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Electron Transfer by *Desulfobulbus propionicus* to Fe(III) and Graphite Electrodes

Dawn E. Holmes, Daniel R. Bond, and Derek R. Lovley*

Department of Microbiology, University of Massachusetts, Amherst, Massachusetts 01003

***Desulfobulbus propionicus* was able to grow with Fe(III), the humic acids analog anthraquinone-2,6-disulfonate (AQDS), or a graphite electrode as an electron acceptor. These results provide an explanation for the enrichment of *Desulfobulbaceae* species on the surface of electrodes harvesting electricity from anaerobic marine sediments and further expand the diversity of microorganisms known to have the ability to use both sulfate and Fe(III) as an electron acceptor.**

Electrical energy can be harvested from marine sediments when a graphite electrode emplaced in marine sediment (anode) is connected by an electrical circuit to another electrode (cathode) in overlying aerobic water (22). Microbial activity in the sediments is required for current production (2, 8, 25, 28), and there is a specific enrichment of δ-*Proteobacteria* on the anode surface (2, 8, 28).

In most freshwater and marine sediment fuel cells, microorganisms in the *Geobacteraceae* family are the predominant microorganisms on the anodes (2, 8, 28). However, in all marine sediment fuel cells evaluated, another group of δ-proteobacterial sequences, most closely related to sulfate-reducing bacteria from the family *Desulfobulbaceae*, was also consistently enriched on the anode (8, 28). In fact, in one field deployment, organisms in this cluster accounted for all of the δ-proteobacterial sequences and ca. 62% of the bacterial 16S rRNA gene sequences recovered from the current-harvesting anode (8).

In order to investigate how *Desulfobulbaceae* might interact with anodes in sediments, studies were conducted with *Desulfobulbus propionicus*. Dissimilatory sulfate reduction via the incomplete oxidation of organic acids such as lactate, propionate, butyrate, and ethanol to acetate is considered the primary metabolism of *D. propionicus* (29, 30). However, *D. propionicus* can oxidize organic electron donors with nitrate (5, 30), nitrite (5), or oxygen (5, 6) and can oxidize inorganic sulfur compounds with the reduction of O_2 (5, 7), nitrite (5), nitrate (5), or Mn(IV) (16). Furthermore, in the absence of an electron acceptor, *D. propionicus* can ferment lactate, pyruvate, or ethanol to a mixture of acetate and propionate via the succinate-propionate pathway (26, 29, 30).

Previous studies also demonstrated that cell suspensions of *D. propionicus* reduced Fe(III)-nitrilotriacetic acid (NTA; 10 mM) and poorly crystalline Fe(III)-oxide (100 mM) when propionate (5 to 10 mM) was provided as the electron donor (22). However, Fe(III) did not support growth in those studies. The ability to grow via reduction of Fe(III) oxides may have relevance to growth on electricity-harvesting electrodes in sediments, because both electrodes and Fe(III) oxides represent insoluble, extracellular electron acceptors.

Dissimilatory Fe(III) reduction. The representative *Desulfobulbaceae* species, *D. propionicus* (DSMZ 2032), was obtained from DSMZ (German Collection of Microorganisms and Cell Cultures; Braunschweig, Germany) and was grown in a slightly modified version of NB Basal medium (14), which contained the following (per liter): 0.42 g of KH_2PO_4, 0.22 g of K_2HPO_4, 0.2 g of NH_4Cl, 0.38 g of KCl, 0.36 g of NaCl, 0.75 g of $CaCl_2 \cdot 2 H_2O$, 0.10 g of $MgCl_2$, 1.8 g of $NaHCO_3$, and 0.5 g of Na_2CO_3, as well as 1 μM Na_2SeO_4 and trace minerals and vitamins. This medium differed from that typically used to culture *D. propionicus* (DSMZ medium 194) in that it contained selenium and fivefold more calcium. Sulfate other than that present in the trace metal solution (300 μM) was omitted to prevent extensive sulfide production with subsequent abiotic reduction of Fe(III). Strict anaerobic techniques were used throughout, and cultures were incubated at 30°C in the dark. Organic acids (21), cell numbers (19), Fe(II), and total iron (19, 20) were monitored as previously described. Growth with various electron donors and acceptors was considered positive only after six consecutive transfers.

