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Graphite electrodes as electron donors for anaerobic respiration

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Summary

It has been demonstrated previously that *Geobacter* species can transfer electrons directly to electrodes. In order to determine whether electrodes could serve as electron donors for microbial respiration, enrichment cultures were established from a sediment inoculum with a potentiostat-poised graphite electrode as the sole electron donor and nitrate as the electron acceptor. Nitrate was reduced to nitrite with the consumption of electrical current. The stoichiometry of electron and nitrate consumption and nitrite accumulation were consistent with the electrode serving as the sole electron donor for nitrate reduction. Analysis of 16 rRNA gene sequences demonstrated that the electrodes supplied with current were specifically enriched in microorganisms with sequences most closely related to the sequences of known *Geobacter* species. A pure culture of *Geobacter metallireducens* was shown to reduce nitrate to nitrite with the electrode as the sole electron donor with the expected stoichiometry of electron consumption. Cells attached to the electrode appeared to be responsible for the nitrate reduction. Attached cells of *Geobacter sulfurreducens* reduced fumarate to succinate with the electrode as an electron donor. These results demonstrate for the first time that electrodes may serve as a direct electron donor for anaerobic respiration. This finding has implications for the harvesting of electricity from anaerobic sediments and the bioremediation of oxidized contaminants.

Introduction

Microorganisms in the family Geobacteraceae can oxidize organic compounds with electrodes serving as the sole electron acceptor, and this respiration shows promise as a strategy for harvesting electricity from aquatic sediments and other sources of waste organic matter (Bond *et al*., 2002; Bond and Lovley, 2003). The first indication that Geobacteraceae had this capability was the finding that microorganisms in this family were highly enriched on the anodes of sediment batteries harvesting electricity from marine and freshwater sediments (Bond *et al*., 2002; Tender *et al*., 2002; Holmes *et al*., 2004). Pure cultures from within this family, such as *Geobacter sulfurreducens*, *G. metallireducens* and *Desulfuromonas acetoxidans*, can conserve energy to support growth by oxidizing acetate or other organic compounds to carbon dioxide and quantitatively transferring the electrons to the surface of a graphite electrode (Bond *et al*., 2002; Bond and Lovley, 2003).

Geobacteraceae appear to transfer electrons directly to the electrode surface (Bond and Lovley, 2003). Scanning electron microscope images of *G. sulfurreducens* growing with acetate as the electron donor and a graphite electrode as the electron acceptor demonstrated that cells nearly covered the electrode surface. These attached cells were responsible for current production (Bond and Lovley, 2003). This contrasts with previously described electron transfer to electrodes in organisms such as *Escherichia coli* (Emde *et al*., 1989; Park and Zeikus, 2000), *Propionibacterium freunreichii* (Emde and Schink, 1990), *Proteus vulgaris* (Kim *et al*., 2000) and *Actinobacillus succinogenes* (Park and Zeikus, 2000), which require mediator compounds to serve as electron shuttles between the cells and the electrodes.

Organisms other than Geobacteraceae may also use electrodes as an electron acceptor without the requirement for an exogenous added electron shuttle. For example, *Rhodoferax ferrireducens* oxidized glucose and other sugars to carbon dioxide with an electrode serving as the sole electron acceptor (Chaudhuri and Lovley, 2003). *Shewanella putrefaciens* incompletely oxidized lactate in cultures in which electrodes were provided as the sole electron acceptor (Kim *et al*., 1999; 2002). In contrast to the nearly quantitative transfer of electrons to the electrode with *Geobacter* sp. (Bond *et al*., 2002; Bond and Lovley, 2003) and *R. ferrireducens* (Chaudhuri and Lovley, 2003), less than 10% of the electrons theoretically available from lactate oxidation were recovered as electricity with *S. putrefactions* (Kim *et al*., 1999; 2002). Similar low current yields have been reported previously for mediator-

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less fuel cells enriched from wastewater (Jang *et al.*, 2004).

The finding that microorganisms can use electrodes as an electron acceptor leads to the question of whether, under the appropriate conditions, electrodes might also serve as electron donors for anaerobic respiration. Here, we report on studies in sediments and with pure cultures that indicate that reduced electrodes can serve as an electron donor for Geobacteraceae, a novel form of anaerobic respiration that has implications for bioremediation and the microbial harvesting of electricity from anaerobic sediments.

