

# Extracellular electron transfer: wires, capacitors, iron lungs, and more

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*‘and by the help of microscopes, there is nothing so small, as to escape our inquiry; hence there is a new visible world discovered to the understanding.’*

Robert Hooke

Terry Beveridge’s broad and insightful studies of microbe–metal interactions, powered in large part by amazing microscopy, have served as an inspiration and provided guidance for many of us working in this field. This perspective deals with just one of the aspects of microbe–metal interactions to which Terry significantly contributed, extracellular electron transfer.

For purposes of this perspective extracellular electron transfer is defined as the process in which electrons derived from the oxidation of electron donors are transferred to the outer surface of the cell to reduce an extracellular terminal electron acceptor. Extracellular electron acceptors may be soluble, yet reduced outside the cell because they are too large to enter the cell, as is probably the case for humic substances (Lovley *et al.*, 1996, 1998). Some soluble metal species, such as U(VI) complexed with carbonate, or Fe(III) chelated with citrate, are often reduced extracellularly (Lovley *et al.*, 1991; Marshall *et al.*, 2006; Coppi *et al.*, 2007; Gralnick & Newman, 2007; Shelobolina *et al.*, 2007) as is dimethyl sulfoxide (Gralnick *et al.*, 2006). It has also been proposed that organisms involved in the syntrophic degradation of organic compounds may make the appropriate connections to directly exchange electrons (Stams *et al.*, 2006). However, extracellular electron transfer to minerals, such as Fe(III) and Mn(IV) oxides (Lovley, 1991; Lovley *et al.*, 2004; Gralnick & Newman, 2007; Shi *et al.*, 2007) and to electrodes (Lovley, 2006a,b), is probably of the greatest interest at present because of their environmental significance and practical applications. Oxidation of organic matter coupled to the reduction of Fe(III) and Mn(IV) oxides plays an important role in the carbon, iron, and manganese

cycles in sedimentary environments, and also influences the fate of a diversity of trace metals and phosphate (Lovley, 1993; Thamdrup, 2000). Anaerobic oxidation of organic contaminants with the reduction of Fe(III) is important in groundwater bioremediation (Lovley *et al.*, 1989; Lovley, 1997), and stimulating dissimilatory metal reduction has shown promise as a method for immobilizing toxic metals in the subsurface (Anderson *et al.*, 2003; Lloyd *et al.*, 2003). Oxidation of organic matter coupled to electron transfer to electrodes is a potential strategy for harvesting electricity from the environment and waste organic matter (Lovley, 2006a).

The primary goal for many investigators of extracellular electron transfer is to determine how electrons are transferred to the electron acceptors and the factors controlling the rate and extent of this process. With this information in hand, it may be possible to optimize practical applications and better model natural processes. Some of the potentially important questions still remaining in extracellular electron transfer are discussed below in relation to key publications of Terry’s that have led to the development of these questions and/or have provided the techniques that may lead to their answers.

## WHAT CONSTITUTES A ‘MICROBIAL NANOWIRE’ AND ARE THEY THE FINAL CONDUIT FOR ELECTRON TRANSFER TO EXTRACELLULAR ELECTRON ACCEPTORS SUCH AS FE(III) OXIDES AND ELECTRODES?

Terry was involved in some of the earliest work on the concept that some structures emanating from microorganisms may be conductive and might serve as a conduit for extracellular electron transfer (Gorby *et al.*, 2006). ‘Microbial nanowires’ (Reguera *et al.*, 2005, 2006; Gorby *et al.*, 2006) are certainly an attractive, easily visualized, explanation for extracellular electron transfer.

However, some caution in this area is probably warranted. It is not uncommon these days for some investigators to confer 'nanowire' status on just about any filament emanating from microorganisms, even those not known to be capable of extracellular electron transfer, often without even examining the filaments to determine if they are, in fact, electrically conductive.

Even for the few instances in which electrically conductive filaments have been documented (Reguera *et al.*, 2005; Gorby *et al.*, 2006), much more investigation is required before it can be definitively stated that electrons actually flow from microorganisms to extracellular electron acceptors along these structures. It is important to recognize that such electron transfer has never been directly demonstrated, only inferred from circumstantial evidence. More information is required on what comprises these structures, the rates of electron transfer through these structures, and the features that confer conductivity.

Mechanistic investigations linking microbial nanowires to mechanisms for extracellular electron transfer have only been carried out with *Shewanella oneidensis* (Gorby *et al.*, 2006) and *Geobacter sulfurreducens* (Reguera *et al.*, 2005). In the case of *S. oneidensis* (Gorby *et al.*, 2006), it is not clear what protein(s) are the scaffold of the conductive filaments. They are described as 'pilus-like appendages' but *S. oneidensis* may produce a variety of pili and pseudo-pili, some of which appear to be nonconductive (Reguera *et al.*, 2005; Gorby *et al.*, 2006). The conductive structures in *S. oneidensis* are much thicker (50 to > 150 nm diameter) and much less numerous (*c.* 1 per cell in figure 1 of Gorby *et al.*, 2006) than most pili and it has been suggested that they are 'bundles' of pili, something which clearly requires further verification. In *G. sulfurreducens*, the nature of the structure seems more clear because deleting the gene for PilA, the structural protein for type IV pili, resulted in cells without the nanowires (*c.* 3–5 nm in diameter), which in the wild-type cells are numerous and localized to one lateral side of the cell (Reguera *et al.*, 2005). Reintroducing the gene for PilA restored the capacity for nanowire production. It seems like similar genetic studies might help identify the structural components of the conductive filaments in *S. oneidensis*.

