Underpinnings and prospects for electrical bacteria

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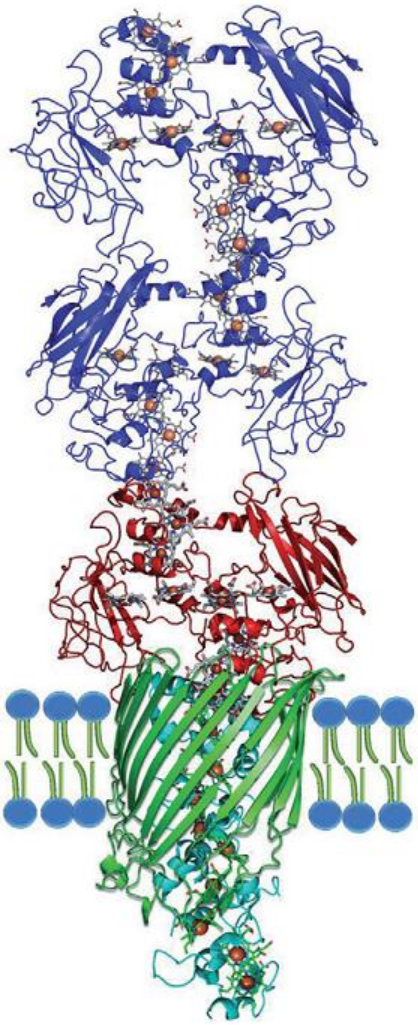

Underpinnings and prospects for electrical bacteria

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On the cover: A possible molecular configuration of a MtrABC complex with the extracellular OmcA attached. Showing MtrA (lilac) MtrB (green) MtrC (red) and OmcA (blue)
Adapted from D.J. Richardson et al., 2012

Abstract

The scarcity and lack of oxygen as a terminal electron acceptor in the seabed and sediments has driven the evolution of specialized anaerobic bacteria. Until recently, it was thought that the natural occurring formation of layers in the sea sediments was connected by diffusion of the redox molecules, causing the reduction of oxygen at the seabed with electrons gained from oxidation of hydrogen sulphide in the deeper sediments. However, the discovery of filamentous bacteria transporting electrons from the sulphidic zone to the seabed by the group of L. P. Nielsen was a major breakthrough in the biogeochemical field. This discovery raises many intriguing questions like can these bacteria be utilized as a living power cord, or as an alternative energy source or improve the existing microbial fuel cells (MFC). Unfortunately our current knowledge is not sufficient and these questions remain unanswered. However, intensive research in the past decade elucidated much of the mechanisms involved in electron conductance and these findings are discussed in this thesis.

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Introduction

Earth's oceans cover over 71 per cent of the planet, however, more than 95 per cent of these underwater regions remain unexplored. The past decades, the seabed has been a topic of intensive research, which resulted in some intriguing findings suggesting that more is happening there than previously thought. The harsh environment on the bottom of the sea drives the evolution of highly specialized microorganisms. One of the major obstacles these organisms have to overcome is finding a suitable electron donor and/or acceptor for their energy production. On land organisms have evolved to use oxygen for this purpose, however, this molecule is limited at the seabed and non-existent in the subsurface. This separation hallmarks the top layer of the seabed, which consists of three different zones with respect to the availability of reductors like oxygen (Figure 1) [1].

The seabed consists of three different zones

In the ocean, oxygen diffuses through the water and into the upper layer of the seabed, creating the first region. This zone is designated the oxic zone and ranges from the sediment surface to approximately 1 mm into the seabed. It is followed by the suboxic (1 mm – 14 mm) and sulphidic (14 mm - 20 mm) zones, both of which are deprived of oxygen [1] [2] [3]. Experimental procedures made it possible to re-create this separation in a laboratory setting; when defaunated sulphidic marine sediment is incubated in the presence of oxygenated seawater, the sulphide gradually disappears from the sediment surface. After some time, the oxic and sulphidic layers become separated by the suboxic zone. This formation of zones is accompanied with some characteristic oxygen, pH and hydrogen sulphide peaks (figure 2). The typical steep oxygen curve shows that this molecule can diffuse approximately 1 mm into the seabed. At this depth the pH also shows a distinct peak of ~8.3 and decreases to a minimum of ~6.3 at the sulphidic zone (figure 2, Oxic). The characteristic peak of the pH indicates a reduction using the protons present at the sediment's surface. However, when defaunated sulphidic marine sediment is incubated

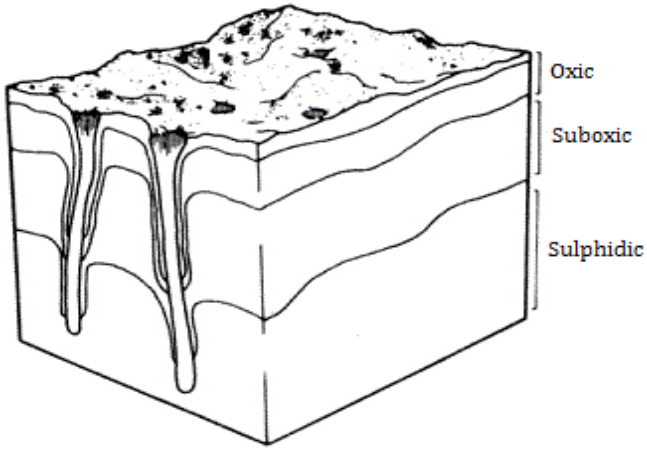

Figure 1 – Zones of the seabed.
The sediment is divided into oxic, suboxic and sulphidic zones. *Adapted from T.M. Fenchel and R.J. Riedl, 1970.*

