Growth of Iron(III)-Reducing Bacteria on Clay Minerals as the Sole Electron Acceptor and Comparison of Growth Yields on a Variety of Oxidized Iron Forms†

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Smectite clay minerals are abundant in soils and sediments worldwide and are typically rich in Fe. While recent investigations have shown that the structural Fe(III) bound in clay minerals is reduced by microorganisms, previous studies have not tested growth with clay minerals as the sole electron acceptor. Here we have demonstrated that a pure culture of Shewanella oneidensis strain MR-1 as well as enrichment cultures of Fe(III)-reducing bacteria from rice paddy soil and subsurface sediments are capable of conserving energy for growth with the structural Fe(III) bound in smectite clay as the sole electron acceptor. Pure cultures of S. oneidensis were used for more detailed growth rate and yield experiments on various solid- and soluble-phase electron acceptors [smectite, Fe(III) oxyhydroxide FeOOH, Fe(III) citrate, and oxygen] in the same minimal medium. Growth was assessed as direct cell counts or as an increase in cell carbon (measured as particulate organic carbon). Cell counts showed that similar growth of S. oneidensis (10⁸ cells ml⁻¹) occurred with smectitic Fe(III) and on other Fe forms [amorphous Fe(III) oxyhydroxide, and Fe citrate] or oxygen as the electron acceptor. In contrast, cell yields of S. oneidensis measured as the increase in cell carbon were similar on all Fe forms tested while yields on oxygen were five times higher, in agreement with thermodynamic predictions. Over a range of particle loadings (0.5 to 4 g liter⁻¹), the increase in cell number was highly correlated to the amount of structural Fe in smectite reduced. From phylogenetic analysis of the complete 16S rRNA gene sequences, a predominance of clones retrieved from the clay mineral-reducing enrichment cultures were most closely related to the low-G+C gram-positive members of the Bacteria (Clostridium and Desulfotobacterium) and the ß-Proteobacteria (members of the Geobacteraceae). Results indicate that growth with smectitic Fe(III) is similar in magnitude to that with Fe(III) oxide minerals and is dependent upon the mineral surface area available. Iron(III) bound in clay minerals should be considered an important electron acceptor supporting the growth of bacteria in soils or sedimentary environments.

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Microbial Fe(III) reduction has been established as an important process catalyzing a large number of natural and contaminant biogeochemical cycles (21, 24, 29). Iron(III) respiration is coupled to a substantial portion of organic matter remineralization in the surface sediments of marine and freshwater environments (18, 35, 48). The fate of organic and inorganic contaminants is intimately tied to the activities of Fe(III)-reducing bacteria (FeRB) in subsurface sediments (24). In addition to its importance in modern environments, Fe(III) respiration is suggested to have been one of the earliest forms of respiration on ancient Earth (21).

The majority of studies of FeRB carried out in pure culture have focused on soluble, complexed Fe forms (such as ferric citrate) or on Fe(III) oxyhydroxide minerals (ferrihydrite and goethite) as the Fe available for reduction. In contrast, most of the oxidized Fe by weight in natural sediments is associated with phyllosilicate clay minerals (48). Clay minerals are abundant and ubiquitous in soils and sediments (41). Along with microorganisms, clays provide some of the most catalytic surfaces in sedimentary environments, which are important to a variety of biogeochemical cycles (41, 43). However, few studies have been carried out on the interactions between these two reactive components of porous media.

Of the organisms currently available in pure culture which are known to conserve energy for growth from the reduction of Fe(III) minerals, members of the shewanellae and of the Geobacteraceae have been characterized in the most detail (21, 29). A small but expanding database has been collected which shows that microorganisms from both of these families catalyze the reduction of clay-bound Fe(III) (16). Microbial clay reduction has been demonstrated at temperatures and pHs common to soils and sediments (15–17). As with Fe oxide minerals, organic chelates and electron transfer agents increased the bioavailability of clay bound Fe(III) for reduction (16, 26). However, no past studies have provided evidence for the growth of bacteria with clay minerals as the sole electron acceptor. Further, it is unclear how respiration and growth on clay-bound Fe(III) compare to those on other Fe mineral forms.