Results

Enrichment from sediment

When 5 ml of sediment was added to 245 ml of medium with nitrate as the potential electron acceptor and only a graphite electrode maintained at –500 mV (versus Ag/AgCl) as the potential electron donor, electrons and nitrate were consumed and nitrite was produced (Fig. 1A). In contrast, there was no loss of nitrate or any nitrite production if no current was supplied to the electrode, or if the microorganisms in the sediment were killed with heat before addition to the culture vessel (data not shown). In a sterile chamber, after a period of flushing with N_2/CO_2, the electrode produced hydrogen at a maximal rate of 6×10^{-7} mmol h^{-1}, which is equivalent to a current of 2.6×10^{-5} mA. This rate of abiotic hydrogen production was 10 000-fold slower than the average rate of current consumption by the nitrate-reducing enrichments (Fig. 1A).

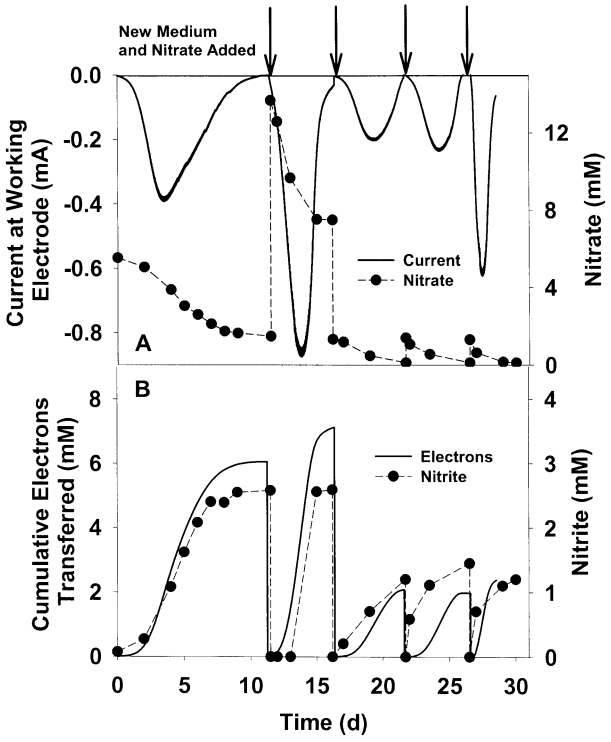

Fig. 1. Sediment enrichment using an electrode poised at –500 mV (versus Ag/AgCl) as the sole electron donor for nitrate reduction.
A. Current flow and nitrate concentrations in an electrode chamber inoculated with sediment.
B. Cumulative electron flow to the working electrode and nitrite concentrations in the electrode chamber inoculated with sediment.
Arrows indicate points at which electrode medium was replaced and nitrate was added.

With the live sediment inoculum, consumption of current and nitrate and nitrite production began almost immediately with little or no lag (Fig. 1A and B). As nitrate was depleted and nitrite accumulated, the rate of current and nitrate uptake slowed. When the medium was replaced with medium containing an initial concentration of 13.8 mM nitrate, current and nitrate consumption resumed and then slowed as nitrite concentrations again approached ≈ 2.5 mM. In this early phase of the enrichment, when electron donors other than the electrode were potentially available from the sediment inoculum, nitrite production accounted for ≈ 50% of the nitrate consumption.

However, once most of the sediment had been diluted from the enrichment, and lower concentrations of nitrate were added (Fig. 1A and B), nitrate loss and nitrite accumulation were nearly equivalent. The ratio of electron consumption and nitrite consumption in this more mature enrichment culture was ≈ 2 (Fig. 1B), which is consistent with the expected stoichiometry of electrons required for nitrate reduction to nitrite:

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \quad (1)$$

These results demonstrated that the mature enrichment culture was reducing nitrate to nitrite with the electrode as the electron donor.

Scanning electron microscopy of enrichment electrode surfaces revealed that electrodes were covered with rod-shaped bacteria, whereas control electrodes not connected to a potentiostat (and thus not providing any current) were not heavily colonized (Fig. 2A and B). Nearly all the microorganisms associated with the electrode appeared to be in direct contact with the electrode surface, rather than in a multilayer biofilm.

