

MINI REVIEW

Happy together: microbial communities that hook up to swap electrons

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The discovery of direct interspecies electron transfer (DIET) and cable bacteria has demonstrated that microbial cells can exchange electrons over long distances (μm – cm) through electrical connections. For example, in the presence of cable bacteria electrons are rapidly transported over centimeter distances, coupling the oxidation of reduced sulfur compounds in anoxic sediments to oxygen reduction in overlying surficial sediments. Bacteria and archaea wired for DIET are found in anaerobic methane-producing and methane-consuming communities. Electrical connections between gut microbes and host cells have also been proposed. Iterative environmental and defined culture studies on methanogenic communities revealed the importance of electrically conductive pili and c-type cytochromes in natural electrical grids, and demonstrated that conductive carbon materials and magnetite can substitute for these biological connectors to facilitate DIET. This understanding has led to strategies to enhance and stabilize anaerobic digestion. Key unknowns warranting further investigation include elucidation of the archaeal electrical connections facilitating DIET-based methane production and consumption; and the mechanisms for long-range electron transfer through cable bacteria. A better understanding of mechanisms for cell-to-cell electron transfer could facilitate the hunt for additional electrically connected microbial communities with omics approaches and could advance spin-off applications such as the development of sustainable bioelectronics materials and bioelectrochemical technologies.

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Introduction

Cooperation is a hallmark of stable microbial communities. The best-known mechanism of interspecies exchange of nutrients, carbon substrates and information is through soluble molecules that are released into the extracellular environment and reach other cells via diffusion (Phelan *et al.*, 2012; Ponomarova and Patil, 2015). Some microorganisms exchange electrons in a similar manner with soluble electron shuttles, such as H_2 or formate, ferrying electrons between electron-donating and electron-accepting cells (Stams and Plugge, 2009; Sieber *et al.*, 2012; Shrestha and Rotaru, 2014).

However, diffusion is a relatively slow and indirect mechanism to relay energy and information. Direct interspecies electron transfer (DIET) may be faster and more specific (Summers *et al.*, 2010; Lovley, 2011). Electrical connections that support DIET include electrically conductive pili (e-pili) and cytochromes (Summers *et al.*, 2010), minerals (Kato *et al.*, 2012a) and abiotic carbon (Liu *et al.*, 2012). Electrically connected microbial communities

(e-communities) recognized to date are primarily restricted to a few anaerobic environments in which bacteria and archaea electrically connect to either produce (Morita *et al.*, 2011; Kato *et al.*, 2012b; Rotaru *et al.*, 2014b) or consume (McGlynn *et al.*, 2015; Wegener *et al.*, 2015) methane. Conductive channels through the anoxic–oxic zone of aquatic sediments also appear to be the result of long-range electrical connections between microbial cells (Pfeffer *et al.*, 2012). A major challenge in identifying other e-communities has been a poor understanding of the types of electrical connections that may exist between cells. For example, although the e-pili of *Geobacter* species have been extensively studied, they are a recent evolutionary event and restricted to only a minority of the wide diversity microorganisms that are known to be capable of electrical communication with their extracellular environment (Holmes *et al.*, 2016). As discussed below, other types of microbial electrical connectors seem possible. The proposed potential for electrical interactions between microbes and human cells (Ericsson *et al.*, 2015) demonstrates the need to think broadly in the early stages of e-community research.

The purpose of this minireview is to describe the function of known e-communities, which may provide information that will aid the search for additional types of communities that function via DIET; and to describe how a developing

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understanding of e-communities is providing new insights into important biogeochemical cycles and the development of novel applications in bioenergy and biomaterials.

