RESEARCH REVIEW

INTERNATIONAL MICROBIOLOGY
OPEN ACCESS

The 25th SEM Congress (Logroño, Spain, July 7-10, 2015)

Microbes, cables, and an electrical touch

Gemma Reguera

Department of Microbiology and Molecular Genetics, Michigan State University, East Lansing, MI, USA

Summary. In nature, highly efficient and diverse consortia of microbes cycle carbon and other elements while generating energy for growth. Driving these reactions are organisms with the ability to extract electrons from the chemical substrates and transfer them to insoluble and soluble electron acceptors. One bacterial group in particular, *Geobacter* spp., can couple their respiratory metabolism to the reduction of insoluble minerals, such as iron and manganese oxides, and soluble toxic metals such as uranium. Key to these activities is the ability of the cells to transfer respiratory electrons extracellularly using an electroactive cell envelope containing abundant metalloproteins, including *c*-cytochromes, and conductive protein appendages or pili (known as nanowires). Thus, in addition to been ecological drivers of the cycling of carbon and metals in nature, these organisms show promise for the bioremediation of environments impacted with toxic metals. The electrical activity of *Geobacter* can also be mimicked in electrochemical reactors equipped with an electrode poised at a metabolically oxidizing potential, so that the electrode functions as an unlimited sink of electrons to drive the oxidation of electron donors and support cell growth. Electrochemical reactors are promising for the treatments of agricultural, industrial, and human wastes, and the electroactivity of these microbes can be used to develop materials and devices for bioenergy and bioremediation applications. [**Int Microbiol** 18(3):151-157 (2015)]

Keywords: *Geobacter* · *c*-cytochromes · electrochemical reactors · microbial fuel cells · nanowires · type IV pili

Introduction

At the most fundamental level, energy transduction in biological systems relies on the transfer of electrons from a donor to an acceptor, in reactions that generate energy for cell growth through processes such as respiration, photosynthesis and fermentation. Microbes are particularly creative at energy generation, having diversified their metabolism in ways so varied that impact the cycling of many elements in nature. The fluxes of the six elements that make up the major building blocks of biological macromolecules—H, C, N, O, S, and P—are, for example, driven mostly by redox reactions catalyzed by microbes [9]. Diverse consortia of microorganisms efficiently cycle these elements by extracting electrons from the chemical substrates and harnessing energy to support their growth and activities. Some microbes have also evolved mechanisms to transfer electrons to extracellular electron acceptors such as insoluble and soluble metals, thus contributing to their cycling as well [12]. Metals are ubiquitous and abundant,

*Correspondence:
Department of Microbiology and Molecular Genetics
Michigan State University
East Lansing, MI, USA
E-mail: reguera@msu.edu

accounting for two thirds of the elements in planet Earth. Thus, metal transformations mediated by microorganisms impact environmental processes at a global scale.

Two of the best-studied metal cyclers are *Geobacter* and *Shewanella* bacteria, particularly the strains *Geobacter sulfurreducens* PCA [2] and *Shewanella oneidensis* MR-1 [24]. The physiological hallmark of these two bacteria is their ability to couple their metabolisms to the reduction of insoluble minerals such as iron (Fe[III]) and manganese (Mn[IV]) oxides [40]. Key to this ability is the presence of numerous metalloproteins, mainly *c*-cytochromes, in their cell envelope, which are organized across the periplasm and outer membrane to efficiently transport respiratory electrons from the menaquinone pool in the inner membrane to extracellular electron acceptors [41]. In *Shewanella*, redox-active flavin compounds are secreted that shuttle electrons between the cell and the insoluble substrates, contributing as much as 75% to the reduction of iron and manganese minerals under laboratory conditions [19] By contrast, *Geobacter* bacteria do not secrete redox mediators and must establish electronic contact with the extracellular electron acceptor during respiration [25].

