(IV) (ref. 23,24, and references therein).

Humic substances are large complex, poorly defined organic compounds, which are often the most abundant form of organic matter in soils. Quinone moieties in humic substances can serve as electron acceptors for microbial respiration.[25–28] Although initial studies focused on microbial reduction of soluble humic substances for technical simplicity, insoluble humic substances can also serve as electron acceptors.[28] The importance of humic substances as electron acceptors in soils and sediments is difficult to quantify, but could be significant in some environments.[6,28–30] One reason for this is that the reduced hydroquinone state of humic substances can abiotically react with Fe(III), reducing it to Fe(II) and regenerating the oxidized form of the humic substance. The addition of humic substances or humic substances analogs, such as anthraquione-2,6-disulfonate, can greatly accelerate the rate of Fe(III) reduction, suggesting that humic substances may be more accessible for microbial reduction than Fe(III) oxides, especially when they are soluble. Therefore, even if the total amount of reactive humic substances is low, a significant amount of electron flow may proceed through humic substances due to continuous recycling of the humics pool.

Other microorganisms may function as extracellular electron acceptors, through a process known as direct interspecies electron transfer (DIET). Initial evidence for DIET was seen in a co-culture of *Geobacter metallireducens* and *Geobacter sulfurreducens*.[31] When grown under conditions that required the two species to exchange electrons in order to metabolize the ethanol that was provided as a substrate, the co-culture evolved over time to produce large (1–2 mm diameter) dual-species aggregates. Multiple lines of evidence ruled out the possibility that the two species were exchanging electrons *via* the well-known process of interspecies hydrogen transfer,[32,33] in which one partner microorganism in the syntrophy disposes of electrons by reducing protons to hydrogen gas and the other partner oxidizes the

hydrogen gas. DIET is expected to conserve more energy for microorganisms exchanging electrons than interspecies hydrogen transfer because it eliminates the multiple enzymatic steps necessary to first produce hydrogen and then consume it.[34]

Some microorganisms have the capacity to directly exchange electrons with electrodes.[35–37] By convention, an electrode serving as an electron acceptor for microbial metabolism is referred to as an anode. Electron flux from the anode to a cathode, typically through metallic wire connection, yields an electrical current in what is commonly referred to as a microbial fuel cell.

Almost every review of microbe-electrode interactions points out that the ability to produce small amounts of electric current with microorganisms has been known for over 100 years. A major paradigm shift in microbe-electrode interactions was the relatively recent discovery that microorganisms could conserve energy to support their growth by completely oxidizing organic compounds to carbon dioxide with direct electron transfer to electrodes.[38–40] One reason that this was an important distinction was that it demonstrated for the first time that organic compounds could be converted to electricity with high columbic efficiency. In previous studies the organic compounds were only partially metabolized leaving most of the electrons available in the initial substrate in organic products, rather than harvesting the electrons as current. The capacity for energy conservation from electron transfer to electrodes is also important because it makes current-producing microbial systems self-sustaining and thus robust.

When electrodes are poised at low electron potentials they can serve as electron donors for microbial respiration.[41–43] In this instance the electrode is acting as a cathode. This form of respiration was first observed in *Geobacter* species using nitrate,[44] fumarate,[44] U(VI),[45] or chlorinated compounds[46] as an electron acceptor. A diversity of microorganisms may be capable of this process with a wide range of electron acceptors,[41–43] but in most instances the microbiology has not been studied in sufficient detail to determine whether there is a direct exchange of electrons between the electrode and the cells.[42]

Mechanisms for DEET

Direct electron transfer to Fe(III) oxides

The finding that *Geobacter* species are often abundant constituents of environments in which Fe(III) reduction is an important process,[18] coupled with the availability of a genetic system for *Geobacter sulfurreducens*[47] established *G. sulfurreducens* as the initial organism of choice for developing a preliminary model for DEET to Fe(III). Gene deletion studies have identified a number of proteins that are essential for Fe(III) oxide reduction, but the model for Fe(III) reduction is far from complete.

The terminal step in the pathway appears to be electron transfer from the multi-heme, c-type cytochrome, OmcS[48] to Fe(III) oxide. OmcS is required for Fe(III) oxide reduction[49] and is specifically localized along the pili of *G. sulfurreducens*,[50] which are also required for Fe(III) oxide reduction.[51] The pili are 3–5 nm in diameter, typically 10–20 μm long, and are electrically conductive,[51,52] and thus are also referred to as 'microbial nanowires'. The simplest explanation for these observations is that the pili deliver the electrons to OmcS which transfers electrons to Fe(III). The fact that cells that produce pili, but not

OmcS, do not reduce Fe(III) oxide suggests that there is a barrier to direct electron transfer from the pili to Fe(III) oxide.