When *D. propionicus* was grown on pyruvate alone, 6.67 ± 0.33 mM ($n = 3$) pyruvate was fermented to 4.22 ± 0.51 mM acetate and 2.23 ± 0.35 mM propionate according to the following reaction: $3CH_3COCOO^- + 3H_2O \rightarrow 2CH_3COO^- + CH_3CH_2COO^- + 2HCO_3^- + 2H^+$.

These results are similar to those of previous studies that have shown that acetate and propionate are formed in a 2:1 ratio when *Desulfobulbus propionicus* is grown on pyruvate in the absence of an electron acceptor (26).

Pyruvate consumption by *D. propionicus* differed significantly from fermentation when a soluble form of iron was provided as an electron acceptor. For example, when pyruvate (mean ± standard deviation, 19.20 ± 0.68 mM; $n = 3$) was provided as the electron donor with Fe(III)-citrate (50 mM) as the electron acceptor, Fe(III) was reduced and 18.65 ± 0.36 mM acetate was formed, accompanied by cell growth (Fig. 1a). The stoichiometry of pyruvate consumption and Fe(III) reduction was consistent with the following reaction: $CH_3COCOO^- + 2Fe^{3+} + 2H_2O \rightarrow CH_3COO^- + HCO_3^- + 2Fe^{2+} + 3H^+$.

D. propionicus was able to grow with several other soluble

* Corresponding author. Mailing address: Department of Microbiology, 106N Morril IV N, University of Massachusetts, Amherst, MA 01003. Phone: (413) 545-9651. Fax: (413) 545-1578. E-mail: dlovley @microbio.umass.edu.
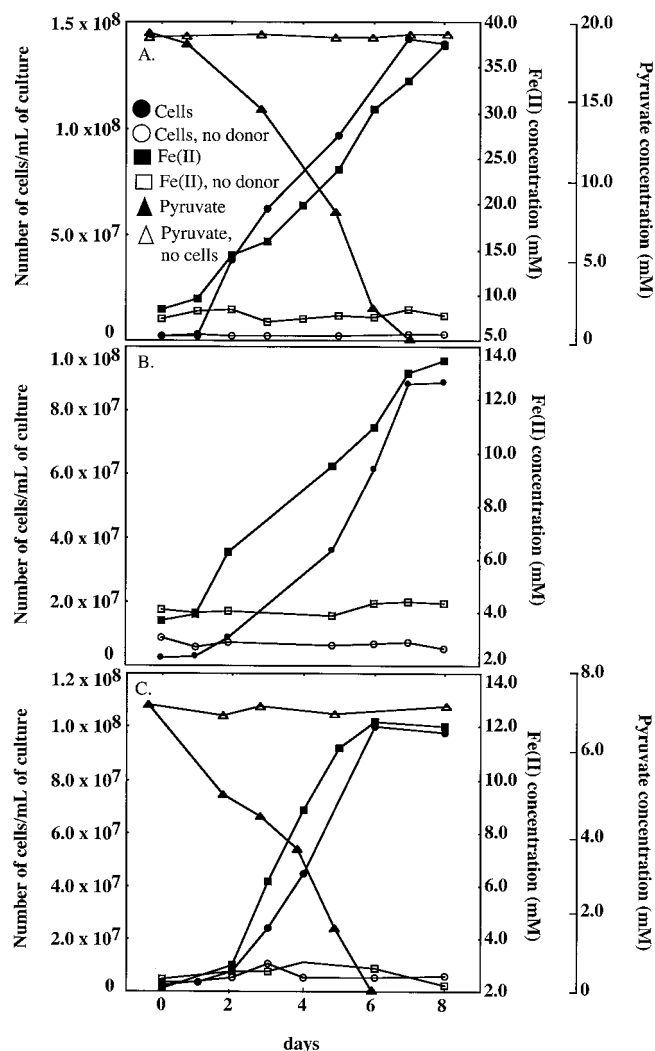
FIG. 1. (A) Growth of *D. propionicus* with pyruvate (19.2 mM) as the electron donor and Fe(III)-citrate (50 mM) as the electron acceptor. (B) Growth with Fe(III)-oxide (100 mmol/liter) as the electron acceptor and hydrogen (101 kPa) as electron donor. (C) Growth with Fe(III)-oxide (100 mmol/liter) as electron acceptor and pyruvate (7.15 mM) as the electron donor. The results are the means of triplicate incubations. ●, cell number; ○, cell number, control (no donor); ■, Fe(II) concentration; □, Fe(II) concentration, control (no donor); ▲, pyruvate concentration; △, pyruvate concentration, control (no cells).