Shewanella and *Geobacter* bacteria have also evolved mechanisms for long-range electron transfer. *S. oneidensis* produces filamentous extensions of its outer membrane and periplasm [27], which organize their *c*-cytochrome complexes as a microbial redox chain that transports charges at rates [7] and with mobilities consistent with charges hopping between the redox-active sites in the chain [28]. *Geobacter sulfurreducens*, on the other hand, have evolved a conductive version of the type IV pili [29]. Other bacterial pili play roles in surface attachment and twitching motility [1]. However, in *Geobacter*, the pili are required to transfer electrons to insoluble electron acceptors such as iron oxides [29].

As illustrated in Fig. 1, the pili of *G. sulfurreducens* extend the redox active surface of the cell beyond the confines of its outer membrane, accessing Fe(III) oxide particles at a distance from the cell and promoting long-range electron transfer. This reduces the Fe(III) oxide minerals, solubilizing some of the iron as Fe(II) and generating a magnetic mineral of mixed iron valence, magnetite. The *Geobacter* pili also bind the soluble uranyl cation (U[VI]) and reductively precipitate it to a mononuclear phase of U(IV) outside the cell [3]. Not only are the pili the primary uranium reductase in this bacterium [30], the pili act as a protective shield that prevents the permeation and mineralization of the toxic metal in the cell envelope [3]. In addition, the pili are required to build electroactive biofilms on electrodes poised at an oxidizable potential [31] and on iron-oxide coatings [32]. Here I discuss

recent work providing novel insights into the mechanism(s) that may allow *G. sulfurreducens* to use the pilus protein filament as a nanowire between the cell and electron acceptors and the ecological and biotechnological implications of these findings.

The electric touch of *Geobacter* pili

Like other type IV pili, the conductive pili of *G. sulfurreducens* are an assembly of a single structural subunit, the pilin or PilA peptide [3]. Like other pilins, it is synthesized as a precursor with a signal peptide carrying the distinctive features of type IVa pilins, which are needed for recognition and cleavage by a dedicated type IV pilin peptidase or PilD in the inner membrane [38]. Upon cleavage, the processed pilin peptide is N-methylated in its first residue, a phenylalanine that participates in pilin assembly [6,26]. Despite conservation in signal peptide processing and assembly, the mature *Geobacter* pilins are shorter (61 amino acids long in *G. sulfurreducens* compared to an average length of ca. 150 amino acids in other bacterial pilins) [6]. Furthermore, phylogenetic analyses comparing the N-terminal regions of *Geobacter* and other bacterial type IVa pilins, places the *Geobacter* pilins in an independent line of descent [29]. Divergence is also found at the structural level [10]. The *Geobacter* pilin peptide retains for example the conserved amino-terminal (N-t) α-helix and amino acids required for assembly but has a short, random-coiled segment at the carboxy-terminus (C-t) instead of the globular head with β-strands of other bacterial pilins [10]. The reduced pilin's size, the absence of β-strands and the predominantly helical peptide all are structural features that promote electron transfer in peptides [13,20,42]. In addition, the *Geobacter* pilins contains aromatic residues (tyrosines and phenylalanines) and charged amino acids predicted to distribute the charges and density of states along the peptide to favor charge transport [10]. Hence, the structural and electronic characteristics of the *Geobacter* pilins are consistent with a peptide environment optimized for electron conduction.