Recent studies have revealed that electron conduction along the length of the pili of *Geobacter sulfurreducens* is similar to the metallic-like conductivity observed with some synthetic organic polymers.[52] In other words, electron conduction along the length of the pili is through conjugated π orbitals of pilin constituents, presumably aromatic amino acids. This mechanism for long-range electron transport is a paradigm shift in biology because, in the previously known mechanisms for biological electron transfer, electrons are always associated with a specific molecule and move from molecule to molecule *via* tunneling or hopping over short distances. In fact, the initial suggestion that electrons could be transferred along *Geobacter* pili[51] was met with significant criticism because a mechanism for electron conduction along a protein filament was not immediately apparent.[3] However, multiple lines of evidence including: electrochemical gating studies; the temperature dependence of conductance; the pH dependence of conductance; and preliminary structural studies; suggest that electron transfer along pili is an organic metallic-like conduction, which might be attributed to π–π interchain stacking between aromatic amino acids conferring conductivity along the length of the pili.

The data demonstrating metallic-like conductivity along the pili rules out electron hopping between OmcS as the mechanism for long-range conduction along the pili. Additional evidence consistent with this conclusion is that the spacing between the OmcS molecules is too great for cytochrome-to-cytochrome electron transfer,[50] a conclusion subsequently confirmed with atomic force microscopy (N. Malvankar, unpublished data). Furthermore, denaturing cytochromes does not impact electron conduction along the pili.[52] Therefore, the proposed role of OmcS is to facilitate electron transfer from the pili to Fe(III) oxides (Fig. 1).

This model for metallic-like electron transfer along the pili of *Geobacter* contrasts with the model that cytochromes are responsible for electron transport along conductive filaments of *Shewanella oneidensis*.[11,12] However, as previously reviewed,[3,53] there are serious criticisms of this cytochrome model, not the
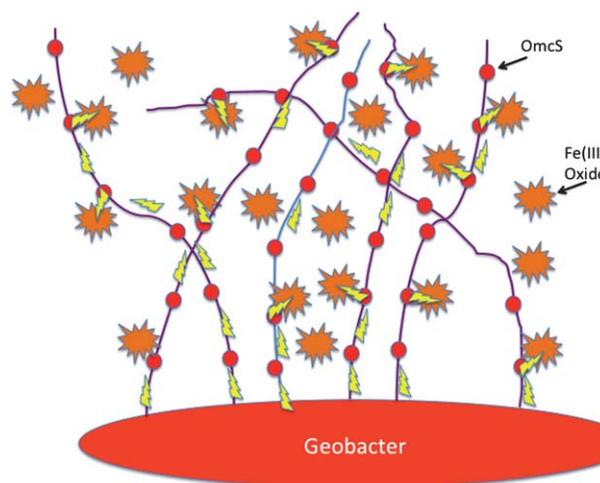

Fig. 1 Hypothesized electron transfer to Fe(III) oxides along conductive pili with the *c*-type cytochrome OmcS mediating electron transfer from pili to Fe(III) oxide. The actual electron transfer pathway may be much more complex and require additional proteins.

least of which is that the cytochromes important in extracellular electron transfer in *S. oneidensis* may not even be localized on the filaments that have been proposed to be conductive. Furthermore, packing of cytochromes close enough to promote electron transfer along the length of the filaments seems unlikely. Genetically removing the capacity for pilin production, and other experimental approaches, suggest that *S. oneidensis* pili do not play an important role in extracellular electron transfer.[9,54,55] Other structures associated with *S. oneidensis* that were previously identified as protein filaments are now thought to polysaccharide artifacts of sample preparation.[56]

Additional evidence for the importance of pili in Fe(III) oxide reduction in *Geobacter* species was the finding that adaptive evolution for enhanced Fe(III) oxide reduction by *G. sulfurreducens* selected for a mutation that enhanced the expression of PilA, the structural pilin protein.[57] Furthermore, a strain that was selected for enhanced current production, which also had significantly greater pilin production,[58] was also a superior Fe(III) oxide reducer (unpublished data).

An alternative model for extracellular electron transfer in *G. sulfurreducens* that does not invoke pili is that a polysaccharide matrix outside the cell is the primary location of electron transfer.[59] However, this model does not account for the finding that the predominant *c*-type cytochrome in the extracellular matrix is OmcZ,[59] which is not required for Fe(III) oxide reduction.[60] OmcS, which is required for Fe(III) oxide reduction, is not found within the matrix.

One mystery is how electrons get transferred to the pili. Given the importance of the outer-surface cytochrome OmcB in electron transfer to Fe(III) oxide, one speculation is that this takes place at the outer cell surface. There are a number of additional *c*-type cytochromes and other putatively redox active proteins associated with the outer surface of the cell that could conceivably facilitate this transfer.[49,61–64] Abundant periplasmic *c*-type cytochromes,[65–67] may shuttle electrons from the inner membrane to the outer membrane or conceivably could transfer electrons to the base of the pili within the cell.

