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# Filamentous bacteria transport electrons over centimetre distances

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**Oxygen consumption in marine sediments is often coupled to the oxidation of sulphide generated by degradation of organic matter in deeper, oxygen-free layers. Geochemical observations have shown that this coupling can be mediated by electric currents carried by unidentified electron transporters across centimetre-wide zones. Here we present evidence that the native conductors are long, filamentous bacteria. They abounded in sediment zones with electric currents and along their length they contained strings with distinct properties in accordance with a function as electron transporters. Living, electrical cables add a new dimension to the understanding of interactions in nature and may find use in technology development.**

Marine sediments become anoxic because oxygen is consumed by microbial processes at the surface. Without available oxygen the microorganisms living below the surface are supposed to depend on energetically less favourable, anaerobic processes[1]. Recently, however, electric currents have been found to directly connect oxygen reduction at the surface with sulphide oxidation in the subsurface[2], even when oxygen and sulphide are separated by more than 1 cm. Half of the sediment oxygen consumption can be driven by electrons transported from below[2,3]. The spatial separation of oxidation and reduction processes invokes steep pH gradients leading to distinct dissolutions and precipitations of minerals[3]. Microbial activity apparently drives the electrochemical half-reactions and the establishment of electron-conducting structures through the sediment[2]. Bacterial nanowires, humus particles and semi-conductive mineral grains are known to conduct electrons over nanometre to micrometre distances, and alone or in combination they have been proposed to facilitate electron transport over centimetre distances in marine sediment[2,4,5]. Experimental tests and observations have failed to confirm these proposals so far, and instead we have unexpectedly found long, filamentous bacteria structured like electric cables as reported below.

## Filamentous Desulfobulbaceae in electric sediment

When sulphidic defaunated marine sediment was incubated in the dark with overlying oxic sea water, the porewater chemistry gradually developed in accordance with the establishment of an electron transport mechanism that coupled oxygen reduction at the sediment surface to sulphide oxidation in deeper anoxic layers (Fig. 1a). As described previously[2,3], the electric coupling of spatially segregated processes was evident from (1) the presence of a distinct pH peak demonstrating aerobic proton consumption—an indicator of electrochemical oxygen reduction[2,3]—and (2) the formation of a 12–15-mm deep suboxic zone separating sulphide oxidation from the associated oxygen reduction[2,3]. When sediment from the top 20 mm was gently washed, tufts of entangled filamentous bacteria appeared (Fig. 1b). Reverse transcription, cloning and sequencing of 16S ribosomal RNA from dissected filaments identified them as novel members of the deltaproteobacterial family Desulfobulbaceae with $<92\%$ sequence identity to any cultured member of this family (Fig. 1d). Almost identical ($>99\%$) 16S rRNA sequences were detected in the suboxic zones of three replicate cores (Fig. 1d), whereas they were absent in the subjacent sulphidic zones. The filaments were identified and quantified in the sediments by fluorescence *in situ* hybridization (FISH) with specific 16S rRNA-targeting probes (Fig. 1c). The length density of filaments was at least 117 m $cm^{-3}$ in the oxic and suboxic sediment zone and no filaments were detected in the deeper sulphidic zone. With an average cell length of 3 μm, this length density corresponded to $4 \times 10^7$ cells $cm^{-3}$. Individual filaments were difficult to dissect out without breaking them, however, fragments up to 1.5 cm long were recorded, supporting that the bacteria had the length to span the entire suboxic zone.

