Hydrogen and Formate Oxidation Coupled to Dissimilatory Reduction of Iron or Manganese by Alteromonas putrefaciens

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The ability of Alteromonas putrefaciens to obtain energy for growth by coupling the oxidation of various electron donors to dissimilatory Fe(III) or Mn(IV) reduction was investigated. A. putrefaciens grew with hydrogen, formate, lactate, or pyruvate as the sole electron donor and Fe(III) as the sole electron acceptor. Lactate and pyruvate were oxidized to acetate, which was not metabolized further. With Fe(III) as the electron acceptor, A. putrefaciens had a high affinity for hydrogen and formate and metabolized hydrogen at partial pressures that were 25-fold lower than those of hydrogen that can be metabolized by pure cultures of sulfate reducers or methanogens. The electron donors for Fe(III) reduction also supported Mn(IV) reduction. The electron donors for Fe(III) and Mn(IV) reduction and the inability of A. putrefaciens to completely oxidize multicarbon substrates to carbon dioxide distinguish A. putrefaciens from GS-15, the only other organism that is known to obtain energy for growth by coupling the oxidation of organic compounds to the reduction of Fe(III) or Mn(IV). The ability of A. putrefaciens to reduce large quantities of Fe(III) and to grow in a defined medium distinguishes it from a Pseudomonas sp., which is the only other known hydrogen-oxidizing, Fe(III)-reducing microorganism. Furthermore, A. putrefaciens is the first organism that is known to grow with hydrogen as the electron donor and Mn(IV) as the electron acceptor and is the first organism that is known to couple the oxidation of formate to the reduction of Fe(III) or Mn(IV). Thus, A. putrefaciens provides a much needed microbial model for key reactions in the oxidation of sediment organic matter coupled to Fe(III) and Mn(IV) reduction.

Fe(III) is an important oxidant of natural and contaminant organic compounds in surface and subsurface aquatic sediments (1, 3, 4, 18, 20, 30). Furthermore, the reduction of Fe(III) and Mn(IV) can influence the inorganic geochemistry of sedimentary environments by greatly increasing the dissolved concentrations of iron, manganese, trace metals, and phosphate (6, 10). Microorganisms appear to catalyze most of the organic matter oxidation coupled to Fe(III) reduction in anaerobic sediments (14, 16, 33). However, the microbiology of Fe(III) reduction has received little attention.

When the studies reported here were initiated, only two microorganisms were definitely known to obtain energy for growth from dissimilatory Fe(III) reduction. A facultatively anaerobic rod, identified as a Pseudomonas sp., grows with hydrogen as the electron donor and Fe(III) as the electron acceptor (5). GS-15, an obligately anaerobic, gram-negative rod, oxidizes simple organic compounds such as acetate, butyrate, and ethanol to carbon dioxide with Fe(III) or Mn(IV) as the sole electron acceptor (23, 24). The metabolism of these two organisms differs significantly from that of other anaerobic Fe(III)-reducing microorganisms, which gain little or no energy from Fe(III) reduction and have primarily a fermentative metabolism (9, 14, 15, 18, 26, 31).

Obuekwe and co-workers (28, 29) found that cell suspensions of aerobically grown cells of Alteromonas putrefaciens (Pseudomonas sp. strain 200) reduced Fe(III) and Mn(IV) under anaerobic conditions, with lactate as a potential electron donor. Respiratory inhibitors inhibited Fe(III) reduction in anaerobic suspensions of aerobically grown cells, which suggested that Fe(III) reduction was linked to electron transport (2, 29). Subsequent investigation demonstrated that cell suspensions of aerobically grown cultures of other A. putrefaciens strains could also reduce Fe(III) (32). It was not demonstrated in any of these studies whether A. putrefaciens could obtain energy for growth from Fe(III) reduction.

In a more recent study, growth of A. putrefaciens was shown to be dependent on Mn(IV) reduction in liquid anaerobic media that contained Mn(IV) as the sole electron acceptor (27). Evidence suggestive of growth with Fe(III) as the electron acceptor was the formation of colonies on agar plates that contained Fe(III), with no growth observed in the absence of Fe(III) or alternative electron acceptors (27). The electron donors for Mn(IV) or Fe(III) reduction were not defined.