In soils and sediments, the insoluble Fe(III) that is available for microbial reduction is expected to be heterogeneously dispersed. The specific expression of flagella during growth on Fe(III) oxide, but not soluble Fe(III)-citrate, as well as apparent chemotaxis to Fe(II) led to the suggestion that *Geobacter* species remain motile during active Fe(III) reduction in order to hunt for new sources of Fe(III).[68] *Geobacter* species are readily collected from groundwater in subsurface environments in which there is rapid Fe(III) reduction[69] and are predominately planktonic when rates of Fe(III) reduction are the highest in the subsurface. Furthermore, recent genetic studies have demonstrated that the presence of flagella greatly enhances Fe(III) reduction in subsurface sediments.[70]

In the planktonic state *Geobacter* species have less access to Fe(III), but their abundant *c*-type cytochromes may function as capacitors, storing electrons and permitting cells to continue to transfer electrons across the inner membrane.[53,71] Once a new source of Fe(III) is found, the electrons stored in the cytochromes can be discharged.

There are still many aspects of *Geobacter* extracellular electron transfer that are not known. However, the current information makes it possible to envision how *Geobacter* species might function in the subsurface.

Direct electron transfer to electrodes

G. sulfurreducens is the only microorganism capable of producing high current densities with direct electron transfer to electrodes in which the mechanisms for electron transfer to electrodes have been investigated. The capacity of *G. sulfurreducens* to transfer electrons to electrodes is not impacted by the exchange of the medium within the anode chamber, which would remove any soluble molecules potentially involved in extracellular electron exchange[39] and electrochemical analyses suggest that *G. sulfurreducens* establishes direct electrical contact with electrodes.[72–78] The mechanisms for electron transfer to electrodes by *G. sulfurreducens* are of special interest because this organism produces the highest current densities of known pure cultures[58,79] and because microorganisms closely related to *G. sulfurreducens* are frequently enriched when electrodes are used to harvest electricity from mixed communities.[21,35–37]

Although Fe(III) oxides and electrodes are both extracellular electron acceptors, the surface of an electrode and a soil containing heterogeneously dispersed Fe(III) are significantly different. Most importantly, an electrode can serve as a permanent electron sink whereas Fe(III) at any particular site will eventually be depleted. During active current production *Geobacter* species form thick (>50 μm) biofilms strongly attached to electrode surfaces.[80–82] In contrast, as noted above, *Geobacter* species are primarily planktonic during active reduction of insoluble Fe(III). Therefore, the components involved in electron transfer to electrodes and insoluble Fe(III) may be the same, but function somewhat differently (Fig. 2).

G. sulfurreducens requires its conductive pili for optimal current production. A mutant strain that can not produce pili can not reduce Fe(III) oxide,[51] but can still produce current, although at much lower levels than wild-type.[60,80] This is associated with much thinner biofilms of the mutant in which only the cells in close
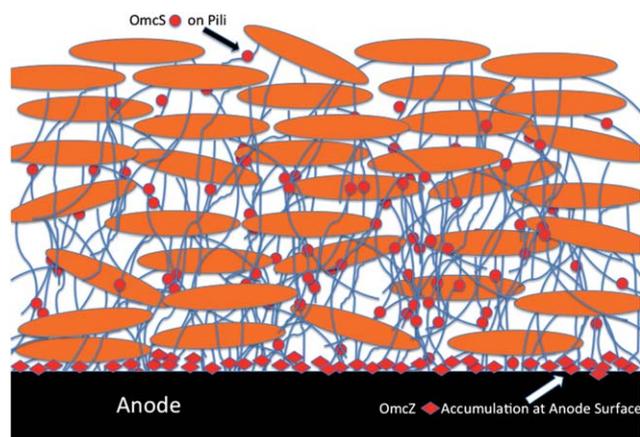

Fig. 2 Model for electron transfer through anode biofilms of *Geobacter sulfurreducens*. OmcS, which is abundant in cells growing on Fe(III) oxide or in syntrophic cultures, is not as abundant in anode biofilms. The *c*-type cytochrome OmcZ is abundant and localized near the anode surface, it is not closely associated with cells or pili. Electrons released from cells move through the conductive biofilm matrix *via* conductive pili. OmcZ is hypothesized to facilitate electron transfer from the biofilm matrix to the anode in a manner similar to OmcS facilitating electron transfer from pili to Fe(III) oxide.

association with the electrode surface appear to be metabolically active. The *pilA*-deficient mutant is still able to produce thick biofilms when fumarate, an alternative electron acceptor, is provided,[60] suggesting that the potential structural role of the pili[83] is not an important consideration. Additional evidence for the importance of pili in electron transfer was the finding that selective pressure for enhanced current production yielded a strain of *G. sulfurreducens* with greater pilin production.[58]

It appears that the pili of *G. sulfurreducens* provide a conductive network through anode biofilms, which have conductivities rivaling those of synthetic organic conductive polymers.[52] There is a strong correspondence between biofilm conductance and the abundance of PilA protein in the biofilms of different strains of *G. sulfurreducens* and the biofilms have an organic metallic-like conductance that is similar to that of the pili.[52] Facile electron conduction through the biofilms makes it feasible for even cells at substantial distance from the electrode to oxidize organic compounds and donate electrons to the conductive biofilm matrix and eventually to the electrode.[52,60,80,81]

