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Dissimilatory Fe(III) Reduction by the Marine Microorganism *Desulfuromonas acetoxidans*

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The ability of the marine microorganism *Desulfuromonas acetoxidans* to reduce Fe(III) was investigated because of its close phylogenetic relationship with the freshwater dissimilatory Fe(III) reducer *Geobacter metallireducens*. Washed cell suspensions of the type strain of *D. acetoxidans* reduced soluble Fe(III)-citrate and Fe(III) complexed with nitriloacetic acid. The *c*-type cytochrome(s) of *D. acetoxidans* was oxidized by Fe(III)-citrate and Mn(IV)-oxalate, as well as by two electron acceptors known to support growth, colloidal sulfur and malate. *D. acetoxidans* grew in defined anoxic, bicarbonate-buffered medium with acetate as the sole electron donor and poorly crystalline Fe(III) or Mn(IV) as the sole electron acceptor. Magnetite (Fe_3O_4) and siderite ($FeCO_3$) were the major end products of Fe(III) reduction, whereas rhodochrosite ($MnCO_3$) was the end product of Mn(IV) reduction. Ethanol, propanol, pyruvate, and butanol also served as electron donors for Fe(III) reduction. In contrast to *D. acetoxidans*, *G. metallireducens* could only grow in freshwater medium and it did not conserve energy to support growth from colloidal S^0 reduction. *D. acetoxidans* is the first marine microorganism shown to conserve energy to support growth by coupling the complete oxidation of organic compounds to the reduction of Fe(III) or Mn(IV). Thus, *D. acetoxidans* provides a model enzymatic mechanism for Fe(III) or Mn(IV) oxidation of organic compounds in marine and estuarine sediments. These findings demonstrate that 16S rRNA phylogenetic analyses can suggest previously unrecognized metabolic capabilities of microorganisms.

Previous studies of microbial Fe(III) reduction have focused primarily on freshwater environments. Studies with pure cultures, enrichment cultures, and natural populations of microorganisms living in freshwater aquatic sediments and aquifers have led to the development of a model for how complex organic matter is oxidized to carbon dioxide with Fe(III) as the electron acceptor (38). In this model, fermentative bacteria metabolize fermentable compounds such as sugars and amino acids to primarily acetate and H_2. The acetate is oxidized to carbon dioxide by Fe(III) reducers with a metabolism similar to that of isolates *Geobacter metallireducens* (41, 47, 52) and strain 172 (40). *Shewanella putrefaciens* (51) and *Pseudomonas* sp. (4) serve as pure culture models for the oxidation of H_2 coupled to Fe(III) reduction.

Oxidation of organic matter coupled to Fe(III) reduction is also an important process in marine and estuarine sediments (1, 15, 26, 30, 45, 59, 61). Although there is the potential for nonenzymatic reduction of Fe(III) by sulfides in marine sediments (8, 28, 58), in many instances enzymatic Fe(III) reduction appears to be the predominant pathway for Fe(III) reduction. For example, geochemical data demonstrate that sulfate reduction is not involved in the reduction of Fe(III) in a wide variety of marine sediments (38). Furthermore, inhibition of sulfate reduction did not inhibit Fe(III) reduction in marine and estuarine sediments or enrichment cultures (15, 46, 59, 67).

If the metabolism of organic matter in marine sediments in which Fe(III) reduction is the predominant terminal electron accepting process is comparable to what has been proposed for similar freshwater environments, then Fe(III)-reducing microorganisms which can oxidize important fermentation products such as acetate and H_2 are likely to play an important role in Fe(III) reduction. Recently, an H_2-oxidizing Fe(III)-reducing microorganism capable of growing at marine and estuarine salinities was recovered from estuarine sediments (13). However, this organism, designated strain BrY, had a limited ability to metabolize organic compounds with Fe(III) as the electron acceptor. It could not oxidize acetate and only incompletely oxidized lactate to acetate and carbon dioxide.

Phylogenetic analysis on the basis of 16S rRNA sequences has indicated that the closest known relative of the freshwater acetate-oxidizing Fe(III) reducer *G. metallireducens* is the marine isolate *Desulfuromonas acetoxidans* (41). *D. acetoxidans* was the first organism found to conserve energy to support growth by oxidizing acetate to carbon dioxide under anoxic conditions (56). It was previously most noted for its ability to couple the oxidation of acetate to the reduction of S^0. However, the results presented here demonstrate that *D. acetoxidans* can also couple the oxidation of acetate to the reduction of Fe(III). Thus, *D. acetoxidans* provides a model for a potentially important mechanism for Fe(III) reduction in marine habitats.

MATERIALS AND METHODS

Source of organisms. The type strain of *D. acetoxidans* (DSM 684) was obtained from the German Collection of Microorganisms, Braunschweig, Germany. The type strain of *G. metallireducens* was cultivated from our laboratory culture collection.

Media and cultivation. Standard anaerobic techniques (5, 32, 54) were used throughout. Gases were passed through a column of hot reduced copper filings to remove traces of oxygen. All transfers and sampling of cultures were performed with syringes and needles that had been flushed with O_2-free gas.

The basal growth medium for *D. acetoxidans* was a

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modification of medium 95 described in the 1989 Catalogue of Strains of the German Collection of Microorganisms and Cell Cultures. The medium contained the following constituents (per liter of distilled water): $NaHCO_3$, 2.0 g; NH_4Cl, 0.3 g; KH_2PO_4, 1.0 g; NaCl, 20 g; yeast extract, 0.5 g; and resazurin, 0.001 g. In some instances, the yeast extract and/or resazurin were omitted. Vitamins and trace minerals were added from stock solutions (42). Prior to inoculation, the following additions were made from sterile, anoxic stocks: $MgCl_2$, 14 mM; $CaCl_2$, 0.68 mM; sodium acetate or ethanol, 10 mM; and, except when noted, Na_2S, 1.5 mM.