The prediction that the *Geobacter* pilin has evolved as a medium for charge transport [10] is in agreement with the conductivity measured on cell-associated and mechanically sheared pili filaments [22,29,47] and, more recently, the demonstration that the pilus protein fiber can transport charges like a nanowire at rates several orders of magnitude greater than the cellular rates of iron respiration [19bis]. However, the mechanism that could allow the pilus
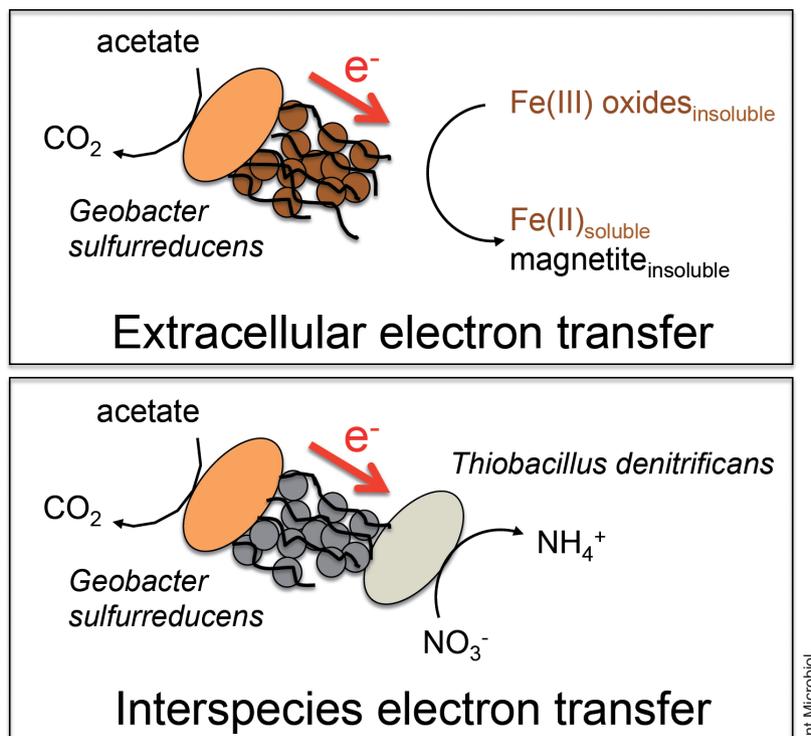
Fig. 1. Extracellular electron transfer via conductive pili (top) allows *Geobacter sulfurreducens* to reduce Fe(III) oxides at a distance, solubilizing some of the iron as Fe(II) and producing a magnetite. The semiconducting properties of magnetite promote interspecies electron transfer between *G. sulfurreducens* and *T. denitrificans* (bottom), and electric syntrophy that couples acetate oxidation to nitrate reduction.

protein fiber to transport charges remains controversial. A metallic model was proposed to explain the metallic-like temperature-dependence of piliated, electrochemically active biofilms and crude preparations of pili and other proteins, including cytochromes, dried on gold electrodes [23]. *In situ* electrical conductivity measurements on living biofilms of *G. sulfurreducens* grown on an electrode later demonstrated the thermal dependence of incoherent redox conductivity [50] Evidence is also emerging for a thermal activation of charge transport across the pili, which is consistent with an incoherent mechanism for pilus conductance [19bis]. Additionally, the metallic model of pilus conductance invokes that the aromatic rings from phenylalanine and tyrosine residues of the pilins dimerize close to each other (~3.5 Å) and in π-π configurations [23]. Structural information provided in an atomic resolution model of the pilus fiber optimized in molecular dynamics simulations [11] confirmed the clustering as a right-handed helix and identified paths for transversal and axial charge transport (Fig. 2). Yet the geometries of aromatic dimers in the pilin assembly were always of the parallel-displaced or T-shaped type, rather than the sandwich type

required for metallic conductance. The molecular dynamics simulations also revealed inter-aromatic distances from 3.5 to 8.5 Å [11], which are optimal for multistep hopping reactions [5,39]. Some of the aromatic side chains in the paths were brought closer together (less than 5 Å) during the simulations, forming aromatic contacts that are required for optimal charge transport in vivo [11]. Yet the aromatic contacts never formed at the same time, as in a metal wire.