In this study, we found that the respiration of structural Fe(III) bound in smectite clay minerals supports the growth of FeRB in pure culture and in enrichment cultures from two very different sedimentary environments. We also compared growth yields of a S. oneidensis strain MR-1 on a variety of Fe(III) forms with that on oxygen. Iron(III) oxide minerals are be-

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† This paper is dedicated to the memory of Dava Dalton, friend and colleague, who passed away during review of the manuscript.
All manipulations of culture samples were carried out under strictly anoxic conditions within a Coy anaerobic chamber (90% N₂, 10% H₂). Inoculum cultures were grown anaerobically on Fe(III) to late log phase on the appropriate minimal medium. Heat-killed controls were heated by microwave radiation until boiling (11).

**Preparation of oxidized Fe.** Amorphous Fe(III) oxyhydroxide (surface area = 600 m² g⁻¹) was prepared as described by Schwertmann and Cornell (38). For all experiments with smectite clay, the 0.5- to 2-μm fraction of the ferruginous smectite Swa-1 from Grant County, Wash. (Source Clays Repository, The Clay Minerals Society), was used. The clay was Na⁺-saturated, fractionated, dialyzed, and freeze-dried prior to use (42). Lear and Stucki (20) reported the structural Fe content of the same dialyzed Swa-1 to be 3.549 mmol of Fe g⁻¹ (with less than 0.1 mmol of this Fe g⁻¹ present as Fe oxide impurities) and the surface area to be 720 m² g⁻¹. All solutions were made anoxic using an updated, commercially available version of the apparatus described by Stucki et al. (42). All Fe minerals were sterilized by heating via microwave radiation (11) before addition to the culture medium. Ferric citrate was prepared as described previously by Kostka and Nealson (14).

**Determination of reduction and growth.** The reduction of Fe(III) was measured as the production of Fe(II) in HCl extracts using the colorimetric reagent ferrozine under strictly anoxic conditions (14, 23). This method was previously validated for use in clay cultures by comparison to HF extracts and Mossbauer spectroscopy (15, 16). Cell numbers were determined by direct counting using acridine orange and epifluorescence microscopy as described previously (10, 23). Bacterial direct counting procedures were modified for solid-containing suspensions according to the work of Proctor and Souza (33). Biomass was determined by measuring the accumulation of particulate carbon using previously described methods (27).

**Phylogenetic characterization of purified Fe(III)-reducing consortia.** Genomic DNA was extracted with an ultraclean soil DNA kit (Mo Bio Laboratories, Inc, Solana Beach, Calif.). Extracted genomic DNA was used as a template for PCR amplification of nearly the entire 16S rRNA gene (~1,400 bp) with the bacterium-specific primers 8F and 1392R as previously described (3). The amplification products were subsequently cloned into *Escherichia coli* with the TOPO TA cloning kit (Invitrogen, Carlsbad, Calif.). The clones were screened by restriction analysis, and unique clones were sequenced with an automated sequencer (Applied Biosystems model 3100) using the terminator cycle sequencing method. The sequences were aligned to comparison strains in the Ribosomal Database Project in accordance with the secondary structure of the 16S rRNA molecule using the ARB software package (40). A phylogenetic distance matrix was calculated from the aligned sequences using distance and maximum likelihood methods.

**RESULTS**

The rapid reduction of soluble and solid Fe(III) forms was observed in cultures of *Shewanella oneidensis* strain MR-1 over 3 days (Table 1). Nearly all of the Fe(III) citrate was reduced, compared to half of the Fe(III) oxyhydroxide (FeOOH) and approximately one-third of the structural Fe(III) bound in smectite. A white, ferrous carbonate precipitate was produced over time in Fe(III) citrate cultures, whereas a black magnetic
precipitate, presumably magnetite, was formed in Fe(III) oxy-
hydroxide cultures. With smectite as the sole electron acceptor,
no solid form other than smectite was observed at the end of
the experiments.