A pressing question for both the *S. oneidensis* and the *G. sulfurreducens* nanowires is: what features make them conductive? For *S. oneidensis* it has been suggested that cytochromes might be associated with the pili because deleting genes for outer-surface cytochromes resulted in nonconductive filaments (Gorby *et al.*, 2006). At present, there is no evidence that cytochromes are associated with the pili of *G. sulfurreducens*. Ongoing genetic studies in which the PilA protein of *G. sulfurreducens* is being modified with amino acid substitutions may provide insights into the mechanisms for conductivity. In the future, more involvement by physicists is going to be required in order to make the sophisticated electrochemical measurements necessary to understand this phenomenon. We have attempted to further evaluate the mechanisms of con-

ductivity in *Geobacter* nanowires by laying the filaments across interdigitated electrodes and querying conductive properties. To date, such studies have proven to be technically challenging and difficult to interpret.

Even after the nature of conductivity of the nanowires is elucidated, the challenge still remains to definitely determine whether these conductive structures are the final conduit to Fe(III) oxides and electrodes. It is tempting to suggest that they are because they could have the potential to greatly extend the electronic reach of the organisms. However, to date the evidence is rather circumstantial and it has been previously noted (Gorby *et al.*, 2006) that 'additional research designed to critically and comprehensively evaluate far-field electron transfer via nanowires is needed to confirm these intriguing observations'. In *G. sulfurreducens*, Fe(III) oxides seem to preferentially associate with the pili, deleting the pilA gene inhibits Fe(III) oxide reduction, and expressing the PilA gene *in trans* in the PilA-deficient mutant restores the capacity for Fe(III) oxide reduction (Reguera *et al.*, 2005). This is all consistent with the nanowires being the final conduit, but does not prove it. In *S. oneidensis*, there is the observation that magnetite accumulates on the surface of some extracellular material when Fe(III) oxide is reduced, but the nature of this extracellular material was not determined and there was significant amounts of magnetite associated with other non-filamentous portions of the cells (Gorby *et al.*, 2006). Another line of evidence for electron transfer via nanowires in *S. oneidensis* is that deleting the genes for the cytochromes that appear to make the *S. oneidensis* nanowires conductive diminishes electron transfer to Fe(III) and electrodes (Gorby *et al.*, 2006). However, it has been reported that these same cytochromes are also associated with the outer membrane of *S. oneidensis* as well as extracellular material that is different from the nanowires (Shi *et al.*, 2007). If so, then the diminished rates of extracellular electron transfer in the cytochrome mutants could just as easily be explained as a consequence of cytochromes that are not associated with filaments directly transferring electrons to Fe(III) oxides and electrodes without mediation of the nanowires. In fact, this is what has recently been proposed (Shi *et al.*, 2007).

In a similar manner, there is an abundance of not only *c*-type cytochromes, but also other redox-active proteins, such as multicopper proteins, on the outer surface of *G. sulfurreducens* (Lovley *et al.*, 2004; Mehta *et al.*, 2005; Mehta *et al.*, 2006; Qian *et al.*, 2007), that are required for Fe(III) oxide reduction and are not attached to pili. The interaction of these proteins with extracellular electron acceptors must be better elucidated before it can be definitively stated that the terminal electron transfer step takes place at the pilin surface. Furthermore, it must be considered that extrusion of pili may be associated with the release of other proteins to outer surface. We are now also carefully examining whether deleting the PilA gene has impacts on the biochemical composition of the outer cell surface beyond taking away the capacity for pilin production.

## DO SOME DISSIMILATORY METAL-REDUCING MICROORGANISMS HAVE 'IRON LUNGS'?

Another fascinating observation made by Terry and colleagues is that *Shewanella putrefaciens* CN32 has the novel capacity to store iron and manganese minerals intracellularly during dissimilatory Fe(III) or Mn(IV) reduction (Glasauer *et al.*, 2002, 2004, 2007). Interestingly, the other *Shewanella* species that have been evaluated do not appear to share this ability (Glasauer *et al.*, 2007). Terry and colleagues have suggested that the intracellular iron oxide deposits might function 'as a reservoir of oxidant' because they are depleted under sustained anoxic conditions (Glasauer *et al.*, 2007). This is an intriguing concept.