Other important components for electron transfer to electrodes by *G. sulfurreducens* include the *c*-type cytochrome OmcZ (Fig. 2) and possibly other outer surface *c*-type cytochromes.[74,76–78,84,85] OmcZ is a multi-heme cytochrome,[86] which is not required for Fe (III) oxide reduction,[60] but is highly expressed in current-producing biofilms and is required for optimal current production.[60,75] Immunogold labeling studies have revealed that OmcZ is located outside the cell and in electrode biofilms OmcZ accumulates at the biofilm-electrode interface. These results have led to the suggestion that OmcZ functions as an "eletrochemical gate" facilitating electron transfer between the biofilm and the anode.[87]

Unfortunately, there has been little investigation of the mechanisms for electron transfer to electrodes in other microorganisms that are known to directly transfer electrons. A preliminary study with *Geobacter metallireducens* did demonstrate that pilin were necessary for significant current production in this species as well.[70]

Gene expression and deletion studies with *G. sulfurreducens* suggested that the pathway for electrons transfer from electrodes to cells was different than electron transfer from cells to an electrode. Genes for components such as pili and OmcZ, that are essential for current production, and highly upregulated in current-producing biofilms,[60] were not upregulated in current-consuming cells.[88] Furthermore, deletion of these genes, which greatly inhibits current production, did not inhibit current consumption. In contrast, deleting a gene for a putative *c*-type cytochrome that was highly upregulated in current-consuming cells inhibited current consumption, but had no impact on current production.[88]

Direct interspecies electron transfer

Mechanisms for direct interspecies electron transfer have, as yet, only been studied in the co-culture of *G. metallireducens* and *G. sulfurreducens* described above. Sequencing the genomic DNA of the aggregates that the two *Geobacter* species formed as they adapted for rapid syntrophic ethanol metabolism revealed a single base pair mutation in *G. sulfurreducens*, which inactivated the regulatory gene *pilR*.[31] One impact of the inactivation of *pilR* is an increased expression of the pilin-associated cytochrome, OmcS,[89] which was much more abundant in the

aggregates than in wild-type cultures of *G. sulfurreducens*.[31] Initiating co-cultures with a strain of *G. sulfurreducens* in which *pilR* had been deleted accelerated aggregate formation and ethanol metabolism. Attempts to initiate co-cultures with strains of *G. sulfurreducens* in which the gene for OmcS or PilA were deleted were unsuccessful.

These results, coupled with the finding that hydrogen or formate did not appear to be involved in electron exchange between the two species, suggested that selective pressure for rapid ethanol metabolism favored the formation of aggregates that could directly exchange electrons through a conductive matrix, rather than producing hydrogen or formate to function as an electron shuttle between the microorganisms. Further evidence for this was the finding that the aggregates were electrically conductive.[31]

There are still many questions about the mechanisms of electron exchange within the *Geobacter* co-culture, which may be better addressed now that methods for genetic manipulation of *G. metallireducens* have been developed.[70] However, the current model (Fig. 3A) is that, much like direct electron transfer to electrodes, the pili of *G. sulfurreducens*, and presumably *G. metallireducens*, form a conductive matrix within the aggregates and cytochromes associated with the pili, and possibly the outer surface of the cells, facilitate electron transfer to and from the pili and cells.

Role of DEET in energy supply and energy-related environmental concerns

The number of ways in which direct extracellular electron transfer (DEET) is known to impact on either the production of bioenergy or in dealing with the legacy of environmental degradation associated with fuel use continues to grow. Various aspects are described below, in rough order of the maturity of understanding of the topic.

Anaerobic hydrocarbon degradation

An environmental consequence of heavy reliance on fossil fuels is the widespread groundwater contamination associated with fuel spills and leaking storage tanks. The contaminants of greatest concern are BTEX (benzene, toluene, ethylbenzenes, and xylenes). These monoaromatic hydrocarbons are not only toxic, but also relatively water-soluble and thus can readily spread in water supplies. Anaerobic oxidation of aromatic hydrocarbons coupled to the reduction of the Fe(III) naturally present in the subsurface takes place without prompting from humans, and can be an important mechanism limiting the spread of BTEX contamination.[90–94]

Furthermore, it is possible to stimulate this process by facilitating electron transfer to Fe(III). This can be accomplished with the addition of chelators that solubilize Fe(III), making the Fe(III) more accessible.[95,96] An alternative is to promote electron transfer to insoluble Fe(III) by amending groundwater with humic substances or other electron shuttles.[25] With either of these techniques, even benzene, which is generally regarded as the monoaromatic hydrocarbon most difficult to degrade under anaerobic conditions, can be rapidly degraded. The degradation of the gasoline additive methyl *tert*-butyl ether (MTBE) was stimulated with a similar strategy.[97]

An alternative strategy for accelerating the degradation of BTEX and other organic contaminants in the anaerobic