After six sequential additions of nitrate, the composition of 16S rRNA gene sequences on the surface of electrodes serving as electron donors was compared with the sequences recovered from control electrodes. There was a significant enrichment of sequences most closely related to the sequences of known Geobacteraceae on the electrode that had been supplying current for nitrate reduction (Fig. 3). All but one of the Geobacteraceae sequences were most closely related to *Geobacter grbi-*

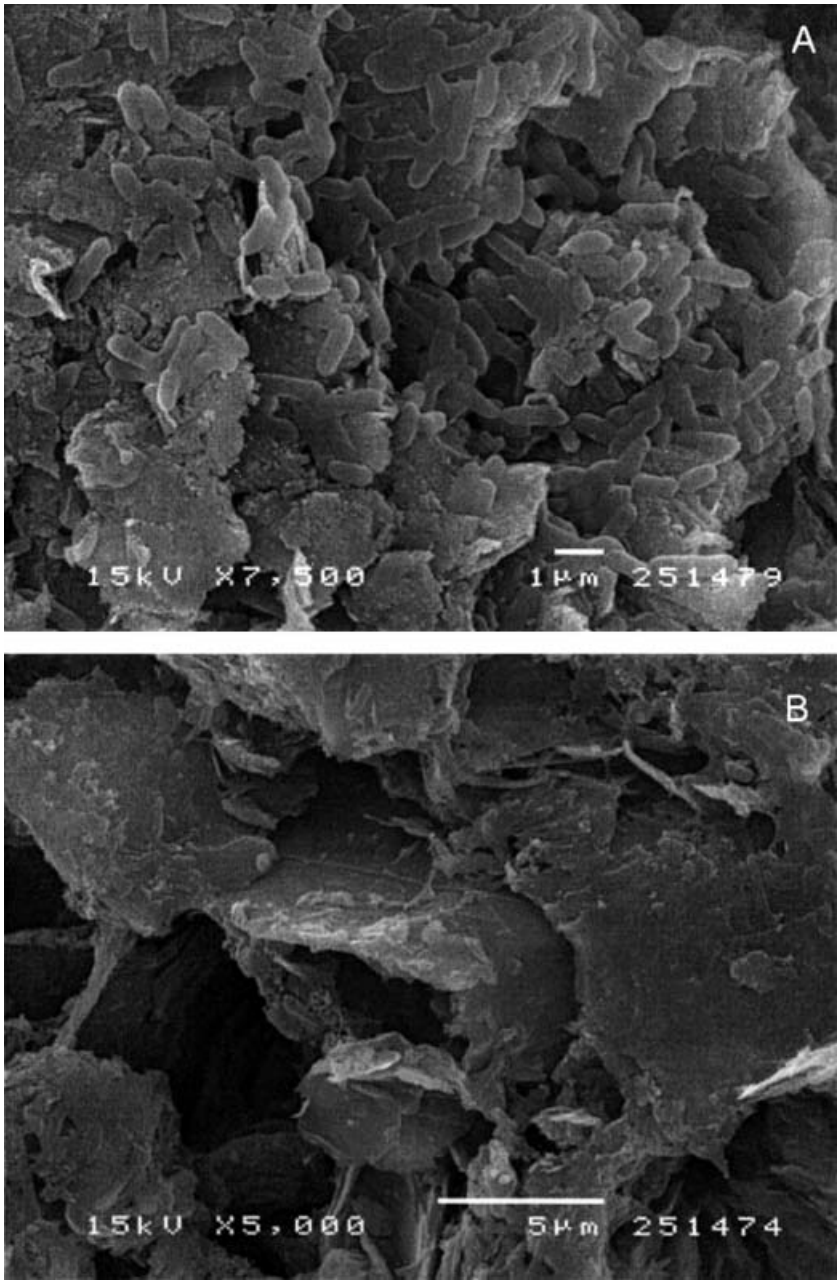

Fig. 2. Scanning electron micrograph showing the surfaces of working chamber electrodes inoculated with river sediment, nitrate and enriched with a poised (−500 mV relative to Ag/AgCl) electrode (A) and a control (unconnected) electrode (B).

ciae. These sequences were 95–99% similar to each other and 95–98% similar to *G. grbiciae*. No δ-proteobacteria, other than *Geobacter* sp., were identified.

In addition to the specific enrichment of Geobacteraceae, there was a small increase in the proportion of sequences from the γ-proteobacteria. This increase was only observed in the clone library constructed from the 8f/519r primer set and not 338f/907r. The γ-proteobacteria sequences recovered were most similar to *Thermomonas* sp. Several members of the genus *Thermomonas* sp. are facultative aerobes and have been isolated from reactors for heterotrophic denitrification of drinking water (Mergaert *et al.*, 2003).