The purpose of the study reported here was to investigate the ability of A. putrefaciens to couple the oxidation of potential electron donors to the reduction of Fe(III) and Mn(IV). The results demonstrate that the metabolism of multicarbon carbon substrates by A. putrefaciens is probably of little environmental significance, because the substrates that can be metabolized at rapid rates are not important extracellular intermediates in organic matter decomposition. However, results of this study also revealed that A. putrefaciens can couple the oxidation of two potentially important intermediates, hydrogen and formate, to the reduction of Fe(III) or Mn(IV). These results have been incorporated into a conceptual model that demonstrates how the cooperative efforts of a microbial food chain might completely oxidize fermentable organic matter to carbon dioxide with Fe(III) or Mn(IV) as the sole electron acceptor.

MATERIALS AND METHODS

Source of organisms. The type strain of A. putrefaciens (ATCC 8071) was obtained from the American Type Culture Collection (Rockville, Md.). A. putrefaciens MR-1, which

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was isolated from sediments of Oneida Lake, N.Y. (27), was provided by Charles Myers (University of Wisconsin-Milwaukee, Milwaukee, Wis.). Although most of the detailed studies on the stoichiometry of substrate metabolism were conducted with the type strain (ATCC 8071), strain MR-1 used the same electron donors for Fe(III) and Mn(IV) reduction and in every instance appeared to have a metabolism identical to that of the type strain.

**Media.** The basic medium used in this study was a slight modification of the previously described medium devised for the culture of the dissimilatory Fe(III)-reducing organism GS-15 (23). In addition to vitamins and trace elements, the medium contained the following constituents (in grams per 900 ml of deionized water): NaHCO₃, 2.5; KCl, 0.1; NH₄Cl, 1.5; NaH₂PO₄ · H₂O, 0.6; L-glutamine, 0.02; and DL-serine, 0.04. *A. putrefaciens* did not grow if the amino acids were omitted. Fe(III) was provided either as Fe(III) citrate (final concentration, 50 mM) or amorphous Fe(III) oxide (final concentration, 200 mmol of Fe(III) per liter) (23). Synthetic MnO₂ was prepared as described previously (23) and added to provide 10 to 30 mmol of Mn(IV) per liter.

The medium was dispensed in 9-ml amounts in anaerobic pressure tubes or in 100-ml quantities in 160-ml serum bottles. The medium was bubbled for at least 5 (pressure tubes) or 15 (serum bottles) min with N₂-CO₂ (80:20) to remove dissolved oxygen. The pH of the autoclaved medium was ca. 6.8. Electron donors and Fe(III) citrate were added from anaerobic stock solutions (100 mM) to provide an initial concentration of 10 mM unless otherwise noted. In the stoichiometry experiments summarized in Fig. 1 and 6, lactate or formate was added to the medium described above along with an additional 100 ml of water before the medium was dispensed, flushed, and autoclaved. Hydrogen was added to provide an initial hydrogen partial pressure of 60 kPa, unless otherwise noted.

**Time course and stoichiometry studies.** Incubations were done at 30°C in the dark. For growth on hydrogen the culture vessels were incubated in a horizontal position with gentle shaking on a wrist-action shaker. All other cultures were incubated statically. Cultures were inoculated with a 3 or 10% inoculum of a culture that was grown in an identical medium or, in the case of studies to determine the ability of *A. putrefaciens* to use electron donors other than lactate or hydrogen, with a culture that was grown with hydrogen as the electron donor. Samples were withdrawn anaerobically with a syringe and needle and were analyzed as outlined below.

![FIG. 1. Growth of *A. putrefaciens* ATCC 8071 in medium with lactate as the electron donor and Fe(III) citrate as the electron acceptor.](http://aem.asm.org/)

The minimum threshold to which hydrogen could be metabolized was determined by growing *A. putrefaciens* under a hydrogen atmosphere until a dense culture was generated. The headspace was flushed with N₂-CO₂ (80:20) until the hydrogen partial pressure decreased 2 to 5 Pa. Uptake of this remaining hydrogen down to the minimum threshold was monitored over time.

**Analytical techniques.** Cell counts were made by using a modification of the epifluorescence microscopy method (12) described previously (23).