Cable bacteria

One of the most amazing phenomena in electro-microbiology are cable bacteria, which appear to facilitate long-range electron transport from reduced sulfidic zones to oxygen in a diversity of marine and freshwater sediments (Pfeffer *et al.*, 2012; Risgaard-Petersen *et al.*, 2012; Larsen *et al.*, 2014; Schauer *et al.*, 2014; Malkin and Meysman, 2015; Nielsen and Risgaard-Petersen, 2015; Risgaard-Petersen *et al.*, 2015). In some instances nitrate, rather than oxygen, may serve as the electron acceptor (Marzocchi *et al.*, 2014). The cable bacteria, which are within the family *Desulfobulbaceae*, form chains, one cell in diameter, extending thousands of cells in length (Pfeffer *et al.*, 2012). Growth of the cable bacteria is associated with an oxidation of sulfide minerals that is much faster than could be accounted for by diffusive processes, and an increase in pH in the oxic zone, consistent with the consumption of protons during oxygen reduction to water (Pfeffer *et al.*, 2012; Risgaard-Petersen *et al.*, 2012). Cutting horizontally through the sediments with a thin tungsten wire eliminated the apparent electron transfer as did emplacement of filters with pore sizes too small to permit growth of the bacteria through the filter (Pfeffer *et al.*, 2012). These and other controls ruled out the possibility of electron transport through conductive minerals, which can confer substantial conductivity in some reduced marine sediments (Malvankar *et al.*, 2015a). The simplest explanation for these observations is that: (1) cable bacteria oxidize sulfide in the anoxic zone; (2) the electrons derived from sulfide are transported through the cable into the oxic zone; and (3) the cable bacteria in the oxic zone transfer the electrons to oxygen (Figure 1). However, it is important to recognize that these three proposed steps are not yet fully verified.

Distinct ridges of unknown composition run along the length of the filaments, which appear to share intercellular periplasmic connections. Electrostatic force microscopy indicated that the ridges have charge storage capacity (Pfeffer *et al.*, 2012). However, the conductivity of these structures has not been measured. There was no measurable current between electrodes placed in contact with the outer cell surface, suggesting that the proposed electron transfer was internal and that the outer membrane served as an insulator (Pfeffer *et al.*, 2012). Additional efforts to connect the cable bacteria with electrodes in a manner that would yield estimates of the conductivity along the length of the filaments have not been successful (Nielsen and Risgaard-Petersen, 2015). Thus, it may be necessary to dissect

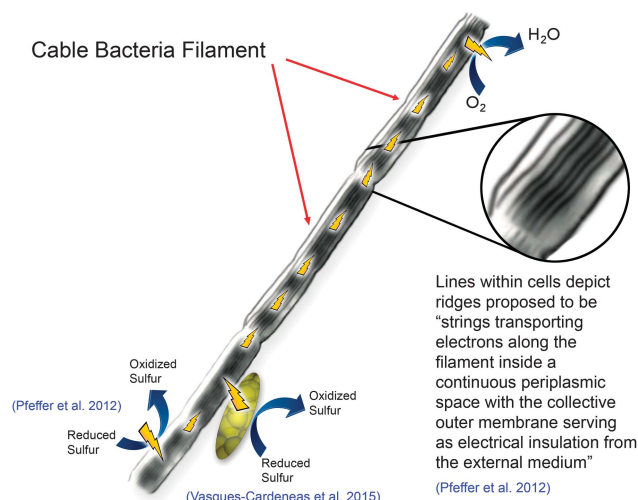


Figure 1 Model for long-range electron transport through the interior of cable bacteria with proposed reactions and relevant references. Lightning bolts depict the direction of electron flow.

the ridges from the cells in order to better evaluate the suggestion that they are electrically conductive.

Quantitative biogeochemical models will also require direct measurements of the contribution of cable bacteria to sediment conductivity because the indirect methods currently in use are probably underestimating electron fluxes from the sulfidic to oxic zones (Vasquez-Cardenas *et al.*, 2015). Electrochemical studies of sediments containing cable bacteria have been reported (Risgaard-Petersen *et al.*, 2014), but the methods employed did not measure the rate of electron transport through the sediment. Techniques for measuring sediment conductivity are available (Malvankar *et al.*, 2015a) and it would be helpful in better understanding this process to directly compare the conductivity of sediments that contain cable bacteria with similar sediments in which cable bacteria are absent.

Cable bacteria may also be involved in DIET (Vasquez-Cardenas *et al.*, 2015). ¹³C-labeling studies suggested that chemolithoautotrophic Epsilon- and Gammaproteobacteria are responsible for the sulfur oxidation that takes place in the presence of cable bacteria (Vasquez-Cardenas *et al.*, 2015). It is hypothesized that the sulfur oxidizers transfer electrons to the *Desulfobulbaceae* filaments via DIET through as yet to be determined electrical connections (Vasquez-Cardenas *et al.*, 2015). Further analysis with a broader range of labeling and omics tools, as have been employed for the study of other sulfur-oxidizing marine populations (Dyksam *et al.*, 2016), may help better define these interactions.

