

## Requirement for a Microbial Consortium To Completely Oxidize Glucose in Fe(III)-Reducing Sediments

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**In various sediments in which Fe(III) reduction was the terminal electron-accepting process, [<sup>14</sup>C]glucose was fermented to <sup>14</sup>C-fatty acids in a manner similar to that observed in methanogenic sediments. These results are consistent with the hypothesis that, in Fe(III)-reducing sediments, fermentable substrates are oxidized to carbon dioxide by the combined activity of fermentative bacteria and fatty acid-oxidizing, Fe(III)-reducing bacteria.**

Geochemical studies have indicated that there are zones in aquatic sediments and aquifers in which either natural or contaminant organic compounds are completely oxidized to carbon dioxide with Fe(III) as the sole electron acceptor (1, 2, 4, 5, 8, 16). Fe(III)-reducing microorganisms which can completely oxidize short-chain fatty acids, aromatic compounds, or hydrogen have been described (3, 8, 11, 13, 14). However, the only Fe(III)-reducing microorganisms known to metabolize fermentable substrates, such as sugars and amino acids, are fermentative microorganisms which only reduce Fe(III) as a minor side reaction in their metabolism (7). Even in the presence of Fe(III), their primary metabolic products are typical fermentation acids, alcohols, and hydrogen. Thus, it was proposed that, in sediments in which Fe(III) reduction is the terminal electron-accepting process, fermentable substrates are oxidized to carbon dioxide by a two-stage process in which fermentative bacteria metabolize the fermentable substrates and then Fe(III)-reducing microorganisms oxidize the fermentation products (7, 13).

However, models for sediment metabolism cannot be based solely on culture data. For example, it is possible that there are Fe(III)-reducing microorganisms in sediments which, by themselves, can completely oxidize fermentable substrates but that these microorganisms are not recovered by standard enrichment or isolation techniques. Therefore, in order to more directly examine the pathways for the metabolism of fermentable substrates by natural populations of Fe(III)-reducing microorganisms, we examined the metabolism of [U-<sup>14</sup>C]glucose in several sediments in which Fe(III) was the predominant terminal electron-accepting process.

**Metabolism of [U-<sup>14</sup>C]glucose in sediments.** As described previously, freshwater sediments in which Fe(III) reduction (12) or methane production (9) was the terminal electron-accepting process were obtained from the Potomac River, Maryland. Furthermore, freshwater sediments in which Fe(III) reduction was the predominant terminal electron-accepting process were artificially generated by adding an anaerobic slurry of a poorly crystalline Fe(III) oxide to the methanogenic sediments to provide ca. 40 mmol of Fe(III) per liter of wet sediment (9). This inhibited methane production by over 95%. Previous studies have demonstrated that this inhibition of methane production is accompanied by a corresponding increase in electron flow to Fe(III) reduction (9, 10). Subsurface sediments in which Fe(III) reduction

was the terminal electron-accepting process were collected from a glacial outwash aquifer located at the Groundwater Toxics Study Site of the U.S. Geological Survey in Bemidji, Minn. (8).

Sediments (10 ml) were transferred under N<sub>2</sub>-CO<sub>2</sub> (93:7; Potomac River sediments) or N<sub>2</sub> (Bemidji subsurface sediments) into anaerobic pressure tubes which were then sealed with butyl rubber stoppers. In order to slow the turnover of the pools of various potential fermentation products, an anaerobic solution (0.1 ml) of a mixture of acetate, propionate, butyrate, formate, lactate, and ethanol was injected to provide a final concentration of ca. 1 mM of each compound. Studies with the methanogenic sediments demonstrated that this was necessary in order to prevent the <sup>14</sup>C-fermentation products that were produced during the metabolism of [<sup>14</sup>C]glucose from being rapidly metabolized further (data not shown). An anaerobic aqueous solution (0.1 ml) of [U-<sup>14</sup>C]glucose (280 mCi/mmol) was injected into the tubes to provide 0.5 μCi (Potomac River sediments) or 0.25 μCi (Bemidji subsurface sediments). Incubations were at in situ temperatures of 20°C (Potomac River sediments) or 9°C (Bemidji subsurface sediments). The Potomac River sediments were incubated for 1 h. Bemidji subsurface sediments were incubated for 47 h because of the slower rates of microbial metabolism in these sediments. Glutaraldehyde (final concentration, 2.5%) was added to the sediments to stop biological activity at the end of the incubations.