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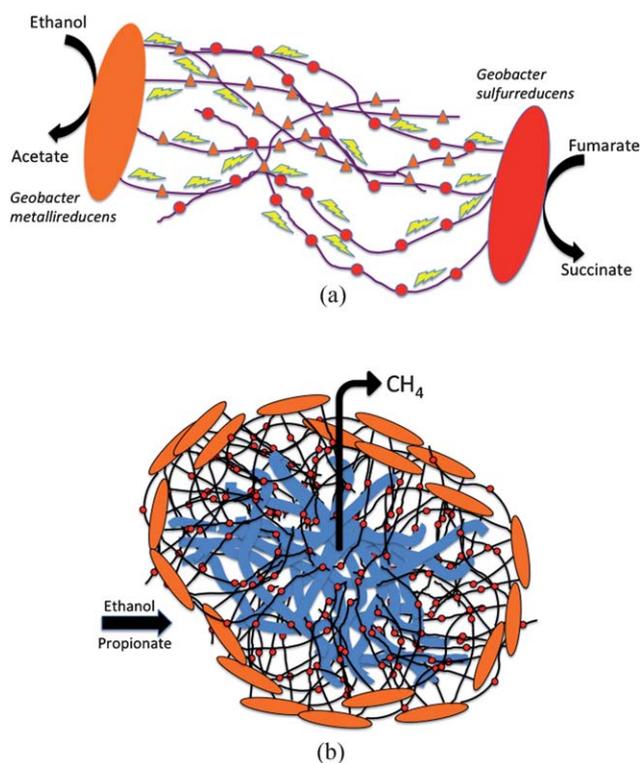This journal is © The Royal Society of Chemistry 2011

Fig. 3 Models for interspecies electron transfer. A. Interspecies electron transfer between *Geobacter* species. Multiple lines of experimental evidence suggest that OmcS and pili are important for the electron exchange and thus OmcS is hypothesized to make electrical contacts with as yet unknown cytochromes of *G. metallireducens* or conductive pili. B. Electron transfer in conductive methanogenic aggregates. *Geobacter* species (orange), and possibly other syntrophic bacteria, are hypothesized to contribute to aggregate conductivity with conductive pili, but the mechanisms by which methanogens (blue) can consume the electrons have yet to be determined.

subsurface is to provide electrodes as an electron acceptor.[98] Aromatic hydrocarbons were anaerobically oxidized with a graphite electrode as the sole electron acceptor, under defined conditions with *G. metallireducens*, as well as with mixed microbial communities in sediments. The graphite electrodes adsorbed the aromatic hydrocarbons prior to degradation.[98] Thus, an advantage of graphite electrodes as an electron acceptor amendment is that they concentrate the contaminants, the degradative microorganisms, and the electron acceptor at the same location. Fe(III) or other electron acceptors that could be added to the subsurface require constant replenishment, but graphite electrodes are a permanent source of electron-accepting capacity. Subsurface microorganisms are capable of transferring electrons to a graphite anode even when the cathode is placed meters above, in the aerobic zone of the subsurface.[99] When it is feasible to extend the system so that it is exposed to the atmosphere, a hollow system that delivers air along the length of a tubular microbial fuel cell may be effective.[100]

An even simpler possibility is to employ a single conductive rod positioned so that it spans the anaerobic and aerobic zones and can function as both the anode and cathode.[101] Such so-called microbial "electrochemical snorkels"[101] were initially developed for wastewater treatment, but we are developing "subsurface

snorkels" which could be an effective strategy for remediating hydrocarbon-contaminated soils and sediments. In this application a conductive material could be driven into the contaminated environment. Microorganisms can colonize the segment in the anaerobic, contaminated zone, oxidizing the benzene and other contaminants (Fig. 4). Electrons will flow through the conductive material to the portion exposed to the aerobic environment where electrons can be transferred to oxygen. When the anode portion is graphite or a similar adsorptive conductive material this will result in the desired co-localization of contaminants, microbes, and electron acceptor. Once installed such a system could be a low-cost, low-maintenance strategy to remove groundwater contaminants. Similar systems should be effective in contaminated aquatic sediments and helpful for enhancing waste removal in constructed wetlands. They could be a useful strategy for preventing methane emissions from landfills or wetlands, diverting carbon and electron flow to the subsurface snorkels and thus away from methane production. The general increase in redox potential associated with electron transfer to electrodes may mitigate other environmental concerns such as the production of sulfide and mercury methylation[102] and might improve the cultivation of crops such as rice.