When malate was the electron acceptor, it was provided as DL-malic acid at a final concentration of 20 mM. When Fe(III) was the electron acceptor [Fe(III)-oxide medium], an aliquot (equal to 10 to 15% of the basal medium volume) of a slurry of poorly crystalline Fe(III)-oxide was added [final Fe(III) concentration, ca. 100 mmol $liter^{-1}$]. For studies with a soluble Fe(III) form, Fe(III)-citrate (50 mM) was provided. When Mn(IV) was used as the electron acceptor [Mn(IV) oxide medium], an aliquot (5 to 10% of basal medium volume) of a slurry of poorly crystalline MnO_2 was added [final Mn(IV) concentration of ca. 15 mmol $liter^{-1}$]. The poorly crystalline Fe(III)- and Mn(IV)-oxides were synthesized as previously described (44, 47).

Anaerobic pressure tubes (10-ml cultures) or glass serum bottles (100-ml cultures) of medium were bubbled with N_2-CO_2 (80:20) for at least 6 or 16 min, respectively, before being capped with thick butyl rubber stoppers, sealed with an aluminum crimp, and autoclaved for 30 min at 120°C. The pH of the medium after autoclaving was 7.0 to 7.2.

G. metallireducens was cultured in the previously described defined medium (47). The medium contained acetate (20 mM) as an electron donor and poorly crystalline Fe(III)-oxide (ca. 100 mmol/liter), ferric citrate (50 mM), $NaNO_3$ (20 mM), or colloidal S^0 (10 mM) as an electron acceptor. Colloidal S^0 was synthesized as described previously (9).

For both organisms, cultures were initiated with a 10% inoculum and the cells were grown in the dark at 30°C.

Cell suspension experiments. In order to determine whether *D. acetoxidans* could reduce Fe(III), cells grown in acetate-malate medium were collected by centrifugation (7,520 × *g*, 10 min, 4°C) under N_2-CO_2, resuspended in anaerobic marine bicarbonate buffer ($NaHCO_3$, 30 mM; NaCl, 342 mM; $MgCl_2$, 14 mM; and $CaCl_2$, 68 mM, pH 7.1), centrifuged again, and then resuspended in ca. 5 ml of marine bicarbonate buffer. An aliquot (0.4 to 0.75 ml) of the cell suspension was added to anaerobic marine bicarbonate buffer (10 ml) in a serum bottle (25 ml) under N_2-CO_2 in order to provide ca. 0.7 mg of cell protein per bottle. The buffer was amended with 10 mmol of Fe(III) per liter and, in some instances, 10 mM acetate. Fe(III)-nitrilotriacetic acid (NTA) was synthesized by combining equimolar concentrations of NTA and Fe(III)-chloride. The potential for U(VI) reduction was assayed in a similar manner, with the exception that U(VI)-chloride (100 μM) was provided as the electron acceptor. The suspensions were incubated at 30°C and sampled over time for Fe(II) or U(VI) with a syringe and needle.

Cytochrome oxidation studies. *D. acetoxidans* cells grown in acetate-malate media were collected by centrifugation as described above and washed twice before being resuspended in an appropriate amount of buffer to provide a final concentration of 0.04 mg of cell protein per ml in quartz cuvettes. Dithionite-reduced minus air-oxidized spectra were recorded with a double-beam spectrophotometer. To investigate the possible reoxidation of cytochromes by various electron acceptors, cell suspensions reduced by addition of acetate (0.8 mM) were placed in the reference beam of the spectrophotometer, and cell suspensions containing acetate plus 0.8 mM electron acceptor were placed in the sample beam. The cell suspensions in the cuvettes were maintained under N_2-CO_2, and the cuvettes were capped to prevent introduction of air into the samples. For these studies, Mn(IV) was solubilized as an Mn(IV)-oxalate complex by dissolving an appropriate amount of poorly crystalline MnO_2 in 10 ml of anoxic oxalate solution (28 g of ammonium oxalate and 15 g of oxalic acid per liter).

Growth and Fe(III) reduction. Growth experiments with acetate as the electron donor and Fe(III) or Mn(IV) as the potential electron acceptor were conducted in 100-ml cultures of basal medium with either Fe(III)- or Mn(IV)-oxide as the electron acceptor. Other electron donors previously shown to support reduction of S^0 or malate by *D. acetoxidans* (56) were tested at 10 mM for their ability to support reduction of Fe(III)-oxide. Medium (10 ml) with each electron donor was inoculated with 1 ml of a 3-day-old acetate-malate culture. After 2 weeks, the cultures (including the controls without added electron donor) were transferred to media of the same composition, and Fe(II) was monitored over time.

Salt requirement experiments. Different amounts of NaCl, $MgCl_2$, and $CaCl_2$ were added from concentrated sterile anaerobic stocks to tubes of acetate-Fe(III)-oxide medium (which included 0.1 g of KCl per liter) in order to provide salt levels ranging from those in the previously described freshwater growth media (47) to those present in seawater. The media were inoculated with acetate-malate-grown *D. acetoxidans* or acetate-Fe(III)-oxide-grown *G. metallireducens*.

Analytical techniques. Fe(II) was measured as previously described (44) by measuring the accumulation of HCl-soluble Fe(II) over time with Ferrozine. Fe(III) was determined by reducing Fe(III) to Fe(II) with hydroxylamine. Mn(II) and Mn(IV) were determined as described previously (47), with the exception that the formaldoxime colorimetric method (10) rather than atomic absorption spectrophotometry was used for manganese determinations. Sulfide levels were determined with the methylene blue method (18). Acetate concentrations were determined with high-pressure liquid chromatography as previously described (48) with a refractive index detector [Fe(III) growth experiment] or a UV detector set at 210 nm [Mn(IV) growth experiment]. U(VI) was measured under anoxic conditions as previously described (29, 50) with a Kinetic Phosphorescence analyzer (KPA-10; Chemchek Instruments). As previously described (47), cell numbers were counted with epifluorescence microscopy after the particulate iron or manganese forms had been dissolved with acidic oxalate or hydroxylamine solutions, respectively.

