Microbial reduction of structural iron in interstratified illite-smectite minerals by a sulfate-reducing bacterium

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ABSTRACT

Clay minerals are ubiquitous in soils, sediments, and sedimentary rocks and could coexist with sulfate-reducing bacteria (SRB) in anoxic environments, however, the interactions of clay minerals and SRB are not well understood. The objective of this study was to understand the reduction rate and capacity of structural Fe(III) in dioctahedral clay minerals by a mesophilic SRB, Desulfovibrio vulgaris and the potential role in catalyzing smectite illitization. Bioreduction experiments were performed in batch systems, where four different clay minerals (nontronite NAu-2, mixed-layer illite-smectite RAr-1 and ISCz-1, and illite IMt-1) were exposed to D. vulgaris in a non-growth medium with and without anthraquinone-2,6-disulfonate (AQDS) and sulfate. Our results demonstrated that D. vulgaris was able to reduce structural Fe(III) in these clay minerals, and AQDS enhanced the reduction rate and extent. In the presence of AQDS, sulfate had little effect on Fe(III) bioreduction. In the absence of AQDS, sulfate increased the reduction rate and capacity, suggesting that sulfide produced during sulfate reduction reacted with the phyllosilicate Fe(III). The extent of bioreduction of structural Fe(III) in the clay minerals was positively correlated with the percentage of smectite and mineral surface area of these minerals. X-ray diffraction, and scanning and transmission electron microscopy results confirmed formation of illite after bioreduction. These data collectively showed that D. vulgaris could promote smectite illitization through reduction of structural Fe(III) in clay minerals.

INTRODUCTION

Since the first isolation of dissimilatory Fe(III)-reducing bacteria (DIRB) more than 20 years ago (e.g. Geobacter metallireducens, Lovley & Phillips, 1988; Shewanella oneidensis, Myers & Nealson, 1988), scientific interest in microbial Fe(III) reduction has increased tremendously. The Fe(III) bioreduction process not only widens the realm of the life-supporting biological reactions but also contributes to energy flux in natural environments (Weber et al., 2006). This Fe cycling also links the carbon cycle to a number of important environmental processes such as retention of nutrients and mobilization or immobilization of toxic metals (Lovley, 1991; Islam et al., 2004; Peretyazhko et al., 2010).

The Fe(III) in solid minerals, such as iron (oxyhydr)oxides and clay minerals, can be reduced to Fe(II) by DIRB (Lovley, 1991; Roden & Zachara, 1996; Dong et al., 2009; Vorhies & Gaines, 2009). The rate and extent of bioreduction of solid phase Fe(III) are typically controlled by similar environmental conditions to those for soluble Fe³⁺ such as solution chemistry (e.g. pH: Zhang et al., 2007a; buffer type: Fredrickson et al., 1998; Dong et al., 2000; carbon source: Dong et al., 2009; Salas et al., 2009), types of DIRB used (Dong et al., 2009; Salas et al., 2010), and the ratio of DIRB to electron acceptor (Jaisi et al., 2007; Dong et al., 2009). However, for solid phase Fe(III) bioreduction, there are additional factors that govern the kinetics and equilbrium of Fe(III) bioreduction such as: iron chelator (Lovley & Woodward, 1996), exogenous and/or endogenous electron mediators (Lovley et al., 1996; Newman & Kolter, 2000; Roden et al., 2010), microbial appendages (Reguera et al., 2005; Gorby et al., 2006), and properties of minerals such as mineral surface area and...
crystallinity (Rodén & Zachara, 1996; Rodén, 2006; Dong et al., 2009). Overall, these biotic and abiotic factors are thought to control the reaction occurring at the interface between iron-containing minerals and micro-organisms. There have been several studies revealing that mineralogical factors strongly influence microbial Fe(III) reduction (Rodén & Zachara, 1996; Jaisi et al., 2005; Rodén, 2006; Bose et al., 2009; Bosch et al., 2010). For example, Bonneville et al. (2004) demonstrated that mineral solubility could be an important parameter in influencing the maximum specific reduction rate of Fe(III) oxides by one typical DIRB, Shewanella putrefaciens. Bose et al. (2009) recently showed that particle size could potentially affect the reduction rate of hematite by S. oneidensis. An experimental study by Rodén & Zachara (1996) demonstrated that the initial rate and long-term extent of bioreduction of Fe(III) in oxides by Shewanella alga were positively correlated with mineral surface area. Subsequent studies by the same author supported the above findings, regardless of the types of DIRB used (Shewanella or Geobacter) (Rodén, 2003, 2006). Although these results collectively suggest that in addition to biotic factors, mineralogical factors of Fe(III) oxides could be important parameters in controlling solid phase Fe(III) bioreduction by DIRB, yet it is poorly known whether or not such mineralogical model can be extended to other micro-organisms and minerals, such as sulfate-reducing bacteria (SRB) and clay minerals.