The local electrostatic environment around the aromatic contacts also influenced their geometric configuration and clustering of the aromatic residues and, in turn, the rates of electron transfer to Fe(III) oxides [11]. Positively charged amino acids were, for example, buried in the pilus fiber core, whereas the negatively charged residues were predominantly exposed on the pilus surface, thus promoting the binding of cationic ligands in the insoluble Fe(III) oxides and the soluble uranyl cation. Interestingly, these exposed carboxyl ligands are close to the most exposed aromatic residue of the pilus, a tyrosine predicted to catalyze the last step in electron transfer to extracellular electron acceptors [11]. In support of this, the carboxylic groups exposed on the pilus surface provide

an atomic environment similar to the atomic coordination of pili-bound uranium modeled from uranium L_{III}-edge extended X-ray absorption spectroscopy spectra [3]. These proximity of the carboxylic ligands to the tyrosine could also permit their transient protonation and proton-coupled electron transfer between the tyrosine and the bound electron acceptor. Such proton-coupled mechanism of electron transfer reduces the oxidation potential of aromatic residues to enable fast rates of electron transfer [46].

Nanowires and the birth of electromicrobiology

The discovery of conductive pili in *G. sulfurreducens* and their role in iron reduction [29] followed by the finding of conductive filamentous structures in *S. oneidensis* [15] changed our understanding of how microbes respire and lead fellow microbiologist, Yuri Gorby, to forecast that we were standing "at the edge of a new scientific frontier": a new field that he coined with the name "electromicrobiology" [16]. His words were indeed prophetic, as evidenced by the many reports published in the last decade demonstrating the creative ways used by microbes to transport charges at micrometer and even centimeter distances to couple spatially separated redox reactions. It is worth mentioning a few, as they relate to *Geobacter* and their nanowires.

Iron oxides are some of the most abundant electron acceptors in soil and sedimentary environments. Their microbial reduction by *Geobacter* bacteria under anaerobic conditions mobilizes the iron as the soluble Fe[II] species (Fig. 1), which can then diffuse to the anoxic/oxic interface and be assimilated as a nutrient or reoxidized by other microbes [8]. The soluble Fe(II) species also influences the transformation of other metals [12]. Thus, dissimilatory iron reducers such as *Geobacter* bacteria, which gain energy for growth by coupling the oxidation of organic matter to the reduction of iron oxide, are key drivers of organic matter decomposition in anaerobic environments and influence the cycling of many other elements, directly or indirectly.

One way *Geobacter* species influence the cycling of other elements is through the establishment of syntrophic associations with other microorganisms [18]. *Geobacter sulfurreducens* can, for example, couple the oxidation of acetate to the reduction of iron oxides but cannot reduce nitrate, a soluble electron acceptor with a more positive reduction potential [2]. However, the reduction of iron oxides by *G. sulfurreducens* generates magnetite, a semiconductor

mineral that permits electron flow to the syntrophic partner *Thiobacillus denitrificans*, which reduces nitrate [17]. Figure 2 illustrates the interactions that establish this electric syntrophy, where electrons rather than metabolites are transferred between species. This allows *G. sulfurreducens* to continue to oxidize acetate, and grow after the iron oxides have been reduced, using an electron acceptor, nitrate, that it cannot respire on its own.

Geobacter pili are also the primary uranium reductase of the cell, binding the uranyl cation and reducing the soluble U(VI) species to the sparingly soluble U(IV) form [3,30]. The uranium reductase activity of the pili is further enhanced in biofilms [4], which provide a physical and chemical protective environment for the sustained immobilization and reduction of uranium. Not only have the biofilms enhanced capacity to immobilize and reduce uranium compared to planktonic cells, they also tolerate exposure to higher concentrations of the contaminant for prolonged periods of time [4]. Evidence indicates that the uranium is reduced extracellularly and mainly in the top biofilm stratum, in a catalytic process influenced by the biofilm structure and the presence of redox components of the electroactive biofilm matrix, most significantly the conductive pili. Thus, *Geobacter* biofilms contribute to the immobilization and reduction of uranium. This radionuclide is often found in complex mixtures with toxic inorganic and organic co-contaminants [37], which can compromise the viability of planktonic cells. However, the reduced susceptibility of the biofilms to the radionuclide makes them particularly suitable for uranium bioremediation applications. Furthermore, as uranium was reductively precipitated on the biofilm surface, the immobilization of the contaminant did not depend on the biofilm biomass and thickness, only on the substrate coverage. This is advantageous for the application of biofilm-based approaches for the *in situ* bioremediation of uranium, as there is no need to stimulate the growth of very thick biofilms to create effective biopermeable barriers.

