Minireview

Exocellular electron transfer in anaerobic microbial communities

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Summary

Exocellular electron transfer plays an important role in anaerobic microbial communities that degrade organic matter. Interspecies hydrogen transfer between microorganisms is the driving force for complete biodegradation in methanogenic environments. Many organic compounds are degraded by obligatory syntrophic consortia of proton-reducing acetogenic bacteria and hydrogen-consuming methanogenic archaea. Anaerobic microorganisms that use insoluble electron acceptors for growth, such as iron- and manganese-oxide as well as inert graphite electrodes in microbial fuel cells, also transfer electrons exocellulary. Soluble compounds, like humic substances, quinones, phenazines and riboflavin, can function as exocellular electron mediators enhancing this type of anaerobic respiration. However, direct electron transfer by cell–cell contact is important as well. This review addresses the mechanisms of exocellular electron transfer in anaerobic microbial communities. There are fundamental differences but also similarities between electron transfer to another microorganism or to an insoluble electron acceptor. The physical separation of the electron donor and electron acceptor metabolism allows energy conservation in compounds as methane and hydrogen or as electricity. Furthermore, this separation is essential in the donation or acceptance of electrons in some environmental technological processes, e.g. soil remediation, wastewater purification and corrosion.

Introduction

Two types of energy sources are employed by microorganisms, light energy by phototrophic microorganisms and chemical energy by chemotrophic microorganisms. To date no microorganisms have been isolated that use other types of energy like thermal or gravitation energy. However, the recent isolation of an obligatory photosynthetic anaerobic bacterium from a deep-sea hydrothermal vent suggests that geothermal radiation can serve as a source of (light) energy (Beatty et al., 2005). In addition, hydrogen produced in igneous rocks can be considered as a physical–chemical energy source for microorganisms. Microbial life in the deep subsurface thrives from geochemically produced hydrogen (Stevens and McKinley, 1995; Freund et al., 2002; Nealson et al., 2005).

This review deals with the exocellular electron transfer in anaerobic environments. With a few exceptions, chemotrophic microorganisms derive energy from coupled oxidation and reduction reactions, resulting in ATP formation by substrate level phosphorylation and electron transport phosphorylation. Besides oxygen (aerobic respiration) microorganisms have the ability to use a variety of alternative electron acceptors for anaerobic respiration (Fig. 1A). Nitrate and sulfate are well-known examples of electron acceptors that are used in the absence of oxygen. Other inorganic electron acceptors for anaerobic microorganisms are oxidized metal ions like iron and manganese and oxy-anions like selenate, arsenate and uranate. In methanogenic environments, bicarbonate and protons act as terminal electron acceptors.

Today, we know much about the structure and function of various membrane-bound proteins involved in the aerobic and anaerobic respiratory chain. However, we know very little about microorganisms that use electron acceptors that do not result in energy conservation, or even need to invest metabolic energy to use an electron acceptor. Such microorganisms play a very important role in methanogenic environments. We also do not fully understand the biochemical mechanism of electron transfer to...
insoluble electron acceptors and the role of small molecules in that exocellular electron transfer. Such knowledge is essential to understand the biochemical diversity of life, and to apply microbial processes for biotechnological purposes. In this review we discuss what is known about the ability of microorganisms to grow by means of exocellular electron transfer via interspecies hydrogen or formate transfer, direct transfer of electrons, or electron transfer to insoluble electron acceptors via organic and inorganic mediators (Fig. 1). Electron transfer that requires complex metabolic pathways is not considered in this review. Biotechnological consequences and environmental relevance of exocellular electron transfer are addressed as well.

Exocellular electron transfer in methanogenic environments

Methanogenesis is, from an environmental and biotechnological viewpoint, a very intriguing microbial process. The formation of methane occurs in environments where organic matter is decomposed in the absence of inorganic electron acceptors other than protons and carbon dioxide, and also in the deep subsurface from geochemically produced hydrogen. Freshwater sediments, swamps, tundras, rice paddy fields, landfills and intestinal tracts of insects and ruminants are important methanogenic environments, which contribute to the increased levels of the ‘greenhouse gas’ methane in the atmosphere. However, methanogenesis that takes place under controlled conditions in landfills and anaerobic bioreactors used for waste and wastewater treatment, conserves chemical energy in organic waste as the ‘green fuel’ methane.