We have yet to identify similar Fe(III) or Mn(IV) oxide deposits in *Geobacter* species, but have discovered what we believe is an alternative 'iron lung' for *Geobacter* species (Esteve-Núñez *et al.*, 2008). This is the abundance of iron in the periplasm and outer membrane of *Geobacter* species, which is found in the form of *c*-type cytochromes. The hypothesis is that these extracytoplasmic cytochromes can act as capacitors to store electrons in the periplasm and outer membrane when external electron acceptors are not immediately available. The reason that this is significant is that energy conservation from organic matter oxidation in *Geobacter* species is expected to result solely from the transport of electrons across the inner membrane with associated proton-pumping, which generates a proton-motive force that drives ATP formation, flagella rotation, etc. (Mahadevan *et al.*, 2006). Once the electrons are transferred to the periplasm, subsequent electron transfer steps to extracellular electron acceptors do not further contribute to the cellular energy yield. This is evident from the finding that *Geobacter* cell yields are comparable with soluble and insoluble forms of Fe(III), even though there are substantial differences in the redox potentials of these electron acceptors. The external electron acceptors merely function to oxidize the electron acceptors earlier in the electron transfer chain to permit continued electron transfer across the inner membrane.

The electron-accepting capacity of the *c*-type cytochromes in *G. sulfurreducens*, determined with two independent methods, was estimated to be *c.*  $1.6 \times 10^{-17}$  mol electrons per cell (Esteve-Núñez *et al.*, 2008). This capacitance can, in the absence of an extracellular electron acceptor, potentially support enough inner membrane electron transfer/proton pumping for *G. sulfurreducens* to satisfy its maintenance energy requirements for 8 min or to swim several hundred cell lengths before all the electron-accepting capacity of the hemes is exhausted (Esteve-Núñez *et al.*, 2008).

The ability to temporarily maintain active electron transfer across the inner membrane in the absence of an extracellular electron acceptor may be key to the survival of *Geobacter* species in the subsurface and the often-noted ability of *Geobacter* species to outcompete a diversity of other dissimilatory Fe(III) reducers in such environments. This is because, in contrast to

*Shewanella* and *Geothrix* species that produce electron shuttles (Newman & Kolter, 2000; Nevin & Lovley, 2002a,b; Lies *et al.*, 2005; Lanthier *et al.*, 2008), *Geobacter* species must directly contact Fe(III) oxides in order to reduce them (Nevin & Lovley, 2000). This presents a challenge to *Geobacter* species in subsurface environments in which Fe(III) sources, such as Fe(III) oxides and structural Fe(III) in clays (Shelobolina *et al.*, 2003, 2006), are heterogeneously dispersed. Once a cell depletes the Fe(III) oxide in one locale it must find an alternative source of the electron acceptor. The current model is that *Geobacter* species are motile and hunt for Fe(III) oxides (Childers *et al.*, 2002). This is consistent with an analysis of the microbial community during *in situ* uranium bioremediation, which indicated that the metabolically active *Geobacter* species are highly planktonic in the subsurface during active Fe(III) oxide reduction (Holmes *et al.*, 2007). However, it presents the quandary of how cells can generate ATP required for motility as well as cell maintenance when they are in a planktonic state searching for a new source of electron acceptor. The electron-accepting capacity of the extracytoplasmic cytochromes provides a potential short-term solution. Respiration can continue and then once a new source of Fe(III) is located the electrons stored in the cytochromes can be discharged from these mini-capacitors.

In addition to the direct measurements of the electron storage capacity of the *c*-type cytochromes in *G. sulfurreducens* there is substantial circumstantial evidence that is consistent with this concept. For example, although genetic studies have indicated that some *c*-types are essential for growth of *G. sulfurreducens* on Fe(III), there is remarkably little conservation of *c*-type cytochromes genes across the six *Geobacter* species whose genomes have been sequenced. This suggests that there has not been evolutionary pressure to maintain specific structures that might promote interaction of the cytochromes with electron acceptors. However, there has apparently been evolutionary pressure for the *Geobacter* species to maintain an abundance of hemes. Not only are there an inordinately high number of *c*-type cytochrome genes in *Geobacter* species (Methé *et al.*, 2003) ([www.jgi.doe.gov](http://www.jgi.doe.gov)), most of these cytochromes have multiple hemes (27 is the maximum yet predicted) giving them relatively high electron-accepting capacity. The vast majority of the cytochrome genes are expressed (Ding *et al.*, 2006) and the cells are intensely red due to the abundance of cytochromes. Further support for the capacitor concept is the finding that the expression of many extracytoplasmic *c*-type cytochrome genes is increased during growth on either synthetic Fe(III) oxides, sediment Fe(III) oxides or electrodes versus growth on soluble electron acceptors (Holmes *et al.*, 2006) (D.E. Holmes and K.P. Nevin, unpublished data). Such a high abundance of cytochromes is not required for Fe(III) reduction, as other Fe(III) reducers, such as *Pelobacter* species (Lovley *et al.*, 1995; Haveman *et al.*, 2006) and the Fe(III)-reducing hyperthermophiles (Vargas *et al.*, 1998; Kashefi & Lovley, 2003; Lovley *et al.*, 2004), reduce Fe(III)

just fine with few, if any *c*-type cytochromes involved. However, the energetic investment that *Geobacter* species make in *c*-type cytochrome production could be very adaptive in providing an 'iron lung' that permits electron transfer in the temporary absence of Fe(III) oxides.