in the absence of oxygenated seawater, no separation of the zones and their accompanied curves is observed (figure 2 Anoxic). The sulphide present in the sediment remains at the surface and no protons are consumed in any reaction. The reason for this remarkable formation of zones are the microorganisms living on and in the marine sediment. The organisms living in the oxic zone use oxygen as the terminal electron acceptor for their energy production by electrochemically reducing oxygen to water: $O_2 + 4e^- + 4H^+ \rightarrow 2H_2O$. The consumption of protons and oxygen in this zone by these microorganisms is the major cause for the distinct hydrogen sulphide, oxygen and pH peaks observed in marine sediments [3]. The consumption of oxygen also leads to the formation of the anoxic layers beneath. In these zones, the microorganisms depend on anaerobic processes and alternative terminal electron acceptors to fulfil their energy needs. As opportunistic creatures, the bacteria living here utilize the sulphuric nature of the soil and have evolved to oxidize hydrogen sulphide to elemental sulphur: $2HS^- + 2H^+ + O_2 \rightarrow 2S + 2H_2O$ [2]. Creating the hydrogen sulphide graph in which the sum of hydrogen sulphide (ΣH_2S) equals the sum of the concentration of other sulphide molecules ($[H_2S] + [HS^-] + [S^{2-}]$)(figure 2) [3].

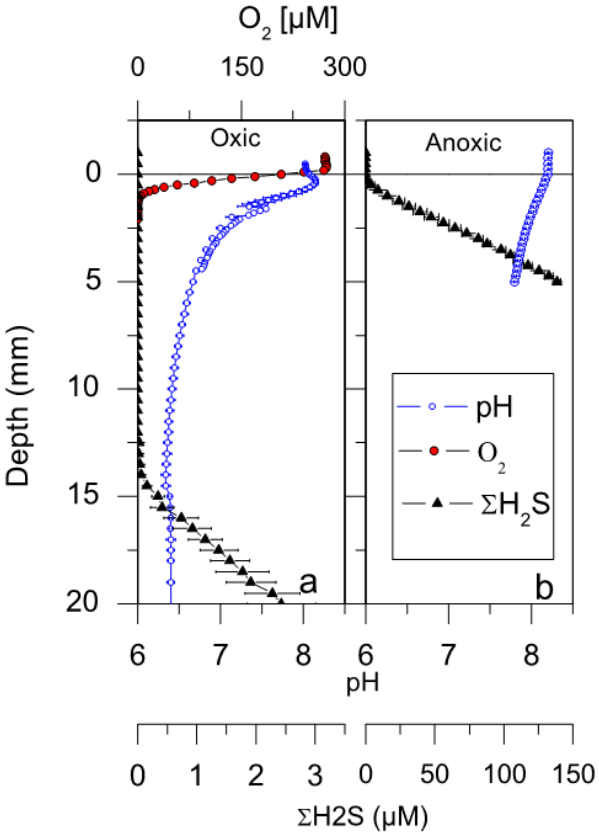

Figure 2 – Hydrogen sulphide, oxygen and pH graphs of marine sediment.
From N. Risgaard-Petersen et al., 2012

Until recently, it was thought that this naturally occurring formation of layers was connected by diffusion of the redox molecules. However, a recent study suggested that the reduction of oxygen at the surface is directly connected to the oxidation of hydrogen sulphide in the sulphidic zone, a distance of more than 15 mm apart, by electrical currents [2]. It has been theorized that a combination of (semi)conductive minerals and specialized bacteria are the driving force of these currents by transferring electrons obtained from the oxidation of hydrogen sulphide to the seabed to reduce oxygen [4]. This conductance of electrons by bacteria might potentially be useful in the near future to provide us with an alternative energy source. However, to utilize these bacteria, the mechanisms by which these organisms conduct electrons must be elucidated and optimized. The question then arises, is it possible to utilize these bacteria or perhaps the underlying mechanisms in the future to provide us

with electricity? To answer this question different electron conduits and their mechanisms will be considered in the following paragraphs.

Interspecies Electron Transfer conducts electron over nanometre distance

In the late '90s, microorganisms were discovered which had evolved molecular mechanisms for discharging electrons to and accepting them from mineral particles surrounding the cells. Characteristic of these bacteria was that they formed intracellular chains of the conductive mineral magnetite in the oxic zone of the seabed [5]. Since then, more bacterial species were discovered that utilize different terminal electron acceptors for their anaerobic respiration, like ferric (oxy)(hydr) oxide minerals [6] [7] and even uranium(VI) [8] [9]. The importance of these bacteria in many biogeochemical processes was made clear by many follow-up studies over the past decades and their ability to overcome the difficulties in anaerobic respiration still surprises biologists. In oxidative phosphorylation, ATP synthesis relies on a proton-motive force, driven by energy from respiratory electron transport and the terminal electron acceptor oxygen. On the seabed and sediments however, oxygen is limited and it is at this place that bacteria have evolved mechanisms to cope with the limited oxygen supply by utilizing other means of generating a proton-motive force. One example is the ability to conduct electrons of anaerobic microorganisms living on and in the sea sediments, of which some are able to exchange electrons between different species of bacteria. These interspecies electron transfers (IET) depend on diffusion of redox chemical species [10] and/or on direct cell-cell contact [11] and/or on naturally occurring minerals in the soil [4], which can be conductive minerals like magnetite or semiconductive like pyrite and hematite [12] [13]. A recent study using a co-culture of *Geobacter sulfurreducens* and *Thiobacillus denitrificans* suggests that the natural minerals in the soil are the driving force behind the electron conductance [4]. Cultured together, acetate oxidation ($CH_3COO^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$) by *G. sulfurreducens* and nitrate reduction ($NO_3^- + 10H^+ + 8e^- \rightarrow NH_4^+ + 3H_2O$) by *T. dentirificans* was only observed in the presence of conducting nanoparticles of magnetite or hematite [4]. This observation suggests an electric connection between these bacteria to facilitate their cooperative catabolism and the dependence of these anaerobic species on each other to fulfil their energy production. The conductive minerals playing a crucial role in these processes, however, are at circumneutral pH (6.5 - 7.5) and without strong complexing ligands, unable to pass the bacterial membranes to the site at which they become reduced. To circumvent this problem these specialized bacteria have developed the ability to transport electrons from the cytoplasmic membrane to the cell surface using outer-membrane proteins,

periplasmic and extracellular cytochromes, often in combination with a network formed by bacterial filaments to conduct the electrons.

