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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 4×10^7 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 ≤ 0.8 μm as indicated from significantly lower
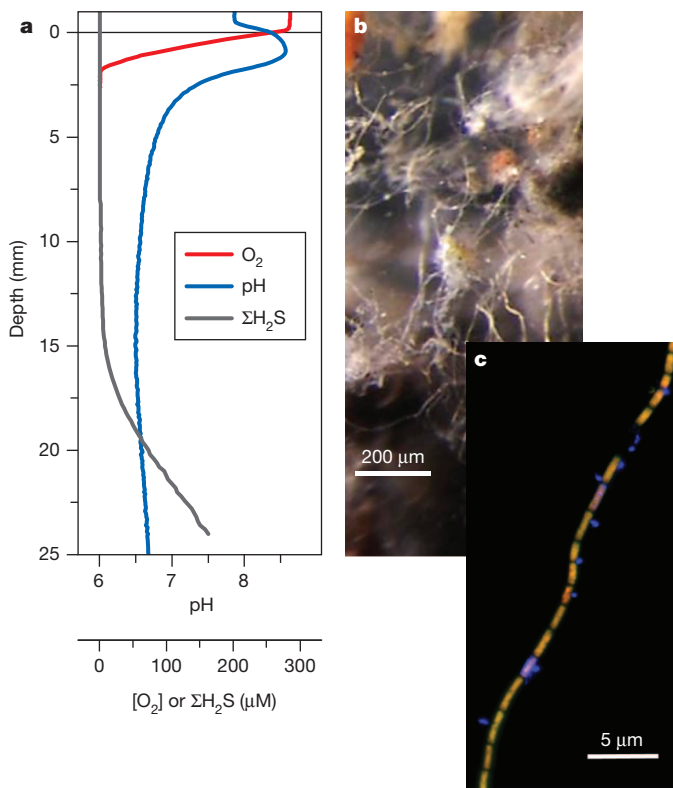
Figure 1 | Filamentous Desulfobulbaceae in current-producing sediments.
a, Microprofiles of O_2, pH and ΣH_2S ($\Sigma H_2S = ([H_2S] + [HS^-] + [S^{2-}])$).
b, Tuft of filamentous Desulfobulbaceae collected from the sulphide-free zone.
c, Filamentous Desulfobulbaceae identified by fluorescence *in situ*
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 micro-
sphere layer as confirmed by FISH and 16S rRNA gene sequencing
(Figs 1c and 4b), and no other connecting structures were observed by
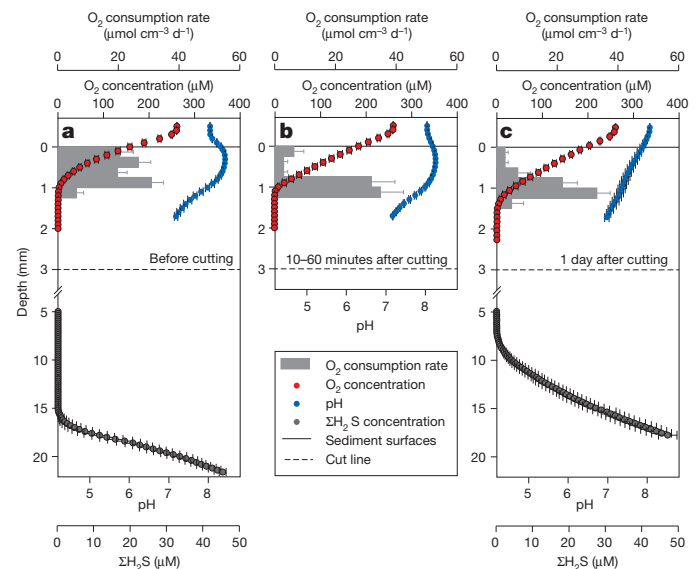light and scanning electron microscopy (SEM) inspection.

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 struc-
ture 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
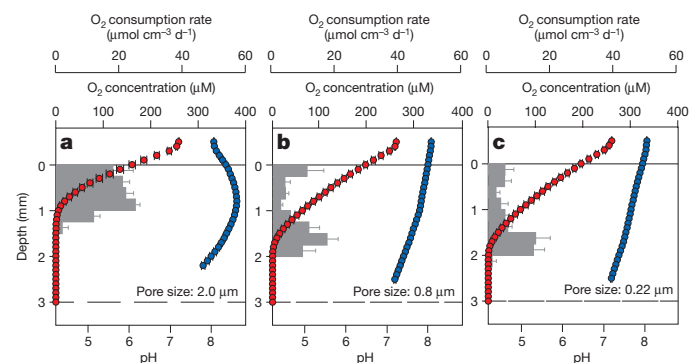electrons. On the basis of all the morphological data, we propose that