In order to optimize strategies for the remediation of BTEX contamination with subsurface snorkels or Fe(III) reduction, it will be important to better understand the mechanisms for benzene activation in those organisms that predominate in the environments of interest. Molecular analysis of environments in which BTEX was being degraded with the reduction of Fe(III) have found that *Geobacter* species were the predominant Fe(III)-reducing microorganisms.[90–94] There are a number of *Geobacter* species available in pure culture capable of degrading aromatic compounds.[103–105] To date aromatics metabolism in *Geobacter* species has primarily been studied in *G. metallireducens*[106] and the recent development of a genetic system in this organism[70] and the discovery that *G. metallireducens* is capable of anaerobic benzene degradation,[107] suggests that this may be the *Geobacter*
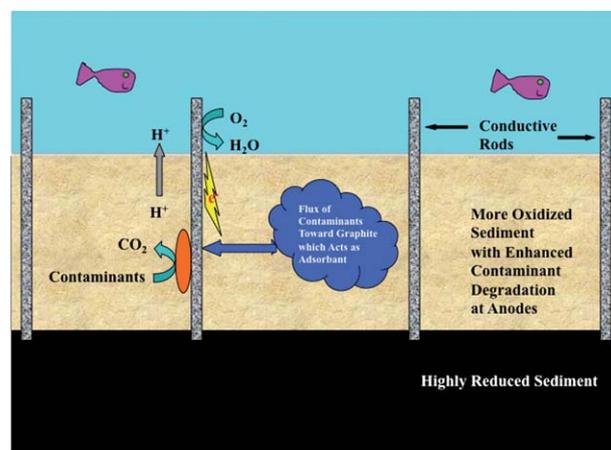

Fig. 4 Subsurface snorkel for stimulating bioremediation of organic contaminants. Organic contaminants are oxidized at the anodic portion of the conductive graphite rod with electron transfer through the rod to aerobic water where electrons can reduce oxygen to form water. The subsurface snorkel functions in a manner similar to a benthic microbial fuel cell with the exception that there is no attempt to harvest current from the electron transfer.

species of choice for further studies of the physiology of anaerobic benzene degradation.

Another aspect of anaerobic hydrocarbon degradation of importance to energy concerns is the potential for some microorganisms to anaerobically oxidize components of hydrocarbon deposits, degrading the quality of the reservoir. It is generally considered that the heating of deposits to 80–90 °C associated with deep burial kills hydrocarbon-degrading microorganisms, sterilizing the deposits.[108] However, it was recently found that *Ferroglobus placidus* anaerobically oxidizes a diversity of straight-chain and branched alkanes, as well as aromatic hydrocarbons with the reduction of Fe(III) at temperatures as high as 94 °C.[109,110] Therefore, further investigation of the mechanisms controlling microbial degradation of petroleum hydrocarbon deposits is warranted.

DEET role in dealing with the legacy of the nuclear fuel cycle

Radionuclides are released as contaminants from a wide diversity of energy-related processes including burning of coal and oil and gas exploration as well as activities more directly associated with the mining, processing, and utilization of nuclear materials.[111] The conversion of oxidized forms of radionuclides to less soluble reduced forms is a promising bioremediation strategy for both *in situ* and *ex situ* approaches. Most investigation has focused on microbial reduction of U(VI), which will be briefly summarized here as an example. Please see other reviews for more extensive coverage of microbial reduction of a wider diversity of radionuclides.[111–113]

Many microorganisms that can reduce Fe(III) can also reduce soluble U(VI) to the much less soluble U(IV).[111,114] Prior to the discovery of microbial U(VI) reduction,[115–117] it was generally considered that reduction of U(VI) in sedimentary environments was primarily an abiotic process. However, evaluation of the possibility of U(VI) reduction by likely abiotic reductants, such as Fe(II) or sulfide, has indicated that such abiotic reduction is unlikely under conditions typically found in subsurface environments. Uranium ores, such as roll-front deposits, are thought to form from the reductive precipitation of uranium. Thus, it seems possible that microorganisms that carry out DEET are responsible for those ores.[118]

The much more intensively studied aspect of microbial U(VI) reduction is the possibility of stimulating the activity of U(VI)-reducing microorganisms to prevent the migration of uranium in contaminated ground water.[114] One potential strategy for this is to feed microorganisms electrons with an electrode.[45] However, the more commonly considered alternative is to provide organic electron donors to enhance the growth of metal-reducing microorganisms.[114] Microbial growth can primarily be attributed to the reduction of Fe(III), or sulfate once Fe(III) is depleted, because even in uranium-contaminated environments the amount of uranium that is available as an electron acceptor is typically quite low (<100 μM).[118] There are many considerations that may influence the effectiveness of *in situ* uranium bioremediation, which are often site-specific and will not be discussed in detail here.

Different organic amendments appear to stimulate the growth of different U(VI)-reducing microorganisms and which microorganisms emerge during *in situ* uranium bioremediation may also be influenced by geochemical or other environmental conditions.