RESULTS

Fe(III) reduction in cell suspensions. Washed cell suspensions of *D. acetoxidans* cells rapidly reduced soluble Fe(III) in the presence of acetate, whereas Fe(III) reduction was negligible in the acetate-free controls (Fig. 1A). The rate of Fe(III)-citrate reduction per milligram of *D. acetoxidans* cell protein (0.57 μmol mg^{-1} min^{-1}) compared favorably with the rate of Fe(III) reduction by *G. metallireducens* cells (0.52 μmol mg^{-1} min^{-1} [36]). Cell suspensions did not reduce poorly crystalline Fe(III)-oxide (data not shown). *D. acetoxidans* did not reduce U(VI), even though there was active Fe(III) reduction in acetate-Fe(III)-NTA controls (Fig. 1B).

Cytochrome oxidation studies. Dithionite-reduced minus

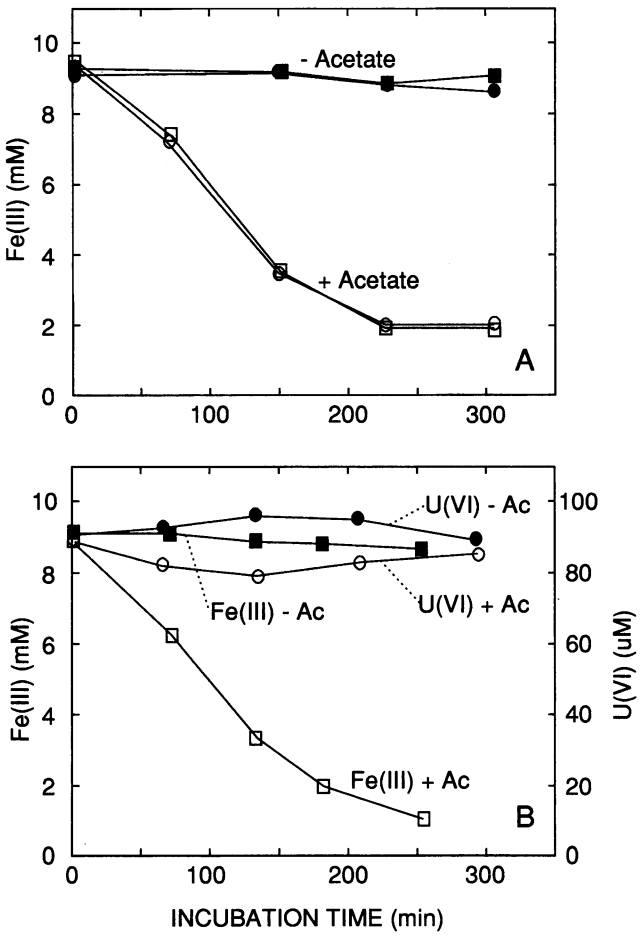

FIG. 1. Fe(III)-citrate (circles) and Fe(III)-NTA (squares) reduction by cell suspensions of *D. acetoxidans* (0.68 mg of cell protein per bottle) in the presence or absence of 10 mM acetate (A) and U(VI) (circles) and Fe(III)-NTA (squares) reduction by cell suspensions of *D. acetoxidans* (0.77 mg of cell protein per bottle) in the presence or absence of 10 mM acetate (Ac) (B).

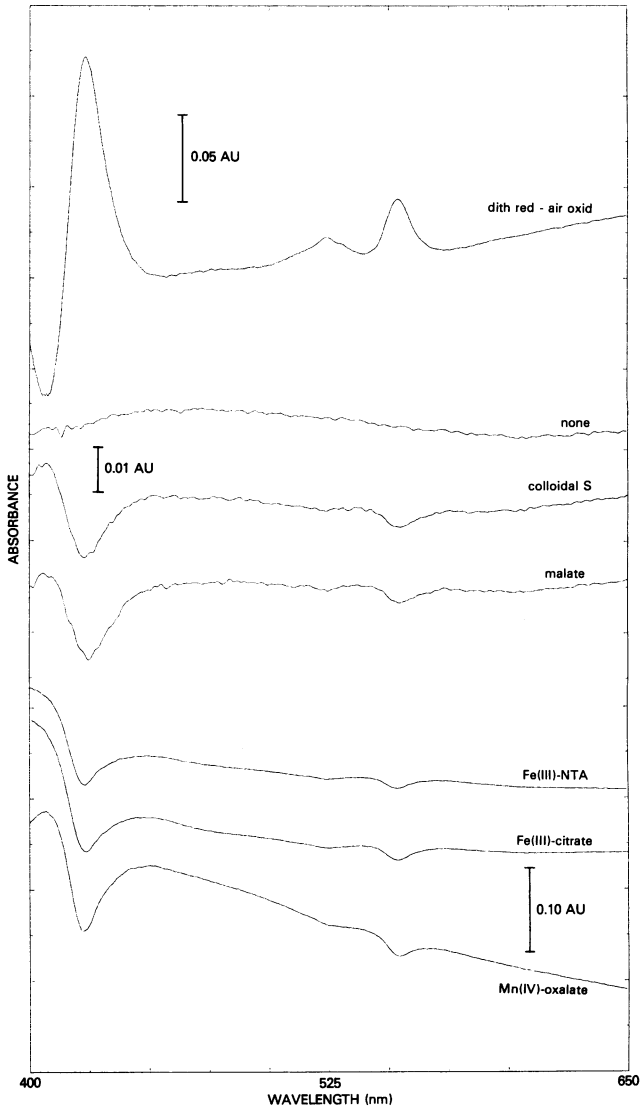

FIG. 2. Difference spectra for whole cell suspensions of *D. acetoxidans*. Dithionite-reduced ([dith red] sample beam) minus air-oxidized ([air oxid] reference beam) difference spectrum is compared with difference spectra of whole cells plus acetate plus the stated electron acceptor (sample beam) minus whole cells plus acetate (reference beam). AU, absorbance units.

air-oxidized difference spectra of acetate-malate-grown *D. acetoxidans* cells indicated the presence of *c*-type cytochrome(s), as has been previously reported (56). *c*-type cytochrome(s) that was reduced in the presence of the physiological electron donor acetate was oxidized when the physiological electron acceptor S^0 or malate was added (Fig. 2B). Fe(III) and Mn(IV) resulted in greater oxidation of the *c*-type cytochrome(s) than with S^0 or malate. This suggested that Fe(III) and Mn(IV) might also serve as a terminal acceptors for electron transport.