Glucose, fatty acids, and ethanol in the sediment pore water were separated by high-pressure liquid chromatography (6) and detected with a refractive index detector. The eluant from the detector was collected over 1-min intervals into 0.1 ml of 0.1 N NaOH in a scintillation vial. Ecolume (15 ml; ICN Biomedical Inc., Costa Mesa, Calif.) was added to the vials, and <sup>14</sup>C was quantified by liquid scintillation counting.

To measure the production of <sup>14</sup>CO<sub>2</sub>, replicate tubes were acidified and the <sup>14</sup>CO<sub>2</sub> was flushed out, trapped in NaOH, and quantified by liquid scintillation counting (8). To measure the <sup>14</sup>CH<sub>4</sub>, replicate tubes were basified with 1 ml of 10 N NaOH to maintain the <sup>14</sup>CO<sub>2</sub> in nonvolatile forms. The tubes were flushed with a stream of O<sub>2</sub>, which was then passed through a column of molecular sieve to ensure that there was no CO<sub>2</sub> in the gas stream. The <sup>14</sup>CH<sub>4</sub> in the gas stream was then combusted to <sup>14</sup>CO<sub>2</sub> in a heated column (800°C) of copper oxide. The <sup>14</sup>CO<sub>2</sub> was trapped and counted as described above.

The metabolism of the [U-<sup>14</sup>C]glucose in methanogenic

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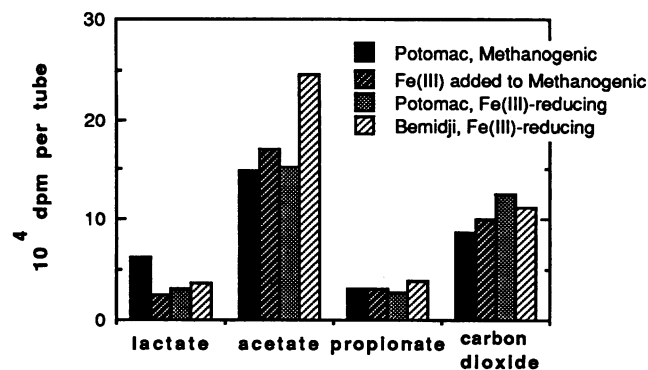


FIG. 1. Production of  $^{14}\text{C}$ -end products from  $[\text{U-}^{14}\text{C}]$ glucose in various sediments. The results are the means of measurements made on duplicate tubes of each sediment type.  $[\text{C-}^{14}]$ butyrate,  $[\text{C-}^{14}]$ ethanol, and  $^{14}\text{CH}_4$  accounted for less than 0.5% of the recovered  $^{14}\text{C}$ -products in all sediments.  $[\text{C-}^{14}]$ lactate may also include  $[\text{C-}^{14}]$ succinate, as these two compounds cochromatographed.

sediments from the Potomac River was similar to that described previously for other freshwater methanogenic sediments (6). Less than 5% of the added  $[\text{U-}^{14}\text{C}]$ glucose could be recovered from the pore water after 10 min of incubation, and the  $[\text{U-}^{14}\text{C}]$ glucose was not removed from the pore water when biological activity was inhibited with glutaraldehyde (data not shown).  $[\text{C-}^{14}]$ acetate and  $^{14}\text{CO}_2$  were the primary initial products of the metabolism of  $[\text{U-}^{14}\text{C}]$ glucose metabolism (Fig. 1). Smaller amounts of  $[\text{C-}^{14}]$ lactate and  $[\text{C-}^{14}]$ propionate also accumulated.