Further investigation into the role of intracellular iron, whether as Fe(III) oxides or as heme groups, in serving as a temporary electron acceptor for dissimilatory Fe(III) reducers is clearly warranted. In addition to *Shewanella* and *Geobacter* species, a diversity of other Fe(III) reducers should be investigated as phylogenetically distinct organisms may have evolved different strategies.

### HOW ARE ELECTRONS PASSED THROUGH THE BIOFILMS ON THE ANODES MICROBIAL FUEL CELLS?

Superficially, this question may appear to be just another version of the nanowire questions above. However, electron flow to conductive anode surfaces is functionally different than electron transfer to Fe(III) oxide. Recent research has suggested that, in the most effective microbial fuel cells, electron transfer must proceed through biofilms, a phenomenon for which there is little prior guidance in the literature. As detailed below, novel techniques for studying biofilms, such as those recently developed by Terry and colleagues, could aid in understanding this special form of extracellular electron transfer.

Although Fe(III) oxides and the anodes of microbial fuel cells both represent insoluble, extracellular electron acceptors, Fe(III) oxide sources, and the clays that contain structural Fe(III) that is microbially reducible, are typically smaller than Fe(III)-reducing microorganisms. Certainly in culture, and possibly in sedimentary environments, it may be more correctly stated that the Fe(III) becomes attached to the microbe rather than vice versa. Furthermore, even when carbon sources are being added to sedimentary environments, such as during *in situ* uranium bioremediation, the density of cells is relatively low and therefore, organized, confluent biofilms are not expected (Nevin & Lovley, 2002b). In contrast, electrodes represent a large surface for cell attachment and microbial fuel cells producing high current densities provide high concentrations of electron donors. This results in the formation of relatively thick (up to *c.* 75  $\mu\text{m}$ ), structured biofilms that are essential for high-density current production in *Geobacter*-powered microbial fuel cells (Reguera *et al.*, 2006; Nevin *et al.*, 2008a). *S. oneidensis* does not build up thick biofilms on anodes, but there is substantial growth of planktonic cells in *S. oneidensis* fuel cells (Lanthier *et al.*, 2008). Thus, in both instances most of the microorganisms are no longer in close contact with the anode and in many instances they are far beyond the conceivable reach of nanowires, yet appear to be viable and metabolically active.

How is this possible? For *S. oneidensis* one key component appears to be soluble electron shuttle molecules (Lanthier

*et al.*, 2008). *Shewanella* species release flavins, most notably flavin mononucleotide and riboflavin, that can function as shuttles, enhancing current production in microbial fuel cells (von Canstein *et al.*, 2008). However, an anonymous reviewer recently suggested that another option for a shuttle between planktonic cells of *S. oneidensis* and fuel cell anodes may be membrane vesicles, similar to those described by Schooling and Beveridge (2006), if these contain appropriate redox-active components. This is an interesting hypothesis that should be further investigated.

The previous finding that *Geobacter* species do not appear to produce an electron shuttle (Nevin & Lovley, 2000), as well as electrochemical measurements (Daniel Bond, University of Minnesota, personal communication), suggests that there is a direct electrical connection between cells of *Geobacter* and anode surfaces. Hunter and Beveridge developed a high-pressure freeze-substitution transmission electron microscopic method that permitted high-resolution imaging of *Pseudomonas aeruginosa* biofilms (Hunter & Beveridge, 2005b). With this method, it may be possible to image *Geobacter* anode biofilms to elucidate how cells might form a conductive network, e.g. *G. sulfurreducens* requires pili to form the thick biofilms on anodes that yield high power densities in microbial fuel cells (Reguera *et al.*, 2006; Nevin *et al.*, 2008b). Do pili from multiple cells intertwine to promote cell-to-cell electron transfer so that cells at substantial distance from the anode surface can establish electrical contact with the anode? Alternatively, pili may play a primarily structural role (Reguera *et al.*, 2007) and one or more of the multiple redox-active proteins displayed on the outer surface of *G. sulfurreducens* may interact to facilitate electron transfer through the biofilm (Nevin *et al.*, 2008b). It is important to know how these outer-surface proteins are distributed throughout the biofilm to determine whether they might contribute to a conductive network. Furthermore, it should be possible to measure the conductivity of the biofilm and via genetic manipulation of the quantities of potential electron transfer facilitators, identify the key components. Such studies are underway.

The flux of nutrients into, and end products out of, anode biofilms is likely to significantly impact on the function of microbial fuel cells. One obvious concern is delivering fuel in an optimum manner. Less obvious, but probably equally important, is the diffusion of end products out of the biofilm. Protons are of particular concern because extracellular electron transfer results in substantially more proton release into the environment than most forms of intracellular reduction of electron acceptors (Mahadevan *et al.*, 2006). Accumulation of these protons may be detrimental for two reasons. The obvious one is that lowering the pH within the biofilm may result in suboptimal conditions for continued respiration. Possibly just as important is the fact that the protons that are produced from substrate oxidation are required on the cathode side of the microbial fuel cell to combine with the electrons released from fuel oxidation, providing charge balance. In the near future

we plan to evaluate the potential for proton accumulation under various fuel cell design strategies with a pH-sensitive fluoroprobe developed by Hunter and Beveridge (2005a). With this probe and confocal scanning laser microscopy, it is possible to determine the pH within the microenvironments of biofilms.