## Desulfobulbaceae filaments qualify as conductors

We demonstrated experimentally that electron transport through the suboxic zone was mediated by a coherent structure like a bacterial filament and not by diffusive electron shuttles or casual contact between conductive elements. Evidence for solid conductors was found by passing a very thin tungsten wire (50 μm diameter) horizontally through sediment 1–2 mm below the oxic–anoxic interface, thus transiently interrupting the sediment continuum (see Supplementary Information for details). The cut resulted in an immediate and lasting halt of the electron transport, as indicated by a significant drop in oxygen consumption (Tukey's test under analysis of variance (ANOVA); $P < 0.001$; alpha $= 0.05$; $n = 27$), attenuation of the pH peak in the oxic zone and contraction of the suboxic zone (Fig. 2), the latter being similar to the response to momentary oxygen removal observed in a previous study[2]. The role of bacteria in establishing a centimetre-long electron transport mechanism was further investigated in incubations, where filters with defined pore sizes were inserted into sediment cores to selectively exclude or permit vertical growth or migration of bacteria. Manifestations of long-distance electron transport appeared in cores containing filter barriers with pore sizes of 2.0 μm (Fig. 3), but did not appear in cores containing filter barriers with pore sizes $\leq 0.8$ μm as indicated from significantly lower

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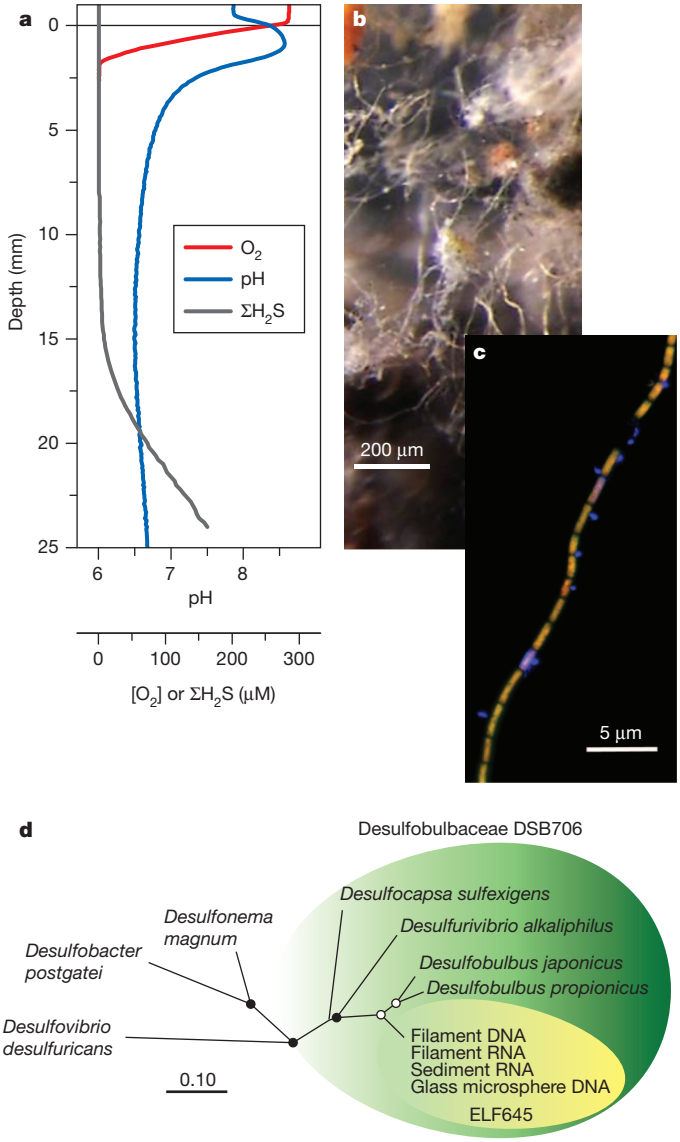


**Figure 1 | Filamentous Desulfobulbaceae in current-producing sediments.** **a**, Microprofiles of $O_2$, pH and $\Sigma H_2S$ ($\Sigma H_2S = ([H_2S] + [HS^-] + [S^{2-}])$. **b**, Tuft of filamentous Desulfobulbaceae collected from the sulphide-free zone. **c**, Filamentous Desulfobulbaceae identified by fluorescence *in situ* hybridization targeting 16S rRNA. Filament cells appear yellow from overlay of images obtained with probe DSB706 (labelled green) and probe ELF654 (labelled red); other cells appear blue from DNA-staining with 4′,6-diamidino-2-phenylindole (DAPI). **d**, Phylogenetic affiliation (by maximum likelihood) of the filaments based on 16S rRNA sequences. Scale bar, 10% estimated sequence divergence; filled and open circles show bootstrap support >80% and >60%, respectively (by maximum parsimony; 1,000 iterations); the specificity of the probes used for FISH is indicated by the green and yellow shading. Accession numbers are given in Supplementary Table 1.