From nature to industry

The reductive activities of *Geobacter* cells via their pili ultimately depend on the availability of electron donors that the cells can oxidize in their respiratory metabolism. Not surprisingly, these microorganisms are adapted to growing within specialized, synergistic consortia that cooperate to degrade and ferment organic matter to carbon dioxide, thus returning the carbon fixed via photosynthesis back to the atmosphere [21]. The fermentation products are rapidly
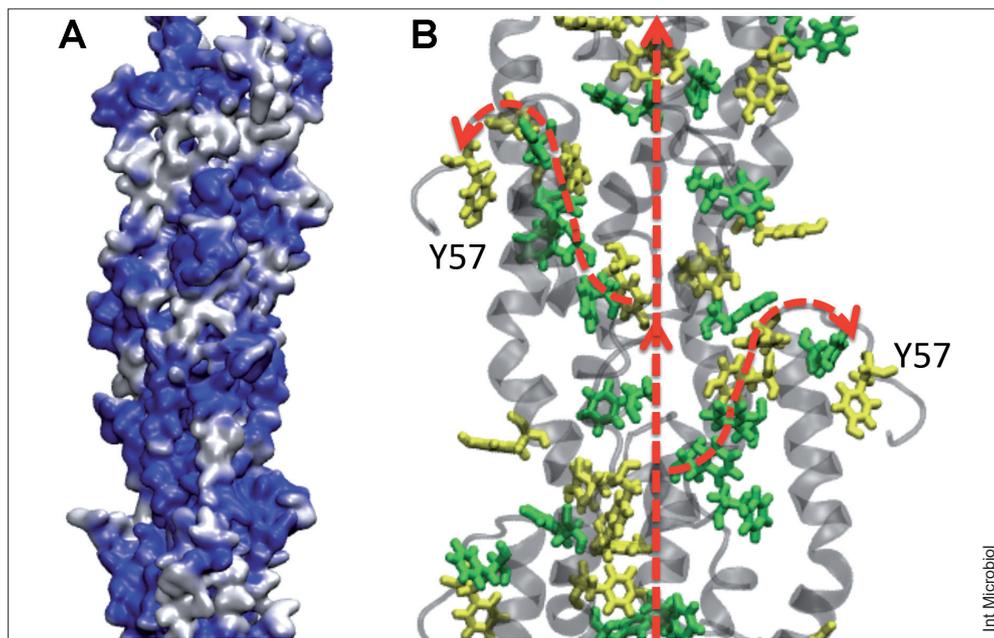
Fig. 2. (A) Isodensity pilus map showing the clustering of aromatic residues (blue) as a right-handed helix. **(B)** Snapshot of an average pilus structure resulting from the molecular dynamics simulations showing the uniform distribution of aromatics (tyrosines, yellow; phenylalanines, green) and potential axial and transversal paths for multistep hopping.

removed by syntrophic partners to prevent feedback inhibition of biomass decomposition and fermentation [49]. Hence, microorganisms such as *Geobacter* bacteria, which use the abundant Fe(III) oxides as final electron sinks, play a key role in organic matter decomposition and the cycling of carbon.

The syntrophic interactions that govern biomass decomposition in nature can be reproduced in the laboratory using electrodes poised at a metabolically oxidizing potential in devices known as microbial electrolysis cells (MECs). These devices are often two chambers containing the growth medium and separated by a proton permeable membrane. Each chamber is equipped with an electrode, which are wired to each other via a potentiostat to set up one of the electrodes (the anode) at an optimal potential for *G. sulfurreducens*. In this manner, the anode electrode provides an unlimited electron sink for the growth of an electrochemically active biofilm on the anode, a process that requires the expression of their conductive pili [31].