Nanowires as conducting filaments to transport electrons over micrometre distance

Besides the conductance of electrons via minerals or direct cell contact, some bacteria have evolved specialized filaments to conduct electrons. The first report about bacterial filaments transporting electrons are of the *Geobacteraceae* genus [7]. The Gram-negative δ-proteobacteria *G. metallireducens* and *G. sulfurreducens* generate energy by using metal ion-mediated electron transport. These bacteria oxidize organic compounds to CO_2 using specialized pilins anchored in the cell's periplasm and outer membrane [14], for electron transfer to extracellular electron acceptors like Fe(III) and Mn(IV)oxides [7], Uranium(VI) [8] [9] and electrodes in biological fuel cells [15]. Although these membrane extensions, commonly called nanowires, are found in a wide diversity of microorganisms, the filaments of the *Geobacteraceae* genus are one of the few proven to conduct electrons to extracellular electron acceptors so far. In other microorganisms, these pilins serve a different function, and are mainly required to establish contact with surfaces [16]. In 2003, the whole genome of *G. sulfurreducens* was sequenced and two genes related to the formation of the type IV pilins, designated *oxpG* and *pilA*, were identified [14] [17]. Functional studies revealed a role for both of these proteins; the gene product of *oxpG* was shown to be involved in protein secretion across the outer membrane [17], whereas *G. sulfurreducens* deletion strains of *pilA* failed to form pilins [7]. Additionally, the deletion strain also could not reduce insoluble electron acceptors. It was, however, capable to reduce soluble acceptors as long as a soluble electron shuttle like anthraquinone-2,6-disulfonate (AQDS) was present. These shuttles transport electrons between the cell's membrane and the surface of the terminal electron acceptor [18]. Furthermore, the growth of the *pilA* deletion strain was impaired compared to wild type cells grown on a solid medium containing only Fe(III)oxides as a terminal electron acceptor. These data suggest that *G. sulfurreducens* does not require the pilins for attaching to oxides, but merely utilizes the nanowires as a network to expand their catalytic surface. The confirmation that the pilins are solely evolved for electron conductance and not for motility was obtained via another deletion model of the *G. sulfurreducens* bacteria. In other microorganisms, the PilT protein is necessarily for a twitching motion and thereby mediates surface motility. Upon deletion of the putative *pilT* gene in *G. sulfurreducens*, no effect on the reduction of Fe(III) oxides was observed compared to wild type cells, additionally the wild type bacteria did not show any twitching motion. These observations all suggest that the *Geobacteraceae* bacteria

do not need the pilins for moving towards oxides, but have evolved specialized pilins for conducting electrons to the oxides. To transfer electrons across the outer membrane to the oxides, bacteria have developed the ability to transport these subatomic particles from the cytoplasmic membrane to the cell surface using outer membrane proteins, periplasmic and extracellular cytochromes, of which the latter are involved in the actual transfer.

C-type cytochromes facilitate the electron discharge in most Fe(III) reducing bacteria

Most Fe(III) reducing bacteria utilize special membrane-bound hemoproteins to facilitate IET or electron conductance via nanowires [7] [19] [20]. These membrane proteins are the c-type cytochromes (c-Cyts). However, not all extracellular electron transfers occur via these proteins as the Fe(III) reducer *Pelobacter carbinolicus* lacks this gene [21]. Cytochromes contain tetrapyrrole aromatic rings which facilitate the binding of a single Fe-ion via the nitrogen atom in every individual ring. Each haem group can conduct one electron via two cysteine residues in the cytochrome "fingerprint" motif CXXCH on the polypeptide chain. This unique sequence makes recognition of cytochrome coding genes relatively straightforward and metal reducing species like *Shewanella* and *Geobacteraceae* are predicted to contain over 40 and 100 of these cytochromes genes per genome respectively [22] [17]. The workings of these c-type cytochromes are intensively studied in the *Geobacteraceae* species and in another metal reducing Gram-negative γ-proteobacterium *Shewanella oneidensis* MR-1, as both complete genomes are sequenced [17] [22]. In the pursuit of elucidating the mechanisms involved in electron conductance, several relevant proteins were identified by deletion and subsequent functional studies. These include four c-type cytochromes in *S. oneidensis*: CymA, MtrA, MtrC, OmcA and a porin-like integral outer membrane protein MtrB, while in *Geobacteraceae* bacteria the c-cytochromes PpcA, OmcB, OmcS, OmcE, OmcZ, OmpB and OxpG were identified to play a role. However, the only proposed electron conductance pathway model existing today comes from the *S. oneidensis* species and will be discussed in the following paragraphs.

The electron conduction pathway of *Shewanella oneidensis* MR-1

The *S. oneidensis* MR-1 Mtr pathway (figure 3) starts with the mobile, lipid-soluble electron shuttle quinone/quinol in the inner membrane. Quinones and molecules containing quinone moieties play an important role in efficient electron transfer in the electron transport chain of every organism. The reduced quinone, quinol, transports

electrons to the inner membrane protein CymA, which oxidizes the quinol back to quinone which can enter the electron transport chain again for electron acceptance. The electron is then by direct contact or an yet to be discovered factor transferred across the periplasmic space and outer membrane to the Fe(III)oxide, with the help of MtrA, MtrB, MtrC and OmcA [23] [24]. Like the *Geobacteraceae* species, *S. oneidensis* can utilize nanowires for this last step [25], but also secretes water-soluble molecules, e.g. extracellular quinones, that facilitate the conductance of electrons from the Mtr pathway to the surface of the electron acceptor [26] [27] [28].