oxygen consumption rates (Tukey's test under ANOVA; $P < 0.001$; alpha = 0.05; $n = 18$) and absence of pH peaks in the oxic zone of these cores. This observation confirms that passage of bacteria-sized objects and not only dissolved or colloidal compounds were required. Sediment particles were not essential as mediators for the electron transport either, because manifestations of long-distance electron transport also developed in incubations where a 5-mm sediment layer had been replaced by non-conductive glass microspheres (Fig. 4). The filamentous Desulfobulbaceae were abundant in the glass microsphere layer as confirmed by FISH and 16S rRNA gene sequencing (Figs 1c and 4b), and no other connecting structures were observed by light and scanning electron microscopy (SEM) inspection.

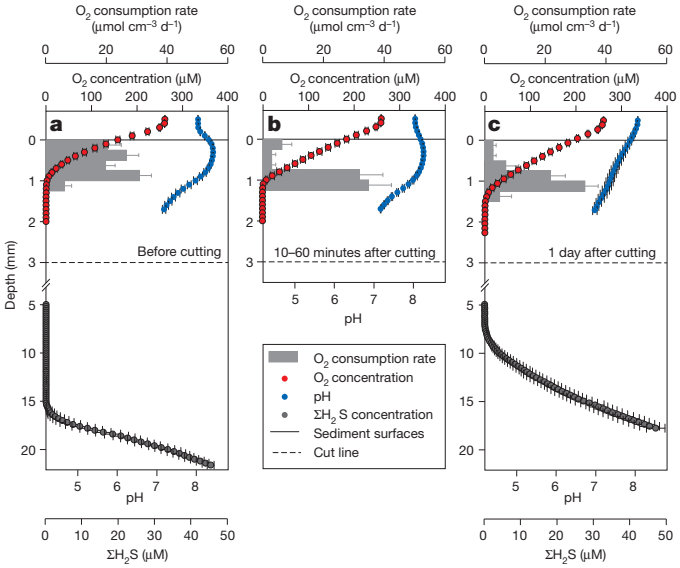


**Figure 2 | Biogeochemical impacts of filament cutting.** **a**–**c**, Microprofiles of oxygen, sulphide and pH measured in undisturbed sediment cores (**a**), 10–60 min (**b**) and 1 day (**c**) after passing a thin tungsten wire (50 μm diameter) horizontally through the sediment near the oxic–anoxic interface. Data are presented as mean values ± s.e.m. (oxygen and pH, $n = 9$; sulphide, $n = 6$). Volume-specific rates of oxygen consumption rates (grey areas) are calculated from numerical modelling of the measured oxygen concentration profiles.

## Structure of Desulfobulbaceae filaments

The multicellular Desulfobulbaceae filaments showed a unique structure with uniform ridges running along their entire length (Fig. 5a). Transmission electron microscopy of thin sections showed two types of filaments that either had 15 ridges and were about 400 nm wide or had 17 ridges and were about 700 nm wide (Fig. 5b, c). Each ridge contained a filled, 70–100 nm wide channel between the cytoplasmic and the outer membrane. The adjacent cells within the filaments were separated by 200 nm wide gaps bridged by the ridge filling and tightly wrapped up by the outer membrane, which seemed to encase the entire filament (Fig. 5d, e). A similar collective outer membrane of the filamentous multicellular cyanobacterium *Anabaena* sp. has been suggested to ease exchange of nutrients between cells without leakage to the surroundings[6]. It is possible that the outer membrane of the Desulfobulbaceae filaments has a similar function with respect to electrons. On the basis of all the morphological data, we propose that