Fe(III) reduction was monitored by measuring the accumulation of Fe(II) over time. As described previously in detail (20, 21), the amount of Fe(II) that was soluble after a 15-min extraction in 0.5 N HCl was determined with ferrozine. Mn(V) reduction was monitored by measuring the accumulation of Mn(II) over time as described previously (23). Briefly, the initial Mn(II) concentration was determined by taking subsamples (0.1 ml) from the tubes and determining the concentration of HCl-soluble manganese after a 10-min extraction in 0.5 N HCl. For subsequent time points, concentrated HCl was added to replicate culture tubes to give a final HCl concentration of 0.5 N, and HCl-soluble manganese was determined after a 10-min extraction. HCI-

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### TABLE 1. Stoichiometry and standard free energy of Fe(III) and Mn(IV) reduction reactions relevant to the metabolism of *A. putrefaciens*

<table>
<thead>
<tr>
<th>Reaction no.</th>
<th>Reactants</th>
<th>Products</th>
<th>ΔG° (kJ/reaction)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Lactate + 2H₂O + 4Fe⁴⁺</td>
<td>Acetate + HCO₃⁻ + 5H⁺ + 4Fe⁴⁺</td>
<td>−461</td>
</tr>
<tr>
<td>2</td>
<td>Lactate + H⁺ + HCO₃⁻ + 2MnO₂</td>
<td>Acetate + 2H₂O + 2MnCO₃</td>
<td>−425</td>
</tr>
<tr>
<td>3</td>
<td>Pyruvate + 2H₂O + 2Fe⁺⁺</td>
<td>Acetate + HCO₃⁻ + 3H⁺ + 2Fe⁺⁺</td>
<td>−276</td>
</tr>
<tr>
<td>4</td>
<td>Pyruvate + MnO₂</td>
<td>Acetate + MnCO₃</td>
<td>−258</td>
</tr>
<tr>
<td>5</td>
<td>H₂ + 2Fe⁺⁺</td>
<td>2H⁺ + 2Fe⁺⁺</td>
<td>−228</td>
</tr>
<tr>
<td>6</td>
<td>H₂ + H⁺ + HCO₃⁻ + MnO₂</td>
<td>2H₂O + MnCO₃</td>
<td>−211</td>
</tr>
<tr>
<td>7</td>
<td>Formate⁻ + H₂O + 2Fe⁺⁺</td>
<td>HCO₃⁻ + 2H⁺ + 2Fe⁺⁺</td>
<td>−227</td>
</tr>
<tr>
<td>8</td>
<td>Formate⁻ + H⁺ + MnO₂</td>
<td>H₂O + MnCO₃</td>
<td>−209</td>
</tr>
</tbody>
</table>

*Free energy is for pH 7 but otherwise standard conditions. Calculations are based on values for lactate, pyruvate, and formate metabolism to hydrogen (36); hydrogen oxidation coupled to Fe⁺⁺ reduction (36); and the free energy of reaction 6, which was calculated from the standard free energy of formation of the reactants and products (35). Mn(II) is shown as MnCO₃ because this was the predominant form of Mn(II) in the cultures (23). Fe(III) and Fe(II) were probably in various forms (23) and are given here as the ions for simplicity.
soluble manganese was defined as the manganese in the HCl extract that passed through a 0.2-μm-pore-size filter (Nuclepore Corp., Pleasanton, Calif.). Manganese was determined with an atomic absorption spectrophotometer with an acetylene flame.

Lactate, formate, and acetate were analyzed by liquid chromatography. Samples were filtered (pore size, 0.45 μm; Gelman Sciences, Inc., Ann Arbor, Mich.), diluted 25-fold in water, and injected onto a column (Dionex HPICE-AS1). The eluant was 1 mM octane sulfonic acid, and it was passed through at a flow rate of 0.8 ml/min. The separated acids were passed through a suppressor (Anion MicroMembrane-ICE) and then quantified with a conductivity detector.

Hydrogen was measured with a reduction gas analyzer (Trace Analytical). Gases were separated on a 0.5-m column of Carboxisieve II (Supelco, Inc.) that was run at room temperature with N2 as the carrier gas.