Methanogenesis is also a fascinating process from a biochemical viewpoint. Methanogenic archaea are the only microorganisms that can produce large amounts of methane. These microorganisms have a very specialized metabolism; they can reduce carbon dioxide with molecular hydrogen, cleave acetate into methane and carbon...
dioxide and ferment a small number of one-carbon compounds (e.g. methanol, formate, methylamines and methylsulfides) to methane and carbon dioxide. As a consequence, methanogenic archaea rely on other anaerobic microorganisms for substrate supply. In a sequence of reactions, complex organic matter (polysaccharides, proteins, lipids, etc.) is degraded by fermenting microorganisms to the methanogenic substrates (hydrogen, formate, acetate) and to a variety of other organic compounds (lactate, ethanol, propionate, butyrate, etc.). The latter compounds are degraded by proton-reducing acetogenic bacteria to the methanogenic substrates, acetate and hydrogen. However, for thermodynamic reasons these bacteria can grow only on these compounds when the hydrogen concentration is kept low by methanogenic archaea. This was first recognized by Bryant and colleagues (1967) when they discovered that the originally believed pure culture *Methanobacillus omelianskii* was a co-culture of an ethanol-degrading bacterium and a methanogen. For detailed overviews of the ecology and physiology of syntrophic consortia in methanogenic environments, the reader is referred to Schink and Stams (2002) and Plugge and Stams (2005).

We have studied the biochemistry and bioenergetics of anaerobic growth on propionate (Fig. 1B). This biotransformation is representative for the way obligate consortia of bacteria and archaea function and cope with the thermodynamic constraints. The Gibbs free energy change of methanogenic propionate degradation is about \(-60 \text{ kJ mol}^{-1}\), which is roughly the amount of energy that is needed to produce 1 mol of ATP (Schink, 1997). However, this amount of energy needs to be shared by three microorganisms. One bacterium that degrades propionate to acetate, carbon dioxide and hydrogen and two methanogenic archaea. One methanogen cleaves acetate and the other one uses hydrogen to reduce carbon dioxide to methane. The model organism that we studied is *Syntrophobacter fumaroxidans*, which was isolated from an anaerobic methanogenic bioreactor. *Syntrophobacter fumaroxidans* degrades propionate via the methylmalonyl-CoA pathway (Fig. 2). In this pathway, one ATP is formed in the conversion of succinyl-CoA to succinate. Reducing equivalents are released at different energetic levels. Reduced ferredoxin is formed in the conversion of pyruvate to acetate, while NADH and FADH$_2$ are formed in the oxidation of oxaloacetate and succinate respectively. These intracellular redox mediators need to be oxidized coupled to proton reduction. The oxidation of reduced ferredoxin [$E^o_{\text{Fd(red)}}$/Fd(ox)] = \(-398 \text{ mV}\) and NADH ($E^o_{\text{NADH/NAD^+}}$ = \(-320 \text{ mV}\)) coupled to proton reduction ($E^o_{\text{H_2/H^+}}$ = \(-414 \text{ mV}\)) is energetically feasible when the hydrogen concentration is kept low by methanogens. However, the oxidation of FADH$_2$ ($E^o_{\text{FADH}_2/FAD}$ = \(-220 \text{ mV}\)) is energetically also not feasible at a hydrogen concentration. The ATP-driven reversed electron transport mechanism is essential to couple FADH$_2$ oxidation to proton reduction.
concentration of 10 nM (1 Pa) that can be reached by methanogens. By means of a reversed electron flow mechanism, ATP needs to be invested to make protons accessible to accept electrons from FADH$_2$ (Schink, 1997; Schink and Stams, 2002). Experimental evidence was obtained that 2/3 ATP is needed for this (Van Kuijk et al., 1998). Thus, the net ATP gain for the bacterium is 1/3 mol ATP per mol of propionate converted.