electron acceptors, including Fe(III)-NTA (5 mM), Fe(III)-pyrophosphate (10 mM), and anthraquinone-2,6-disulfonate (AQDS; 5 mM), with pyruvate as the electron donor. Propionate, lactate, and hydrogen also served as electron donors for growth on all forms of soluble Fe(III) evaluated as well as AQDS. The mechanism(s) for Fe(III) reduction appeared to be independent of the mechanism(s) for sulfate reduction, because *D. propionicus* continued to reduce Fe(III)-citrate in the presence of 1 to 10 mM molybdate, an inhibitor of sulfate reduction.

There was a mixture of pyruvate fermentation and Fe(III) reduction when poorly crystalline Fe(III)-oxide (100 mM) (17) was provided as the electron acceptor with pyruvate (7.15 mM) as the electron donor. In the presence of Fe(III)-oxide approximately twice as much acetate was formed from pyruvate oxi-

dation than would be expected from pyruvate fermentation alone; 6.87 ± 0.75 mM ($n = 3$) of Fe(III)-oxide was reduced, and 5.13 ± 0.36 mM acetate and 1.84 ± 0.25 mM propionate accumulated (Fig. 1c). In addition, Fe(III)-oxide reduction during pyruvate metabolism appeared to yield energy to support cell growth, as the final cell numbers in cultures grown in the presence of Fe(III)-oxide, $1.0 \times 10^8 \pm 1.53 \times 10^7$ cells/ml (starting cell number was $1.20 \times 10^6 \pm 2.32 \times 10^5$; $n = 3$), were significantly higher than those in cultures grown with pyruvate alone ($3.72 \times 10^7 \pm 2.5 \times 10^6$ cells/ml of culture; starting cell number was $2.43 \times 10^6 \pm 6.35 \times 10^5$; $n = 3$). The stoichiometry of this mixed reaction was consistent with the following reaction: $3.4CH_3COCOO^- + 6.8Fe^{3+} + 6.8H_2O \rightarrow 3.4CH_3COO^- + 3.4HCO_3^- + 6.8Fe^{2+}$ and $3.75CH_3COCOO^- + 3.75H_2O \rightarrow 2.5CH_3COO^- + 1.25CH_3CH_2COO^- + 2.5HCO_3^- + 2.5H^+$ for a combined reaction of $7.15CH_3COCOO^- + 6.8Fe^{3+} + 10.55H_2O \rightarrow 5.9CH_3COO^- + 5.9HCO_3^- + 6.8Fe^{2+} + 1.25CH_3CH_2COO^- + 12.7H^+$.

D. propionicus could also be continually cultured with hydrogen as the electron donor and poorly crystalline Fe(III)-oxide as the electron acceptor when acetate (0.1 mM) was provided as a carbon source (Fig. 1b).