The electron entry point of the Mtr pathway, CymA

The 21 kDa integral cytoplasmic membrane protein A (CymA) is a tetrahaem c-type cytochrome and a member of the NapC/NirT family of quinol dehydrogenases [29]. It is an essential mediator of quinone moiety cycling during anaerobic respiration as it catalyses the oxidation of quinol (QH_2) to quinone (Q) and subsequently transports the two released electrons into the periplasm [30]. The structure of CymA consists of an N-terminal cytoplasmic and transmembrane domain, which catalyses the oxidation of quinol and anchors the protein, which is followed by a large periplasmic domain containing four haem groups and a catalytic site for reduction of DMSO, fumarate and nitrite [31]. Functional studies thus far have not elucidated the mechanism by which quinol binds and gets oxidized in the cytoplasmic domain, however, weak protein interaction with the CymA periplasmic domain suggests that it might be flexible regarding its partners involved in quinol oxidation [32] [33]. Deletion of *cymA* revealed its gene product's crucial role in extracellular electron transport. Mutants demonstrated a strong

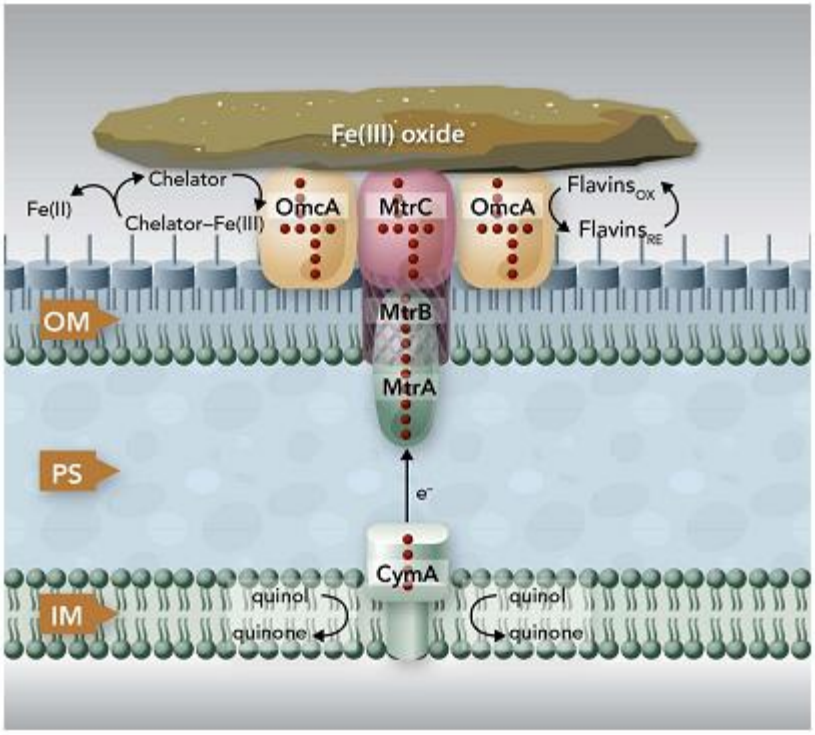

Figure 3 – Proposed model of the Mtr pathway of *Shewanella oneidensis* MR-1 with the current understandings of functional properties of the electron conducting proteins involved.
From: L. Shi et al. 2012

phenotype with respect to extracellular oxidation; these mutant cells were unable to use ferric iron, nitrate, nitrite, fumarate and DMSO as well as an anode as terminal electron acceptors [29] [31] [34]. This deletion emphazises the important role of CymA as donor for periplasmic reductases. Several periplasmic reductases and periplasmic electron-carrying proteins have been proposed to receive these electrons from CymA, however, so far *in vivo* chemical cross-linking failed to demonstrate a physical interaction [32]. In contrast, investigating direct and reverse electron transfer reactions revealed the transfer of electrons from CymA to the periplasmic proteins MtrA, CctA or FccA [32] [33] [35]. Further investigation revealed that upon deletion of the genes encoding the CctA and FccA proteins, the capacity of *S. oneidensis* for Fe(III)oxide reduction was not affected, indicating a minor role for these proteins in the electron conductance process from CymA to MtrA.

MtrA together with MtrB facilitates electron conductance across the outer membrane

The prokaryote's general machinery for inserting and secreting proteins across the cytoplasmic membrane, is a highly conserved heterotrimeric complex consisting of the SecY, -E and -G proteins that works in concert with a set of cytosolic proteins [36] [37] [38]. One monomer of the homodimer 35 kDa decahaem periplasmic metal-reducing protein A (MtrA) contains a signal peptide which is recognized by the SecYEG translocon, this system transports the monomer across the cytoplasmic membrane into the periplasmic space. The MtrA gene is encoded by a polycistronic operon that also contains the other components of the electron conduction pathway MtrB, MtrC and OmcA [39]. Functional analysis of this operon showed that the MtrABC proteins form a complex that is capable of transporting electrons across the lipid bilayer of proteoliposomes [40]. Furthermore, heterologous expression of these proteins in *E. coli* gave the cells the capacity to anaerobically reduce solid phase Fe(III)oxides [41]. *In vivo* and *in vitro* cross-linking studies in pursuit of elucidating the mechanisms of the Mtr pathway identified MtrB and –C as interaction partners of MtrA in a stoichiometry ratio of 1:1:1 [42]. Structural studies of MtrA revealed a flat and elongated protein with the dimensions of 20Å x 50Å x 104Å (LxWxH) [43]. Functionally and sequentially similar proteins have been identified in *E. coli* and *Desulfovibrio vulgaris*, NrfB and NrfH respectively. The molecular structures of these proteins show a similar conformation and placement of their haem groups; to facilitate rapid electron conductance through or along the protein, the distance between the individual haem groups is not more than 6Å [44] [45]. This arrangement of haem groups might also be applicable for MtrA, and would be in line with the proposed role of MtrA to conduct electrons from CymA to

MtrC. However, MtrA alone cannot insert and conduct electrons in the outer membrane. Interaction studies between the outer membrane Mtr pathway components showed that a stable complex is formed between MtrA and -B in the absence of MtrC, however, no MtrAC nor a MtrBC complex has been observed in the absence of MtrB or MtrA respectively [40].