There is a clear need to develop strategies to culture cable bacteria in order to study in more detail the inferences that have been made about their conductivity, their mechanisms of long-range electron transport and potential growth modes. Furthermore, identification of their conductive structures has the possibility to make an important contribution

to the development of bio-inspired electrically conductive materials.

Biological nanotubes and related cell-to-cell connections

Electrically conductive structures might not be the only strategy for transferring electrons between the interiors of adjacent cells. *Desulfovibrio vulgaris* and *Clostridium acetobutylicum* growing in co-culture in a sulfate-free glucose medium forged connections that permitted interspecies transfer of proteins and allowed *D. vulgaris* to grow even though sulfate was not available as an electron acceptor (Benomar *et al.*, 2015). One possible explanation is that electron carriers, such as ferredoxin, function as an interspecies electron shuttle (Figure 2): the shuttle is reduced within *D. vulgaris*; transferred to *C. acetobutylicum* via the intercellular connection; and oxidized with the reduction of organic electron acceptors or protons within *C. acetobutylicum* (Benomar *et al.*, 2015). However, further study is required to verify this hypothesized novel method of interspecies electron exchange.

The connections between *D. vulgaris* and *C. acetobutylicum* appeared to be a fusion of the two cells, but special nanotube structures are another strategy for interspecies exchange of cellular components (Dubey and Ben-Yehuda, 2011; Pande *et al.*, 2015; Dubey *et al.*, 2016). Membrane vesicles can also ferry a range of cellular components between cells (Hasegawa *et al.*, 2015). Electron transport via diffusion of electron shuttles through nanotubes and related intercellular connections would be expected to be slower than electron transfer through electrically conductive structures shared between cells, as proposed for cable bacteria, but would be more selective and potentially faster than the release of an electron shuttle into the extracellular environment, such as in interspecies hydrogen transfer.

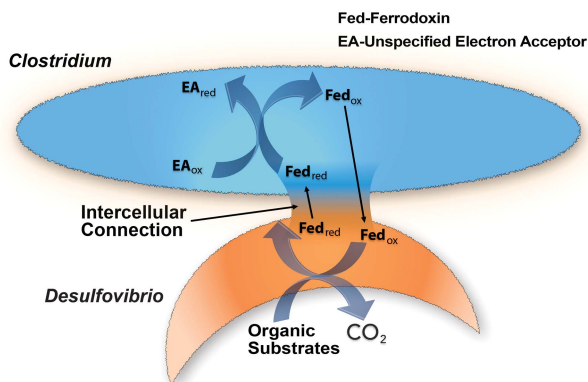


Figure 2 Model for electron exchange between *Desulfovibrio vulgaris* and *Clostridium acetobutylicum* proposed in Benomar *et al.*, 2015. An electron shuttle, such as ferredoxin, provides interspecies electron exchange by moving between the two species through an intercellular connection.

Shewanella oneidensis produces outer membrane extensions (Pirbadian *et al.*, 2014) that look very similar to the nanotubes of other bacteria. It is often stated that the *S. oneidensis* outer membrane extensions form electrical connections between cells (Benomar *et al.*, 2015; Blumberger, 2015; Shen *et al.*, 2015), presumably because the *S. oneidensis* outer membrane extensions are referred to as microbial nanowires (Pirbadian *et al.*, 2014). However, as previously reviewed (Lovley and Malvankar, 2015), there is no evidence that the *S. oneidensis* membrane extensions facilitate cell-to-cell electron transfer or any other form of long-range electron transport under physiologically relevant conditions.

The formation of nanotubes in *Bacillus subtilis* can be diminished by deleting the gene for YmdB, a phosphodiesterase (Dubey *et al.*, 2016). Ymb is highly conserved in gram positive and gram negative bacteria (Dubey *et al.*, 2016) and thus may provide a target for determining whether nanotubes are involved in extracellular electron exchange through gene deletion studies.