Acetate is often used as a carbon source and electron donor to grow and establish *G. sulfurreducens* anode biofilms [43]. However, the anode biofilms have been shown to have a broader range of electron donors in MECs than originally recognized, being able to oxidize organic acids such as formate, and lactate when provided in mixes with acetate [43]. The electrical input used to poise the anode electrode in MECs is also used to react the electrons with protons at the cathode,

simultaneously producing hydrogen fuel at much higher yields than those achieved fermentatively [14]. Furthermore, the applied potential removes cathodic limitations [35,36,48] and promotes the growth of exoelectrogenic biofilms on the anode electrode [31]. This maximizes the conversion of fermentation products to cathodic hydrogen while preventing the accumulation of feedback inhibitors of fermentation [43].

The ability of the anode biofilms to completely oxidize fermentation products to carbon dioxide with an electrode serving as the sole electron acceptor opens opportunities to use MECs for the conversion of complex substrates, such as organic wastes and renewable biomass, to electricity and/or biofuels in MECs. The efficient consolidated bioprocessing of corn stover to ethanol and cathodic hydrogen was demonstrated in a MEC driven by *G. sulfurreducens* [44]. Electron losses were minimized by selecting a consolidated bioprocessing strain, *Cellulomonas uda*, which hydrolyzed and fermented chemically-pretreated corn stover to ethanol and produced fermentation byproducts that served as electron donors for *G. sulfurreducens*. The synergistic interactions between the two partners promoted the removal and electrical conversion of all the fermentation byproducts and stimulated ethanol and cathodic hydrogen production, increasing the total energy recovery as ethanol and hydrogen fuel from corn stover to approximately 73%. A similar approach but using a strain of *Clostridium cellobioparum* adaptively evolved

for robust growth and fermentation of glycerol was used to develop a *Geobacter*-driven MEC for ethanol production from glycerin [45]. Thus, robust platforms can be developed for the treatment of and energy recoveries from agricultural and industrial wastes, reducing costs and carbon emissions associated with the treatment of wastes and biofuel production.

Microbial conversations with an electrical touch?

The discovery that microorganisms such as *Geobacter* bacteria can electrically wire their interactions demonstrates that cells can exchange electrical signals. In 2008, I raised the question "*Are microbial conversations being lost in translation?*" [33]. Physical modes of cell-cell communication were for long considered an esoteric subject, yet recent advances in electromicrobiology suggest that electronic communication may be widespread [34]. Cell growth reorientation (galvanotropism), swimming patterns (galvanotaxis or electrotaxis) and the electrical reorientation of cells are well known phenomena. Voltage-activated ion channels are widespread in the three domains of life and could act as membrane receptors for electrical signals. Indeed, physical modes of communication may have played a significant role in the evolution of life on Earth and the metabolic diversification of microorganisms, because they require minimum energy investment in the form of energy released from natural cellular processes. Physical signals—not only electrical—also have other advantages when compared with chemical signals: they propagate faster and can bypass the requirement for a cognate receptor on the plasma membrane [34]. Thus, I would like to end this commentary raising a new question: *Are we finally hearing microbial conversations that have for long been lost in translation?* Stay tuned! More is surely yet to come.

Competing interests. None declared.