Addition of the humic acid analog anthraquinone disulfon-
atate (AQDS) generally stimulated reduction of the FeOOH to
a larger extent than clay-bound Fe(III) (Table 1). Control
cultures (heat killed, exposed to HgCl2, or cultured aerobi-
cally) showed little or no reduction of clay-bound Fe(III) to
Fe(II). As has been demonstrated previously (16, 25), lactate
was depleted and carbon dioxide was produced according to
the 4:1 stoichiometry of Fe(III) reduced to carbon oxidized in
all cultures (data not shown).

Growth of *S. oneidensis*, measured by an increase in cell
number, paralleled the reduction of all Fe(III) forms tested
(Table 1; Fig. 1 and 2). Little or no growth was observed in
killed controls (with heat or HgCl2) or in control cultures to
which no electron acceptor had been added (Fig. 1). Interest-
ingly, the increase in cell number exhibited a similar range for
all electron acceptors tested (Table 1; Fig. 2). Within 3 days,
cell densities of *S. oneidensis* were observed to increase to a
range between 9.0 × 10^7 and 16.5 × 10^7 cells ml⁻¹. On the
third day, cultures were sacrificed for particulate organic car-
bon analysis and the yield within each culture was determined.
It was not possible to resolve the particulate organic carbon
yield in smectite cultures due to the presence of background
carbon impurities (data not shown). However, in contrast to
the increase in cell number, accumulation of *S. oneidensis*
biomass (measured as grams of C cell⁻¹) ranged over a factor
of 5 depending upon the electron acceptor utilized [O₂, Fe
citrate, and Fe(III) oxyhydroxide] (Table 1).

*S. oneidensis* conserved energy for growth by coupling the
reduction of smectite to the oxidation of lactate (Fig. 1). *S.
oneidensis* was then inoculated into minimal basal media sup-
plemented with various particle concentrations of smectite as
the sole electron acceptor. Not only was the degree of struc-
tural Fe(III) reduction proportional to the initial particle load,
but a corresponding proportional increase in cell density was
also observed (Fig. 3).

In enrichment cultures freshly purified from rice paddy soil
and from contaminated subsurface sediment, Fe(III)-reducing
consortia were shown to conserve energy for growth by cou-
pling the reduction of structural Fe(III) in smectite to the
oxidation of acetate. Growth paralleled the reduction of Fe(III) with
smectite added as the sole electron acceptor, while
little or no growth was observed in control cultures to which no
electron acceptor had been added (Fig. 4). Representative
growth curves are shown for parallel enrichment cultures en-
riched from the same subsurface sediment core (Fig. 4), and
approximately twice the growth was observed for duplicate rice
paddy enrichments \([13 \times 10^7 \text{ cells mL}^{-1} \text{ and } 2.5 \text{ mM smectite-bound Fe(III) reduced; data not shown}]\). Using a cloning and sequencing approach of the 16S rRNA genes in these enrichment cultures, the dominant members of the Fe(III)-reducing consortia were identified. In the subsurface enrichment (Ac032) (Fig. 5), five clones were obtained, and the dominant sequences retrieved showed the highest sequence similarity to “Geobacter akaganeitredirectens” (three clones, 90% similarity) and Desulfotobacterium chlororespirans (two clones, 90% similarity). In the rice paddy enrichment (LacRPS) (Fig. 5), six clones were obtained and the dominant sequences retrieved showed the highest sequence similarity to Clastrium celerecrescens (three clones, 96% similarity) and Geobacter sulfurreducens (two clones, 98% similarity).

**DISCUSSION**

Growth with smectite clay minerals as the sole electron acceptor. The growth of FeRB in culture has mostly been quantified by measuring the increase in cell number. Using the maximum cell number increase in comparison to control cultures, the range of growth measured for \(S. \text{oneidensis} \) coupled to smectite respiration reported here (\(1 \times 10^8 \text{ to } 2 \times 10^8 \text{ cells ml}^{-1}\)) is very similar to the range reported in previous studies for the growth of pure cultures of FeRB coupled to the respiration of synthetic Fe(III) oxide minerals (\(1 \times 10^8 \text{ to } 2 \times 10^8 \text{ cells ml}^{-1}\) under similar culture conditions (7, 23, 25, 34). Our observation that microorganisms can gain energy for growth by catalyzing the respiration of smectite clay minerals is not surprising when the energy available from these reactions is considered (Table 2). The standard free energy at pH 7 that was