Growth of *D. acetoxidans* coupled to Fe(III) or Mn(IV) reduction. There was rapid Fe(III) reduction in preliminary experiments in which ethanol-Fe(III)-oxide medium was inoculated with ethanol-malate-grown *D. acetoxidans* (data not shown). It seemed possible that the addition of the sulfide reductant to Fe(III)-oxide medium could promote an indirect reduction of Fe(III) through a cyclic process in which Fe(III) nonenzymatically oxidized sulfide to form S^0 and Fe(II) and then *D. acetoxidans* rereduced the S^0 to sulfide. Such a recycling process could result in continual reduction of Fe(III) without *D. acetoxidans* enzymatically reducing Fe(III). However, there was no decrease in the rate of Fe(III) reduction with continued repeated transfer of *D. acetoxidans* in medium without the added sulfide. Furthermore, addition of 1.5 mM sulfide did not stimulate the rate of Fe(III) reduction, suggesting that Fe(III) reduction was directly coupled to cell metabolism.

Detailed growth studies were conducted with acetate because, of all the electron donors *D. acetoxidans* is known to utilize, acetate is likely to be the most environmentally significant. When *D. acetoxidans* was inoculated into acetate-Fe(III)-oxide medium, there was a consumption of acetate and concurrent accumulation of Fe(II) as the result of Fe(III) reduction (Fig. 3A). There was an increase in cell numbers during the first few days of incubation, but after the 4th day, cell numbers remained roughly constant, while Fe(II) production and acetate consumption continued. The

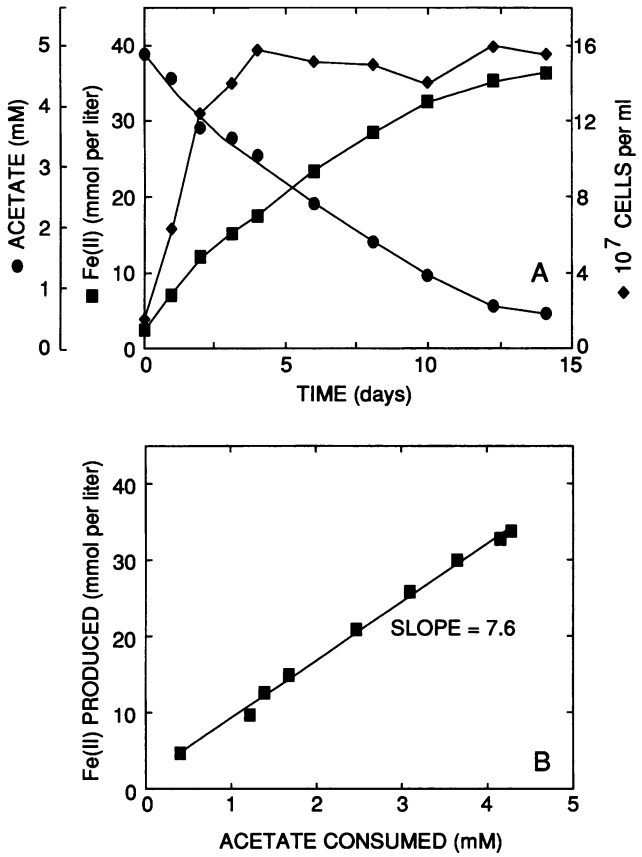

FIG. 3. Concentrations of acetate, cell numbers, and Fe(II) over time in Fe(III)-oxide medium inoculated with *D. acetoxidans* that had been grown in acetate-Fe(III)-oxide medium (A) and Fe(II) production versus acetate consumption for data in panel A (B). The results are the means of duplicate determinations on duplicate cultures.

ratio of Fe(II) production to acetate consumption (7.6) (Fig. 3B) was close to the value of 8 expected from the complete oxidation of acetate coupled to Fe(III) reduction (Table 1, reactions 1 and 2). This suggests that, as previously observed in *G. metallireducens* (47), only a small amount (ca. 5%) of the acetate consumed was incorporated into cellular material. Only ca. 40% of the added Fe(III) was reduced because mixed Fe(II)-Fe(III) solids were the primary product of Fe(III) reduction (see below).

D. acetoxidans reduced Fe(III) in acetate-Fe(III)-citrate medium at a rate comparable to that in acetate-Fe(III)-oxide medium (data not shown). This is in sharp contrast to *G. metallireducens*, which reduces Fe(III)-citrate ca. five times more rapidly than poorly crystalline Fe(III) (47). To test whether or not citrate inhibited the metabolism of *D. acetoxidans*, sodium citrate (50 mM, final concentration) was added to tubes of acetate-malate media before inoculation. Cell growth (as evidenced by turbidity of the media) was much slower in the citrate-containing medium, suggesting that citrate is inhibitory to *D. acetoxidans*. Citrate also inhibits Fe(III) reduction in *Desulfovibrio desulfuricans* (53).