Electrodes as electron donors for Geobacter metallireducens

In order to evaluate further whether the specific enrichment of Geobacteraceae on the electrode might be the result of Geobacteraceae using the electrode as an electron donor for nitrate reduction, studies were conducted with *G. metallireducens*, which has the ability to reduce

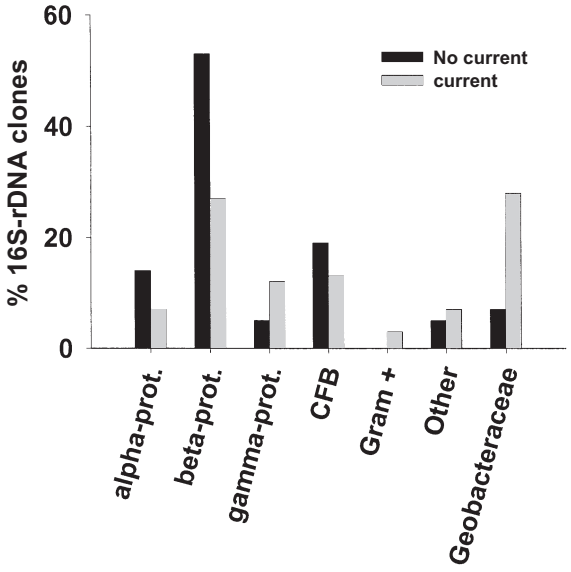

Fig. 3. Relative proportions of 16S rRNA gene sequences from clone libraries of the attached microbial community on a poised electrode and control electrode. Clones were placed into groups according to the closest percentage similarity to sequences in the database as determined by the BLASTN algorithm.

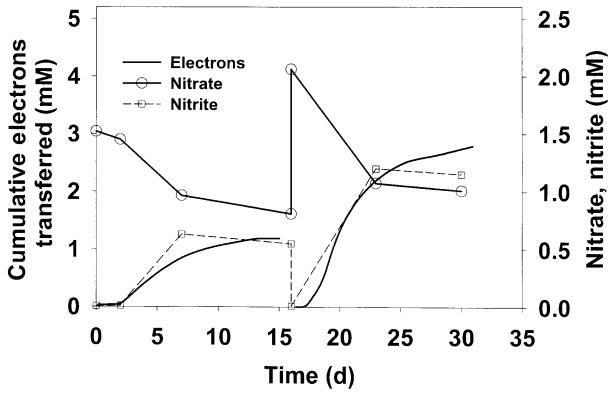

Fig. 4. Reduction of nitrate by *G. metallireducens* with an electrode (–500 mV versus Ag/AgCl) as the sole electron donor.

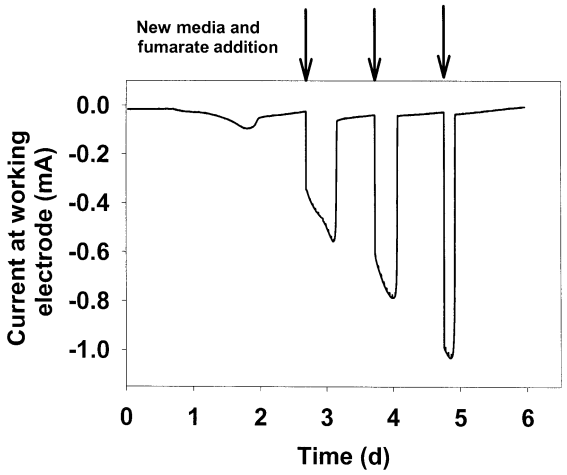

Fig. 5. Rate of current flow to an electrode for the reduction of fumarate by *G. sulfurreducens*. Sequential additions of fumarate over time are indicated by arrows.

nitrate with acetate as the electron donor (Lovley *et al.*, 1993) and is not able to use hydrogen as an electron donor. When a washed culture of nitrate-grown *G. metallireducens* was inoculated into a chamber with the electrode as the sole potential electron donor and nitrate as the potential electron acceptor, current was consumed and nitrate was reduced after a brief lag (Fig. 4). Nitrite accumulation accounted for 90% of the nitrate consumed, and the amount of current consumed was consistent with reduction of nitrate to nitrite. Ammonia was not detected.

