Electrode-Reducing Microorganisms That Harvest Energy from Marine Sediments

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Energy in the form of electricity can be harvested from marine sediments by placing a graphite electrode (the anode) in the anoxic zone and connecting it to a graphite cathode in the overlying aerobic water. We report a specific enrichment of microorganisms of the family Geobacteraceae on energy-harvesting anodes, and we show that these microorganisms can conserve energy to support their growth by oxidizing organic compounds with an electrode serving as the sole electron acceptor. This finding not only provides a method for extracting energy from organic matter, but also suggests a strategy for promoting the bioremediation of organic contaminants in subsurface environments.

The organic matter stored in anoxic subsurface environments and aquatic sediments represents a large potential source of energy. Although some of this organic matter, most notably petroleum, is in a concentrated form that can readily be extracted and used, most of the organic matter cannot be converted to useful energy by current technologies. However, anaerobic microorganisms capable of using organic matter inhabit anoxic subsurface environments and continually tap into this energy reservoir.

It was recently shown that electrical current could be harvested from anoxic marine sediments by embedding an electrode (the anode) into the sediment and connecting it through electronic circuits to a similar electrode in the overlying aerobic seawater (the cathode) (1).

Even with a simple, unmodified graphite electrode, the magnitude of current produced, ~0.01 W/m², was sufficient to theoretically power marine-deployed electronic instrumentation. Killing the microorganisms in the sediments inhibited current flow (1).

To further evaluate the potential role of microorganisms in electron transfer to the anode, we constructed sediment batteries in laboratory aquaria in a manner similar to that described in (1), with graphite anodes in the anoxic marine sediment and graphite cathodes in overlying aerobic water (2). Electrical power output from these batteries was continuous, averaging 0.016 W per square meter of electrode surface area in three independent experiments during 6 months of current harvesting. After this energy-harvesting phase, microbial communities attached to the anodes were compared to communities on identical control electrodes that had been placed in the same sediments for the same length of time but were not electrically connected to the electrode in the overlying water.

Analysis of 16S ribosomal RNA (rDNA) genes (3–6) demonstrated that there was a pronounced enrichment of microorganisms from the δ-subgroup of the Proteobacteria colonizing energy-harvesting anodes (7). Whereas only 17 ± 4.3% (mean ± SD, n = 3) of 16S rDNA sequences from control electrodes were in the δ-subgroup of the Proteobacteria, 71.3 ± 9.6% (mean ± SD, n = 3) of the 16S rDNA sequences from microorganisms colonizing anodes of the current-producing batteries were in this subgroup. Furthermore, 70% of the increase in δ-Proteobacterial sequences was due to a single cluster of bacteria in the family Geobacteraceae, a group of anaerobic microorganisms that can couple the oxidation of organic compounds to reduction of insoluble Fe(III) oxides (8, 9). The organism in pure culture most closely related to the sequences repeatedly enriched on anodes (7) was Desulfuromobas acetoxydans, a marine microorganism known to grow anaerobically by oxidizing acetate with concomitant reduction of elemental sulfur (10) or Fe(III) (11). Enumeration of Desulfuromonas 16S rDNA sequences by a most-probable-number polymerase chain reaction (MPN-PCR) technique (12) revealed that Desulfuromonas target sequences on anodes from current-generating batteries were enriched by a factor of 100 relative to those on anodes from control batteries.

To further investigate the specific enrichment of microorganisms on anodes, we inoculated the anoxic side of a two-chambered microbial battery with sediment; the seawater was periodically changed with fresh acetate-amended anoxic seawater (13). Replacing the medium diluted the sediment and microorganisms from the inoculum that were not growing, while re-supplying acetate, the primary intermediate in the degradation of organic carbon in anoxic sediments (14). After 85 days, 16S rDNA sequences from bacteria attached to the anode were examined. All of the anode-attached bacteria detected were members of the 6-Pro-

References and Notes

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teobacteria, with the majority (70%) of the sequences most closely related to the genus *Desulfuromonas*.