Although the medium used for the acetate-Fe(III) growth experiment contained 0.05% yeast extract, *D. acetoxidans* also grew in acetate-Fe(III) medium without added yeast extract. However, growth was ca. 30% slower in the medium without yeast extract (data not shown). There was no Fe(III) reduction in medium containing yeast extract but not acetate (Fig. 4). In addition to ethanol and acetate, propanol and pyruvate served as electron donors for Fe(III) reduction (Fig. 4). Butanol alone did not stimulate Fe(III) reduction, but butanol combined with succinate supported Fe(III) reduction at a rate comparable to that occurring with acetate or pyruvate. This result is similar to the previously reported requirement of succinate as a carbon source to support butanol reduction of S^0 (56).

D. acetoxidans also grew in acetate-Mn(IV)-oxide medium that did not contain yeast extract (Fig. 5A). The increase in cell numbers was associated with acetate consumption and Mn(II) production. As previously observed with growth of *G. metallireducens* on Mn(IV) (47), a white $MnCO_3$ precipitate (rhodochrosite) was formed during Mn(IV) reduction. The ratio of Mn(II) production to acetate consumption (3.4) (Fig. 5B) is consistent with the stoichiometry expected for acetate oxidation to carbon dioxide with Mn(IV) as the electron acceptor (Table 1, reaction 3), assuming that some acetate was incorporated into cell material. There was no cell growth or acetate consumption in medium without Mn(IV).

Magnetite and siderite formation. Less than 3% of the Fe(II) that was produced from Fe(III) reduction was present

TABLE 1. Stoichiometry and free energy of reactions related to the metabolism of *D. acetoxidans*

Reaction no.	Reactants	Products	$\Delta G'$ (kJ/reaction)	
			Standard[a]	Culture[b]
1	$CH_3COO^- + 8Fe(OH)_3(s) + 6HCO_3^- + 7H^+$	$8FeCO_3(s) + 20H_2O$	−316	−247
2	$CH_3COO^- + 24Fe(OH)_3(s)$	$8Fe_3O_4(s) + 2HCO_3^- + 36H_2O + H^+$	−707	−710
3	$CH_3COO^- + 4MnO_2(s) + 2HCO_3^- + 3H^+$	$4MnCO_3(s) + 4H_2O$	−738	−705
4	$CH_3COO^- + 4S^0(s) + 4H_2O$	$2HS^- + 2H_2S + 2HCO_3^- + 3H^+$	−7	−62[c]
5	$CH_3COO^- + 4$ malate^{2-}	4 Succinate^{2-} + $2HCO_3^- + H^+$	−225	−228[d]
6	$S^0(s) + 6Fe(OH)_3(s) + 6HCO_3^- + 4H^+$	$6FeCO_3(s) + SO_4^{-2} + 14H_2O$	−191	−155[e]
7	$S^0 + 3MnO_2(s) + 3HCO_3^- + H^+$	$3MnCO_3(s) + SO_4^{-2} + 2H_2O$	−507	−499[e]

[a] Free energy calculated from the standard free energies of formation of the products and reactants (64, 65) and by assuming standard conditions except for pH 7.

[b] Free energy calculated from the standard free energies of formation of the products and reactants (pH 7) and by assuming the following substrate concentrations after 50% of an initial 5×10^{-3} mol of acetate per liter of culture medium had been oxidized: acetate, 2.5×10^{-3} M; HCO_3^-, 29×10^{-3} M. The initial HCO_3^- concentration in the culture medium was assumed to be 24×10^{-3} M.

[c] HS^- and H_2S concentrations were assumed to be 5.75×10^{-3} M (the initial sulfide concentration in reduced media was 1.5×10^{-3} M).

[d] Malate^{2-} and succinate^{2-} concentrations were assumed to be 10×10^{-3} M (the initial malate concentration was assumed to be 20×10^{-3} M).

[e] HCO_3^- and SO_4^{2-} were assumed to be 25×10^{-3} and 4×10^{-4} M, respectively (the initial concentrations in the medium).

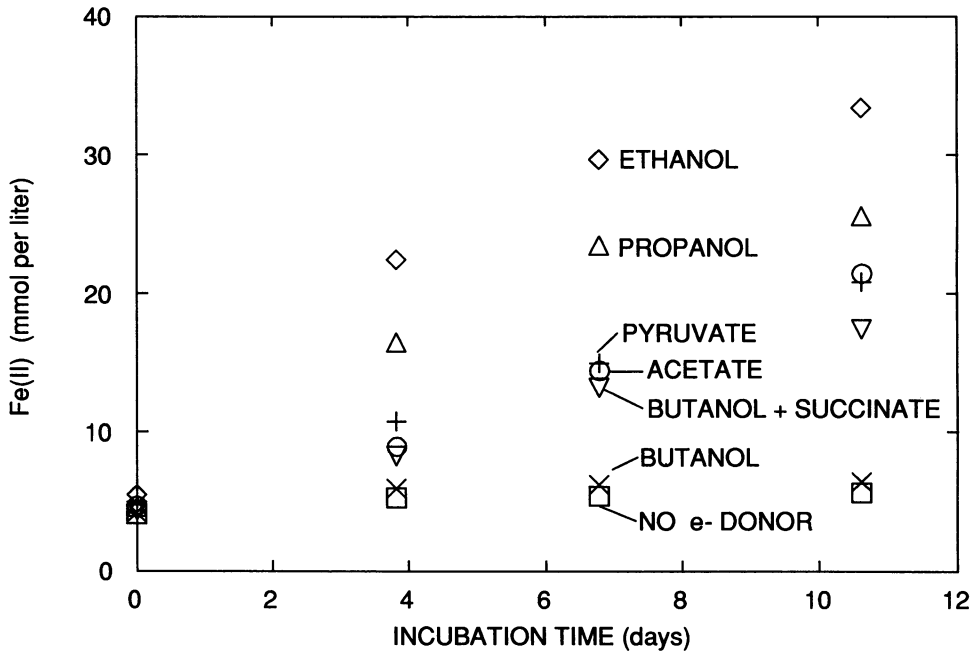

FIG. 4. Fe(II) production by *D. acetoxidans* in Fe(III)-oxide media with various potential electron donors and/or carbon sources. The results are the means of duplicate determinations on duplicate cultures for each treatment.

in a form that would pass through a 0.2-μm-pore-diameter filter prior to acidification. This indicated that most of the Fe(II) products of Fe(III) reduction were predominantly insoluble Fe(II) phases. This is consistent with the observation that Fe(III) reduction did not dissolve the Fe(III)-oxide but rather converted it to a black, magnetic precipitate (Fig. 6). X-ray diffraction analysis indicated that the precipitate contained magnetite (Fe_3O_4) as well as siderite ($FeCO_3$). This is similar to the production of fine-grained magnetite and siderite that is observed when *G. metallireducens* is grown in a bicarbonate-buffered medium (62).