Based on the current data available, it is proposed that MtrB, as a porin-like integral outer membrane protein, forms a β-barrel with the dimensions of >30Å x 40Å (WxH) [43]. Due to this pore size, it is theorized that MtrA is inserted into the outer-membrane by entering and interacting with the pore to create a conduit for the electron transport from CymA to MtrC [43]. As the typical Gram-negative outer membrane thickness is approximately 70Å [46], MtrA, with its height of 104Å, is long enough to span the distance of this bilayer and would create a conduit from the periplasm to the cell's surface. However, until today studies have failed to explain the electron transfer mechanism from the integral cytoplasmic membrane CymA to the outer membrane protein MtrA. Due to the distance between the two lipid bilayers of 235Å ± 37Å [47] a direct connection cannot be possible and diffusion of MtrA through the periplasmic space and the MtrB pore or other periplasmic factors seem to be necessary to close the circuit. Whether one of these mechanisms or a combination is involved remains to be elucidated.

MtrC and OmcA facilitate electron discharge to the terminal electron acceptor

Following the oxidation of MtrA by the electrons acquired from CymA directly or indirectly, MtrA in turn oxidizes MtrC at the cell's surface by transferring the electrons to it. MtrC was first assigned to the Mtr pathway after the discovery of the Mtr operon [39] and subsequent isolation and analysis showed that it forms a complex with MtrAB but also coprecipitates with a stoichiometry ratio of 1:2 with OmcA [48]. Increasing evidence emerges that the extracellular decahaem cytochromes of the MtrC family, to which the *S. oneidensis* MR-1 homologues OmcA and MtrF also belong, are crucial for direct contact with or function as electron shuttles to the terminal electron acceptor [49]. As MtrF shares 30% identity and 46% similarity with MtrC plus the non-haem and penta-heam domains are conserved, MtrF is suggested to serve as the prototypical structure for the MtrC family [50]. MtrF as a homologue of MtrC, associates with a MtrDE porin-cytochrome complex, homologous to the MtrAB complex. This MtrDEF holoenzyme is suggested to be functionally similar to the MtrABC complex, and therefore functions as a prototypical complex for elucidating the electron-conducting pathways. Until recently the only structural data available about the MtrC family came

from spectro-potentiometrical characterizations and showed that each member had 10 haem groups, however, new insights into the structural conformations came from a X-ray crystallography study about the MtrC homologue MtrF. Its structure was determined with a resolution of 3.2Å and showed a unique "wire-cross" conformation [50] (figure 4) and provides compelling evidence supporting the terminal reductase role of this cytochrome in Fe(III)oxide reduction. The structure of MtrF comprises four distinct domains; domain I and III consist of seven anti-parallel β-strands folded together to form split-β-barrel structure, while five tightly packed haems are covalently bound to the domains II and IV. These four domains fold together in such manner that the overall haem co-ordination of the 10 haem groups resemble a structure referred to as a "wire-cross" [51]. A staggered 65Å octahaem chain consisting of haems 10, 9, 8, 6, 1, 3, 4 and 5 transects the length of the protein through domains IV and II and is crossed at the middle by a 45Å tetrahaem chain consisting of haems 2, 1, 6 and 7 which connects the two split β-barrel domains I and III. Due to a maximum distance of 7Å between individual haem groups, rapid electron conductance through or along the protein can be achieved. It is proposed that domain I and III are involved in binding and reduction of extracellular electron shuttles e.g. secreted quinones and flavins, and