The metabolism of  $[\text{U-}^{14}\text{C}]$ glucose in anaerobic sediments is complex (6). The rate and pathways of glucose metabolism cannot be strictly defined by monitoring the accumulation of  $^{14}\text{C}$  in extracellular products, as once the  $[\text{U-}^{14}\text{C}]$ glucose is taken up by the sediment microorganisms, the  $^{14}\text{C}$  is diluted in various intracellular pools which are probably not in isotopic equilibrium (6). For example, even though the added  $[\text{U-}^{14}\text{C}]$ glucose was rapidly removed from the pore water of the Potomac River sediments, only about one-third of the added  $^{14}\text{C}$  was recovered in fermentation products and  $\text{CO}_2$  after a 1-h incubation. Similar results were reported previously (calculated from Fig. 3 and 4 of reference 6). Despite the complexities of the metabolism of  $[\text{U-}^{14}\text{C}]$ glucose in anaerobic sediments, the accumulation of  $^{14}\text{C}$ -metabolic products gives a qualitative indication of how glucose is metabolized. There is little doubt that fermentation is the

first step in glucose mineralization in methanogenic sediments (6). Therefore, it was assumed that if the production of  $^{14}\text{C}$ -fatty acids in Fe(III)-reducing sediments was similar to that in methanogenic sediments, then the  $[\text{U-}^{14}\text{C}]$ glucose was also fermented in the Fe(III)-reducing sediments.

In all of the Fe(III)-reducing sediments examined,  $[\text{U-}^{14}\text{C}]$ glucose was fermented to  $^{14}\text{C}$ -fatty acids in a manner similar to that observed in the methanogenic sediments (Fig. 1). This was true whether the sediments were artificially switched to Fe(III) reduction by the addition of synthetic Fe(III) oxide or whether naturally occurring Fe(III) oxides served as the electron acceptor. The metabolism of  $[\text{U-}^{14}\text{C}]$ glucose in the Fe(III)-reducing sediments was consistent with the hypothesis (7) that, in sediments in which Fe(III) reduction is the predominant terminal electron-accepting process, fermentable substrates, such as sugars and amino acids, are first metabolized to fatty acids and then the fatty acids are oxidized to carbon dioxide.

**Thermodynamic considerations.** It was previously argued (7) that an organism which could completely oxidize glucose to carbon dioxide with Fe(III) as the electron acceptor should have a competitive advantage over organisms that ferment glucose, because the oxidation of glucose with the reduction of Fe(III) can potentially yield more energy per mole of glucose metabolized than can fermentation. However, McInerney and Beaty (15) have proposed that the important thermodynamic consideration is not the amount of energy available per mole of glucose metabolized but, rather, the amount of energy released per electron transferred. They have suggested that the reason that glucose is fermented in sulfate-reducing and methanogenic systems is that more energy is available per electron transferred in typical fermentation reactions than is available from the complete mineralization of glucose by sulfate reducers or methanogens. One of the potential advantages of a greater potential energy yield per electron transferred is that it may allow fermentative organisms to grow faster than respiratory organisms could if they mineralized glucose by sulfate reduction or methane production (15).

If the hypothesis of McInerney and Beaty (15) is correct, a similar thermodynamic rationale may explain why glucose is fermented in Fe(III)-reducing sediments. Per electron transferred, typical fermentations of glucose (Table 1, reactions 3 and 5 to 8) are more energetically favorable than the complete oxidation of glucose with Fe(III) oxide as the electron acceptor (Table 1, reaction 1). This is true both at standard conditions and at the reactant and product concen-

TABLE 1. Energy potentially available from various pathways for glucose metabolism