References

1. Burrows LL (2012) *Pseudomonas aeruginosa* twitching motility: type IV pili in action. Annu Rev Microbiol 66:493-520
2. Caccavo F Jr, et al. (1994) *Geobacter sulfurreducens* sp. nov., a hydrogen- and acetate-oxidizing dissimilatory metal-reducing microorganism. Appl Environ Microbiol 60:3752-3759
3. Cologgi DL, et al. (2011) Extracellular reduction of uranium via *Geobacter* conductive pili as a protective cellular mechanism. Proc Natl Acad Sci USA 108:15248-15252
4. Cologgi DL, et al. (2011) Enhanced uranium immobilization and reduction by *Geobacter sulfurreducens* biofilms. Appl Environ Microbiol 80:6638-6646
5. Cordes M, et al. (2008) Influence of amino acid side chains on long-distance electron transfer in peptides: electron hopping via "stepping stones". Angew Chem Int Ed Engl 47:3461-3463
6. Craig L, Pique ME, Tainer JA (2004) Type IV pilus structure and bacterial pathogenicity. Nat Rev Microbiol 2: 363-378
7. El-Naggar MY, et al. (2010) Electrical transport along bacterial nanowires from *Shewanella oneidensis* MR-1. Proc Natl Acad Sci USA 107:18127-18131
8. Emerson D, Roden E, Twining BS (2012) The microbial ferrous wheel: iron cycling in terrestrial, freshwater, and marine environments. Front Microbiol 3:383
9. Falkowski PG, Fenchel T, Delong EF (2008) The microbial engines that drive Earth's biogeochemical cycles. Science 320:1034-1039
10. Feliciano GT, et al. (2012) The molecular and electronic structure of the peptide subunit of Geobacter sulfurreducens conductive pili from first principles. J Phys Chem A 116:8023-8030
11. Feliciano GT, Steidl RJ, Reguera G (2015) Structural and functional insights into the conductive pili of *Geobacter sulfurreducens* revealed in molecular dynamics simulations. Phys Chem Chem Phys 17:22217-22226
12. Gadd GM (2010) Metals, minerals and microbes: geomicrobiology and bioremediation. Microbiology 156:609-643
13. Galoppini E, Fox MA (1996) Effect of the electric field generated by the helix dipole on photoinduced intramolecular electron transfer in dichromophoric α-helical peptides. J Am Chem Soc 118:2299-2300
14. Geelhoed JS, Hamelers HV, Stams AJ (2010) Electricity-mediated biological hydrogen production. Curr Opin Microbiol 13: 307-315
15. Gorby YA, et al. (2006) Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms. Proc Natl Acad Sci USA 103:11358-11363
16. Gorby Y (2009) Op-Ed: Microbes may be more networked than you are, in From the Fields Wired Science
17. Kato S, Hashimoto K, Watanabe K (2012) Microbial interspecies electron transfer via electric currents through conductive minerals. Proc Natl Acad Sci USA 109:10042-10046
18. Kouzuma A, Kato S, Watanabe K (2015) Microbial interspecies interactions: recent findings in syntrophic consortia. Front Microbiol 6:477
19. Kotloski NJ, Gralnick JA (2013) Flavin electron shuttles dominate extracellular electron transfer by *Shewanella oneidensis*. MBio 4(1)
19bis. Lampa-Pastirk S, Veazey JP, Walsh KA, Feliciano GT, Steidl RJ, Tessmer SH, Reguera G (2016) Thermally activated charge transport in microbial protein nanowires. Sci. Rep 6:23517. doi: 10.1038/srep23517
20. Long YT, Abu-Irhayem E, Kraatz HB (2005) Peptide electron transfer: more questions than answers. Chem Eur J 11:5186-5194
21. Lynd LR, et al. (2002) Microbial cellulose utilization: fundamentals and biotechnology. Microbiol Mol Biol Rev 66: 506-577
22. Malvankar NS, et al. (2014) Visualization of charge propagation along individual pili proteins using ambient electrostatic force microscopy. Nat Nanotechnol 9:1012-1017
23. Malvankar NS, et al. (2011) Tunable metallic-like conductivity in microbial nanowire networks. Nat Nanotechnol 9:573-579
24. Myers CR, Nealson KH (1988) Bacterial manganese reduction and growth with manganese oxide as the sole electron acceptor. Science 240:1319-1321
25. Nevin KP, Lovley DR (2000) Lack of production of electron-shuttling compounds or solubilization of Fe(III) during reduction of insoluble Fe(III) oxide by *Geobacter metallireducens*. Appl Environ Microbiol 66:2248-2251
26. Pelicic V (2008) Type IV pili: e pluribus unum? Mol Microbiol 68:827-837

27. Pirbadian S, et al. (2014) *Shewanella oneidensis* MR-1 nanowires are outer membrane and periplasmic extensions of the extracellular electron transport components. Proc Natl Acad Sci USA 111**:**12883-12888