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
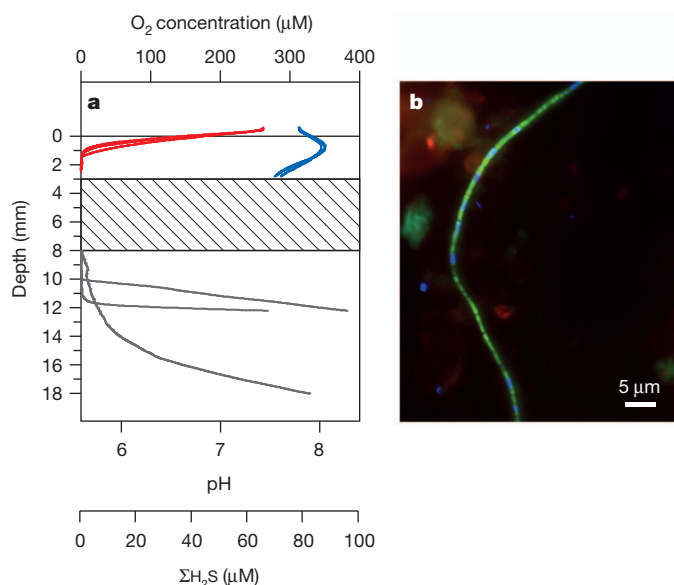values ± s.e.m. ($n = 6$).

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 con-
trast 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 pre-
viously 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 con-
ductive structures present in the periplasmic continuum are electric-
ally 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 bac-
teria of the Desulfobulbaceae family. As the simplest metabolic model
consistent with our data, we propose that the micro-cables are multi-
cellular, 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 envir-
onment balances the internal electron transport, thereby completing
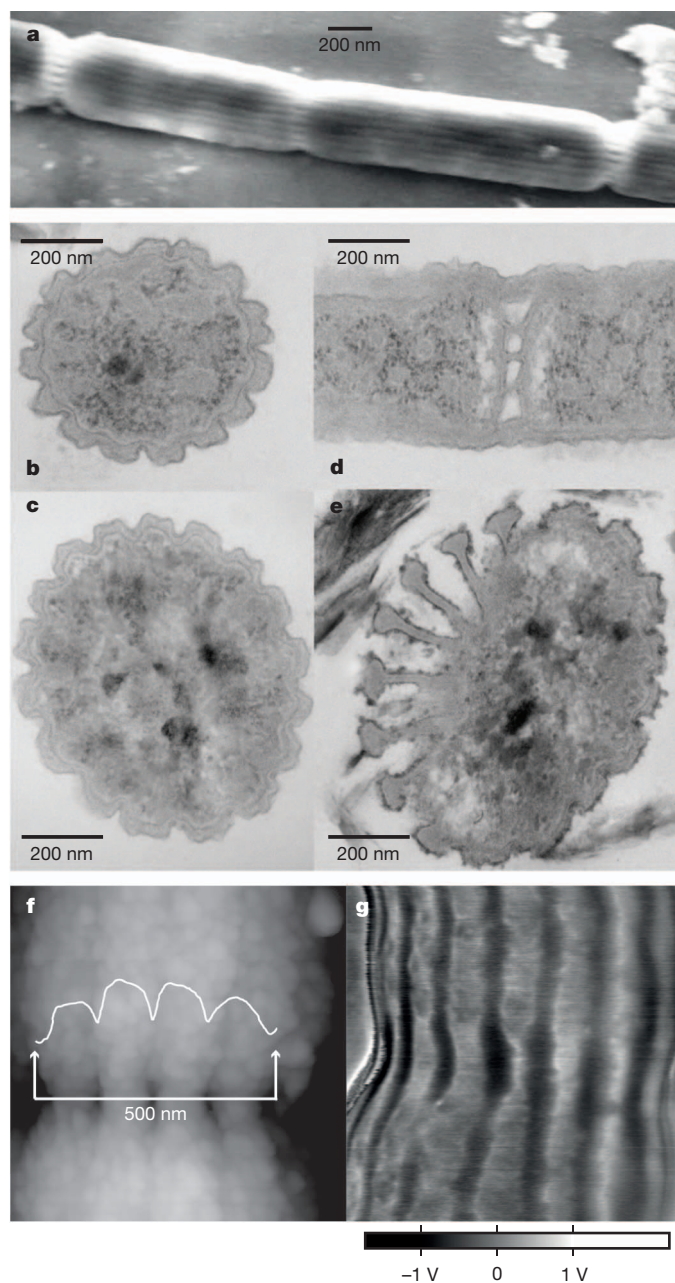the electric circuit and retaining charge balance[2,3].

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 ex-
change as a method to control metabolic interactions with external
solids or cells. Electrons are exchanged with insoluble electron accep-
tors and donors like iron and manganese minerals[9] or electrodes[10].
Furthermore, syntrophic interspecies electron exchange via nano-
wires 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 sepa-
rate 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 micro-profiles 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; ω, 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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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).