RESULTS

Metabolism of lactate and pyruvate. A. putrefaciens grew rapidly in anaerobic medium with lactate as the electron donor and Fe(III) citrate (Fig. 1) or amorphous Fe(III) oxide (data not shown) as the sole electron acceptor. Growth was accompanied by the metabolism of lactate to acetate and the concomitant reduction of Fe(III) to Fe(II) (Fig. 1). Growth stopped when lactate or Fe(III) was depleted. Lactate was metabolized to acetate and, presumably, carbon dioxide. The metabolism of lactate was consistent with reaction 1 (Table 1), in which 1 mol of acetate was produced and 4 mol of Fe(III) was reduced for each 1 mol of lactate that was consumed. Thermodynamic calculations indicated that this reaction can be expected to support growth, as it is highly exergonic under standard conditions (Table 1).

A. putrefaciens also oxidized lactate to acetate with Mn(IV) as the electron acceptor (Fig. 2). In accordance with reaction 2 (Table 1), 1 mol of acetate was produced for each 1 mol of lactate that was consumed, and this resulted in the reduction of 2 mol of Mn(IV) to Mn(II).

Pyruvate could also serve as an electron donor for Fe(III) reduction (Fig. 3). Pyruvate was not quantified because medium constituents interfered with the chromatographic analysis. The accumulation of Fe(II) and acetate was consistent with the stoichiometry of reaction 3 (Table 1). There was no reduction of Fe(III) by pyruvate in unoinoculated controls.

The ability of A. putrefaciens to couple the oxidation of pyruvate to Mn(IV) reduction (Table 1, reaction 4) was difficult to determine because pyruvate chemically reduced Mn(IV), as has been reported previously (34). This was most evident under the acidic conditions of the Mn(II) assay in which 10 mM Mn(II) was immediately recovered from uninoculated Mn(IV)-medium, which initially contained 10 mM pyruvate. However, there was a more rapid loss of the brown MnO2 precipitate and faster and more extensive formation of white rhodochrosite (MnCO3) crystals in medium containing A. putrefaciens than in uninoculated medium, and more acetate was formed in inoculated medium (Table 2). Thus, although Mn(II) production could not be monitored in the medium containing pyruvate, there was qualitative evidence that A. putrefaciens catalyzed the reduction of Mn(IV) with pyruvate faster than it proceeded under abiotic conditions.

Oxidation of hydrogen and formate. A. putrefaciens oxidized hydrogen with the reduction of Fe(III), and this metabolism was associated with cell growth (Table 3 and

![FIG. 2. Metabolism of lactate by A. putrefaciens ATCC 8071 in medium with lactate as the electron donor and MnO2 as the electron acceptor.](image)

![FIG. 3. Fe(III) reduction and acetate production by A. putrefaciens ATCC 8071 with pyruvate as the electron donor and Fe(III) citrate as the electron acceptor. AC designates the amount of acetate that accumulated in the medium after 15 days of incubation.](image)

<table>
<thead>
<tr>
<th>Electron donor</th>
<th>Inoculated</th>
<th>Acetate produced (mmol/liter)</th>
<th>Formate produced (mmol/liter)</th>
<th>Mn(II) produced (mmol/liter)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate</td>
<td>-</td>
<td>3.3</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>Pyruvate</td>
<td>+</td>
<td>7.0</td>
<td>NA</td>
<td>ND</td>
</tr>
<tr>
<td>Formate</td>
<td>-</td>
<td>NA</td>
<td>0.2</td>
<td>0.7</td>
</tr>
<tr>
<td>Formate</td>
<td>+</td>
<td>NA</td>
<td>6.7</td>
<td>6.4</td>
</tr>
</tbody>
</table>

* The initial pyruvate concentration was 10 mM; the initial formate concentration was 8 mM. Pyruvate-containing medium was incubated for 11 days; formate-containing medium was incubated for 10 days.

* Values are means of duplicate cultures.

* NA, Not applicable.

* ND, Mn(II) production in the presence of pyruvate was not determined because of the rapid abiotic reduction of Mn(IV) in the acidic extractant.
TABLE 3. Fe(III) reduction by A. putrefaciens ATCC 8071 with hydrogen and succinate as potential electron donors

<table>
<thead>
<tr>
<th>Electron donor(s)</th>
<th>Fe(II) produceda</th>
<th>Hydrogen consumeda</th>
<th>Fe(II)/H₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.3</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hydrogen</td>
<td>150</td>
<td>78</td>
<td>1.9</td>
</tr>
<tr>
<td>Succinate</td>
<td>0.5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Hydrogen and succinateb,c</td>
<td>152</td>
<td>81</td>
<td>1.9</td>
</tr>
</tbody>
</table>

a: Micromoles of Fe(II) produced or hydrogen consumed during a 20-h incubation period; values are means of duplicate culture tubes for each treatment.
b: Initial hydrogen partial pressure, ca. 11 Pa.
c: Initial succinate concentration, 20 mM.