Electrodes as electron donors for Geobacter sulfurreducens

In order to determine whether electrodes might serve as an electron donor for the reduction of electron acceptors other than nitrate, studies were conducted with *G. sulfurreducens*, using fumarate as the electron acceptor. Although *G. sulfurreducens* is able to grow with acetate as the electron donor and fumarate as the electron acceptor (Caccavo *et al.*, 1994), it cannot grow with an inorganic electron donor, i.e. hydrogen, and fumarate as the electron acceptor without the addition of a carbon source. Therefore, *G. sulfurreducens* cultures were initially established with10 mM fumarate and 2 mM acetate. After a brief lag, a small amount of current flow was observed (Fig. 5). Subsequent additions of fumarate (10 mM) resulted in consumption of current from the working electrode and reduction of fumarate to succinate. The maximum rate of current consumption increased with each pulse, suggesting the establishment of an electrode-utilizing community of *G. sulfurreducens* in the working electrode chamber. Not only was current consumption dependent upon the presence of fumarate but, in the absence of cells, fumarate was not reduced by graphite electrodes. Furthermore, fumarate reduction was dependent on current supply. Cells did not reduce or ferment fumarate in the presence of an electrode that was not supplying current (data not shown).

When the medium was removed from chambers containing established electrode-utilizing, fumarate-reducing cultures, and replaced with non-growth medium lacking electron donors or acceptors, addition of fumarate again resulted in immediate current consumption and fumarate reduction (Fig. 6A). The medium could be repeatedly removed and replaced with fresh non-growth medium without impairing the rate of fumarate reduction, demon-

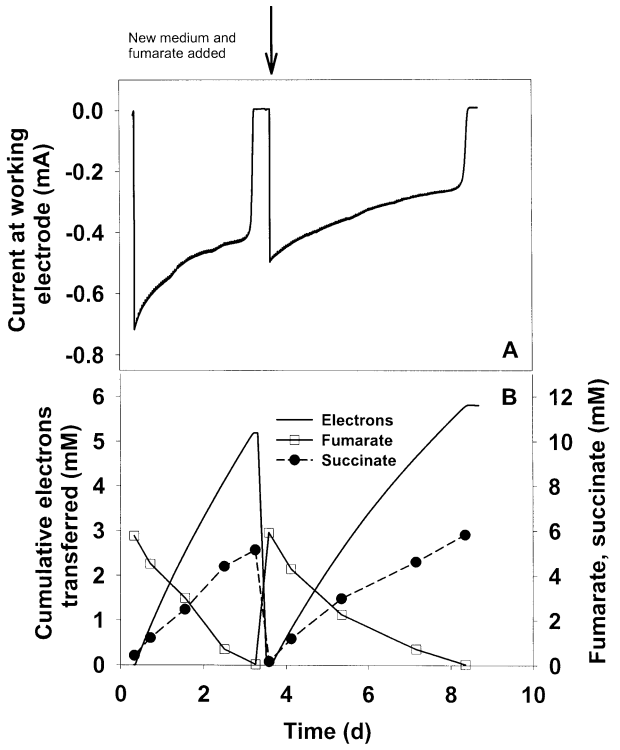

Fig. 6. Stoichiometry of electrode oxidation during fumarate reduction by *G. sulfurreducens*.
A. Current flow to the working electrode.
B. Cumulative electron flow to the working electrode and fumarate and succinate concentrations. Arrow indicates points at which electrode medium was replaced and fumarate was added.

strating that cells attached to the electrode were responsible for the fumarate-dependent current consumption. The stoichiometry of electron consumption and succinate production was ≈ 1:1 (Fig. 6B), whereas the reduction of fumarate to succinate requires two electrons per succinate produced.

$$\text{Fumarate} + 2H^+ + 2e^- \rightarrow \text{succinate} \quad (2)$$

These results are consistent with a partial oxidation of fumarate when an alternative electron donor, such as an electrode, is also present. Further evidence that some of the organic acids were metabolized was the finding that minor concentrations (<0.5 mM) of organic acids such as formate, citrate and 2-oxoglutarate accumulated in the medium. These products would be expected to accumulate if a portion of the fumarate were oxidized via anapleurotic reactions allowing flux of metabolites into the TCA cycle.

Unlike *G. metallireducens*, *G. sulfurreducens* can use hydrogen as an electron donor (Caccavo *et al*., 1994). However, there was no evidence that hydrogen production by the poised electrode was responsible for the electron consumption by attached cultures. As noted previously, the electrode only produced hydrogen at a rate of 6×10^{-7} mmol h^{-1}, which was much slower than the rate of fumarate reduction. Headspace hydrogen concentrations in chambers using electrodes were typically 10^{-5}–10^{-6} atm, and levels of hydrogen in the chambers did not decrease or increase significantly when fumarate was added as an electron acceptor. Flushing of chambers to reduce hydrogen below 10^{-7} atm had no effect on current consumption or fumarate reduction by active cultures. Furthermore, a mutant lacking the key uptake hydrogenase (Coppi *et al*., 2004) consumed electrons and reduced fumarate at the same rate and extent as wild type (data not shown).