Reaction no.	Reactants	Products	$\Delta G'$ (kJ/electron transferred) <sup>a</sup>	
			Standard	In situ
1	$\text{C}_6\text{H}_{12}\text{O}_6 + 24\text{Fe}(\text{OH})_3(\text{s}) + 42\text{H}^+$	$24\text{Fe}^{2+} + 6\text{HCO}_3^- + 60\text{H}_2\text{O}$	-12	-30
2	$\text{C}_6\text{H}_{12}\text{O}_6 + 8\text{Fe}(\text{OH})_3(\text{s}) + 12\text{H}^+$	$8\text{Fe}^{2+} + 2\text{CH}_3\text{COO}^- + 2\text{HCO}_3^- + 20\text{H}_2\text{O}$	-37	-62
3	$\text{C}_6\text{H}_{12}\text{O}_6 + \text{H}_2\text{O}$	$\text{CH}_3\text{COO}^- + \text{CH}_3\text{CH}_2\text{COO}^- + \text{HCO}_3^- + \text{H}_2 + 3\text{H}^+$	-71	-93
4	$\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{Fe}(\text{OH})_3(\text{s}) + \text{H}^+$	$2\text{Fe}^{2+} + \text{CH}_3\text{COO}^- + \text{CH}_3\text{CH}_2\text{COO}^- + \text{HCO}_3^- + 5\text{H}_2\text{O}$	-76	-98
5	$\text{C}_6\text{H}_{12}\text{O}_6 + 2.6\text{H}_2\text{O}$	$0.6\text{CH}_3\text{COO}^- + 0.7\text{CH}_3\text{CH}_2\text{CH}_2\text{COO}^- + 2\text{HCO}_3^- + 2.6\text{H}_2 + 3.3\text{H}^+$	-60	-93
6	$\text{C}_6\text{H}_{12}\text{O}_6$	$\text{OOCCH}_2\text{CH}_2\text{COO}^{2-} + \text{CH}_3\text{COO}^- + \text{H}_2 + 3\text{H}^+$	-66	-85
7	$\text{C}_6\text{H}_{12}\text{O}_6$	$2\text{CH}_3\text{CHOHCOO}^- + 2\text{H}^+$	-50	-61
8	$\text{C}_6\text{H}_{12}\text{O}_6 + 2\text{H}_2\text{O}$	$2\text{CH}_3\text{CH}_2\text{OH} + 2\text{HCO}_3^- + 2\text{H}^+$	-57	-73

<sup>a</sup> The free energy changes at pH 7 ( $\Delta G'$ ) were calculated from standard free energies of formation of the products and reactants (17, 18). Fermentation reactions 3 and 5 to 8 are from McInerney and Beaty (15). Standard designates the  $\Delta G'$  with reactants and products at standard conditions, except for pH 7. Estimates of in situ concentrations in sediments with Fe(III) as the terminal electron-accepting process are based on previous literature values for acetate and hydrogen (10) or estimates of probable values for other compounds. In situ concentrations were as follows:  $\text{Fe}^{2+}$ ,  $1 \times 10^{-3}$  M; hydrogen,  $2.5 \times 10^{-7}$  atm; acetate,  $5 \times 10^{-7}$  M; glucose,  $5 \times 10^{-7}$  M; propionate,  $1 \times 10^{-7}$  M; butyrate,  $1 \times 10^{-7}$  M; lactate,  $1 \times 10^{-7}$  M; succinate,  $1 \times 10^{-7}$  M; ethanol,  $1 \times 10^{-7}$  M;  $\text{HCO}_3^-$ , 0.015 M.

trations expected in Fe(III)-reducing sediments. Although the partial oxidation of glucose to acetate and carbon dioxide with Fe(III) as the electron acceptor (Table 1, reaction 2) is more energetically favorable than complete glucose oxidation (Table 1, reaction 1), it is less energetically favorable than fermentation. Microorganisms which can use Fe(III) as a minor electron sink during fermentation are expected to have a slight energetic advantage over strictly fermentative organisms (compare reactions 3 and 4 in Table 1). This may explain why the only microorganisms that are known to metabolize glucose with the reduction of Fe(III) have this metabolic strategy.

In summary, it appears that fermentation is an important process for the metabolism of organic matter in sediments in which Fe(III) reduction is the terminal electron-accepting process. If there are Fe(III)-reducing microorganisms which, by themselves, can completely oxidize fermentable substrates to carbon dioxide, their metabolism does not appear to be an important factor in the metabolism of sediment organic matter. The conclusion that organic matter is first fermented in Fe(III)-reducing sediments suggests that most of the Fe(III) reduction in sediments is catalyzed by Fe(III)-reducing microorganisms such as GS-15 and *Alteromonas putrefaciens*, which can completely oxidize fermentation products or aromatic compounds (8, 11, 13, 14).

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