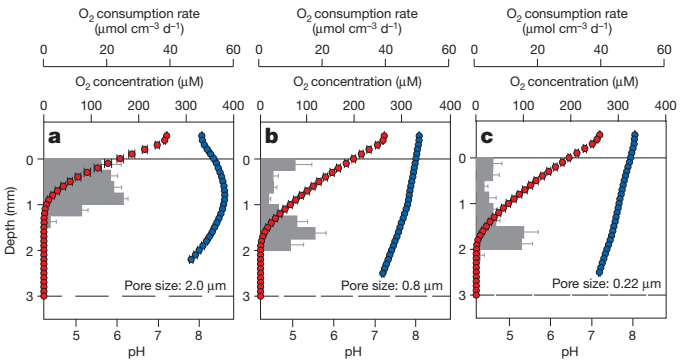


**Figure 3 | Biogeochemical effects of filter pore size.** **a**–**c**, Depth distributions of oxygen concentrations (red circles) and pH (blue circles) measured after 20 days of incubation in sediment cores containing polycarbonate filters with different pore sizes: 2.0 μm (**a**), 0.8 μm (**b**) and 0.22 μm (**c**). The filter position is indicated by the dashed line. Volume-specific rates of oxygen consumption (grey bars) were estimated from numerical modelling of the oxygen concentration profiles. Data are presented as mean values ± s.e.m. ($n = 6$).

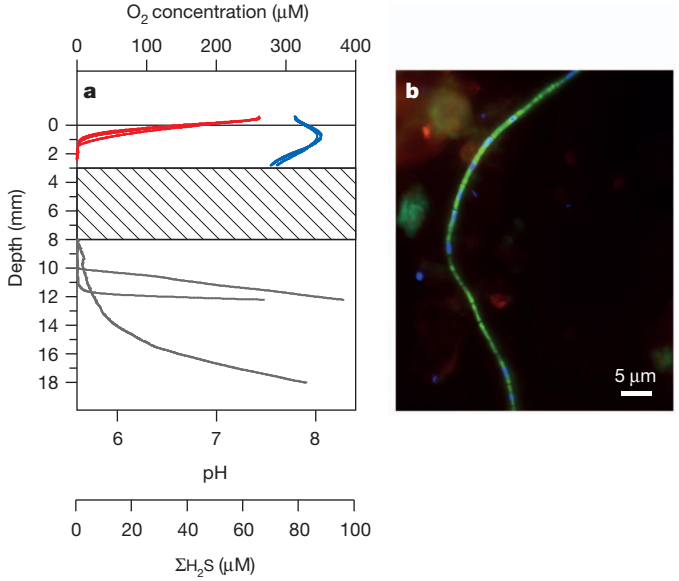


**Figure 4 | Effect of layer of glass beads intercalated in the sediment.** **a**, Oxygen concentrations (red lines), pH (blue lines) and sulphide concentrations (black lines) in three sediment cores with an intercalated layer of electrically inert glass microspheres reaching from 3 mm down to 8 mm sediment depth (hatched area). **b**, Micrograph showing filamentous Desulfobulbaceae extracted from a glass bead section and hybridized with the specific ELF654 FISH probe. DAPI-stained chromosomes are visible in some cells of the filament.

the ridges are strings transporting electrons along the filament inside a continuous periplasmic space, with the collective outer membrane serving as electrical insulation from the external medium. To explore the conductive properties of the strings, electrostatic force microscopy (EFM)[7] was applied, as this technique does not require direct contact and may therefore trace electric properties of insulated materials. A distinct elevation of electrostatic force on the ridges as compared to the intermittent areas (Fig. 5g) indicates that the underlying strings possess significant polarizability or charge storage capacity along their length as well as across cell–cell junctions. The highly organized contrast pattern excludes significant contribution from random leakage of polymers like extracellular DNA. These results are consistent with the proposed charge distribution role. Furthermore, we tested the hypothesis using a nanofabrication approach, similar to that previously used to demonstrate charge transfer along the length of a bacterial nanowire[8]. Current–voltage measurements were performed on individual filaments that were bridging two gold electrodes and fixed with platinum leads. In contrast to what should be observed if the conductive structures were exposed to the surface, sweeping the voltage from −10 V to 10 V was not accompanied by any measurable electrical currents. This observation supports the hypothesis that conductive structures present in the periplasmic continuum are electrically insulated by the collective outer membrane.