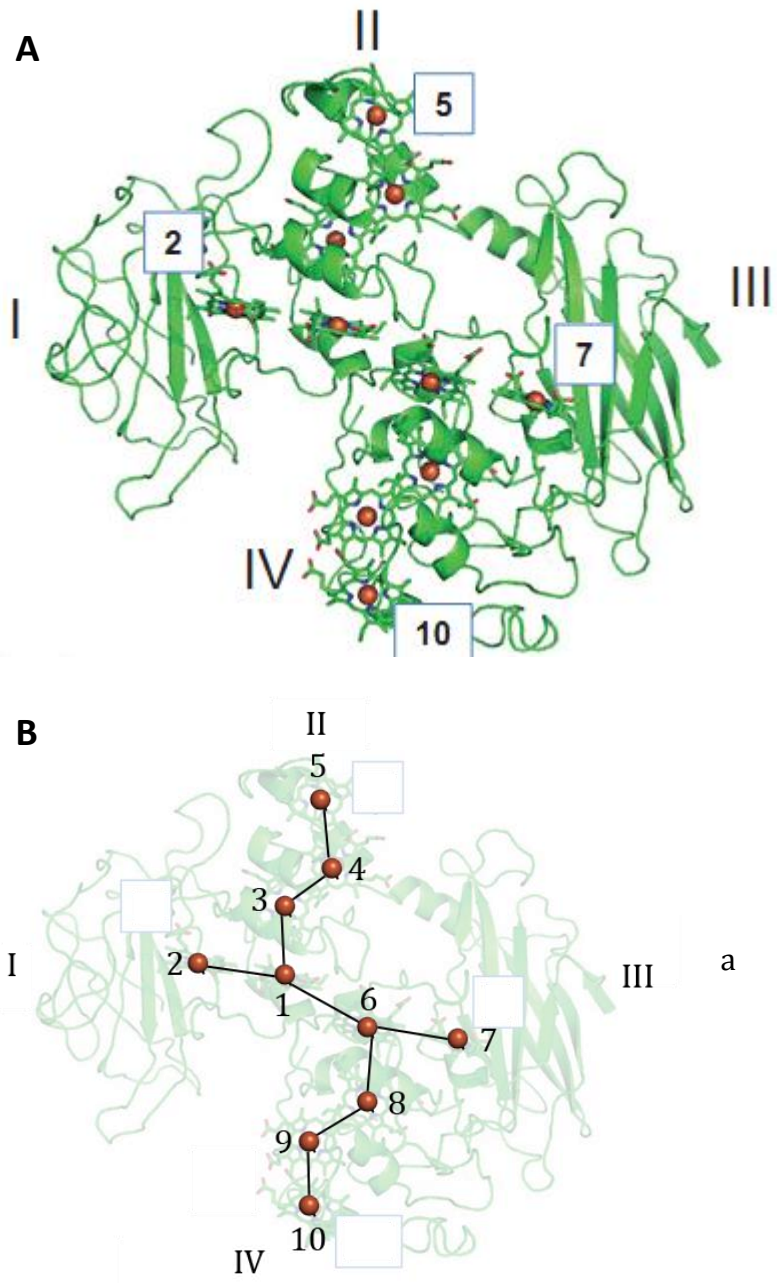

Figure 4 – Molecular MtrF structure from *S. oneidensis* MR-1 (a) The crystal structure of *S. oneidensis* MtrF showing the peptide chain (green), haem groups (blue) and iron atoms (orange). (b) Arrangement of the MtrF haem groups numbered through iron atom positions according to the unique CXXCH peptide motif *Adapted from Clarke et al., 2011*

soluble metals like chelated Fe(III). Interaction and reduction of solid-phase Fe(III) is suggested to be facilitated by domain II, while domain IV is predicited to physically interact and exchange electrons with the MtrDE complex [50]. Due to the homology of MtrF with MtrA and OmcA and the overlapping functions in Fe(III)oxide reductions, the MtrA and OmcA proteins might fold in a similar "wire-cross" structure, facilitating electron discharge to the terminal electron acceptor and ensuring ATP synthesis by generating a proton-motive force in the absence of oxygen. Although extensive research has been carried out and major breakthroughs have been made throughout the past decades, researchers were always sceptic about electron conductance over centimetre distances, coupling the oxidation of sulphidic compounds in the sea sediments to oxygen reduction at the seabed. However, a recent discovery of a new kind of electron-conducting bacteria stunned all of science.

Desulfobulbaceae: a new genus of filamentous bacteria transporting electrons over centimetre distances

The on-going debate about biogeochemical reactions and zone formations in the top layer of the seabed is still a driving force to investigate these topics. Until recently, it was thought that the formation of these zones was caused by the transport of electrons across the suboxic zone by electron discharge to extracellular terminal electron acceptors from anaerobic bacteria, working in concert with diffusion of (semi)conductive minerals, yet experimental procedures have failed to confirm this so far. However, a recent breakthrough has been made in the biogeochemical field as the group of L. P. Nielsen discovered filamentous bacteria which transport electrons from the sulphidic to the oxic zone in a way never observed before (figure 5) [52].

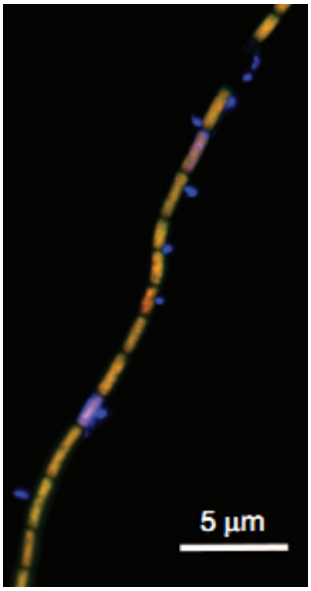

Figure 5 – FISH 16S rRNA identification of the bacterial filament.
From Pfeffer et al., 2012

The filament length of the recently discovered filamentous bacteria of the *Desulfobulbaceae* genus have reported to be up to 15 mm long, spanning the entire suboxic layer and providing a direct connection between the sulphidic and oxic zones [52]. The first compelling evidence that this genus of bacteria is involved in the connection between the reduction at the seabed and oxidation of hydrogen sulphide in the sediment, came from placing different pore size filters at 3 mm beneath the seabed to permit or exclude migration of bacterial cells. While the filters with a pore size of 2.0 µm showed the formation of the different layers accompanied with the characteristic pH peak and oxygen consumption

(figure 2), the effect was not observed anymore with smaller pore size filters, indicating an involvement of microorganisms because potential redox molecules could diffuse freely. Additional evidence came from passing a 50 µm diameter tungsten wire horizontally through the sediment approximately 2 mm below the oxic-anoxic interface. This would disrupt any structure formed in the sediment, but would still allow diffusion of molecules. After examining the sediments, the distinct pH peak and oxygen consumption was not observed anymore, indicating a disruption of the electron flow. Redox molecules were not the cause of the observed peaks for pH and oxygen as sediments containing non-conducting glass microspheres showed the formation of zones, which might only be explained by the filamentous *Desulfobulbaceae* bacteria observed in these sediments.