Discussion

These results demonstrate that electrodes can serve as an electron donor for microbial reduction of electron acceptors in *Geobacter* species. Although electrodes have previously been used to regenerate Fe(II) from Fe(III) as an electron donor in the metabolism of *Acidithiobacillus* (formerly *Thiobacillus*) (Blake *et al*., 1994), to our knowledge, the results presented here demonstrate for the first time that electrodes have been found to serve as direct electron donors. As discussed below, this finding has potential applications for manipulating microbial respiration in anaerobic sedimentary environments.

Electrodes as electron donors for nitrate and fumarate reduction

The results clearly demonstrate that the electrode provided electrons for the reduction of nitrate and fumarate. In the studies with *G. metallireducens*, as well as the adapted enrichment cultures, the consumption of nitrate and electrons and the production of nitrite were consistent with the reduction of nitrate to nitrite with the electrode serving as the sole electron donor. Nitrate was not reduced in the absence of *G. metallireducens*. The significant increase in cells on the electrode surface in the enrichment cultures in which the electrode was donating electrons compared with the control, non-current electrode suggested that nitrate reduction provided energy to support cell growth.

The ability of some *Geobacter* species effectively to use an electrode as an electron donor for nitrate reduction appears to have aided them in outcompeting other microorganisms in colonizing the electrode surface in the enrichment studies. 16S rRNA gene sequences most closely related to known *Geobacter* species were the most abundant in clone libraries constructed with 16S rRNA gene polymerase chain reaction (PCR) amplicons from electrode-associated microbial DNA. Although cell morphology cannot be used as a phylogenetic characteristic,

the electrode surfaces were colonized by rod-shaped microorganisms with a morphology similar to that of known *Geobacter* species. Other known nitrate reducers did not appear to be significantly enriched on the electron-donating electrodes. It is not clear whether this was because other species of nitrate reducers cannot use electrodes as an electron donor or because the conditions of the enrichment favoured the pre-emptive colonization of the electrode by *Geobacter* species. The enrichment of *Geobacter* on the electrodes serving as an electron donor is similar to the colonization of *Geobacter* on electrodes serving as an electron acceptor in a variety of aquatic sediments (Bond *et al*., 2002; Tender *et al*., 2002; Bond and Lovley, 2003; Holmes *et al*., 2004).

The *Geobacter* species in the enrichment and *G. metallireducens* only partially reduced nitrate to nitrite. *G. metallireducens* reduces nitrate to ammonia with acetate (Lovley and Phillips, 1988) or the reduced humic acid analogue, anthrahydroquinone-2,6-disulphonate (AHQDS) (Lovley *et al*., 1999) as the electron donor. The low (−500 mV) potential of the electrode should have been sufficient to permit complete reduction to ammonia. Further investigation is required to determine what factor(s) limit nitrite reduction.

Geobacter sulfurreducens consumed more fumarate than was expected based upon the amount of current consumed. This was probably attributable to some concurrent fermentation of fumarate. Although fumarate is not fermented when it is provided as the sole potential electron donor, studies with hydrogen have suggested that, when there is another electron donor available, *G. sulfurreducens* can ferment fumarate at a slow rate (Coppi *et al*., 2004). The finding that there was no fumarate reduction in the absence of current and that the electrode could not reduce fumarate in the absence of cells indicate that *G. sulfurreducens* was reducing ≈50% of the fumarate using the electrode as the electron donor.

Mechanisms for electron transfer from electrodes

The available data suggest that *Geobacter* species directly accept electrons from the electrode surface. In the instances in which the medium in the chambers was replaced to remove planktonic cells, current consumption continued, indicating that attached cells were responsible for current uptake. Furthermore, the attached cells formed an apparent monolayer on the electrode surface, indicating that cells needed to be in direct contact with the electrode. This is similar to when *Geobacter* species use electrodes as an electron acceptor (Bond *et al*., 2002; Bond and Lovley, 2003). Electron transfer to electrodes has been postulated to be comparable to the reduction of insoluble Fe(III) oxides in which *Geobacter* species appear to have a highly evolved system for searching for and directly attaching to Fe(III) oxides in order to reduce them rather than producing soluble electron-shuttling compounds that can transfer electrons from the cells to Fe(III) oxides that the cell is not contacting (Childers *et al*., 2002).