Salt requirement. *D. acetoxidans* reduced Fe(III) in acetate-Fe(III)-oxide medium with salt levels that were 25% of those in seawater (i.e., with 0.6% NaCl and 0.3% $MgCl_2$) (Fig. 7). *D. acetoxidans* also grew in acetate-malate over the same range of salinities (data not shown). The minimum salt requirement for growth of *D. acetoxidans* found in this study is 2.5-fold lower than previously reported (56). *G. metallireducens* reduced Fe(III) only in the freshwater medium.

Colloidal S^0 metabolism. In order to determine whether *D. acetoxidans* could oxidize S^0 with the reduction of Fe(III) or Mn(IV), acetate-malate-grown *D. acetoxidans* was inoculated into medium containing colloidal S^0 as the potential electron donor, Fe(III)- or Mn(IV)-oxide as the potential electron acceptor, and 1 mM acetate as a potential carbon source. In cultures with Fe(III), a small amount (5 mmol/liter) of Fe(II) was produced within 3 days. Although Mn(II) was not measured, a small amount of Mn(IV) reduction was visually apparent in the medium with Mn(IV). However, the reduction of Fe(III) and Mn(IV) was not the result of S^0 oxidation, as there was no sulfate formation over a period of 3 weeks. The Fe(III) and Mn(IV) reduction presumably resulted from *D. acetoxidans* oxidizing the small quantity of acetate that was present.

When acetate-colloidal S^0 medium was inoculated with Fe(III)-citrate-grown *G. metallireducens* cells, formation of a black ferrous-sulfide precipitate was accompanied by consumption of acetate (Fig. 8). However, only ca. 20% of the initial 10 mM colloidal S^0 was reduced, and there was no increase in cell numbers. A similar rate of S^0 reduction was observed in a similar experiment with an acetate-NO_3^--grown inoculum (data not shown). In contrast, under similar cultural conditions *D. acetoxidans* reduced 10 mM S^0 within several days.

DISCUSSION

The results demonstrate that *D. acetoxidans* can obtain energy for growth by coupling the oxidation of acetate to the dissimilatory reduction of Fe(III) or Mn(IV). This is the first description of a marine microorganism capable of such metabolism. The only other organisms known to carry out a similar metabolism are the freshwater organisms *G. metallireducens* (41, 47, 52) and strain 172 (40). Preliminary experiments have indicated that a freshwater strain of *Desulfuromonas*, *D. acetexigens* (DSM 1397), can also grow by coupling the oxidation of acetate to the reduction of Fe(III) (36).

Energetics and physiology of Fe(III) and Mn(IV) reduction by *D. acetoxidans*. Calculating the potential energy yield from Fe(III) reduction is complicated by the fact that both Fe(III) and Fe(II) can potentially exist in many poorly identified or quantified forms (47). The form of Fe(III) that microorganisms actually reduce and the initial forms of the Fe(II) products are unknown. For example, it is assumed that ionic Fe(II) is the initial product of Fe(III) reduction and that the dissolved Fe(II) then combines with Fe(III) or bicarbonate to form magnetite or siderite (37). However, the actual mechanisms for magnetite and siderite formation have not been elucidated. Thus, for purposes of discussion, thermodynamic calculations (Table 1) were based on the forms of

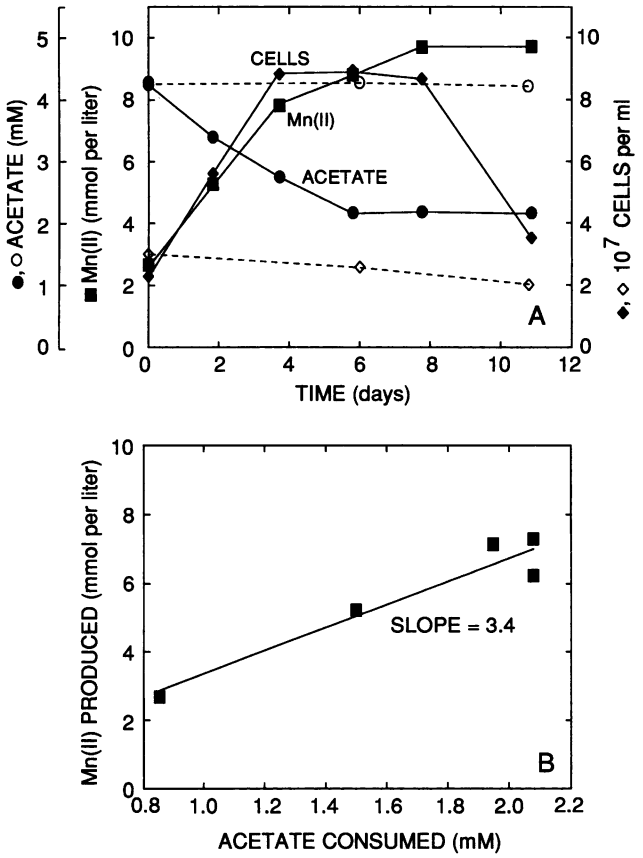

FIG. 5. Concentrations of acetate (circles), cell numbers (diamonds), and Mn(II) (squares) over time in Mn(IV) medium inoculated with *D. acetoxidans* that had been grown in acetate-malate medium (A). Solid symbols, medium with both acetate and Mn(IV), open symbols, medium with acetate only. (B) Mn(II) production versus acetate consumption for data in panel A. The results are the means of duplicate determinations on duplicate cultures.