Outer surface cytochrome matrix

A conductive network of *c*-type cytochromes was proposed to support DIET in the consortia of methane-oxidizing ANME-2 archaea and sulfate reducers that have an important role in consuming methane in marine environments (McGlynn *et al.*, 2015). It was speculated that the ANME-2/sulfate reducer aggregates were electrically conductive because estimates of microbial activity did not fit a model consistent with a diffusive interspecies electron carrier. Heme staining indicated the presence of *c*-type cytochromes not only in the membranes of ANME-2 and their sulfate-reducing partners, but also in the tight intercellular space between the cells (McGlynn *et al.*, 2015). A model was suggested (Figure 3) in which ANME-2, which can oxidize methane with extracellular electron transfer (Scheller *et al.*, 2016), transfer electrons to extracellular cytochromes that then form an electrical connection with outer surface cytochromes on the sulfate reducer, which can then transfer electrons into the cell for sulfate reduction (McGlynn *et al.*, 2015). It was also proposed that the cytochromes provided a conductive matrix so that sulfate reducer cells not in close proximity to ANME-2 could receive electrons to support sulfate reduction. Major assumptions in this model have not yet been validated including: (1) whether the aggregates are electrically conductive; (2) whether cytochromes are sufficiently abundant to form a conductive matrix throughout the aggregates; and (3) whether the sulfate reducers are capable of using extracellularly derived electrons for sulfate reduction.

In evaluating this model, it is important to recognize that there is no instance in which a

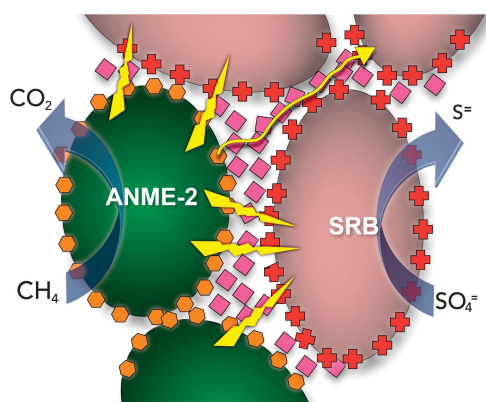


Figure 3 Model for direct interspecies electron transfer in consortium of ANME-2 (green) and syntrophic sulfate-reducing partner (pink) anaerobically oxidizing methane with the reduction of sulfate. Cytochrome (hexagons, diamonds and crosses)-based electron transfer proposed for adjacent cells (lightning bolts) and longer percolation path (yellow line) proposed for electron transfer to non-adjacent cells.

cytochrome-based biological conductive matrix capable of transporting electrons over μm distances has been verified. At one time it was thought that cytochrome-based electron exchange could account for the long-range electron transport through the conductive anode biofilms of *Geobacter sulfurreducens* microbial fuel cells (Strycharz *et al.*, 2011b). However, subsequent studies demonstrated that, although cytochromes were highly abundant in the biofilms, the cytochrome density was not sufficient for cytochromes to make electrical connections over multiple cell lengths (Malvankar *et al.*, 2012b). For example, the biofilms of a strain of *G. sulfurreducens* designed with poorly conductive pili have low conductivity despite the production of abundant *c*-type cytochromes (Vargas *et al.*, 2013). Attempts to build artificial consortia that can perform DIET solely via cytochrome-to-cytochrome electron transfer with co-cultures of *G. metallireducens* and *G. sulfurreducens* have been unsuccessful, unless a soluble electron shuttle to mediate the extracellular electron transfer is also provided (Smith *et al.*, 2015). Thus, the DIET model for the ANME-2 methane-oxidizing consortia is attractive, but there is as of yet little direct evidence to support the model.