## Discussion

Taken together, the data presented here strongly indicate that long-distance electron transport from sulphide to oxygen in the sediment is mediated by living micro-cables in the form of long filamentous bacteria of the Desulfobulbaceae family. As the simplest metabolic model consistent with our data, we propose that the micro-cables are multicellular, aerobic, sulphide oxidizers where electrons generated by sulphide oxidation in cells at one end can be passed through internal, insulated wires to cells at the other end and here are consumed by oxygen reduction. Charge transport by ions in the surrounding environment balances the internal electron transport, thereby completing the electric circuit and retaining charge balance[2,3].

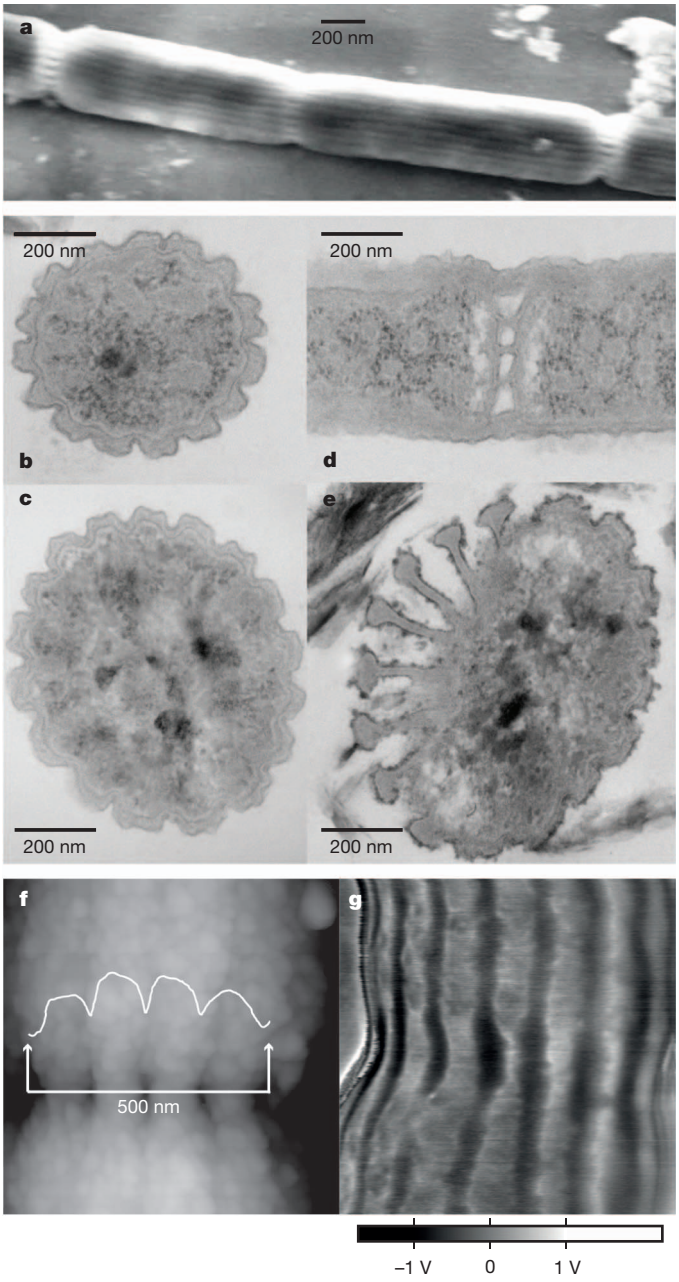


**Figure 5 | AFM, SEM, TEM and EFM micrographs of the filamentous Desulfobulbaceae.** **a**, SEM image of four cells. **b**, **c**, Thin-section TEM images of filament cross sections. **d**, **e**, Longitudinal section including a cell–cell junction (**d**) and oblique section of cell–cell junction (**e**). **f**, Height AFM image of cell–cell junction with inserted topographic curve along 500 nm line with the same scaling of *x*, *y* and *z* axes. **g**, 1 × 1 μm EFM image above cell–cell junction with lighter contrast closely following the ridge topography mapped in the preceding AFM scan (not shown).