Fe(III) and Mn(IV) that were added to the medium and the predominant Fe(II) and Mn(II) products.

These calculations indicate that the potential energy yield from acetate oxidation coupled to Fe(III) or Mn(IV) reduction (Table 1, reactions 1 through 3) is greater than that from acetate oxidation coupled to other electron acceptors such as S^0 and malate (reactions 4 and 5) which were previously shown to support the growth of *D. acetoxidans* (56). Thus, given *D. acetoxidans*' ability to reduce Fe(III) and Mn(IV), it is not surprising that it can conserve energy to support growth from this metabolism. However, the growth rates on Fe(III) and Mn(IV) reported here are significantly slower than with S^0 (56). A possible explanation for this is that although both S^0 and Fe(III) are considered to be insoluble, in the presence of sulfide, S^0 is solubilized as polysulfides which may be the direct electron acceptors (9, 14).

D. acetoxidans has several similarities with other organisms which conserve energy to support growth from dissimilatory Fe(III) and Mn(IV) reduction. *c*-type cytochrome(s) appears to be involved in electron transport to Fe(III) and Mn(IV) in *G. metallireducens* (41) and possibly in *S. putrefaciens* (3, 23, 55). Recent studies have demonstrated that cytochrome c_3 can function as a terminal Fe(III) and U(VI) reductase in *Desulfovibrio vulgaris* (36). *D. acetoxidans* has

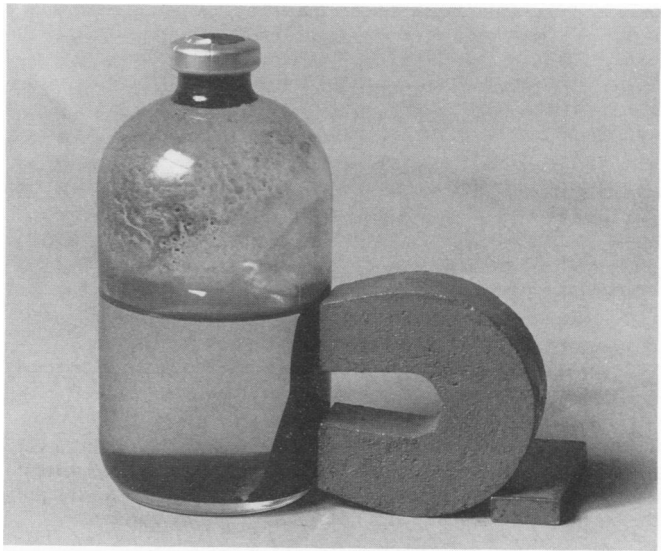

FIG. 6. Magnetite accumulation in an acetate-Fe(III)-oxide culture of *D. acetoxidans*.

a triheme c_7 cytochrome which bears some similarities to the tetraheme c_3 cytochrome of *D. vulgaris* (24, 57). Whether *c*-type cytochromes are the metal reductase in *D. acetoxidans* and other dissimilatory metal reducers is yet to be determined. Further investigation into the mechanisms of metal reduction in *D. acetoxidans* may explain why this organism does not reduce U(VI) whereas all other respiratory Fe(III)-reducing microorganisms that have been evaluated do reduce U(VI) (13, 49, 50).

Another similarity between *D. acetoxidans* and *G. metallireducens* is that both oxidize acetate to carbon dioxide via the citric acid cycle (16, 27), whereas most other strictly anaerobic organisms that oxidize acetate do not use this pathway (66). *D. acetoxidans*, *G. metallireducens*, and sulfate reducers are also the only gram-negative, obligately

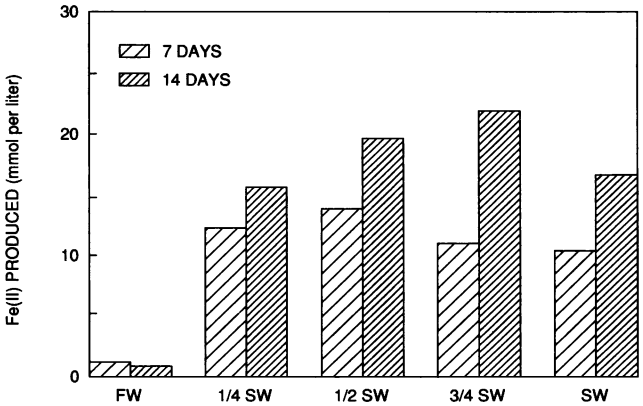

FIG. 7. Production of Fe(II) by *D. acetoxidans* in acetate-Fe(III)-oxide medium with different levels of NaCl, $MgCl_2 \cdot 6H_2O$, and $CaCl_2 \cdot 2H_2O$. Seawater (SW) levels of these salts were 23.4, 10.6, and 1.5 g per liter, respectively. The freshwater (FW) medium contained no added NaCl, $MgCl_2 \cdot 6H_2O$, or $CaCl_2 \cdot 2H_2O$. The results are the means of duplicate determinations on duplicate cultures.