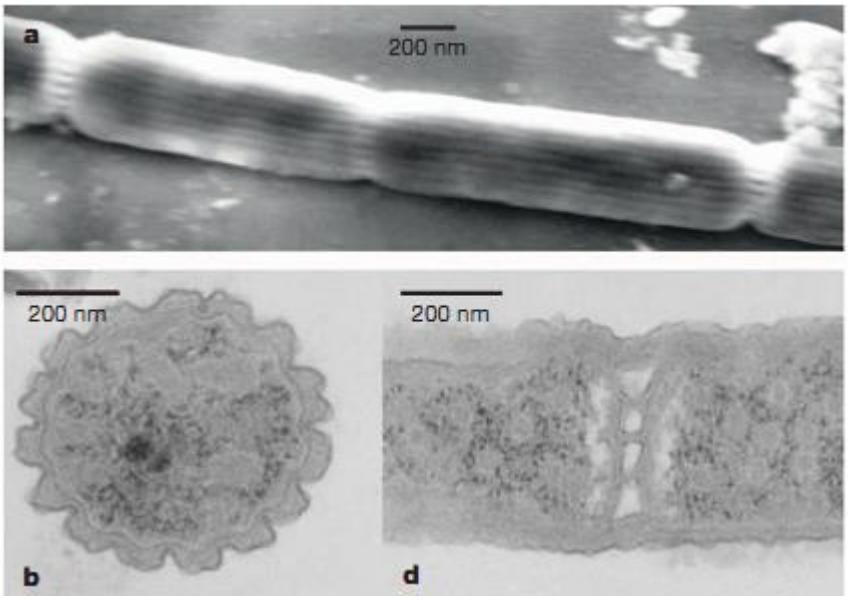

Figure 6 - Electron microscopy pictures of the *Desulfobulbaceae* filaments (a) SEM image of *Desulfobulbaceae* filament showing bacteria encased in the outer membrane. (b) TEM image of a filament cross section clearly showing ridges and grooves. (c) TEM image of a filament longitudinal section showing two individual bacteria, the gap between them and the tubular channel junction. *Adapted from Pfeffer et al., 2012*

The Gram-negative *Desulfobulbaceae* family contains both morphologically and functionally diverse members, previously shown to generate and consume hydrogen sulphide and capable of using extracellular terminal electron acceptors for their energy production [53] [54] [55]. Interestingly, the filaments discovered by L. P. Nielsen and colleagues, showed to be yet again an unknown member with an unique structure. The multicellular filaments contained a collectively shared outer membrane with uniform ridges along the entire length (figure 6a). Cells encased in the outer membrane are separated by 200 nm wide gaps and can exists in two types with respect to their diameter and ridges; filaments either 400 nm in diameter containing 15 ridges, or 700 nm in diameter with 17 ridges have been identified by electron microscopy. The ridges fulfil a special role as each of them contained a highly charged periplasm-filled 70-100 nm wide tubular channel, which runs between the cytoplasmic and outer membrane alongside the entire length of the filament and continues as junctions between neighbouring cells (figure 6d). This remarkable feature might be the missing electron conductance link between the different zones in the sea sediments. It is proposed that

cells on the sulphidic side of the filament oxidize hydrogen sulphide to gain the electrons for the oxygen reduction by the cells of the oxigenic side of the filament. The charge transport by ions in the environment surrounding the bacteria might counterbalance the electron transport and close the electric circuit to retain charge balance [2] [3] [52]. Unfortunately, since these bacteria are only just discovered, mechanisms by which these cells reduce hydrogen sulphide, conduct electrons gained from this process and oxidize oxygen remains to be elucidated. It is, however, not uncommon in bacteria to form long filaments, as cyanobacteria also form multicellular structures with one collective outer membrane. It is suggested that these microorganisms utilize the joint periplasm to ease the exchange of nutrients between cells without leakage and other influences from the environment [56] [57]. Perhaps to some extent this applies also to the filaments formed by the *Desulfobulbaceae* bacteria. Current-voltage measurements of the outer membrane showed that it does not conduct electrons, but insulates the exterior from the interior, which might indicate that these bacteria utilize the joint periplasm in the channels to ease and optimize conductance of electrons, enabling them to monopolise major energy sources in a harsh environment. Additionally, our current understanding of the reduction and oxidation processes and the wide-spread c-type cytochrome involvement in these, might also be applicable to the *Desulfobulbaceae* bacteria, however, further research is necessary to elucidate the molecular mechanisms involved in the reduction, oxidation and electron conductance processes.

Prospects for the use of electrical bacteria

One of the interesting aspects of the discovery of the *Desulfobulbaceae* filaments, the electron conductance over centimetre distances, raises many intriguing questions like can this bacterium be utilized as a living power cord, or as an alternative energy source or to improve the existing microbial fuel cells (MFC). It is believed that besides the traditional renewable energy sources e.g. solar, wind, geothermal and hydro power, the use of MFC might become an important source of bioenergy. One of the strong points is that these fuel cells have the capability of producing electrons from a wide range of complex organic by-products of waste fermentation [58] [59] [60] [61] and microbial sources [62]. However, up until today, all existing laboratory prototypes show large variations in power production and sustainability, which prevents their wide-spread utilization as an alternative energy source. Today's only practical utilization of these microbial fuel cells are those on the seabed, where electrons from hydrogen sulphide reduction are used to create a current between two grids to power offshore electronic monitoring devices like a meteorological buoy [63]. For a MFC to work, the

microorganisms extracting the electrons from the substrate source need to interact with an electrode for electron discharge. Several mechanisms have been proposed for this interaction with the anode of fuel cells (figure 7) based on the intensively studied ferric iron reducers *G. sulfurreducens* and *S. oneidensis*. The genome sequence of both species revealed abundant c-type cytochrome genes [17] [22], of which the gene products have been shown to reduce a diverse range of terminal electron acceptors, like an anode, via direct contact (figure 7a). The transfer of electrons from the cells to the anode creates a current, which can be used to power electronic devices.