![FIG. 4. Growth (A) and structural Fe(III) reduction (B) of an FeRB consortium purified from subsurface sediments with acetate as the electron donor and smectite as the electron acceptor. Results are expressed as means ± 1 standard deviation for triplicate culture treatments from parallel enrichment cultures generated from the same sediment core. Symbols: squares and triangles, smectite added; diamonds and circles, no electron acceptor added. Squares and diamonds represent core sample A; triangles and circles represent core sample B.](image)

**FIG. 5. Phylogenetic tree for enrichment cultures of Fe(III)-reducing consortia and selected strains of eubacteria, based on a distance matrix analysis (see Materials and Methods). Consortia purified from rice paddy soil and subsurface sediments are designated LacRPS and Ac032t, respectively. Scale bar, 5 substitutions per 100 bases. Parsimony and maximum likelihood analyses yielded equivalent results with respect to the phylogenetic positions of the Fe-reducing strains.**
calculated to be available from lactate oxidation coupled to smectite reduction (Table 2, equation 1) is very similar to that calculated to be available with Fe(III) oxyhydroxide or soluble ferric citrate as the electron acceptor (Table 2, equations 2 and 3). Our findings suggest that a similar amount of energy for growth is generated during Fe(III) reduction, regardless of the form of Fe being utilized in cultures of *S. oneidensis*.

Earlier culture studies indicated that the availability of Fe(III) minerals for reduction by microorganisms was determined by their crystallinity or mineral form (22). Amorphous Fe(III) oxyhydroxides were shown to be rapidly and extensively reduced by microbes, whereas crystalline Fe(III) minerals were reduced slowly and incompletely. More recent studies by Kostka and Nealson (13) and Roden and Zachara (36) revised this view by showing that FeRB were capable of growth on the crystalline Fe(III) oxide minerals magnetite and goethite, respectively. It was further suggested that the potential for cell growth and Fe(III) reduction was determined by the Fe(III) oxide surface area and not by crystallinity (36). Though Fe-containing clay minerals are operationally defined as crystalline Fe(III) minerals, rates of microbial clay reduction have been observed to be comparable to rates of reduction for poorly crystalline or amorphous Fe(III) oxyhydroxides (16). In this study, we observed that growth was highly correlated to the particle concentration of smectite added to cultures of *S. oneidensis* (Fig. 3). The percentage of structural Fe(III) reduced in smectite remained at 20% throughout the range of particle loading tested (Fig. 3). Thus, our results support previous observations which suggested that reduction and growth on crystalline Fe(III) minerals is determined by mineral surface area. We extend this concept to smectite clay minerals and suggest that their high surface area, comparable to that of amorphous Fe(III) oxyhydroxides (∼700 m² g⁻¹) (38, 41), results in an increased availability for microbial reduction and growth.

Organic compounds such as humic acids are believed to facilitate the reduction of Fe(III) minerals by serving as an electron shuttle or by chelating and solubilizing Fe(III), thereby making the Fe more available for reduction. Our results concur with past studies to show that the reduction of smectite (26) or Fe(III) oxyhydroxide (21) is enhanced in the presence of the humic acid analog AQDS (Fig. 1; Table 1). However, we observed a minimal enhancement of cell growth in the presence of AQDS (Fig. 1 and 2).

Growth with smectite as the sole electron acceptor was also observed in Fe(III)-reducing consortia enriched from two very different environments (contaminated subsurface sediment and rice paddy soil) where Fe-rich clay minerals are abundant and potentially important electron acceptors (41, 52). The 16S rRNA gene sequences retrieved from these consortia were dominated by those closely related to the δ-Proteobacteria (*Geobacteraceae*) and low-G+C gram-positive members of the *Bacteria* (Desulfobacterium and Clostridium). Retrieval of *Geobacteraceae* sequences is not unexpected, since these organisms have been established as important members of FeRB consortia in sediments (5, 37, 46). Detection of low-G+C gram-positive members of FeRB consortia is more surprising and intriguing. Members of the low-G+C *Bacteria* (*Bacillus, Desulfobacterium, and Desulfotomaculum*) have been shown to be capable of Fe(III) respiration (6, 30, 31, 32, 45, 47). Gram-positive organisms are thought to be more resistant to environmental extremes such as desiccation in soils and sediments.