Networks of electrically conductive pili (e-pili)

Conductive, multi-species aggregates participating in DIET are possible with networks of e-pili (Figure 4). This was first recognized when co-cultures of *G. metallireducens* and *G. sulfurreducens* were adaptively evolved to grow in a medium with ethanol as the electron donor and fumarate as the electron acceptor (Summers *et al.*, 2010). *G. metallireducens* conserves energy to support growth by oxidizing ethanol to acetate with electron transfer to *G. sulfurreducens*, which can grow with electrons

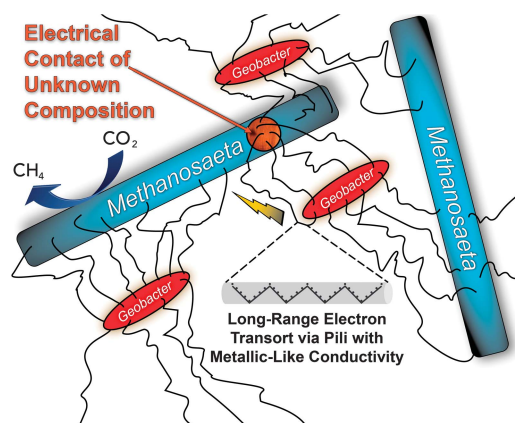


Figure 4 Model for direct interspecies electron transfer between *Geobacter* and *Methanosaeta* species. The gray tube illustrates the proposed metallic-like mechanism for conduction along the length of the e-pili via delocalized electrons associated with tightly packed aromatic amino acids.

solely derived from DIET (Shrestha *et al.*, 2013a), but also consumes acetate released by *G. metallireducens*. As the co-culture adapted for syntrophic growth it formed large (1 mm diameter) electrically conductive aggregates that were filled with a dense network of e-pili (Summers *et al.*, 2010). The two species were not uniformly distributed throughout the aggregates, suggesting that DIET does not require close interspecies associations for the interspecies electron exchange. Instead, it appears that the e-pili network within the aggregates forms a conductive matrix similar to that described in current-producing *Geobacter* biofilms (Reguera *et al.*, 2006; Malvankar *et al.*, 2011). As previously reviewed in detail (Malvankar and Lovley, 2014; Lovley and Malvankar, 2015), multiple lines of evidence suggest that the e-pili within *Geobacter* biofilms have a 'metallic-like' conductivity similar to that observed in synthetic organic conducting polymers (Malvankar *et al.*, 2011), which can be attributed to closely packed aromatic amino acids that facilitate electron transport along the length of the pili (Vargas *et al.*, 2013; Malvankar *et al.*, 2014, 2015b; Adhikari *et al.*, 2016; Xiao *et al.*, 2016). There are alternative theoretical models for e-pili conductivity (see Feliciano *et al.*, 2015 and Malvankar *et al.*, 2015b for additional references and discussion), but the feasibility of DIET mediated by e-pili is not materially influenced by which model for electron transport along the length of the e-pili eventually prevails. The multi-heme *c*-type cytochrome OmcS, which is associated with e-pili (Leang *et al.*, 2010), is important for DIET and is hypothesized to serve as an electrical contact to promote electron transfer to and from the pili (Summers *et al.*, 2010). Diverse experimental approaches have supported this DIET model and have ruled out alternative models such as interspecies electron transfer via hydrogen or other electron shuttles (Summers *et al.*, 2010; Rotaru *et al.*, 2012; Shrestha *et al.*, 2013a,b).

The first environment in which DIET supported by e-pili was detected was anaerobic digesters converting brewery wastes to methane (Morita *et al.*, 2011; Shrestha *et al.*, 2014; Rotaru *et al.*, 2014b). These upflow anaerobic sludge blanket digesters treat a relatively simple waste in which ethanol is a major constituent (Shrestha *et al.*, 2014). *Methanosaeta* species were the dominant methanogens and *Geobacter* species were the most abundant and most metabolically active bacteria (Morita *et al.*, 2011; Rotaru *et al.*, 2014b). Metatranscriptomics revealed that the *Methanosaeta* species in the digester were highly expressing genes for the reduction of carbon dioxide to methane (Rotaru *et al.*, 2014b), a surprising finding because *Methanosaeta* were not known to produce methane from carbon dioxide and do not use hydrogen or formate, typical electron donors for carbon dioxide reduction (Smith and Ingram-Smith, 2007). Defined co-culture studies with *G. metallireducens* and the digester isolate *Methanosaeta harundinacea* demonstrated that *M. harundinacea* could reduce carbon dioxide to methane with electrons received from *G. metallireducens* via DIET (Rotaru *et al.*, 2014b). The conductivity of the digester aggregates and genetic studies with the defined co-culture demonstrated the importance of e-pili for DIET (Morita *et al.*, 2011; Shrestha *et al.*, 2014; Rotaru *et al.*, 2014b).