Spectroelectrochemical studies have shown that *G. sulfurreducens* indeed interacts in this manner with an anode [64], however, cultures of these bacteria are also known to form over 50 µm thick biofilms on anodes, in which each cell contributes to the current derived from the discharge of electrons [65]. It is proposed that the electron conducting nanowires produced by this species facilitates the transport to the anode upon discharge (figure 7b). The biofilm itself formed by *G. sulfurreducens* might be conductive as preliminary data shows (N. Malvankar, unpublished data). Therefore it might be possible that this conductive matrix is also needed for the transport of electrons to the anode, and that cytochromes may be recruited as electron shuttles to facilitate the conductance (figure 7c). The usage of biofilms might expand the capacity of MFCs as the current density increases with the number of cells (in)directly attached to the anode. However, further research is necessary to investigate the possible beneficial properties of enhancing biofilm formation onto the anode. The last proposed mechanism by which electrons are transferred from the cells to the anode is the use of soluble electron shuttles secreted by the *S. oneidensis* bacteria (figure 7d). Studies have indicated that *G. sulfurreducens* and *S. oneidensis* differ

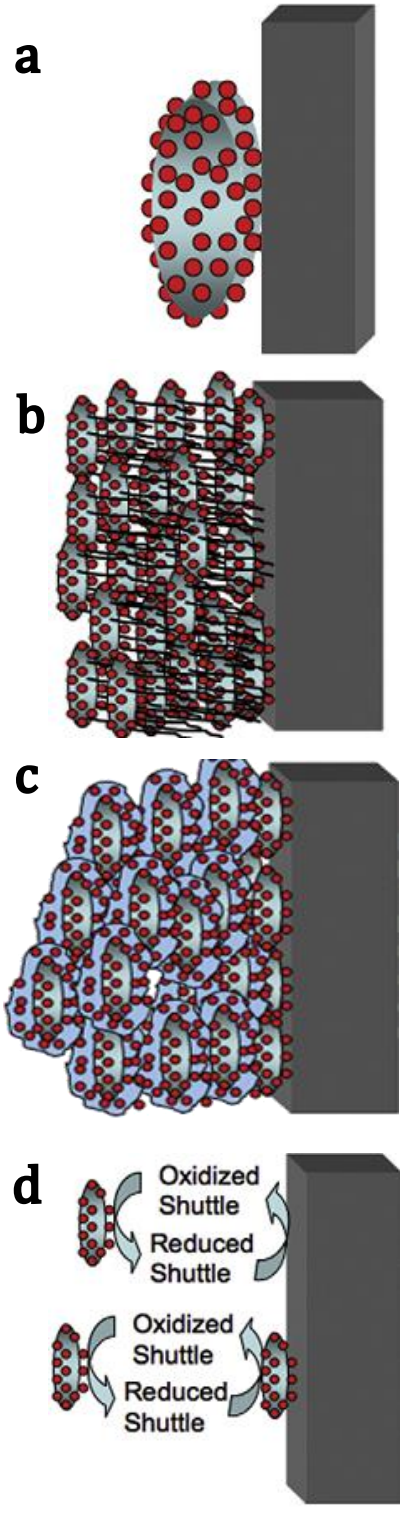

Figure 7 - Mechanisms by which ferric iron reducers might facilitate electron discharge to the anode. (a) Direct contact of the cell with the anode. (b) *G. sulfurreducens* biofilm formation on an anode in which nanowires facilitate the discharge. (c) *G. sulfurreducens* conductive biofilm in which the electron shuttles facilitate the discharge. (d) Discharge of electron by electron shuttles secreted by *S. oneidensis*
adapted from D.R. Lovley 2008

in the extracellular electron conducting mechanisms to anodes [66]. In contrast to *G. sulfurreducens* which depends on nanowires for long-distance electron transfer to anodes, *S. oneidensis* cells have evolved the ability to utilize excreted quinones and riboflavins that mediate the electron conductance over similar distances [27] [67].

Elucidating the mechanisms by which the newly discovered *Desulfobulbaceae* filaments interact with their terminal electron acceptor, could give new insights into the use of these electrical wires for the conductance of electrons or utilization for an alternative energy source. It is suggested that these filaments are the electrical coupling between the oxidation of hydrogen sulphide and reduction of oxygen in the sea sediments by conduction of the electrons gained from oxidation. The non-conductive collectively shared outer membrane spans over 15 mm isolating the individual cells from outside influences and optimizing nutrient exchange in a hostile environment. It might be possible after further research to utilize these bacteria in a new type of microbial fuel cell in which the cells are highly organized attached to the anode as a terminal electron acceptor (figure 8). However, the current understanding of the generation, conductance and discharge of electron to electrodes for generating a current, is not sufficient to engineer such bacteria [68]. The complexity of these fuel cells and the molecular mechanisms involved requires more intensive research to make this type of energy source suitable for powering vehicles, electronic devices or households. Nevertheless, the utilization of microorganisms to generate electricity from waste waters or other organic sources remains fascinating with the ultimate goal of creating a new renewable energy source.

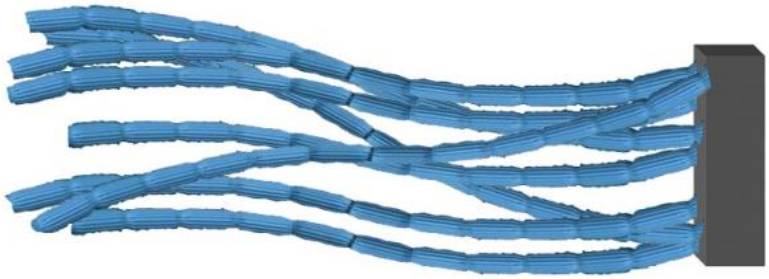

Figure 8 - Possible layout of a microbial fuel cell utilizing *Desulfobulbaceae* filaments for generating a current.

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