Although the electrodes produced a small amount of hydrogen, it is unlikely that hydrogen was a major electron donor for nitrate or fumarate reduction. The rate of hydrogen production was 10 000-fold less than the rates of nitrate and fumarate reduction. In addition, *G. metallireducens* is unable to use hydrogen as an electron donor (Lovley *et al*., 1993), probably because it lacks the genes for the hydrogenase found to be essential for hydrogen-based respiration in *G. sulfurreducens* (Coppi *et al*., 2004). Furthermore, a mutant of *G. sulfurreducens* that was deficient in this uptake hydrogenase (Coppi *et al*., 2004) reduced fumarate as well as wild type.

The finding that *Geobacter* can use electrodes as either an electron donor or an electron acceptor, depending upon the potential of the electrode, is reminiscent of other instances in which *Geobacter* species can use the oxidized form of a compound as an electron acceptor and the reduced species as an electron donor. *G. metallireducens* is not only capable of oxidizing organic compounds with the reduction of Fe(III) to Fe(II), but can also oxidize Fe(II) with nitrate as the electron acceptor (Finneran *et al*., 2002). In a similar manner, U(VI) can be reduced to U(IV), or U(IV) can be anaerobically oxidized to U(VI) (Finneran *et al*., 2002). As the biochemical mechanisms of reduction of metals by *Geobacter* species are only poorly understood (Lloyd *et al*., 1999; Magnuson *et al*., 2000; Leang *et al*., 2003), it is difficult to evaluate whether the oxidation of reduced species involves a reversal of the reductive electron transport pathway or an alternative strategy.

Potential environmental applications

The use of electrodes as direct electron donors could have several environmental applications. For example, sediment batteries (Reimers *et al*., 2001; Tender *et al*., 2002) harvesting electricity from organic matter now rely on a cathode that reduces oxygen in the water overlying the sediments. The ability of *Geobacter*, and possibly other species, to accept electrons from a cathode under anaerobic conditions suggests that sediment batteries could, in some instances, use a cathode placed within anoxic sediments or water in which nitrate is available. Furthermore, many groundwater contaminants, such as chlorinated solvents and toxic metals, persist in groundwater because of a lack of electrons required for reductive dechlorination or reductive precipitation of the metals. Electron transfer from electrodes may serve as a mechanism for supporting these bioremediation reactions, espe-

cially when it is considered that the capacity for electron transfer to chlorinated compounds (McCormick *et al.*, 2002; Sung *et al.*, 2003) and toxic metals (Lloyd and Lovley, 2001) is a characteristic of many Geobacteraceae.

Experimental procedures

Electrodes and electrode chambers

A dual-chambered fuel cell was constructed with 54 mm outer diameter (OD) glass tubing and a 22 mm OD pinch clamp assembly as described previously (Bond and Lovley, 2003). The volume of each chamber, with electrode, was ≈250 ml of medium with a 150 ml headspace. The chambers were separated with a cation-selective membrane (Nafion 117; Electrosynthesis). Electrodes were $1 \times 3 \times 0.5$ inch sticks of unpolished graphite (grade G10; Graphite Engineering and Sales). New electrodes were soaked in 1 N HCl, which was changed daily until extractable Fe(II) was below detection. After each use, electrodes were washed in 1 N HCl and 1 N NaOH to remove possible metal and biomass contamination. All connections between the electrodes and the potentiostat have been described previously (Bond and Lovley, 2003).

The cathode (working) and anode (counter) chambers were flushed with sterile, anaerobic gas (80:20 N_2:CO_2), filled with the appropriate buffer medium (described below) and connected to a potentiostat. The anode chamber was bubbled continuously with N_2:CO_2. The medium in the cathode chambers was stirred at 180 r.p.m. with a magnetic stir bar. The chambers were allowed to equilibrate at –500 mV (versus Ag/AgCl) for 24 h before inoculating with cultures or sediment. Control chambers were treated identically, but were not connected to a potentiostat, and no current was able to flow to the working electrode.

Current measurements for studies were collected directly from potentiostat outputs every 15 s with a Power Laboratory 4SP unit connected to a Power Macintosh computer, and data were logged with CHART 4.0 software (AD Instruments). Current (mA) was integrated over time and converted to electrons recovered using the conversions 1 C = 1 Amp * second, 1 C = 6.24×10^{18} electrons and 1 mol = 6.02×10^{23} electrons (96 500 C mol^{-1}). Background current (current at the working electrode in the absence of cells, typically 0.03–0.04 mA) was determined for each experiment and subtracted from all values before calculating total electron recovery.