In most syntrophic methanogenic associations, hydrogen plays a predominant role. Hydrogen is small in size and easily diffusible, and hydrogenases are present in many different anaerobic bacteria and archaea. However, interspecies hydrogen transfer is not the only mechanism of exocellular electron transfer in syntrophic methanogenic consortia. As hydrogen is poorly soluble in water (the maximal solubility at atmospheric pressure and room temperature is about 1 mM), it was speculated from theoretical considerations that formate may also act as electron shuttle in methanogenic consortia (Thiele and Zeikus, 1988; Boone et al., 1989). Clear evidence for the role of formate transfer was obtained in studies with Syntrophobacter fumaroxidans. This bacterium is able to grow with propionate in the presence of methanogens that can use hydrogen and formate but not with methanogens that can only use hydrogen (Dong and Stams, 1995; Stams and Dong, 1995). In addition, the bacterium possesses two tungsten-dependent formate dehydrogenases, which exhibit very high activity both in the formate oxidation and the CO$_2$ reduction direction (De Bok et al., 2002; 2003). The latter is essential for interspecies formate transfer. In contrast, the more common molybdenum-containing formate dehydrogenases have only high formate oxidation activity, but have never been shown to have CO$_2$ reduction activity. We also have obtained evidence for the occurrence of formate transfer in butyrate-degrading co-cultures (Dong and Stams, 1995; Stams and Dong, 1995). Presently, the genomes of Syntrophobacter fumaroxidans and Syntrophomonas wolfei are being sequenced by the JGI-DOE in Walnut Creek, CA, USA (http://www.jgi.doe.gov). This will provide deeper insight into the relative role of hydrogen and formate transfer in propionate and butyrate oxidation.

The role of interspecies hydrogen transfer is well recognized when it concerns the degradation of compounds that cannot be fermented. However, several substrates that are known to be easily fermentable may require obligate syntrophic consortia as well. In the absence of an electron acceptor, Syntrophococcus sacromutans is only able to grow on sugars in the presence of hydrogenotrophic methanogens (Krumholz and Bryant, 1986). Similarly, several anaerobic bacteria can only degrade certain amino acids, including alanine, valine, aspartate and glutamate in syntrophic association with methanogens (Stams and Hansen, 1984; Zindel et al., 1988; Plugge et al., 2002). Furthermore, when subcultured with methanogens many anaerobic bacteria that grow fermentatively on sugars and amino acids switch their metabolism in favour of hydrogen formation. The energetic advantage of such a shift in metabolism might be energy conservation by means of a special class of [Ni-Fe] hydrogenases termed energy-converting hydrogenases. These hydrogenases reduce protons with electrons derived from reduced ferredoxin coupled to energy conservation by means of electron-transport phosphorylation (Hedderich, 2004). We believe that many bacteria that have been detected with molecular biological techniques in anaerobic environments are dependent on interspecies hydrogen transfer. Therefore, adapted cultivation strategies are required to get insight into their function in the environment. A relatively simple but elegant way is to culture such microorganisms in media to which hydrogen-consuming methanogens have been added.

Interspecies hydrogen transfer also plays an important role in the reductive conversion of chlorinated organic compounds in methanogenic environments. Anaerobic bacteria can use chlorinated compounds as terminal electron acceptor for growth. For recent reviews on anaerobic dehalogenating bacteria, the reader is referred to Smidt and de Vos (2004) and Bradley (2003). Although some dehalogenating bacteria can use organic substrates as electron donors, molecular hydrogen (H$_2$) seems to be the major electron donor for dehalogenation of chlorinated compounds in mixed microbial communities (Distefano et al., 1992; Gibson and Sewell, 1992; Luijten et al., 2004a). Most dehalogenating bacteria, e.g. Dehalobacter restrictus and Dehalococcoides ethenogenes, are dependent on hydrogen as electron donor. It was observed in mixed cultures that organic compounds such as short-chain fatty acids and alcohols are oxidized by anaerobic microorganisms, and that the H$_2$ produced serves as electron donor for reductive dehalogenation (Fennel et al., 1997). Thus, dehalogenating bacteria depend on hydrogen-producing acetogenic bacteria like Syntrophomonas and Syntrophobacter for growth and they have to compete with hydrogen-consuming methanogenic archaea for hydrogen. Half-maximum velocity constants, $K_v$(H$_2$) values and hydrogen thresholds are generally five to 10 times lower for reductive dechlorination than for methanogenesis (Smatlak et al., 1996; Ballapragada et al., 1997; Yang and McCarty, 1998; Löfﬂer et al., 1999). Due to these lower $K_v$(H$_2$) and threshold values for hydrogen, dechlorinating bacteria will outcompete methanogens at low H$_2$ concentrations.