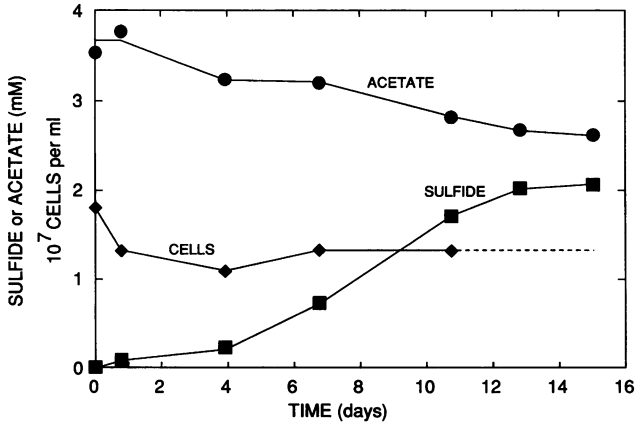

FIG. 8. Concentrations of acetate, cell numbers, and sulfide over time in colloidal S^0 medium inoculated with *G. metallireducens* that had been grown in acetate-Fe(III)-citrate medium. The results are the means of duplicate determinations on duplicate cultures.

anaerobic respiratory microorganisms known to possess menaquinone as an electron carrier (21, 41).

D. acetoxidans and *G. metallireducens* are significantly different in other aspects of their physiology. *G. metallireducens* does not reduce sublimed S^0 powder (47) and only slowly couples the oxidation of acetate to the reduction of colloidal S^0. *G. metallireducens* does not appear to conserve energy to support growth from S^0 reduction, whereas *D. acetoxidans* grows rapidly by the same reaction (56). Another contrast is that *D. acetoxidans* cannot use nitrate as a terminal electron acceptor (56) and *G. metallireducens* readily grows via nitrate reduction to ammonia (47). *D. acetoxidans* and *G. metallireducens* are similar in that both use acetate, ethanol, and propanol as electron donors for Fe(III) reduction. However, *G. metallireducens* can oxidize a variety of monoaromatic compounds to carbon dioxide with Fe(III) as the electron acceptor (39, 43), but *D. acetoxidans* cannot.

Biogeochemical significance. Fe(III) reduction may be a quantitatively important pathway for organic matter oxidation in some continental shelf (1, 30) and tropical estuarine sediments (31). Furthermore, geochemical data indicate that there are extensive zones in pelagic marine sediments in which Fe(III) or Mn(IV) reduction are the predominant terminal electron accepting processes (6, 19, 20, 22, 26, 68). In freshwater sediments in which Fe(III) reduction is the predominant terminal electron accepting process, acetate is the major fermentation product, as it is in sulfate-reducing and methanogenic freshwater sediments (48). Acetate is also the major fermentation product in sulfate-reducing marine sediments (17, 60). It seems likely then that acetate is also a major intermediate in organic carbon mineralization in marine sediments in which Fe(III) or Mn(IV) reduction is the predominant terminal electron accepting process. Thus, organisms with a metabolism like that of *D. acetoxidans* could play a significant role in organic carbon diagenesis in such sediments. Given the wide range of salinities over which *D. acetoxidans* can reduce Fe(III), this organism could potentially mediate organic carbon oxidation coupled to Fe(III) reduction in a broad range of coastal and estuarine environments.

The formation of ultrafine-grained magnetite in the sediment layer in which Fe(III) reduction is the predominant terminal electron accepting process may make a significant contribution to the magnetic remanence of some pelagic marine sediments (34). It has been suggested that magnetite formation in anaerobic marine sediments could result from the activity of magnetotactic bacteria (7, 34). However, magnetite formation during dissimilatory Fe(III) reduction is an alternative explanation (37, 52). The magnetite formation as the result of Fe(III) reduction by *D. acetoxidans* provides a potential model for such magnetite accumulations. Comparisons of the morphologies of magnetite found in marine sediments with that formed by pure cultures of dissimilatory Fe(III)-reducing and magnetotactic bacteria are needed to distinguish the mechanism for magnetite formation in these sediments (37, 63).

Both Fe(III) and Mn(IV) react chemically with sulfide to form S^0 (8, 12). Thus, S^0 and Fe(III) or Mn(IV) are likely to coexist in anaerobic sediments where sulfide diffusing in from the sulfate reduction zone meets with Fe(III) or Mn(IV). In fact, the highest levels of S^0 in marine sediments may be within the Fe(III) and Mn(IV) reduction zones (61). The ability of *D. acetoxidans* to utilize both (i) S^0 and (ii) Fe(III) and Mn(IV) as electron acceptors for organic carbon oxidation makes it particularly well suited for life in this type of environment.

In anoxic marine sediments, reduced sulfur compounds are oxidized to sulfate by unknown mechanisms (2, 25, 33, 35). Oxidation of S^0 with either Fe(III) or Mn(IV) is thermodynamically favorable (Table 1, reactions 6 and 7). Although *Thiobacillus thiooxidans*, *Thiobacillus ferrooxidans*, and *Sulfolobus acidocaldarius* are capable of oxidizing S^0 with the reduction of Fe(III) under acidic conditions (11), no microorganisms have been reported to catalyze this reaction at circumneutral pH. However, metabolic inhibitors do inhibit the Mn(IV)-dependent oxidation of S^0 to sulfate in marine sediments (1). Because *D. acetoxidans* contains enzymes for metabolizing sulfur as well as Fe(III) and Mn(IV), it was of interest to determine whether it could couple S^0 oxidation to Fe(III) or Mn(IV) reduction. However, *D. acetoxidans* did not oxidize S^0 to sulfate under the conditions employed.

In summary, the studies presented here demonstrate that the marine strain of *D. acetoxidans* is capable of dissimilatory Fe(III) and Mn(IV) reduction. Acetate oxidation coupled to Fe(III) and Mn(IV) reduction is likely to be an important component in the pathways for the oxidation of organic matter in the Fe(III)- and Mn(IV)-reducing zones of marine sediments. *D. acetoxidans* was evaluated for its potential to reduce Fe(III) because of its close phylogenetic relationship with *G. metallireducens*. These results emphasize the potential utility of phylogenetic analyses in suggesting novel metabolic capabilities in organisms previously isolated for other properties. However, more direct studies on the microbial communities in marine sediments in which Fe(III) or Mn(IV) reduction is the predominant terminal electron accepting process are required in order to fully understand the processes and microorganisms involved in Fe(III) and Mn(IV) reduction in marine sediments.

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