### WHAT IS THE PHYSIOLOGICAL STATE OF METAL-REDUCING MICROORGANISMS UNDER ENVIRONMENTALLY RELEVANT CONDITIONS?

Terry and colleagues demonstrated that the environmental conditions under which Fe(III)-reducing microorganisms are cultured may significantly influence their physiology, even impacting which forms of Fe(III) oxides can serve as electron acceptors (Glasauer *et al.*, 2003). *S. putrefaciens* CN32, grown in minimal medium designed to more accurately reflect environmental conditions in the subsurface, could reduce poorly crystalline Fe(III) oxide, but not the highly crystalline forms which CN32 can reduce if grown in rich media (Glasauer *et al.*, 2003). The lack of reduction of the crystalline Fe(III) forms in more environmentally relevant media is consistent with data demonstrating that these minerals tend to persist, even in organic-rich sedimentary environments (Phillips *et al.*, 1993).

It is an important concept that we must understand the environmentally relevant physiological status of Fe(III)-reducing microorganisms in order to make accurate predictions about process in environments in which Fe(III) reduction is prevalent. One additional step toward this goal is growing the organisms under nutrient-limiting conditions in chemostat cultures (Esteve-Núñez *et al.*, 2005). Another possibility is to grow pure cultures in sterilized sediments from the site of interest (D.E. Holmes, unpublished data). However, the ultimate is to evaluate the physiological status of the natural populations of Fe(III)-reducing microorganisms *in situ* (Lovley, 2003; Lovley *et al.*, 2008).

It is now apparent that this is possible, at least for the many subsurface environments in which Fe(III) reduction is an important process and *Geobacter* species are the predominant Fe(III)-reducing organisms. Genome sequences for a number of *Geobacter* species are available and from a combination of gene expression, proteomic, and genetic studies, it has been possible to identify genes, whose level of expression is diagnostic of specific physiological conditions and/or the rates of cell metabolism. When coupled with techniques for quantifying numbers of gene transcripts in the environment this makes it feasible to evaluate the *in situ* physiological status of the microorganisms (Holmes *et al.*, 2004, 2005, 2008; O'Neil *et al.*, 2008) (D. E. Holmes *et al.* unpublished data). With this approach it is possible to determine: what nutrients are likely to be limiting the activity of *Geobacter* species during *in situ* bioremediation of uranium-contaminated groundwater; whether oxygen, toxic metals, etc. are stressing the organisms; and

relative rates of metabolism in the subsurface under different conditions imposed during bioremediation. The ultimate goal is to be able to predict how dissimilatory metal-reducing microorganisms will behave under different possible bioremediation strategies, making it possible to select an optimized approach prior to implementing field trials (Lovley, 2003; Lovley *et al.*, 2008). One of the first steps toward this goal was the development of a genome-scale *in silico* model of the metabolism of *G. sulfurreducens*, which demonstrated that it is possible to predictively model the physiology of a *Geobacter* species under different environmental conditions (Mahadevan *et al.*, 2006). Subsequent steps include making an *in silico* model that reflects the physiology of the *Geobacter* species that predominate in a diversity of subsurface environments, known as subsurface clade I (Holmes *et al.*, 2007), and coupling this with geochemical and hydrological models. These studies are underway.

These are, of course, only a few of the many potential lines of inquiry about extracellular electron transfer that may be promising in the future. Undoubtedly, direct observation and measurement of microbes interacting with minerals and each other will play an important role in answering these questions. One can only hope that someone with the talent and insight of Terry will come along to fill this critical niche.

### REFERENCES

- Anderson RT, Vrionis HA, Ortiz-Bernad I, Resch CT, Peacock A, Dayvault R, Marutzky S, Metzler DR, Karp K, Lowe M, White DC, Long PE, Lovley DR (2003) Stimulating the *in situ* activity of *Geobacter* species to remove uranium from the groundwater of a uranium-contaminated aquifer. *Applied Environmental Microbiology* **69**, 5884–5891.
- von Canstein H, Ogawa J, Shimizu S, Lloyd JR (2008) Secretion of flavins by *Shewanella* species and their role in extracellular electron transfer. *Applied Environmental Microbiology* **74**, 615–623.
- Childers SE, Ciuffo S, Lovley DR (2002) *Geobacter metallireducens* accesses Fe(III) oxide by chemotaxis. *Nature* **416**, 767–769.
- Coppi MV, O'Neil RA, Leang C, Kaufmann F, Methe BA, Nevin KP, Woodard TL, Liu A, Lovley DR (2007) Involvement of *Geobacter sulfurreducens* SfrAB in acetate metabolism rather than intracellular, respiration-linked Fe(III) citrate reduction. *Microbiology* **153**, 3572–3585.
- Ding YR, Hixson K, Giometti CS, Stanley A, Esteve-Nunez A, Khare T, Tollaksen SL, Zhu W, Adkins JN, Lipton MS, Smith RD, Mester T, Lovley DR (2006) The proteome of the dissimilatory metal-reducing microorganism *Geobacter sulfurreducens* under various growth conditions. *Biochimica et Biophysica Acta* **1764**, 1198–1206.
- Esteve-Núñez A, Rothermich MM, Sharma M, Lovley DR (2005) Growth of *Geobacter sulfurreducens* under nutrient-limiting conditions in continuous culture. *Environmental Microbiology* **7**, 641–648.
- Esteve-Núñez A, Sosnik J, Visconti P, Lovley DR (2008) Fluorescent properties of *c*-type cytochromes reveal their potential role as an extra-cytoplasmic electron sink in *Geobacter sulfurreducens*. *Environmental Microbiology* **10**, 497–505.
- Glasauer S, Langley S, Beveridge TJ (2002) Intracellular iron minerals in a dissimilatory iron-reducing bacterium. *Science* **295**, 117–119.