Very interesting interspecies hydrogen-dependent syntrophic associations are involved in the degradation of chlorinated aromatic compounds. The degradation of 3-chlorobenzoate has been studied extensively. Four organisms participate in the complete mineralization of 3-chlo-
robenezolate; a bacterium that converts 3-chlorobenezolate to benzoate, a benzoate degrader, a hydrogenotrophic methanogen and an acetotrophic methanogen (Shelton and Tiedje, 1984) (Fig. 1C). The dechlorinating microorganism in this consortium, Desulfomonile tiedjei DCB1 (DeWeerd et al., 1990), is able to couple the reductive dechlorination of 3-chlorobenezolate to growth by dehalorespiration (Dolfing and Tiedje, 1987; Dolfing, 1990). Of special interest is the competition between the dechlorinator and the hydrogenotrophic methanogen for the reducing equivalents produced by the benzoate-degrading bacterium. Hydrogen measurements in the consortium (without the acetotrophic methanogen) growing on 3-chlorobenezolate versus the consortium growing on benzoate indicated that the dechlorinating organism created a lower hydrogen concentration than the methanogen (Dolfing and Tiedje, 1991). Via this lower hydrogen concentration, which was accompanied by a higher benzoate degradation rate, part of the electrons that otherwise would have gone to the methanogen is used by the dechlorinating bacterium. A similar type hydrogen-dependent interaction may also occur in the anaerobic fermentative degradation of chlorinated alkenes and alkanes, e.g. vinylchloride and 1,2 dichloroethane (Bradley and Chapelle, 1999; Gerritse et al., 1999).

Exocellular electron transfer in non-methanogenic environments

Interspecies hydrogen transfer is also important in sulfate-reducing environments. The metabolic interactions between sulfate-reducing bacteria and methanogenic consortia were reviewed recently (Stams et al., 2003; 2005). As sulfate-reducing bacteria are able to grow on organic acids like propionate and butyrate, the impact of obligate syntrophic consortia is less in sulfate-rich environments than in methanogenic environments. In the presence of sulfate, Desulfovibrio species outcompete methanogenic archaea for the available hydrogen (Robinson and Tiedje, 1984). On the other hand, in the absence of sulfate, Desulfovibrio species can grow in syntrophic association with methanogens (Bryant et al., 1977). Under these conditions, they grow as proton-reducing acetogenic bacteria. Unfortunately little is known about the regulation of this shift in metabolism of sulfate reducers. Recently, the genome sequence of Desulfovibrio vulgaris has become available (Heidelberg et al., 2004). This may be helpful to get insight into the regulation of the metabolism of sulfate reducers in the presence and absence of sulfate. Some of the Syntrophobacter species are able to reduce sulfate in pure culture with propionate as electron donor. However, in co-culture with a Desulfovibrio species, Syntrophobacter wolinni grows acetogenically (Boone and Bryant, 1980). It is not known whether a similar type of syntrophic association of sulfate reducers is possible during growth on lactate and sulfate. Desulfovibrio produces hydrogen when grown on lactate and sulfate. In the hydrogen-cycling mechanism, demonstrated by Odom and Peck (1981), the hydrogen formed by Desulfovibrio is scavenged by the same microorganism. However, in the presence of a sulfate reducer with a higher affinity for hydrogen, it could also result in interspecies hydrogen transfer.

Other mechanisms of interspecies electron transfer than hydrogen and formate transfer exist, particularly in anaerobic environments that are characterized by a higher redox potential. The occurrence of a cysteine–cystine shuttle has been demonstrated for a co-culture of Geobacter sulfurreducens and Wolinella succinogenes growing on acetate and nitrate (Kaden et al., 2002). This co-culture can also be sustained with sulfide as electron shuttle. One of the reasons to look for electron carriers other than hydrogen and formate in this consortium was that the hydrogen concentration was too low to explain the growth rates of the two partner organisms (Cord-Ruwisch et al., 1998).