The electrical contact(s) for *Methanosaeta* to accept electrons via DIET are unknown. However, *Methanosarcina barkeri*, which is closely related to *Methanosaeta* species and is genetically tractable, can also participate in DIET (Rotaru *et al.*, 2014a; Wang *et al.*, 2016) and thus may represent the best model organism for elucidating mechanisms for extracellular electron exchange in methanogens involved in DIET.

The ability of *Methanosaeta* to participate in DIET has potential importance in the global carbon cycle because of the abundance of *Methanosaeta* in terrestrial wetlands and aquatic sediments that significantly contribute to atmospheric methane (Smith and Ingram-Smith, 2007). For example, metatranscriptomic analysis of methanogenic rice paddy sediments revealed that *Methanosaeta* were highly expressing genes for carbon dioxide reduction and that there was also high expression of genes for e-pili by abundant *Geobacter* species (DE Holmes, personal communication). Expression of genes for carbon dioxide reduction in *Methanosaeta* was also noted in incubations of arctic peat soils (Tveit *et al.*, 2015). Furthermore, *Geobacter* species were among the most metabolically active bacteria in Fe(III)-depleted methanogenic rice paddy soils (Hori *et al.*, 2007; Kim and Liesack, 2015), consistent with the concept that the *Geobacter* might be growing as syntrophic partners with methanogens.

DIET between bacteria and archaea partners facilitated by e-pili was also proposed for a thermophilic enrichment culture dominated by a methane-oxidizing archaeon in the ANME-1 phylogenetic

clade and its electron-accepting partner, the hydrogenotrophic, sulfate reducer previously referred to as hot-seep-1 and now known as *Candidatus Desulfotribus auxilii* (Wegener *et al.*, 2015; Krukenberg *et al.*, 2016). Even though the sulfate reducer effectively uses hydrogen as an electron donor, slow rates of hydrogen production by the consortium and a lack of hydrogenase gene sequences in the ANME-1 genome suggested that hydrogen was not the interspecies electron carrier (Wegener *et al.*, 2015). Both partners greatly increased expression of genes for outer surface *c*-type cytochromes when cooperating to oxidize methane. Furthermore, the sulfate reducer also highly expressed genes for type IV pili when grown syntrophically, but not when grown on hydrogen (Wegener *et al.*, 2015). Abundant pili were apparent with transmission electron microscopy in aggregates oxidizing methane (Wegener *et al.*, 2015). The conductivity of the pili or the aggregates was not measured, but it was speculated that the pili might be electrically conductive in a manner similar to *Geobacter* e-pili (Wegener *et al.*, 2015).

Other types of electrically conductive filaments have been documented and could potentially serve as electrical contacts for DIET. For example, the Fe(III)-reducing microorganism *Aeromonas hydrophila* (Castro *et al.*, 2013), the Fe(II) oxidizer *Acidithiobacillus ferrooxidans* (Li and Li, 2014) and the sulfate reducer *D. desulfuricans* (Eaktasang *et al.*, 2016) produce filaments distinct from the *Geobacter* e-pili that are conductive across their diameter. *Rhodospseudomonas palustris*, specifically expresses electrically conductive filaments of unknown composition under Fe(III)-reducing conditions (Venkidusamy *et al.*, 2015). It does not appear that the ability of any of these organisms to exchange electrons with other species has been investigated yet. There is a wide diversity of other filaments in the microbial world that might be conductive and warrant investigation.