For example, acetate additions at a former uranium-mining site in Rifle, CO have consistently resulted in significant enrichments of *Geobacter* species, which can account for over 90% of the microorganisms in the groundwater during the height of bioremediation.[64,69,119] This predominance of *Geobacter* species, and the known ability of *Geobacter* species to reduce U(VI), suggests that *Geobacter* species are the agents for U(VI) reduction. However, given the low concentrations of U(VI) (<10 μM) in the groundwater, it is conceivable that some microorganisms at a very low abundance could be responsible for U(VI) removal. At other sites U(VI)-reducing microorganisms such as *Anaeromyxobacter*, *Desulfovibrio*, and *Desulfosporosinus* as well as *Geobacter* species, are abundant during *in situ* uranium bioremediation.[120–122]

Microbial U(VI) reduction may be a fortuitous process given that U(VI) concentrations in natural environments are very low and there is little incentive for microorganisms to evolve specific mechanisms for U(VI) reduction in order to enhance their growth. Many Fe(III)- and sulfate-reducing microorganisms contain *c*-type cytochromes, which can readily reduce U(VI) *in vitro*.[123] Gene deletion studies have implicated *c*-type cytochromes either on the outer surface of the cells[124,125] or in the periplasm[126] in U(VI) reduction.[111]

Production of electricity from organic wastes with DEET

Microbial fuel cells are the application of DEET to the bioenergy field for which there have been by far the most publications in the last decade. These publications have been reviewed on a regular basis (see for example[37,127–130]) and will not be comprehensively reviewed here.

It is a relatively simple matter to produce small amounts of electric current from organic wastes with a microbial fuel cell, which makes it a very popular science fair project. The often-stated driver for more sophisticated microbial fuel cell research is large-scale conversion of wastes to electricity. Unfortunately, the inability to generate high current densities under conditions that could be scaled to the large volumes necessary for waste water treatment has raised doubts whether this goal is practical.[129,131,132] The economics of wastewater treatment with microbe-electrode interactions could be improved with the production of valuable products at the cathode such as hydrogen, caustic soda, or peroxide[133] but these applications will also require that the scaling issue be resolved.

The short-term application of microbial fuel cells appears to be in localized production of small amounts of power. For example, benthic microbial fuel cells harvest electrical power from marine sediments and may be used to power underwater electronic devices, eliminating the expense and technical difficulty of continuous replacement of traditional batteries.[134–136] The anode is buried in the sediment and is colonized by microorganisms that can oxidize electron donors in the sediment with electron transfer to the anode. In organic-rich sediments this may be members of the *Geobacteraceae* family[38,137] or other organisms.[138] Sulfide can be an important electron donor in sulfide-rich sediments. The abiotic oxidation of sulfide to elemental sulfur can provide some electrons, and then microorganisms such as *Desulfobulbus*,[139] can oxidize the elemental sulfur to sulfate, increasing the current contribution from sulfide. Other near term applications of microbial fuel cells are likely to be autonomous robots[140] and sensors.[141]

Producing fuels and other desirable chemicals with DEET

The ability of microorganisms to accept electrons from electrodes as an electron donor offers the possibility of converting electrical energy to other energy forms. This may be an attractive option when electricity is derived from renewable, but intermittent technologies such as solar and wind. One solution to storing electrical energy is to capture the energy in covalent chemical bonds,[142] preferably in chemicals that can be stored, distributed, and utilized within the existing infrastructure.

Microbial electrosynthesis is one possibility for such an energy conversion. As originally defined[143] microbial electrosynthesis is the process in which microorganisms use electrons provided with electrodes to reduce carbon dioxide to organic products that are then released from the cells.[42,143,144] A diversity of acetogenic microorganisms were used to generate acetate in proof-of-concept studies, but diversion of the central intermediate, acetyl-CoA, to the production of other more valuable products should readily be feasible with genetic engineering. For example, acetyl-CoA can be converted to butanol, which is an attractive transportation fuel because it can be stored and distributed within the existing fossil fuel infrastructure and can be combusted in automobile engines designed to combust gasoline without engine modification.

If microbial electrosynthesis can be scaled up it may prove to have advantages over biomass-based strategies for the production of fuels and other useful organic chemicals. When powered with solar energy microbial electrosynthesis is essentially an artificial form of photosynthesis with the same net overall reaction as plant-based photosynthesis in which solar energy powers the conversion of carbon dioxide and water to organic compounds with oxygen as a byproduct. However, as previously discussed in detail,[42,143,144] microbial electrosynthesis is expected to be more efficient than biomass-based strategies because microbial electrosynthesis: 1) offers much greater efficiencies in harvesting solar energy; 2) directs energy inputs to the production of desired organic products rather than generating biomass, eliminating the further energy inputs and waste generatation associated with processing biomass; and 3) avoids the substantial energy inputs of fertilization and cropping associated with biomass production. Furthermore, microbial electrosynthesis is expected to be more environmentally sustainable than biomass-based approaches. Growing plants of any kind requires arable land and potentially removes biomass from the food supply. Intensive agriculture consumes significant water resources and pollutes water with nutrients. Converting biomass to fuels can also consume large quantities of water. In contrast, microbial electrosynthesis is possible anywhere the sun shines (or wind or geothermal energy is available), will not divert resources from the food supply, and can be run as a continuous closed system, consuming little water. However, much research is required before microbial electrosynthesis can become a practical large-scale process.

Alternatives to electrosynthesis of liquid transportation fuels include microbial reduction of carbon dioxide to methane[145,146] or microorganisms catalyzing the reduction of protons to hydrogen.[147–149] *Geobacter sulfurreducens* is the only pure culture yet found to catalyze hydrogen production with a cathode as the sole electron donor[150] and provides a genetically tractable model organism with which to study and potentially optimize this process further.