Electron transfer to a graphite electrode. In order to evaluate the ability of *D. propionicus* to transfer electrons to an electrode, the inoculum was grown fermentatively on lactate (10 mM). The cells were pelleted via centrifugation, washed, and then resuspended in 20 ml of fresh anoxic medium lacking electron donor or acceptor. Ten milliliters of this cell suspension was inoculated into the anaerobic anodic chamber (250 ml of medium) of a two-chambered electrode system, constructed as previously described (2, 3). The electrodes were (in centimeters) 2.34 by 7.02 by 1.17 sticks of unpolished graphite (grade G10; Graphite Engineering and Sales, Greenville, Mich.). The anode was poised with a potentiostat (AMEL instruments, Milan, Italy) at a constant potential of $+0.52$ V in reference to a standard H_2 electrode, which preliminary studies demonstrated was the optimal potential to support growth. *D. propionicus* was able to use the electrode surface as an electron acceptor when pyruvate (2.42 mM) (Fig. 2), lactate (1 mM) (Fig. 3), propionate (2 mM) (data not shown), or hydrogen (data not shown) was provided as electron donor. Almost identical current profiles were observed when lactate (Fig. 3) or propionate (data not shown) was provided as electron donor; current rose rapidly and then declined as lactate or propionate was depleted. Maximum current production with lactate and propionate reached 28.35 ± 4.72 mA/m^2 (mean $\pm$ standard deviations; $n = 3$) and 26.77 ± 7.87 mA/m^2, respectively, and between 21 to 27% of the electrons available from the incomplete oxidation of lactate and propionate were transferred to the electrode. Current produced with pyruvate and hydrogen was significantly lower: only 8.27 ± 1.97 mA/m^2 from pyruvate (Fig. 2) and 5.91 ± 1.18 mA/m^2 of current from hydrogen (data not shown). Similar to Fe(III) reduction, the addition of molybdate (5 mM) to cultures grown in the anodic chamber with lactate (2 mM) did not have a significant effect on current production (data not shown).

Evaluation of end products and electron recovery from pyruvate oxidation suggests that electron transfer to an insoluble graphite electrode can be attributed to the same mixed metabolism as observed with insoluble Fe(III)-oxide. For example, when
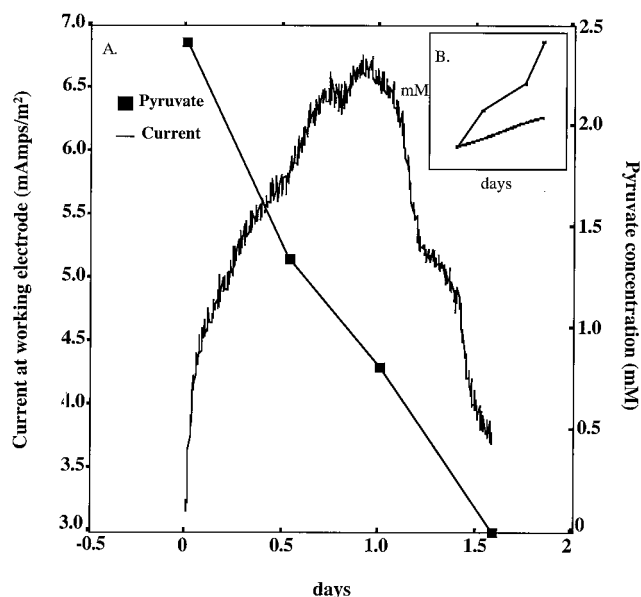
FIG. 2. (A) Current production by *D. propionicus* when pyruvate (2.42 mM) was provided as the electron donor, and a poised electrode (+0.52 V in reference to a standard H₂ electrode) served as the electron acceptor. (B) Number of electrons available from the incomplete oxidation of pyruvate that were transferred to the electrode surface. The results are the means of triplicate incubations.