Interestingly, it was proposed that a similar sulfur–sulfide shuttle is involved in the reductive dehalogenation of trichloroacetate by Trichlorobacter thiogenes growing in pure culture (De Weyer et al., 2000) (Fig. 1E). This indicates that sulfur compounds may also play an important role in the conversion of chlorinated compounds in anaerobic environments. The electron transfer mechanism is not clear, but evidence was provided that an unknown cell-associated catalyst is required.

Respiration with naturally occurring insoluble electron acceptors

Iron and manganese are abundantly present in the biosphere. Anaerobic bacteria can use oxidized iron, Fe(III), and oxidized manganese, Mn(IV), as terminal electron acceptor for growth. For an overview on the ecophysiology of iron- and manganese-reducing bacteria, the reader is referred to Lovley (2000), Lovley and colleagues (2004) and Nealson and Saffarini (1994). Bacteria that respire with Fe(III) and Mn(IV) have to cope with the poor solubility of iron and manganese minerals. Nevertheless, manganese and iron reduction play an important role in biogeochemical cycles and in bioremediation of polluted soils.

A wide variety of Fe(III)- and Mn(IV)-reducing microorganisms has been described (Lovley et al., 2004). Geobacter and Shewanella are well-known genera with the ability to respire with minerals. Also members of other physiological groups can reduce iron, e.g. microorganisms that we know for their ability to grow by dehalorespiration (Niggemeyer et al., 2001; Cervantes et al., 2002;
Luijten et al. (2004b), sulfate reduction (Coleman et al., 1993; Cervantes et al., 2002; Holmes et al., 2004) and methane formation (Cervantes et al., 2002; Bond and Lovley, 2003; Van Bodegom et al., 2004).

Lovley and colleagues (1996a,b) showed that humic substances can enhance the microbial reduction of insoluble Fe(III). Humic substances contain quinone structures, which can be reduced to a corresponding hydroquinone form. This hydroquinone reacts chemically with Fe(III), but it can also be an electron donor for anaerobic microbial respiration (Lovley et al., 1999). Humic substances are thus mediating in the exocellular electron transfer from a bacterium to an insoluble electron acceptor or to another bacterium (Fig. 1D).

In many studies that followed, soluble quinones (anthraquinone sulfonate and anthraquinone disulfonate) were used as model compounds. The ability to use quinones as terminal electron acceptor is widespread in the microbial world. Based on the available literature and our own experience, respiration with quinones seems to be a common property among anaerobes rather than an exception. Humic substances and soluble quinones have been shown to mediate and enhance other reduction processes as well, like azo-dye cleavage (Van der Zee et al., 2000), reduction of nitroaromatics (Schwarzenbach et al., 1999), reductive dehalogenation (Curtis and Reinhard, 1994) and the reduction of uranium in iron minerals (Frederickson et al., 2000). In addition, such compounds can act as terminal electron acceptor in the anaerobic oxidation of aromatic hydrocarbons like benzene and toluene (Lovley et al., 1996b; Cervantes et al., 2001). These examples emphasize the environmental relevance of quinones as mediators of exocellular electron transfer.

Other soluble organic compounds can act in a similar way as quinones. Examples of naturally occurring redox mediators are phenazines, e.g. pyocyanin, a pigment produced by Pseudomonas aeruginosa (Hernandez and Newman, 2001; Rabaey et al., 2005), melanin (Turick et al., 2002) and riboflavin (Dos Santos et al., 2004) (Fig. 1D). Riboflavin is an even more efficient electron mediator in azo-dye reduction than soluble quinones. In principle, nontoxic synthetic redox active molecules can also mediate exocellular electron transfer. Sulfur compounds can act as exocellular mediators as well, e.g. the cysteine–cystine shuttle and the sulfide–sulfur(poly)sulfide shuttle (Kaden et al., 2002; Straub and Schink, 2003; 2004).