When a pure culture of *D. acetoxidans* was placed in the anaerobic compartment of the two-compartment cell, with only sterile oxygenated buffer in the aerobic compartment, the electron acceptor. Cells (180 mg of protein per liter) were placed directly into the aerobic compartment of a two-chambered poised-potential system, using only a graphite electrode as the electron acceptor and acetate as the electron donor. The working electrode was poised at +200 mV versus an Ag/AgCl reference electrode, and a 3% inoculum (grown with fumarate as the electron acceptor) was introduced to initiate the experiment. No electron mediator compounds were added in the medium.

**Fig. 1.** Electricity production by *D. acetoxidans* in a two-chambered fuel cell, using only a single graphite electrode as the electron acceptor. The aerobic chamber was sterile, and the two electrodes were connected by a 500-ohm resistor.

**Fig. 2.** Growth of *D. acetoxidans* in a two-chambered poised-potential system, using only a graphite electrode as the electron acceptor and acetate as the electron donor. The working electrode was poised at +200 mV versus an Ag/AgCl reference electrode, and a 3% inoculum (grown with fumarate as the electron acceptor) was introduced to initiate the experiment. No electron mediator compounds were added in the medium.

**Fig. 3.** Growth of *G. metallireducens* in a two-chambered poised-potential system, using only a graphite electrode as the electron acceptor and benzoate as the electron donor. The working electrode was poised at +200 mV versus an Ag/AgCl reference electrode. Cells for inoculation were grown in a similar device under identical conditions, and a 10% inoculum of electrode-grown bacteria was used to initiate growth in the experiment shown.

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ment (15), addition of acetate resulted in immediate current flow at a density (0.014 W/m²) comparable to that observed in sediment microbial batteries (Fig. 1). The addition of anthraquinone-2,6-disulphonate (AQDS), a known electron transfer mediator between Geobacteraeaceae and Fe(III) oxides (16), increased current production only 24%, even though the electrode readily accepted electrons from reduced AQDS in cell-free controls. Killing the culture by heating to 65°C rapidly inhibited current production.

We found that *D. acetoxidans* conserved energy to support growth from electron transfer to the electrode (17), as evidenced by an increase in electrical current, and growth of the organism over time (Fig. 2). The increase in cell protein (12.4 mg/liter) was accompanied by the consumption of 1.05 mM acetate and the harvesting of 6.92 meq (per liter) electrons. The cell yield from growth with the electrode as the electron acceptor (1.79 mg of protein per milliequivalents of electrons) was similar to yield values observed when *D. acetoxidans* was grown on more common electron acceptors. There was no growth in incubations containing electrodes and acetate in the absence of electrical current harvesting. The oxidation of acetate to carbon dioxide yields eight electrons; thus, 82% of the acetate loss could be accounted for as acetate oxidation with the electrode serving as the terminal electron acceptor. When it is considered that some acetate is required for cell biosynthesis, this finding demonstrates that *D. acetoxidans* was growing as a result of acetate oxidation coupled to reduction of the electrode. Similar studies with *Geobacter sulfurreducens*, a member of the Geobacteraeaceae family that grows in freshwater environments, demonstrated that this close relative also oxidized acetate with the electrode serving as the sole electron acceptor.

Another close relative, *Geobacter metallireducens*, was previously shown to oxidize a variety of aromatic contaminants with the reduction of Fe(III) (18). When *G. metallireducens* was inoculated into a vessel containing benzoate as the electron donor and only a graphite electrode as the electron acceptor, current was produced and benzoate was consumed. When a portion (10%) of this culture was transferred to a new vessel containing only benzoate and an electrode, current production and benzoate oxidation were again initiated (Fig. 3). In these experiments, benzoate (0.48 mM) was completely oxidized to CO₂, and 84% of the electrons derived from benzoate oxidation were recovered with the electrode.

This ability of Geobacteraeaceae to oxidize organic compounds with an electrode serving as the electron acceptor has implications for harvesting energy from waste organic matter, and also suggests a means for bioremediation of subsurface environments contaminated with organic compounds. Production of electricity by bacteria has previously been reported, but only in highly artificial systems amended with mediators such as neutral red, potassium ferricyanide, or thionine, which shuttle electrons from the cells to the surface of the electrode (19–21). It would be impractical to maintain sufficient concentrations of electron-shuttling compounds for...
long-term electricity harvesting in open systems such as sedimentary environments.