D. propionicus was grown in the electrode chamber with 2.42 ± 0.23 mM pyruvate provided as the electron donor with a poised electrode as the sole electron acceptor, 1.79 ± 0.33 mM acetate and 0.64 ± 0.29 mM propionate was formed. Thus, 26.4% of the
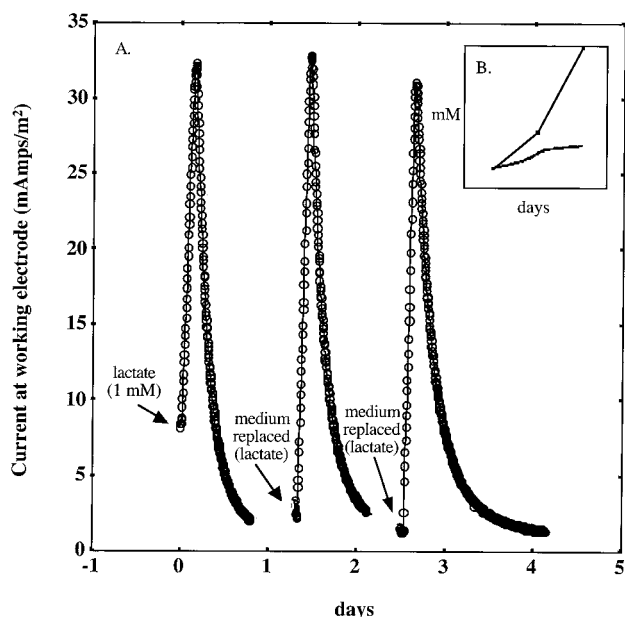

FIG. 3. (A) Continued current production when lactate-depleted medium was replaced with fresh medium and lactate (1 mM) at times designated by the arrows. (B) Number of electrons available from the incomplete oxidation of lactate that were transferred to the electrode surface. The results are the means of triplicate incubations.
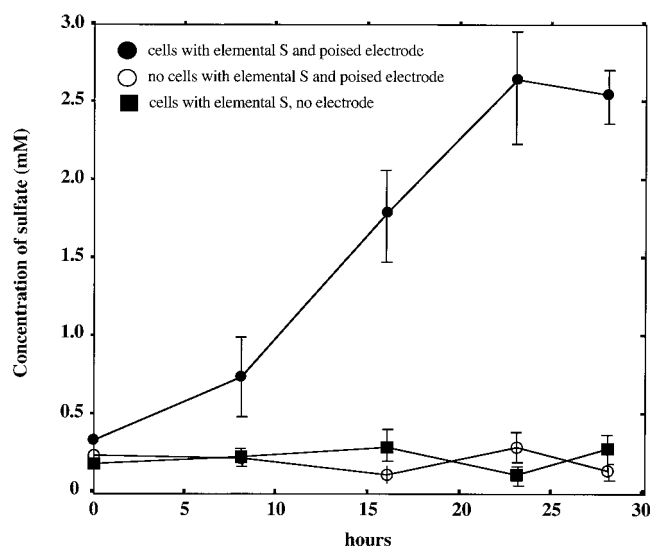

FIG. 4. Sulfate production by *D. propionicus* when elemental S^0 was provided as the electron donor, and an electrode poised at +0.522 V (in reference to a standard H₂ electrode) was the electron acceptor. Sulfate was measured with ion chromatography as previously described (16). The results are the means of triplicate incubations.

pyruvate metabolized was associated with oxidation of pyruvate to acetate coupled to electron transfer to the electrode.

When spent medium from a lactate-grown culture was replaced with fresh medium containing more lactate (1 mM), current production immediately resumed (Fig. 3). This suggested that, as was previously seen with *Geobacter sulfurreducens* (3) and *Rhodoferax ferrireducens* (4), cells of *D. propionicus* attached to the electrode, rather than planktonic cells, were primarily responsible for the current production.

When S^0 (20 g/liter) was added as a potential electron donor for electron transfer to the electrode, sulfate was produced in the presence of *D. propionicus* but not in the absence of cells (Fig. 4). Cells did not produce sulfate in the absence of the electrode.