Fig. 4). There was no growth under a hydrogen atmosphere in the absence of Fe(III). Succinate could not serve as the sole electron donor for Fe(III) reduction, and the addition of succinate and hydrogen did not stimulate Fe(III) reduction more than the addition of hydrogen alone did. Approximately 2 mol of hydrogen was consumed for each 1 mol of Fe(III) reduced (Table 3), indicating that hydrogen oxidation coupled to Fe(III) reduction follows reaction 5 (Table 1). With Fe(III) as the electron acceptor, A. putrefaciens metabolized hydrogen down to a minimum threshold of 0.04 ± 0.007 Pa (mean ± standard deviation; n = 3).

Hydrogen could also serve as an electron donor for Mn(IV) reduction (Fig. 5). This metabolism provided energy to support cell growth as, after 7 days of incubation, six cultures grown under conditions similar to those for the cultures for which the results are given in Fig. 5 had produced an average of 3 × 10⁶ cells (standard deviation, 5 × 10⁵) per µmol of Mn(IV) reduced. There was no cell growth when electron donor was not added or when succinate was added.

A. putrefaciens could obtain energy for growth by coupling the oxidation of formate to the reduction of Fe(III) (Fig. 6). The stoichiometry of formate consumption and Fe(III) reduction indicated that the reaction followed reaction 7 (Table 1). A. putrefaciens readily metabolized the added 10 mM formate down to concentrations below 100 µM. There was no reduction of Fe(III) with formate in controls that were not inoculated with A. putrefaciens.

A. putrefaciens also coupled the oxidation of formate to the reduction of Mn(IV) (Table 2). In accordance with reaction 8 (Table 1), the metabolism of each 1 mol of formate resulted in the reduction of ca. 1 mol of Mn(IV). Even under the acidic conditions of the Mn(II) assay, there was no abiotic reduction of Mn(IV) by formate.

Other electron donors. Neither strain of A. putrefaciens was able to couple the oxidation of the following electron donors to Fe(III) reduction at significant rates: succinate, citrate, acetate, butyrate, propionate, oxalate, ethanol, glycerol, benzoate, glucose, and fructose. When amino acids or
peptides were added in substrate quantities, there was a slow reduction of Fe(III), with the accumulation of small quantities of acetate (Table 4). This slow rate of Fe(III) reduction appeared to be due to the metabolism of A. putrefaciens, as Fe(II) or acetate did not accumulate in uninoculated media. However, given the slow rates of Fe(III) reduction, amino acid metabolism was not investigated in detail.

**DISCUSSION**

The results of this study extend the known metabolic capabilities of dissimilatory Fe(III)- and Mn(IV)-reducing microorganisms. *A. putrefaciens* is the only third microorganism that is known to obtain energy for growth via dissimilatory Fe(III) reduction and is one of only two microorganisms that is known to gain energy from dissimilatory Mn(IV) reduction. As discussed in detail below, the metabolism of *A. putrefaciens* differs significantly from that of the previously described Fe(III)- or Mn(IV)-reducing microorganisms, and *A. putrefaciens* provides an important pure-culture model for the catalysis of several key reactions in the oxidation of sediment organic matter coupled to the reduction of Fe(III) or Mn(IV).

**Hydrogen oxidation coupled to Fe(III) and Mn(IV) reduction.** The results of this study demonstrate that *A. putrefaciens* can obtain energy for growth from the oxidation of hydrogen coupled to the reduction of Fe(III). There are two other reports in which it was concluded that microorganisms can grow by oxidizing hydrogen with the reduction of Fe(III) (5, 14). In the first study (5), several lines of evidence indicated that the growth of a *Pseudomonas* sp. was the result of hydrogen metabolism with Fe(III) reduction. The organism reduced millimolar quantities of Fe(III) and grew to high culture densities with hydrogen as the electron donor, hydrogen uptake with the reduction of the expected amount of Fe(III) was documented, and growth coincided with Fe(III) reduction. In the second study (14), an unidentified gram-negative coccobacillus reduced less than 50 µM Fe(III) over a 28-day incubation period. This contrasts with the production of over 50 mM Fe(II) by *A. putrefaciens* in less than 24 h when hydrogen was used as the electron donor. Furthermore, the data presented previously (14) in support of the ability of the pure culture of the coccobacillus to metabolize hydrogen demonstrated that hydrogen did not consistently stimulate Fe(III) reduction and indicated that growth in the presence of hydrogen was not always significantly greater than that in its absence. Thus, the *Pseudomonas* sp. described by Balashova and Zavarzin (5) and *A. putrefaciens* appear to be the only known organisms which significantly couple hydrogen oxidation to Fe(III) reduction to obtain energy for growth.