Microbial communities consisting of fermentative microorganisms and Geobacteraceae are capable of degrading complex assemblages of organic matter (22). Thus, it seems likely that Geobacteraceae could aid in the harvesting of energy not only from aquatic sediments and subsurface environments, but also from a wide variety of organic waste materials. Molecular studies have shown that microorganisms in the Geobacteraceae are important members of the microbial community involved in the anaerobic degradation of aromatic hydrocarbons in petroleum-contaminated aquifers (23, 24), but this metabolism is often limited by the availability of Fe(III) (25). It may be possible to simply use electrodes to increase the electron-accepting capacity of contaminated sediments and enhance bioremediation of the subsurface. Further study of the mechanisms for electron transfer from the Geobacteraceae to electrodes should aid in designing strategies to optimize these processes.

References and Notes
2. Electrodes used in sediment incubations were solid, unpolished graphite with a total surface area of 62.8 cm². Connections were made with threaded marine-grade water-tight connectors filled with silver epoxy and sealed with marine epoxy. Sediments and seawater were from Boston Harbor, MA.
5. Electrodes were rinsed under a stream of sterile marine water to remove all visible sediment and debris, then scraped with sterile razor blades to remove adherent bacteria. DNA was extracted from this graphite slurry by a standard lysosome–SDS–hexadecyltrimethyl ammonium bromide (CTAB) non-chloroform method and further cleaned using a Wizard DNA purification kit (Promega). DNA was independently extracted from electrodes used in four different incubations.
6. To reduce PCR bias, we amplified 16S rDNA genes in two different reaction mixtures, using the bacterial primers 27F and 519R or 63F and 519R. The products of these reactions were pooled before cloning with a Wizard DNA purification kit (Promega). Sequences were aligned to demonstrate this effect experimentally in a natural Daphnia metapopulation in which genetic bottlenecks and local inbreeding are common. We estimate that in this metapopulation, hybrid vigor amplifies the rate of gene flow several times more than would be predicted from the nominal migration rate. This can affect the persistence of local populations and the entire metapopulation.

A Selective Advantage to Immigrant Genes in a Daphnia Metapopulation

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Immigrants to habitats occupied by conspecific organisms are usually expected to be competitively inferior, because residents may be locally adapted. If residents are inbred, however, mating between immigrants and residents results in offspring that may enjoy a fitness advantage from hybrid vigor. We demonstrate this effect experimentally in a natural Daphnia metapopulation in which genetic bottlenecks and local inbreeding are common. We estimate that in this metapopulation, hybrid vigor amplifies the rate of gene flow several times more than would be predicted from the nominal migration rate. This can affect the persistence of local populations and the entire metapopulation.

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annealing temperature 51°C. Testing showed that only Desulfuromonas and closely related Geobacteraceae sequences were amplified from environmental samples. These primers were used for five-tube MPN-PCR enumerations using DNA extracted from three different electrodes.
13. A dual-chambered fuel cell was constructed using 54-mm OD (outside diameter) glass tubing and a 22-mm OD pinch clamp assembly. Electrodes used in dual-chamber fuel cells had a surface area of 25.4 cm². The volume of each chamber was 225 ml. The chambers were separated by a cation-selective membrane (Nafion 117).
15. The aerobic chamber of a two-chambered cell was filled with an aerobic marine buffer; the anaerobic chamber was filled with Desulfuromonas growth medium lacking electron donor or acceptor. Common electron shuttles were avoided by omitting resazurin and cysteine, and by culturing cells using fumarate as the electron acceptor. The headspace of the anaerobic chamber was flushed with oxygen-free N₂,CO₂ (80:20). All fuel cells were maintained at 30°C. Current and voltage measurements were collected with a multimeter.
17. For poised-potential growth, both chambers were filled with anaerobic Desulfuromonas (marine) or Geobacter (freshwater) growth medium lacking electron donors or acceptors. Resazurin and cysteine were omitted from the medium. Both chambers were maintained under oxygen-free N₂,CO₂ (80:20). The reference electrode was a standard 3M NaCl Ag/AgCl electrode. Potential at the working electrode was maintained at ±200 mV versus the reference electrode by a potentiotat interfaced with a Macintosh computer, which also recorded current flowing to the working electrode.
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