Implications. The ability of *D. propionicus* to transfer electrons to Fe(III), AQDS, and electrodes has implications for anaerobic respiration in sedimentary environments. Only one other organism, the gram-positive bacterium *Desulfotomaculum reducens*, has been reported to grow with both sulfate and Fe(III) as terminal electron acceptors (27). *D. propionicus* is the first sulfate-reducing organism found to conserve energy to support growth from the reduction of insoluble Fe(III)-oxide, the most abundant form of microbially reducible Fe(III) in most sedimentary environments (12). Microorganisms that can use both sulfate and Fe(III) as electron acceptors may be most competitive at the interface between the zones of Fe(III) reduction and sulfate reduction in aquatic sediments where Fe(III) is still available but is not in sufficient concentrations to inhibit sulfate reduction (15, 18).

The ability of *D. propionicus* to use a graphite electrode as an electron acceptor provides an explanation for the consistent enrichment of closely related organisms in the family *Desulfobulbaceae* on electrodes harvesting electricity from marine sediments. As previously observed with such dissimilatory Fe(III)-reducing microorganisms as species within the family *Geobacteraceae* (2, 3), *Shewanella putrefaciens* (9, 10), *Clostridium butyricum* (23), *Rhodoferax ferrireducens* (4), *Aero-*

monas hydrophila (24), and *Geothrix fermentans* (D. R. Bond and D. R. Lovley, unpublished data). *D. propionicus* did not require the addition of exogenous electron-shuttling compounds for electricity production.

In addition, the finding that *D. propionicus* is able to oxidize elemental S^0 with an electrode serving as the electron acceptor provides further insight into the biological mechanisms involved in current production by the marine sediment fuel cell. It has previously been shown that when Mn(IV) oxides are added to anoxic marine sediments containing sulfides, there is a production of sulfate that requires biological activity (1, 11). Mn(IV) is able to chemically oxidize sulfides to S^0, which is then oxidized by microorganisms to sulfate with the reduction of Mn(IV). When a current-harvesting electrode is placed in anoxic sediments, similar processes are observed. Elemental S^0 is known to precipitate on the anodes of marine sediment fuel cells as the result of abiotic sulfide oxidation at the anode surface (28), and elevated sulfate levels have been measured in sediments closest to the current-harvesting anode (28). These geochemical results coupled with the fact that S^0 oxidation with an electrode serving as the electron acceptor was observed in pure-culture studies of *D. propionicus* suggests that the oxidation of elemental sulfur on the anode surface may be an important biological process in the marine sediment fuel cell.

However, the results also suggest that *Desulfobulbaceae* species are unlikely to play an important role in coupling the oxidation of organic matter to the reduction of the electrode in marine sediment fuel cells. *Desulfobulbaceae* are not known to oxidize acetate, which is likely to be the primary electron donor for electricity production. Rather, *D. propionicus* can only metabolize such organic acids as propionate, lactate, and pyruvate, which are less likely to be important extracellular intermediates in sediments (13). Furthermore, electron transfer to the electrode from the mixed metabolism of organic acids by *D. propionicus* is relatively inefficient; only ca. 25% of the electrons available from the incomplete oxidation of pyruvate, lactate, and propionate were transferred to the electrode surface. In contrast, several *Geobacteraceae* species can oxidize acetate and are able to quantitatively transfer all of the electrons available from the complete oxidation of organic acids to CO_2 to an electrode (2, 3). Therefore, the consistent enrichment of *Desulfobulbaceae* 16S rRNA gene sequences on current-harvesting marine anodes (2, 8, 28) is probably due to their ability to oxidize S^0 on the electrode surface.

In summary, this study provides the first example of a sulfate-reducing organism that can also conserve energy to support growth via electron transfer to insoluble electron acceptors, such as Fe(III) oxide and electrodes. Further studies are warranted to determine whether the mechanisms involved in electron transfer by *D. propionicus* to these extracellular electron acceptors are similar to those in more well-studied Fe(III)-reducing microorganisms.

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