DIET mediated by abiological conductive materials

The need for conductive filaments for DIET can be alleviated with abiotic conductive materials (Figure 5). Conductive carbon materials such as granular activated carbon (Liu *et al.*, 2012), biochar (Chen *et al.*, 2014b) and carbon cloth (Chen *et al.*, 2014a) all effectively wire different species together for DIET. Cells of both species attach to the conductive material, but are often not in close physical contact with other cells (Chen *et al.*, 2014a, b; Liu *et al.*, 2012). In the presence of conductive materials, it is possible to initiate cocultures of *G. metallireducens*/*G. sulfurreducens* or *G. metallireducens*/*M. barkeri* with *Geobacter* strains that lack the ability to produce either e-pili or the e-pili cytochrome OmcS (Chen *et al.*, 2014a, b; Liu *et al.*, 2012; Rotaru *et al.*, 2014a). These results

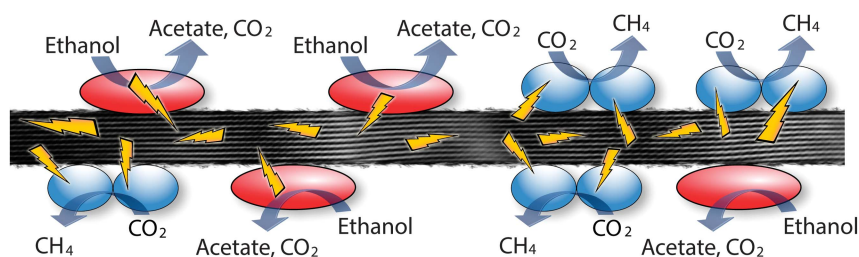


Figure 5 Model for electron exchange between *Geobacter* and *Methanosarcina* species mediated by carbon cloth. Lightning bolts depict electron transfer from *Geobacter* (red rods) through the cloth and to *Methanosarcina* (blue coccoids).

suggest that electron transport carriers associated with the outer surface of the cell are able to make the required electrical contacts with the conductive material.

The conductive iron mineral magnetite also promotes DIET (Kato *et al.*, 2012a, b). The magnetite, which is typically smaller than the cells, has been observed to attach to the cells participating in DIET. Illustrations of magnetite-enhanced DIET often show chains of magnetite bridging between the electron-donating and the electron-accepting partner, but direct experimental evidence for this appears to be lacking. Genetic analysis of *G. metallireducens*/*G. sulfurreducens* co-cultures and visualization of magnetite suggested that magnetite promoted DIET by attaching to e-pili, functioning as a surrogate for the e-pili cytochrome OmcS (Liu *et al.*, 2015). In the presence of magnetite, a strain of *G. sulfurreducens* in which the gene for OmcS had been deleted was nearly as effective in DIET as the wild-type strain and wild-type cells downregulated expression of the *OmcS* gene, presumably conserving energy when an OmcS substitute was available (Liu *et al.*, 2015).

Harnessing cell-to-cell electrical connections for practical applications

Many technologies in which microbes interact with electrodes as either an electron acceptor or an electron donor are in development (Lovley and Nevin, 2013; Rosenbaum and Franks, 2014). The current-producing capabilities of *Geobacter* species were linked to their ability to participate in DIET, not their effectiveness in Fe(III) oxide reduction, suggesting that understanding DIET mechanisms may aid in promoting strategies for enhancing microbe–electrode interactions (Rotaru *et al.*, 2015). e-pili, and possibly other electrically conductive components of e-communities, may be sources of sustainably produced electronic materials (Malvankar *et al.*, 2012a; Adhikari *et al.*, 2016; Tan *et al.*, 2016).

Promoting DIET might enhance the anaerobic digestion of organic wastes, one of the few proven, effective, large-scale bioenergy strategies. Many studies have now shown that adding materials known to facilitate DIET either to anaerobic digesters, or methanogenic enrichment cultures

metabolizing important intermediates in anaerobic digestion, can accelerate the rate of methane production; increase the methane content of the gas produced; and/or stabilize the digestion to permit higher organic loading rates (Viggi *et al.*, 2014; Baek *et al.*, 2015; Li *et al.*, 2015a, b; Xu *et al.*, 2015; Yamada *et al.*, 2015; Zhao *et al.*, 2015; Zhuang *et al.*, 2015a, b; Beckmann *et al.*, 2016; Lee *et al.*, 2016). None of these studies have directly demonstrated DIET with detailed studies of microbial metabolism, but DIET was inferred from enrichment of microorganisms known to participate in DIET and/or increased rates of methane production. Genera of methanogens that are known to use H_2 as an electron donor are abundant in anaerobic digesters treating complex organic wastes such as sewage sludge, manure, industrial waste and food waste (Regueiro *et al.*, 2012; Vanwonterghem *et al.*, 2014; Cai *et al.*, 2016; Luo *et al.*, 2016). The available evidence suggests that many of these methanogens are unlikely to participate in DIET (Rotaru *et al.*, 2014b). These findings raise two important questions: (1) is DIET ever an important mechanism of electron exchange during the degradation of these complex wastes; and (2) will promoting DIET with the addition of conductive materials have a significant long-term impact on the performance of such digesters.