Many prokaryotes are known to perform extracellular electron exchange as a method to control metabolic interactions with external solids or cells. Electrons are exchanged with insoluble electron acceptors and donors like iron and manganese minerals[9] or electrodes[10]. Furthermore, syntrophic interspecies electron exchange via nanowires and magnetite grains has recently been confirmed[4,11]. External deposition of electrons preventing internal accumulation of insoluble and potentially harmful metabolites has also been found[12]. However, different from these prokaryotes using external electron transport, the competitive advantage of micro-cables seems to be their ability to separate soluble electron acceptors and donors in space, thereby enabling them to monopolise major energy sources. This is done by transport of

electrons from donors to acceptors over long distances via insulated internal wires with no need for electron transfer across the outer membrane. Oxidation of sulphide is a major driver of oxygen consumption in marine sediments, and the question arises how successful the filamentous bacteria are in competing with other sulphide oxidizing bacteria and in controlling the process. Separation of oxygen and sulphide is common in marine sediment and conventionally explained by iron and manganese biogeochemistry driven by particle mixing or by bacterial nitrate transport[13–16]. Few studies have however directly quantified these pathways.

Bacterial micro-cables represent a hitherto unknown lifestyle, which immediately raises many intriguing questions for further research: How are energy conservation and growth allocated among the cells? What is their genetic and metabolic diversity? How are filament division and dispersal controlled? What is the molecular and electronic basis of the electron transport? How widespread are they in nature? Transmission improvement and control of electric currents have been major drivers for electronic innovation. It appears that biological evolution has worked successfully in the same direction.

## METHODS SUMMARY

Microbiological and geochemical data were obtained from sulphidic sediment sampled in Aarhus Bay, Denmark. The sediment was sieved, transferred to glass core liners and incubated for three to four weeks in aquaria with circulating air-saturated sea water as described previously[2]. Oxygen, sulphide and pH microprofiles were measured as described previously[17–19].

Nucleic acids were extracted from sediment, glass microspheres and from single filaments collected by micromanipulation. RNA was reverse-transcribed, and 16S rRNA complementary DNA fragments were PCR-amplified, cloned and sequenced. Oligonucleotide probes for FISH were designed specific for the retrieved sequences and used for microscopic identification and quantification of the filamentous Desulfobulbaceae by FISH in fixed sediment samples.

AFM images of rinsed air-dried cells were obtained on a Nanowizard II (JPK Instruments) in intermittent contact mode under ambient conditions with an OMCL-AC160TN cantilever (Olympus).

EFM images of rinsed air-dried cells were performed on a Bruker Dimension 3100 (Bruker Corporation) equipped with a Pt/Ir-coated tip (SCMPIC, Veeco Metrology) using the two-pass scan technique[20]. On the second pass, the probe was lifted at 5 nm with respect to the sample topography, while an oscillating bias potential $V = V_{dc} + V_{ac}\sin(\omega t)$ was applied between the tip and the substrate ($V_{dc}$, direct current voltage; $V_{ac}$, alternate current voltage; $\omega$, modulation frequency (500 Hz)). The electrostatic force can be represented by volts.

SEM of air dried cells were obtained on a NanoSEM (FEI, Nova 600 NanoSEM) operated in low-vacuum (60 Pa) and low-voltage (3 kV) mode to apply the charge contrast imaging data.

TEM of 40–60 nm cell sections were obtained on an FEI CM100 (FEI CM100). Electric conductivity of the cell surface was addressed with nano-fabricated electrodes.

A full description of the methodology applied in the present study is given in the Supplementary Information.

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**Supplementary Information** is available in the online version of the paper.

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**Author Information** All sequences are deposited in GenBank/EMBL/DDBJ under accession numbers JX091023–JX091073. Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to N.R.-P. (nils.risgaard-petersen@biology.au.dk) or L.P.N. (biolpn@biology.au.dk).