- Glasauer S, Weidler PG, Langley S, Beveridge TJ (2003) Controls on Fe reduction and mineral formation by a subsurface bacterium. *Geochimica et Cosmochimica Acta* **67**, 1277–1288.
- Glasauer S, Langley S, Beveridge TJ (2004) Intracellular manganese granules formed by a subsurface bacterium. *Environmental Microbiology* **6**, 1042–1048.
- Glasauer S, Langley S, Boyanov M, Lai B, Kemner K, Beveridge TJ (2007) Mixed-valence cytoplasmic iron granules are linked to anaerobic respiration. *Applied Environmental Microbiology* **73**, 993–996.
- Gorby YA, Yanina S, McLean JS, Rosso KM, Moyles D, Dohnalkova A, Beveridge TJ, Chang IS, Kim BH, Kim SK, Culley DE, Reed SB, Romine MF, Saffarini DA, Hill EA, Shi L, Elias DA, Kennedy DW, Pinchuk G, Watanabe K, Ishii S, Logan B, Neelson KH, Fredrickson JK (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. *Proceedings of the National Academy of Sciences of the USA* **103**, 11358–11363.
- Gralnick JA, Newman DK (2007) Extracellular respiration. *Molecular Microbiology* **65**, 1–11.
- Gralnick JA, Vali H, Lies DP, Newman DK (2006) Extracellular respiration of dimethyl sulfoxide by *Shewanella oneidensis* strain MR-1. *Proceedings of the National Academy of Sciences of the USA* **103**, 4669–4674.
- Haveman SA, Holmes DE, Ding YR, Ward JE, DiDonato RJ, Lovley DR (2006) *c*-type cytochromes in *Pelobacter carbinolicus*. *Applied Environmental Microbiology* **72**, 6980–6985.
- Holmes DE, Chaudhuri SK, Nevin KP, Mehta T, Methe BA, Ward JE, Woodward TL, Webster J, Lovley DR (2006) Microarray and genetic analysis of electron transfer to electrodes in *Geobacter sulfurreducens*. *Environmental Microbiology* **8**, 1805–1815.
- Holmes DE, Mester T, O'Neil RA, Adams LA, Larrahando MJ, Glaven R, Sharma M, Ward JE, Nevin KP, Lovley DR (2008) Genes for two multicopper proteins required for Fe(III) oxide reduction in *Geobacter sulfurreducens* have different expression patterns both in the subsurface and on energy-harvesting electrodes. *Microbiology* (in press).
- Holmes DE, Nevin KP, Lovley DR (2004) *In situ* expression of *nifD* in Geobacteraceae in subsurface sediments. *Applied Environmental Microbiology* **70**, 7251–7259.
- Holmes DE, Nevin KP, O'Neil RA, Ward JE, Adams LA, Woodward TL, Lovley DR (2005) Potential for quantifying expression of Geobacteraceae citrate synthase gene to assess the activity of Geobacteraceae in the subsurface and on current harvesting-electrodes. *Applied Environmental Microbiology* **71**, 6870–6877.
- Holmes DE, O'Neil RA, Vrionis HA, N'Guessan LA, Ortiz-Bernad I, Larrahando MJ, Adams LA, Ward JA, Nicoll JS, Nevin KP, Chavan MA, Johnson JP, Long PE, Lovley DR (2007) Subsurface clade of Geobacteraceae that predominates in a diversity of Fe(III)-reducing subsurface environments. *ISME Journal* **1**, 663–677.
- Hunter RC, Beveridge TJ (2005a) Application of a pH-sensitive fluoroprobe (CSNARF-4) for pH microenvironment analysis in *Pseudomonas aeruginosa* biofilms. *Applied Environmental Microbiology* **71**, 2501–2510.
- Hunter RC, Beveridge TJ (2005b) High-resolution visualization of *Pseudomonas aeruginosa* PAO1 biofilms by freeze-substitution transmission electron microscopy. *Journal of Bacteriology* **187**, 7619–7630.
- Kashefi K, Lovley DR (2003) Extending the upper temperature limit for life. *Science* **301**, 934.
- Lanthier M, Gregory KB, Lovley DR (2008) Growth with high planktonic biomass in *Shewanella oneidensis* fuel cells. *FEMS Microbiology Letters* **278**, 29–35.