Sediment studies

Sediments were collected from the Connecticut River at a shallow, near-edge sampling point in Sunderland, MA, USA. The sediment slurry (5 ml) was inoculated into the working chamber containing the poised electrode containing 245 ml of anaerobic medium. Unless otherwise stated, the medium contained (per l) 0.1 g of KCl, 0.2 g of NH_4Cl and 0.3 g of NaH_2PO_4. The medium was adjusted to pH 6.8, 2.0 g of $NaHCO_3$ was added, and the medium was flushed with 80:20 N_2:CO_2 to remove oxygen before autoclaving in sealed bottles. After current flow was established, the medium in both working and counter chambers was replaced each time nitrate was added to wash out planktonic cells and sediment debris.

Electron microscopy

Electrodes were removed from the electrode chambers and rinsed with sterile medium. Several small (0.5 $cm^2 \times 2$ mm thick) pieces of the electrode were cut from the surface of a connected and an unconnected control electrode. The samples were immediately immersed in a fixative solution of deionized water and 1% glutaraldehyde/1% formaldehyde. Dehydrating, mounting and imaging procedures have been described previously (Bond and Lovley, 2003).

Amplification of 16S rRNA genes, construction of clone libraries and phylogenetic analysis

Electrodes were rinsed with sterile medium and scraped with a sterile razor blade into 1.5 ml of TE buffer (pH 8) to produce a slurry of graphite and cells. DNA was extracted from the graphite using a modified version of the Miniprep of bacterial genomic DNA protocol (Ausubel, 1999). The method was conducted according to previous descriptions (Bond *et al.*, 2002; Holmes *et al.*, 2004). 16S rRNA genes were amplified with the primer 8 forward (Lane *et al.*, 1985) with 519 reverse (Lane *et al.*, 1985) or 338 forward (Amann *et al.*, 1995) and 907 reverse (Lane, 1991). PCR mixtures and conditions were followed as detailed elsewhere (Holmes *et al.*, 2004). Clone libraries were constructed from the 16S rRNA genes using the TOPO TA cloning© kit version P (Invitrogen) according to the manufacturer's instructions.

A total of 60 clones (30 from 8f/519r and 30 from 338f/915r libraries) under each condition (with and without current) were selected for sequencing. The 16S rRNA gene was amplified from each clone using M13 forward and reverse primers (Invitrogen) using whole-colony PCR. PCR products were purified using the QIAquick© PCR purification kit (Qiagen). Sequencing of the inserts was performed at the Umass Biotechnology Center's sequencing facility. Sequences were compared with the GenBank database with the BLAST (Altschul *et al.*, 1990) algorithm. Multiple sequence alignments were performed using the CLUSTAL W algorithm (Thompson *et al.*, 1994) and were visualized using MVIEW (Brown *et al.*, 1998). Local sequence alignments were conducted using LFASTA (Pearson, 1990).

Pure culture studies

Geobacter sulfurreducens strain PCA (ATCC #51573) and *G. metallireducens* strain GS-15 (ATCC #53774) were obtained from our laboratory culture collection. All incubations were at 30°C. Growth medium was described previously (Bond and Lovley, 2003). Acetate served as the electron donor unless otherwise indicated. To obtain cells well adapted to using insoluble electron acceptors, cells were maintained on this medium amended with 100–120 mM poorly crystalline Fe(III) oxide as the electron acceptor. Working cultures of *G. sulfurreducens* were then transferred (10% inoculum) three times in medium containing 40 mM fumarate as the electron

acceptor before inoculation into electrode-containing chambers. The non-growth medium for *G. sulfurreducens* omitted sulphur, nitrogen, vitamin and mineral solutions as described previously (Bond and Lovley, 2003). The working cultures of *G. metallireducens* were transferred three times in medium containing nitrate (NO_3^-) as the electron acceptor before inoculation into the electrode chambers. The medium in the electrode chambers for experiments with *G. metallireducens* was identical to the medium described above for the sediment experiments.

Analytical methods

Organic acids were determined via high-performance liquid chromatography (HPLC) with an Aminex HPX-87H column (Bio-Rad) operated with 0.008 mM H_2SO_4 as the eluent (0.5 ml min^{-1}) and UV detection. Nitrate and nitrite (NO_2^-) were quantified using a Dionex DX-100 ion chromatograph with a Dionex AS4-SC IonPac column. For hydrogen analysis, gas samples were injected into a 0.5 ml sampling loop, separated on a Supelco 100/120 Carbosieve S-II column at room temperature with N_2 gas as the carrier and detected with a reduction gas analyser (RGD2; Trace Analytical).

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