Hydrogen metabolism by *A. putrefaciens* differs from that of the hydrogen-oxidizing, Fe(III)-reducing *Pseudomonas* sp. The extent of Fe(III) reduction by the *Pseudomonas* sp. is limited by the fact that Fe(II) inhibits Fe(III) reduction at Fe(II) concentrations as low as 1.3 mM (5). In contrast, *A. putrefaciens* readily reduced Fe(III) at Fe(II) concentrations of 50 mM. The *Pseudomonas* sp. requires yeast extract for growth on hydrogen and Fe(III) (5), whereas *A. putrefaciens* could be grown on a completely defined medium.

In contrast to the *Pseudomonas* sp., which did not reduce Mn(IV) (5), *A. putrefaciens* also reduced Mn(IV), with hydrogen as the sole electron donor, and obtained energy for growth from this reaction. It was recently reported (27) that the growth of *A. putrefaciens* was coupled to the reduction of Mn(IV) in a defined medium that contained succinate as the carbon source. The findings presented here suggest that in that study (27), *A. putrefaciens* was actually using the hydrogen in the culture gas mixture as the electron donor for Mn(IV) reduction. Cell extracts of microorganisms were previously shown to reduce Mn(IV) with hydrogen (40). However, the extracts also reduced many nonphysiological electron acceptors, and there was no evidence that Mn(IV) could serve as an electron acceptor for hydrogen oxidation in intact organisms. Thus, *A. putrefaciens* is the first organism in which hydrogen oxidation coupled to Mn(IV) reduction has been shown to be an energy-generating reaction carried out by whole cells.

Hydrogen is an intermediate in the oxidation of organic matter in sediments in which Fe(III) reduction is the terminal electron-accepting process, as hydrogen is both produced and consumed in such sediments (22; D. R. Lovley and S. Goodwin, Geochim. Cosmochim. Acta, in press). A biologically catalyzed reaction also rapidly consumes hydrogen in sediments in which Mn(IV) reduction is the terminal electron-accepting process (Lovley and Goodwin, in press). Results of previous studies (19, 22; Lovley and Goodwin, in press) with the mixed microbial assemblages in sediments have suggested that hydrogen-oxidizing, Fe(III)- and Mn(IV)-reducing bacteria can outcompete sulfate reducers and methanogens for hydrogen in sediments by maintaining the hydrogen concentration at levels that are too low for sulfate reducers or methanogens to metabolize the hydrogen. When grown on hydrogen, pure cultures of sulfate-reducing and methanogenic bacteria have been found to have minimum thresholds for hydrogen uptake of ca. 1 and 6 Pa, respectively (7, 17; S. Goodwin and D. Lovley, unpublished data). The finding that *A. putrefaciens* can metabolize hydrogen down to levels that are 25-fold lower than those metabolized by sulfate-reducing bacteria provides pure culture evidence for the hypothesis that when Fe(III) availability is not limiting, Fe(III)-reducing bacteria have a higher affinity for hydrogen than do sulfate-reducers and methanogens.

**Oxidation of organic compounds.** In addition to hydrogen, formate may be an important reduced fermentation product in some anaerobic environments (8, 11, 13, 25, 37, 38). Microorganisms that are capable of oxidizing formate with Fe(III) or Mn(IV) as the electron acceptor have apparently not been described previously. However, under standard conditions, the energy that is potentially available from formate oxidation coupled to Fe(III) or Mn(IV) reduction is approximately the same as that which is available from hydrogen oxidation (Table 1). The ability of *A. putrefaciens* to metabolize formate to low levels suggests that, as with

Table 4. Fe(III) reduction and acetate production with amino acids or peptides as potential electron donors