Outlook

The study of e-communities is clearly in its infancy, but their potential importance in biogeochemical cycles, as well as in practical applications such as bioenergy, is readily becoming apparent. With an increasing understanding of the mechanisms involved in DIET it will become easier to recognize e-communities in diverse environments. For example, based on the microorganisms present, it was inferred (Shrestha and Rotaru, 2014) that conversion of coal to methane (Jones *et al.*, 2010) was carried out by a DIET-based community. As discussed above, the expression of key genes associated with DIET in *Methanosaeta* and *Geobacter* species has suggested that DIET is important in some rice paddy soils and as other electrical connectors are identified, it should be possible to more confidently identify additional e-communities from meta-omics data.

Studies to date have focused on microbial communities in which one primary substrate (ethanol, methane) is fueling most of the microbial activity and DIET is essential for metabolism of that substrate. Additional substrates known to be metabolized via DIET are, so far, restricted to simple alcohols and short-chain fatty acids (Wang *et al.*, 2016). Thus, in more complex environments DIET may be just one of multiple types of interspecies interactions and detecting DIET will be more technically difficult.

As more information becomes available to advance the modeling of e-communities (Nagarajan *et al.*, 2013; Storck *et al.*, 2015), it will become more feasible to predict which conditions favor direct electrical connections over electron exchange via hydrogen or other shuttles. e-communities are analogous to the electrification of human civilization. Rapid transportation of energy via electrons through the grid has greatly improved the quality of human life, but in some instances economic or technical considerations still favor the slower conveyance of energy between providers and consumers in the form of chemical bonds (wood, coal and petroleum). The same must be true for microbial communities—the abundance of microorganisms that function via interspecies hydrogen transfer demonstrates that speed of electron transfer is not always the only consideration in choosing a route to share energy.

The emergence of many studies engineering DIET with conductive materials is one example of how a functional understanding can facilitate directing e-communities toward better performance. On the horizon may be construction of synthetic e-communities. There are a diversity of materials such as redox-active polymers, inorganic nanoparticles, carbon nanotubes and graphene that improve the ability of some microorganisms to electrochemically interact with their extracellular environment, especially when those microorganisms lack an inherent effective capacity for extracellular electron exchange (Ajo-Franklin and Noy, 2015; Du *et al.*, 2014). Electrification of designer microbial communities may make processes less susceptible to contamination with competing microorganisms than synthetic communities that rely on shared metabolites for metabolic cooperation.

Just as the electrification of human civilization for energy exchange was followed by the internet for information exchange, it may be that microbial communities can share information as well as energy through their electrical connections. This suggestion (Reguera, 2011) has yet to be experimentally verified, but it has already been demonstrated that electrical communication within biofilms is possible through ion channels (Masi *et al.*, 2015; Prindle *et al.*, 2015). Thus, electrical communication via electron exchange seems feasible. Whether or not this is an important phenomenon in natural communities, there is certainly the possibility of artificially

controlling the activity of e-communities with electrical inputs that are already known to influence gene expression (Strycharcz *et al.*, 2011a).

Another intriguing concept is the possibility that microorganisms in the human gut may electrically interact with cells in the gut epithelium (Ericsson *et al.*, 2015). This, and the strong possibility of microbe-microbe electrical communication in this anaerobic environment, suggests that the human gut may be one of the next exciting frontiers in the study of e-communities. Furthermore, there is a growing appreciation of the possibility that electrical communication via conductive protein filaments has an important role in the function and development of humans (Friesen *et al.*, 2015). Electrically conductive microbial aggregates may provide a primitive model for better understanding the evolution and function of these larger multi-cellular electrical systems.

Conflict of Interest

The author declares no conflict of interest.

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