Although a number of studies point to the role of soluble organic compounds in the transfer of electrons from the bacteria to the insoluble iron and manganese, some iron-reducing bacteria appear to have different strategies. As pointed out in the review of Lovley and colleagues (2004) when comparing different iron-reducing bacteria, the addition of organic redox mediators does not always enhance the reduction of insoluble iron to the same extent. The observation that bacteria can adhere to iron minerals may indicate that direct donation by means of redox mediating proteins is possible. The biochemistry of iron reduction of Geobacter sulfurreducens was studied in detail. Cytochrome-dependent and NAD-dependent Fe(III) reductions have been purified and characterized, and evidence was provided that a parin-like protein and a special cytochrome c are important in the reduction of iron (Gaspard et al., 1998; Seeliger et al., 1998; Kaufmann and Lovley, 2001; Magnuson et al., 2001; Afkar et al., 2005; Kim et al., 2005). Yet, the exact mechanism of electron transfer from this bacterium to the insoluble electron acceptor is not clear. Recently, evidence for a direct electron transfer by Geobacter sulfurreducens via conductive pili, called nanowires, was obtained (Reguera et al., 2005). It may well be that electron transfer from Shewanella algae to hydrous ferric oxide proceeds via an analogous mechanism. Shewanella produces the extracellular heteropolymer melanin. The polyquinonoidal nature of melanin has the electrochemical properties of an amorphous semiconductor capable of redox cycling, and there is evidence that cell-associated melanin acts as an electrical conduit for iron mineral reduction by Shewanella algae (Turick et al., 2003).
Electrodes as electron mediators

Chemically rather inert graphite electrodes may both donate electrons to and accept electrons from microorganisms. The ability of microorganisms to transfer electrons to electrodes is known already for a long time (Turner et al., 1983; Delaney et al., 1984). Only recently the challenge to conserve chemical energy in the form of electricity by microbial processes was realized (Bond et al., 2002; Tender et al., 2002). Since then, much research with microbial fuel cells was performed (Chaudhuri and Lovley, 2003; Rabaey et al., 2003; 2004; 2005; Holmes et al., 2004; Liu and Logan, 2004; Min and Logan, 2004; He et al., 2005; Rabaey and Verstraete, 2005). By means of anaerobic digestion in bioreactors, chemical energy is conserved as methane which can be used as a fuel or to produce electricity, but in microbial fuel cells chemical energy in waste components is directly converted to electricity (Fig. 3). As pointed out by Lovley and colleagues (2004), this process can be very efficient. It seems that some Fe(III)-reducing bacteria have the ability to transfer more than 80% of the electrons available in the organic substrates to electricity. Typically, these bacteria do not require soluble mediators, but can donate electrons directly to the electrode surface by attachment (Bond and Lovley, 2003; Lovley et al., 2004). However, phenazines produced by bacteria were recently shown to enhance electron transfer in microbial fuel cells (Rabaey et al., 2005). The observation that bacteria can grow by the oxidation of organic compounds (e.g. glucose, acetate) by using electrodes as terminal electron acceptor is an intriguing example of exocellular electron transfer, which resembles electron transfer to insoluble electron donors and electron-accepting microorganisms.

Besides producing electricity from the decomposition of organic matter, microbial fuel cells may also be used to produce hydrogen from organic compounds like acetate using electricity (Liu et al., 2005; Rozendal et al., 2006) (Fig. 3). Electricity is used to enhance microbial hydrogen production and to allow hydrogen production to occur at a high hydrogen partial pressure. However, the exact mechanism is not yet clear.

Microbial communities that were enriched in microbial fuel cells have been characterized (Gregory et al., 2004; Holmes et al., 2004; Rabaey et al., 2004). The types of bacteria that are enriched in microbial fuel cells are strongly dependent on the substrate and environmental conditions. At the anode side of a fuel cell with marine sediments Desulfuromonas species were enriched and in one case a Desulfobulbus species. In contrast, in a freshwater fuel cell Geothrix species were enriched. The presence of bacteria belonging to the Desulfobulbus/Desulfocapsa group was thought to be related to the ability of this group of bacteria to oxidize elemental sulfur (which precipitates at the anode) to sulfate with a suitable electron acceptor or to disproportionate sulfur to sulfide.

Fig. 3. Schematic representation of a microbial fuel cell to produce electricity from organic compounds (upper mode). Acetate is oxidized to carbon dioxide. The electrons move through the circuit, while protons diffuse via a proton exchange membrane (PEM) to the cathode. The difference in redox potential of the couples O2/H2O and acetate/CO2 is the driving force for electricity production. In a similar fashion (lower mode) a power supply (PS) delivers energy to enable proton reduction with electrons derived from acetate oxidation.
and sulfate (Finster et al., 1998). Desulfuromonas species are known for their ability to reduce sulfur. The occurrence of these types of bacteria suggests that a similar sulfide—sulfur (polysulfide) shuttling system as observed with ferrihydrite is also important in microbial fuel cells.