Another possibility for exploiting microbe-electrode interactions for production of fuels and other desirable organic chemicals is to use an electrode to alter the endproducts of metabolism. For example, providing an electrode as an electron acceptor made it feasible for a genetically engineered strain of *Shewanella oneidensis* to convert glycerol, a waste product of biodiesel production, to ethanol.[151] Electron transfer to the electrode consumed the two electrons that needed to be disposed of to make this reaction feasible. Reduction of organic acids to alcohols that can serve as fuels with electrodes serving as a cathode is another alternative.[133]

Potential role of DEET in converting wastes to methane

The conversion of organic wastes to methane in upflow anaerobic sludge blanket (UASB) reactors and related systems is one of the most mature and effective bioenergy technologies.[132,152] Like many other methanogenic environments, the degradation of complex mixtures of organic matter in anaerobic digestors requires an effective exchange of electrons between the microorganisms that degrade fatty acids and alcohols to acetate and the methanogens that can consume the electrons released in this metabolism.[32,33] It has generally been considered that hydrogen or formate function as the interspecies electron carriers and there are many defined, multispecies co-cultures in which this is the case.[32,33] However, it is difficult to track hydrogen and formate metabolism within the more complex aggregates of cells that form in methanogenic digestors.

Recent evidence suggests that direct interspecies electron transfer (DIET) is a likely alternative, at least in some UASB aggregates.[153] Aggregates from a UASB reactor treating brewery waste were electrically conductive, in a manner similar to the aggregates, discussed above, that *G. metallireducens* and *G. sulfurreducens* produced while directly exchanging electrons to metabolize ethanol.[31] The temperature dependence of the conductance in the methanogenic aggregates was consistent with the organic metallic-like conductivity observed in the pili and biofilms of *G. sulfurreducens*. The methanogenic aggregates did not significantly metabolize hydrogen added at high levels, suggesting that the microbial community was not adapted to transfer hydrogen. The aggregates had some potential to convert formate to methane, suggesting that there was the possibility for interspecies formate transfer, but the potential for formate metabolism was well below what would be required for interspecies formate transfer to be the major form of electron exchange. *Geobacter* species accounted for 25% of the 16S rRNA gene sequences recovered with PCR primers designed to amplify the sequences of all microorganisms, suggesting that *Geobacter* species were significantly contributing to the UASB aggregate conductivity. The probable role of the *Geobacter* species was to function as syntrophic microorganisms oxidizing ethanol and fatty acids larger than acetate to acetate (Fig. 3B). It is likely that other microorganisms were also contributing to the UASB aggregate conductivity. Methanogens that can directly accept electrons from metallic iron have been previously described,[154,155] consistent with the concept that methanogens might be able to directly accept electrons released into a conductive matrix by other microorganisms. As is typical for wastewater aggregates,

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Methanosaeta species were the predominant methanogens in the conductive aggregates[153] and it seems likely, based on the recent discovery of a complete carbon dioxide reduction pathway in *Methanosaeta* genomes that *Methanosaeta* might have the capacity to accept electrons with the production of methane.[153]

If DIET is an important component of electron flow in methanogenic wastewater aggregates, this understanding could lead to the design of even more effective and stable methanogenic syntrophic associations. However, study of aggregates from a wider diversity of digesters and the electron transfer capabilities of methanogens and other microorganisms within aggregates is warranted. This should include an investigation of electron flow in wetlands and other natural methanogenic environments that contribute to the global carbon cycle. Furthermore, DIET is expected to play an important role with microbial associations involved in the anaerobic oxidation of methane,[31,34] which also has a significant impact on the global methane cycle.[156]

Future contributions of DEET to energy and related environmental concerns

Although DEET is a relatively unexplored form of microbial respiration, findings in just the last couple of years have demonstrated that as new basic discoveries are made they suggest new practical applications. More work on the ecology of microorganisms responsible for DEET is likely to aid in the design of better bioremediation strategies for energy-related environmental contamination. Rapid advances in molecular biology and computational analysis have made it feasible to address bioremediation with a systems biology approach, which is expected to accelerate the design of optimized restoration strategies.[157,158] The rapidly evolving understanding of DEET *via* highly conductive protein networks is likely to aid in the further development of mature bioenergy approaches, such as conversion of wastes to methane, as well as developing novel strategies such as microbial electrosynthesis. Furthermore, the possibility of producing highly conductive materials with inexpensive organic feed stocks such as acetate[52] may lead to new developments in the emerging field of bioelectronics, reducing costs or introducing new applications. Studies on the possibility that a wider diversity of microorganisms are capable of DEET and the mechanisms of electron transfer is warranted. In general, it seems likely that more in-depth investigation of the vast, as yet untapped metabolic potential of the wide diversity of microorganisms on earth could provide additional solutions to energy-related needs.

Acknowledgements

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