**Growth rate and yield of *S. oneidensis* strain MR-1 on various electron acceptors.** *S. oneidensis* is a facultative anaerobe which grows well on oxygen and is incapable of fermentative growth (29, 30, 39). Thermodynamic calculations of free energy are often used as the basis for comparing the energetics of Fe(III) respiration to aerobic respiration by FeRB of the *Shewanella* family, though few quantitative growth data are available to support this comparison. Soluble Fe(III) forms such as ferric citrate are also believed to be more available for reduction and therefore may provide more energy for growth than solid Fe(III) minerals. To our knowledge, growth rates or yields of FeRB on solid versus soluble Fe forms have not been compared, and no studies have compared growth coupled to Fe(III) respiration versus aerobic respiration. We observed growth yields of *S. oneidensis* as the increase in total cell carbon in the following order: O₂ > ferric citrate > Fe(III) oxyhydroxide. When normalized per mole of electron acceptor (e⁻) utilized, growth yields were nearly identical for cells growing on Fe(III) oxyhydroxide (0.17 g of C mol of e⁻⁻¹) compared to ferric citrate (0.20 g of C mol of e⁻⁻¹), while the growth yield on oxygen was larger by a factor of 5 (Table 1). By measuring an increase in cell protein, Bazylinski et al. (4) observed a growth yield, similar to that in our study, of 0.40 g of cell C mol of Fe(III) reduced for *Geobacter* cells with ferric citrate as the electron acceptor.

In support of the growth yield data, cell sizes visualized under epifluorescence microscopy appeared to be substantially larger in cultures grown on soluble electron acceptors (oxygen or ferric citrate) than in those grown with solid electron acceptors [Fe(III) oxyhydroxide or smectite]. Therefore, we conclude that cell counts are not always directly proportional to growth yield in cultures of *S. oneidensis*. Such an observation

### Table 2. Stoichiometry and free energy of reactions related to the metabolism of *S. oneidensis* strain MR-1 with various electron acceptors

<table>
<thead>
<tr>
<th>Equation</th>
<th>Reactants</th>
<th>Products</th>
<th>Std G°&lt;sup&gt;a&lt;/sup&gt;</th>
<th>kJ/reaction</th>
<th>kJ/mol of e⁻</th>
</tr>
</thead>
<tbody>
<tr>
<td>1⁷</td>
<td>1.47[smectite] + CH₃CHOHCOO⁻ + 2H₂O</td>
<td>1.47[smectite] + CH₃COO⁻ + HCO₃⁻ + 5H⁺</td>
<td>-436.09</td>
<td>-109.02</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>12 Fe(OH)₃ + CH₃CHOHCOO⁻</td>
<td>4Fe₂O₃ + CH₃COO⁻ + HCO₃⁻ + H⁺ + 18H₂O</td>
<td>-409.70</td>
<td>-102.42</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>4Fe²⁺ + CH₃CHOHCOO⁻ + 2H₂O</td>
<td>4Fe⁺ + CH₃COO⁻ + HCO₃⁻ + 5H⁺</td>
<td>-460.34</td>
<td>-115.08</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>O₂ + CH₃CHOHCOO⁻</td>
<td>CH₃COO⁻ + HCO₃⁻ + H⁺</td>
<td>-478.28</td>
<td>-115.08</td>
<td></td>
</tr>
</tbody>
</table>

* a Free energy calculated from the standard free energies of formation of the products and reactants (44, 49) and by assuming standard conditions except for pH 7.
* b Free energy of formation for smectite was calculated from the standard reduction potential estimated by Amonette (2).
* c The chemical formula for smectite used in the calculations was Na₀.₈₁(Si₇.₃Al₀.₇)(Al₁.₀₆Fe₂.₇₃(OH)₄). (79).
suggests that the increase in biomass should be measured more often for these anaerobes. Given that previous perceptions on the growth of FeRB are heavily dependent upon direct counts in pure cultures, this observation could well revise our views on the energy obtained for growth via Fe(III) respiration. This conclusion is supported by the observation that both the predicted thermodynamic energy yield (Table 2) and the measured cell yield (Table 1) were higher by a factor of 5 when S. oneidensis cells were grown on oxygen than on various Fe(III) forms as the electron acceptor.