Future perspectives

There are fundamental differences but also similarities between the above-mentioned types of exocellular electron transfer. They are similar in the sense that the electron donor is physically separated from the electron acceptor; the latter being a microorganism, an insoluble mineral or an electrode. Methanogenic environments are low in redox potential, while iron and manganese reduction as well as electron transfer to electrodes takes place at a higher redox potential as a consequence of chemical and biological sulfide oxidation. So far, only hydrogen and formate were shown to act as electron shuttles in methanogenic environments, while a variety of shutting compounds are used in the reduction of insoluble electron acceptors. The presence of hydrogenases or formate dehydrogenases is not essential in the last type of transfer; bacteria that lack hydrogenases like Propionibacterium are able to transfer electrons to an electrode (Emde and Schink, 1990), while Desulfobulbus which is involved in electron transfer at electrodes has never been shown to grow in syntrophy with methanogens (Samain et al., 1986).

The differences between exocellular electron transfer to a microorganism or to a solid electron acceptor are not as clear-cut as they seem. Soluble shuttle compounds may also be involved in interspecies electron transfer in methanogenic environments. Some natural redox mediators have a midpoint redox potential that is sufficiently low (< −100 mV) to be relevant for methanogenic environments, e.g. riboflavin and phenazines (Abken et al., 1998; Murakami et al., 2001). In some methanogenic environments, proton-reducing acetogenic bacteria and methanogenic archaea have direct physical contact and a direct transfer of electrons without the involvement of hydrogen or formate cannot be excluded. On the other hand, membrane-associated hydrogenases and periplasmic hydrogenases are present in many different anaerobic bacteria. So, a role of hydrogenases in electron transfer to insoluble electron acceptors cannot be excluded either. In fact, hydrogenases are applied in biofuel cells (Karyakin et al., 2002; Lamle et al., 2003; Vincent et al., 2005). An interesting observation with RNA-based stable isotope probing was the identification of Geobacter, as one of the microorganisms involved in propionate oxidation in rice paddy soils that produced methane (Lueders et al., 2004). This may indicate that Geobacter species are able to grow in syntrophic association with methanogens.

In methanogenic environments, acetate can be converted by anaerobic microorganisms to hydrogen and carbon dioxide, but because of thermodynamical reasons this conversion is only possible at a low hydrogen partial pressure maintained by methanogens. Recent research has shown that electricity can be used to enhance hydrogen formation from organic acids. For future electrochemical hydrogen formation, it is important to note that hydrogen formation from organic compounds is energetically much more favourable than hydrogen formation by electrolysis of water. The Gibbs free energy change of bioelectrolysis of acetate is about +26 kJ per mol H₂, while the electrolysis of water requires an energy input of about 237 kJ per mol H₂ (Fig. 3). A better understanding of exocellular electron transfer will help to optimize bioelectrolysis from organic waste compounds for large-scale hydrogen production.

We believe that future research will show the enormous physiological flexibility of specific phylogenetic microbial groups with respect to exocellular electron transfer. It is clear from recent literature that certain physiological properties are not confined to certain phylogenetic groups. Besides reducing sulfate, sulfate-reducing bacteria have a function as proton-reducing acetogens in methanogenic environments. Methanogens form methane, but they are also able to reduce iron. In addition, Geobacter species that we know from the ability to respire with minerals are also able to transfer electrons to other microorganisms. The observation that many phylogenetic groups of anaerobic bacteria are able to use quinones as terminal electron acceptors and that hydroquinones are chemically oxidized with Fe(III), indicates that assignment of a physiological function to a phylogenetic group in iron-reducing environments should be done with caution. Modern molecular biological techniques enable us to shed light on the presence of phylogenetic microbial groups in anaerobic environments in which exocellular electron transfer is a key process. However, to understand their function, scientists need to put efforts in developing novel and innovative strategies to isolate novel microorganisms to make them available for detailed physiological studies. This will improve our understanding of their role in nature. Therefore, we fully agree with the call for balanced approaches in geomicrobial studies as advocated recently by Oremland and colleagues (2005).

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