- Lies DP, Hernandez ME, Kappler A, Mielke RE, Gralnick JA, Newman DK (2005) *Shewanella oneidensis* MR-1 uses overlapping pathways for iron reduction at a distance and by direct contact under conditions relevant for biofilms. *Applied Environmental Microbiology* **71**, 4414–4426.
- Lloyd JR, Lovley DR, Macaskie LE (2003) Biotechnological application of metal-reducing microorganisms. *Advances in Applied Microbiology* **53**, 85–128.
- Lovley DR (1991) Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiology Review* **55**, 259–287.
- Lovley DR (1993) Dissimilatory metal reduction. *Annals of Review of Microbiology* **47**, 263–290.
- Lovley DR (1997) Potential for anaerobic bioremediation of BTEX in petroleum-contaminated aquifers. *Journal of Industrial Microbiology* **18**, 75–81.
- Lovley DR (2003) Cleaning up with genomics: applying molecular biology to bioremediation. *Nature Review of Microbiology* **1**, 35–44.
- Lovley DR (2006a) Bug juice: harvesting electricity with microorganisms. *Nature Review of Microbiology* **4**, 497–508.
- Lovley DR (2006b) Microbial fuel cells: novel microbial physiologies and engineering approaches. *Current Opinions in Biotechnology* **17**, 327–332.
- Lovley DR, Baedecker MJ, Lonergan DJ, Cozzarelli IM, Phillips EJP, Siegel DI (1989) Oxidation of aromatic contaminants coupled to microbial iron reduction. *Nature* **339**, 297–299.
- Lovley DR, Phillips EJP, Gorby YA, Landa ER (1991) Microbial reduction of uranium. *Nature* **350**, 413–416.
- Lovley DR, Phillips EJP, Lonergan DJ, Widman PK (1995) Fe(III) and S<sup>0</sup> reduction by *Pelobacter carbinolicus*. *Applied Environmental Microbiology* **61**, 2132–2138.
- Lovley DR, Coates JD, Blunt-Harris EL, Phillips EJP, Woodward JC (1996) Humic substances as electron acceptors for microbial respiration. *Nature* **382**, 445–448.
- Lovley DR, Fraga JL, Blunt-Harris EL, Hayes LA, Phillips EJP, Coates JD (1998) Humic substances as a mediator for microbially catalyzed metal reduction. *Acta Hydrochimica et Hydrobiologica* **26**, 152–157.
- Lovley DR, Holmes DE, Nevin KP (2004) Dissimilatory Fe(III) and Mn(IV) reduction. *Advances in Microbial Physiology* **49**, 219–286.
- Lovley DR, Mahadevan R, Nevin KP (2008) Systems biology approach to bioremediation with extracellular electron transfer. In *Microbial Degradation: Genomics and Molecular Biology* (Diaz, E., ed.), pp. 71–96. Norfolk, UK: Caister Academic Press.
- Mahadevan R, Bond DR, Butler JE, Esteve-Nunez A, Coppi MV, Palsson BO, Schilling CH, Lovley DR (2006) Characterization of metabolism in the Fe(III)-reducing organism *Geobacter sulfurreducens* by constraint-based modeling. *Applied Environmental Microbiology* **72**, 1558–1568.
- Marshall MJ, Beliaev AS, Dohnalkova AC, Kennedy DW, Shi L, Wang Z, Boyanov MI, Lai B, Kemner KM, McLean JS, Reed SB, Culley DE, Bailey VL, Simonson CJ, Saffarini DA, Romine MF, Zachara JM, Fredrickson JK (2006) *c*-Type cytochrome-dependent formation of U(IV) nanoparticles by *Shewanella oneidensis*. *PLoS Biology* **4**, e26B. DOI: 10.1371/journal.pbio.0040268.
- Mehta T, Coppi MV, Childers SE, Lovley DR (2005) Outer membrane *c*-type cytochromes required for Fe(III) and Mn(IV) oxide reduction in *Geobacter sulfurreducens*. *Applied Environmental Microbiology* **71**, 8634–8641.
- Mehta T, Childers SE, Glaven R, Lovley DR, Mester T (2006) A putative multicopper protein secreted by an atypical type II secretion system involved in the reduction of insoluble electron acceptors in *Geobacter sulfurreducens*. *Microbiology* **152**, 2257–2264.