In agreement with the yield results (Table 1), growth rates of S. oneidensis calculated from the increase in cell number (Fig. 2) were higher for the soluble electron acceptors (0.489 × 10^{-7} [O_2] and 0.520 × 10^{-7} [Fe citrate] cells m^{-1} h^{-1}) in comparison to the solid electron acceptors (0.125 × 10^{-7} [smectite] and 0.174 [FeOOH] cells m^{-1} h^{-1}) tested. Growth rates for smectite and FeOOH were similar to those observed in previous studies of S. putrefaciens growing on FeOOH (25). In contrast, our growth rates were 1 to 2 orders of magnitude higher than those observed in past studies of Shewanella strains growing on the crystalline Fe(III) oxide minerals goethite (36) and maghemite (13). It appears that the growth rate of Shewanella on smectite more closely resembles rates on poorly crystalline Fe(III) oxides (FeOOH) than those on crystalline Fe(III) oxides. We suggest that at least 20% of the Fe(III) bound in smectite is available as FeOOH for relatively rapid growth. However, the fact that the growth rate of S. oneidensis on smectite was 25% lower than that on FeOOH suggests that there is some metabolic cost associated with smectitic Fe(III) utilization.

**Biogeochemical significance.** The form and concentration of reactive Fe(III) minerals are of paramount importance to the environmental significance of microbial Fe(III) reduction in sedimentary environments. Clay minerals are particularly important because they are highly reactive and account for a large fraction of Fe-containing minerals in nature (16, 41, 43, 48). In aquatic and marine sediments, silicates or clay minerals comprise 65% of all Fe minerals (48). Geochemical evidence has shown that Fe(III) in clay minerals is rapidly reduced and may constitute a significant fraction of the redox-active Fe from terrestrial environments to the deep sea (43, 52). Evidence also suggests that Fe-rich smectites comprise an important electron acceptor available for dissimilatory metal reducing metabolism in some surface (marine and aquatic) (12) and terrestrial subsurface sediments (52). In some contaminated subsurface sediments within the Department of Energy complex, iron-rich clay minerals are the primary electron acceptor available for microbial Fe(III) reduction (52). Thus, the microbial reduction of Fe-rich clay minerals is thought to have a significant impact on nutrient cycles, agricultural productivity, and the environmental fate of contaminants (8, 9, 41, 43, 51). However, the role of microbial clay reduction in natural environments has not been extensively determined.

Here we have demonstrated that FeRB, in a well-characterized pure culture and in enriched enrichments cultures, can conserve energy for growth by coupling the reduction of structural Fe(III) bound in clay minerals to the oxidation of organic acids, lactate, or acetate. This is the first description of any organism capable of such metabolism. Given their abundance and ubiquity, Fe-containing clay minerals may be important and previously overlooked electron acceptors for the growth of bacteria in natural environments. Our results showing rapid bacterial growth via smectite respiration support past geochemical evidence (8, 9, 16, 17, 48) to indicate that a substantial portion (20 to 50%) of the Fe(III) bound in smectite is easily accessible to FeRB. Furthermore, our results show that the growth rate and yield on smectitic Fe(III) are comparable to those on poorly crystalline Fe(III) oxide minerals (FeOOH), which suggests that FeRB respire smectite in parallel with FeOOH. In other words, our data suggest that bacteria do not preferentially reduce Fe oxides over clay minerals. Microbial clay reduction may therefore be an important, but little-studied, process limiting natural and contaminant biogeochemical cycles.

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**REFERENCES**


47. Tebo, B. M., and A. Y. Obraztsova. 1996. Sulfate-reducing bacteria grows with Cr(IV), U(VI), Mn(IV), and Fe(III) as electron acceptors. FEMS Microbiol. Lett. 162:193–198.