28. Pirbadian S, El-Naggar MY (2012) Multistep hopping and extracellular charge transfer in microbial redox chains. Phys Chem Chem Phys 14**:**13802-13808

29. Reguera G, et al. (2005) Extracellular electron transfer via microbial nanowires. Nature 435:1098-1101

30. Reguera G (2012) Electron transfer at the cell-uranium interface in *Geobacter* spp. Biochem Soc Trans 40:1227-1232

31. Reguera G, et al. (2006) Biofilm and nanowire production lead to increased current in microbial fuel cells. Appl Environ Microbiol 72:7345-7348

32. Reguera G, et al. (2007) Possible nonconductive role of *Geobacter sulfurreducens* pilus nanowires in biofilm formation. J Bacteriol 189:2125-2127

33. Reguera G (2008) Are microbial conversations being lost in translation? Microbe 4:506-512

34. Reguera G (2011) When microbial conversations get physical. Trends Microbiol 19:105-113

35. Ren Z, et al. (2007) Characterization of the cellulolytic and hydrogen-producing activities of six mesophilic *Clostridium* species. J Appl Microbiol 103:2258-2266

36. Rezaei F, et al. (2009) Simultaneous cellulose degradation and electricity production by Enterobacter cloacae in a microbial fuel cell. Appl Environ Microbiol 75:3673-3678

37. Riley RG, Zachara JM, Wobber FJ (1992) Chemical contaminants on DOE lands and selection of contaminant mixtures for subsurface science research, S.S.P. Office of Energy Res US Dept of Energy. Washington, DC.

38. Richter LV, Sandler SJ, Weis RM (2012) Two isoforms of *Geobacter sulfurreducens* PilA have distinct roles in pilus biogenesis, cytochrome localization, extracellular electron transfer, and biofilm formation. J Bacteriol 194:2551-2563

39. Shih C, et al. (2008) Tryptophan-accelerated electron flow through proteins. Science 320:1760-1762

40. Shi L, et al. (2007) Respiration of metal (hydr)oxides by *Shewanella* and *Geobacter*: a key role for multihaem c-type cytochromes. Mol Microbiol 65:12-20

41. Shi LA, et al. (2009) The roles of outer membrane cytochromes of *Shewanella* and *Geobacter* in extracellular electron transfer. Environ Microbiol Reports 1:220-227

42. Shin Y-g. K, Newton MD, Isied SS (2003) Distance dependence of electron transfer across peptides with different secondary structures: The role of peptide energetics and electronic coupling. J Am Chem Soc 125:3722-3732

43. Speers AM, Reguera G (2012) Electron donors supporting growth and electroactivity of *Geobacter sulfurreducens* anode biofilms. Appl Environ Microbiol 78:437-444

44. Speers AM, Reguera G (2012) Consolidated bioprocessing of AFEX-pretreated corn stover to ethanol and hydrogen in a microbial electrolysis cell. Environ Sci Technol 46:7875-7881

45. Speers AM, Young JM, Reguera G (2014) Fermentation of glycerol into ethanol in a microbial electrolysis cell driven by a customized consortium. Environ Sci Technol 48**:**6350-6358

46. Stubbe J, et al. (2003) Radical initiation in the class I ribonucleotide reductase: long-range proton-coupled electron transfer? Chem Rev 103:2167

47. Veazey JP, Reguera G, Tessmer SH (2011) Electronic properties of conductive pili of the metal-reducing bacterium *Geobacter sulfurreducens* probed by scanning tunneling microscopy. Phys Rev E 84**:**060901

48. Wang X, et al. (2009) Bioaugmentation for electricity generation from corn stover biomass using microbial fuel cells. Environ Sci Technol 43:6088-6093

49. Wolin MJ (1982) Hydrogen transfer in microbial communities. In Bull AT, Slater JH (eds), Microbial interactions and communities. Academic Press, London

50. Yates MD, et al. (2015) Thermally activated long range electron transport in living biofilms. Phys Chem Chem Phys 17:32564-32570