- Methé BA, Nelson KE, Eisen JA, Paulsen IT, Nelson W, Heidelberg JF, Wu D, Wu M, Ward N, Beanan MJ, Dodson RJ, Madupu R, Brinkac LM, Daugherty SC, DeBoy RT, Durkin AS, Gwinn M, Kolonay JF, Sullivan SA, Haft DH, Selengut J, Davidsen TM, Zafar N, White O, Tran B, Romero C, Forberger HA, Weidman J, Khouri H, Feldblyum TV, Utterback TR, Van Aken SE, Lovley DR, Fraser CM (2003) The genome of *Geobacter sulfurreducens*: insights into metal reduction in subsurface environments. *Science* **302**, 1967–1969.
- Nevin KP, Kim B-C, Glaven RH, Johnson JP, Woodard TL, Methe BA, DiDonato RJ Jr, Covalla SF, Franks AE, Liu A, Lovley DR (2008b) Differences in physiology between current-producing and fumarate-reducing biofilms of *Geobacter sulfurreducens*: identification of a novel outer-surface cytochrome essential for electron transfer to anodes at high current densities. (submitted).
- Nevin KP, Lovley DR (2000) Lack of production of electron-shuttling compounds or solubilization of Fe(III) during reduction of insoluble Fe(III) oxide by *Geobacter metallireducens*. *Applied Environmental Microbiology* **66**, 2248–2251.
- Nevin KP, Lovley DR (2002a) Mechanisms for accessing insoluble Fe(III) oxide during dissimilatory Fe(III) reduction by *Geothrix fermentans*. *Applied Environmental Microbiology* **68**, 2294–2299.
- Nevin KP, Lovley DR (2002b) Mechanisms for Fe(III) oxide reduction in sedimentary environments. *Geomicrobiology Journal* **19**, 141–159.
- Nevin KP, Richter H, Covalla SF, Johnson JP, Woodard TL, Orloff AL, Jia H, Zhang M, Lovley DR (2008a) Power output of *Geobacter sulfurreducens* comparable to that observed with mixed communities in microbial fuel cells. (submitted).
- Newman DK, Kolter R (2000) A role for excreted quinones in intracellular electron transfer. *Nature* **405**, 94–97.
- O'Neil RA, Holmes DE, Coppi MV, Adams LA, Larrahando MJ, Ward JE, Nevin KP, Woodard T, Vironis HA, N'Guessan AL, Lovley DR (2008) Gene transcript analysis of assimilatory iron limitation in *Geobacteraceae* during groundwater bioremediation. *Environmental Microbiology* (in press).
- Phillips E, Lovley DR, Roden EE (1993) Composition of non-microbially reducible Fe(III) in aquatic sediments. *Applied Environmental Microbiology* **59**, 2727–2729.
- Qian X, Reguera G, Mester T, Lovley DR (2007) Evidence that OmcB and OmpB of *Geobacter sulfurreducens* are outer membrane surface proteins. *FEMS Microbiology Letters* **277**, 21–27.
- Reguera G, McCarthy KD, Mehta T, Nicoll J, Tuominen MT, Lovley DR (2005) Extracellular electron transfer via microbial nanowires. *Nature* **435**, 1098–1101.
- Reguera G, Nevin KP, Nicoll JS, Covalla SF, Woodard T, Lovley DR (2006) Biofilm and nanowire production leads to increased current in *Geobacter sulfurreducens* fuel cells. *Applied and Environmental Microbiology* **72**, 7345–7348.
- Reguera G, Pollina RB, Nicoll JS, Lovley DR (2007) Possible non-conductive role of *Geobacter sulfurreducens* pili nanowires in biofilm formation. *Journal of Bacteriology* **189**, 2125–2127.
- Schooling SR, Beveridge TJ (2006) Membrane vesicles: an overlooked component of the matrices of biofilms. *Journal of Bacteriology* **188**, 5945–5957.
- Shelobolina ES, Anderson RT, Vodyanitskii YN, Yuretich R, Lovley DR (2003) Importance of clay size minerals for Fe(III) respiration in a petroleum-contaminated aquifer. *Geobiology* **2**, 67–76.
- Shelobolina ES, Nevin KP, Blakeney-Hayward JD, Nevin KP, Blakeney-Hayward JD, Johnsen CV, Plaia TW, Krader P, Woodard T, Holmes DE, VanPraagh CG, Lovley DR (2006) *Geobacter pickeringii*, sp. nov., *Geobacter argillaceus*, sp. nov. and *Pelosinus fermentans*, gen. nov., sp. nov., isolated from subsurface kaolin lenses. *International Journal of Systematic and Evolutionary Bacteriology* **57**, 126–135.
- Shelobolina ES, Coppi MV, Korenevsky AA, DiDonato LN, Sullivan SA, Konishi H, Xu H, Leang C, Butler JE, Kim BC, Lovley DR (2007) Importance of the role of *c*-type cytochromes for U(VI) reduction by *Geobacter sulfurreducens*. *BMC Microbiology* **7**, 16.
- Shi L, Squier TC, Zachara JM, Fredrickson JK (2007) Respiration of metal (hydr)oxides by *Shewanella* and *Geobacter*: a key role for multihaem *c*-type cytochromes. *Molecular Microbiology* **65**, 12–20.
- Stams AJM, de Bok FAM, Plugge CM, van Eekert MHA, Doling J, Schraa G (2006) Exocellular electron transfer in anaerobic microbial communities. *Environmental Microbiology* **8**, 371–382.
- Thamdrup B (2000) Bacterial manganese and iron reduction in aquatic sediments. *Advances in Microbial Ecology* **16**, 41–84.
- Vargas M, Kashafi K, Blunt-Harris EL, Lovley DR (1998) Microbiological evidence for Fe(III) reduction on early Earth. *Nature* **395**, 65–67.