<table>
<thead>
<tr>
<th>Substrate added</th>
<th>Fe(II) produced (mmol/liter) after incubation for*:</th>
<th>Acetate produced (mmol/liter) after 49 days</th>
<th>8 days 21 days 49 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>None*</td>
<td>1.6</td>
<td>5.2</td>
<td>0.4</td>
</tr>
<tr>
<td>Casamino Acids</td>
<td>5.6</td>
<td>18.5</td>
<td>19.4</td>
</tr>
<tr>
<td>Proteose Peptone</td>
<td>9.3</td>
<td>17.3</td>
<td>20.9</td>
</tr>
<tr>
<td>Trypticase peptone</td>
<td>4.1</td>
<td>10.7</td>
<td>19.8</td>
</tr>
</tbody>
</table>

*Values are means of duplicate culture tubes for each treatment.

*Basic medium contained: vitamins; 50 mM ferric citrate; and L-arginine hydrochloride, L-glutamine, and DL-serine at 0.02, 0.02, and 0.04 g/liter. Casamino Acids (Difco Laboratories, Detroit, Mich.), Proteose Peptone (Difco), and Trypticase (BBL Microbiology Systems, Cockeysville, Md.) were added at a final concentration of 2 g/liter.
hydrogen, formate is rapidly consumed in sediments in which Fe(III) or Mn(IV) reduction is the terminal electron-accepting process. Lactate and pyruvate were the only other organic compounds that were found to support good growth of \textit{A. putrefaciens}. Lactate and pyruvate are not expected to be important products of fermentation in anaerobic environments with low hydrogen concentrations (39). Thus, it is unlikely that lactate and pyruvate could be major substrates for Fe(III) reduction in most natural environments. Although \textit{A. putrefaciens} appeared to couple the metabolism of amino acids to Fe(III) reduction, the rates of metabolism were so slow that it is doubtful that \textit{A. putrefaciens} could successfully compete with fermentative bacteria for amino acids in sediments.

Other than the recently described strain GS-15 (23, 24), \textit{A. putrefaciens} is the only dissimilatory Fe(III)- or Mn(IV)-reducing organism that is known to obtain energy for growth by oxidizing organic compounds. Unlike GS-15, which completely oxidizes volatile fatty acids and ethanol to carbon dioxide, \textit{A. putrefaciens} only incompletely oxidized its multicarbon substrates to acetate.

\textbf{Ecological niche.} The potential ecological niche of \textit{A. putrefaciens} in sediments with Fe(III) or Mn(IV) reduction as the predominant terminal electron-accepting process is shown in Fig. 7. The available evidence suggests that Fe(III)- and Mn(IV)-reducing organisms that are capable of metabolizing fermentable substrates oxidize little of their substrate with the reduction of Fe(III) or Mn(IV) and that they generate hydrogen and fatty acids as the primary products of their metabolism (18, 23). The activity of organisms with a metabolism like that of \textit{A. putrefaciens} is necessary for the oxidation of hydrogen and formate as well as the small quantities of lactate that might be produced. GS-15 or similar organisms are required to metabolize the other important fermentation products, most notably acetate. Figure 7 illustrates that pure cultures of organisms are now available that, when grown together, should be capable of completely oxidizing fermentable substrates to carbon dioxide with Fe(III) or Mn(IV) as the sole electron acceptor. Since \textit{A. putrefaciens} and organisms with a metabolism like that of GS-15 have been recovered from surface (23, 27) and subsurface (32; D. Lovley and F. Chapelle, manuscript in preparation) environments, such model systems constructed from pure cultures might be useful tools for determining the factors which control the rate and extent of microbial Fe(III) and Mn(IV) reduction in a variety of sedimentary environments.

In summary, results of this study with \textit{A. putrefaciens} have demonstrated that Fe(III)- and Mn(IV)-reducing bacteria can obtain energy for growth from the oxidation of hydrogen or formate and may have high affinities for these electron donors. Results of recent studies on hydrogen metabolism in sediments and other anaerobic environments have demonstrated that physiological data collected with pure cultures of hydrogen-consuming microorganisms are useful in interpreting hydrogen dynamics in natural environments (Lovley and Goodwin, in press). \textit{A. putrefaciens} provides a readily available organism with which to study hydrogen metabolism coupled to Fe(III) or Mn(IV) reduction under defined conditions.

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\textbf{LITERATURE CITED}

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