Clay minerals are ubiquitous on the Earth’s surface and they are the most abundant mineral constituents of marine sediments with various ferric iron contents in mineral structures (Vorhies & Gaines, 2009). For example, smectite, one of the largest classes of clay minerals, contains various ferric iron contents varying from 0.4 mmol g⁻¹ for Wyoming Na-montmorillonite (SWy-1) to 4.2 mmol g⁻¹ for nontronite (Zhang et al., 2007a). These structural Fe(III) in clay minerals can be biologically reduced by certain micro-organisms, mostly by DIRB (Dong et al., 2009 and references therein).

Although the Fe(III)-reducing capability of SRB has been long recognized (Coleman et al., 1993), there are only a limited number of reports on the interaction between SRB and clay minerals. To our knowledge, Li et al. (2004) is the only study which presented the evidence that SRB can grow on structural Fe(III) in nontronite (an iron-rich smectite) as the sole electron acceptor. Despite the cosmopolitan co-existence of SRB and various clay minerals in anoxic environments, it is still unknown whether or not SRB are capable of reducing structural Fe(III) in clay minerals other than nontronite.

Among the clay minerals with structural Fe(III), the smectite-to-illite reaction (the S-I reaction or smectite illitization, smectite + Al³⁺ + K⁺ → illite + silica) is of special significance during sediment diagenesis of mudstones and shales (Peacor, 1992; Kim et al., 2004; Dong et al., 2009) because the degree of the S-I reaction, termed ‘smectite illitization’ is linked to maturation, migration, and trapping of hydrocarbons (Pevear, 1999). The S-I reaction proceeds through mixed-layer illite-smectite (I-S) intermediates in which the percentage of illite layers in the mixed-layer phases increases with increasing temperature, time, K concentration, and water/rock ratio (Dong, 2005). These intermediate phases include pure smectite, smectite-rich R0, ordered R1, R2, R3 I-S (R, Reichweite ordering parameter), and end-member illite.

The roles of various DIRB in promoting the smectite to illite reaction have been increasingly recognized (e.g. Shewanella strains: Kim et al., 2004; Zhang et al., 2007b; Gaines et al., 2009; Jaisi et al., 2011; Thermococcus, Thermus, Thermophilus) (Jaisi et al., 2011). However, up till now, it is still not clear whether or not SRB can promote smectite illitization via Fe(III) reduction at ambient temperature. In the present study, we conducted experiments to address the following questions: (i) whether or not SRB can reduce structural Fe(III) in multiple clay minerals; (ii) what is the effect of clay mineral properties on the bioreduction extent and rate; (iii) whether or not SRB can catalyze the S-I reaction. Desulfovibrio vulgaris, a mesophilic SRB, and a suite of interstratified dioctahedral clay minerals including smectite, mixed-layer I-S, and illite, were chosen for this study. Our results demonstrated that SRB were capable of reducing structural Fe(III) in these clay minerals and promoting smectite illitization at 30 °C and ambient pressure.

**MATERIALS AND METHODS**

**Clay mineral preparation**

A suite of clay minerals were selected: nontronite NAu-2 (smectite group), illite IMt-1, and rectorite RAr-1 (50:50 illite:smectite), and R2 ISCz-1 (70:30 illite:smectite). Bulk samples of NAu-2, RAr-1, ISCz-1, and IMt-1 were purchased from the Source Clays Repository of the Clay Minerals Society (West Lafayette, IN, USA). Each sample was manually ground and thoroughly soaked in 0.5 M NaCl solution followed by centrifugation to obtain a final size fraction of 0.02–0.5 μm according to Stock’s law (given specific centrifugation time and speed). The chloride anion was removed by repeated washing in doubly distilled water and its complete removal was tested with AgNO₃. Previous studies in our laboratory have measured total Fe and Fe(III) contents in these minerals. Briefly, total Fe and Fe(III) contents were 23.4% and 23.3% for NAu-2, 4.9% and 3.8% for RAr-1, 0.7% and 0.6% for ISCz-1, and 12.3% and 11.1% for IMt-1 (Jaisi et al., 2005; Bishop et al., 2011). The BET surface area for these four clay minerals was 271,144, 129, and 5 m² g⁻¹, respectively (Bishop et al., 2011).

**Bacterial culturing**

Desulfovibrio vulgaris ATCC29579, a common member in the widespread Desulfovibrionaceae family, was a gift from...
Dr. Mary Ann Bruns (Pennsylvania State University). Prior to the bioreduction experiments, *D. vulgaris* was cultured in a simple lactate medium, which is frequently employed for cultivation of *Desulfovibrio* (Widdel & Bak, 1992). In brief, the lactate medium consists of (per liter) 1.0 g CaSO₄, 1.0 g NH₄Cl, 0.5 g KH₂PO₄, 2.0 g MgSO₄·7H₂O, 1.0 g yeast extract, 20 mmol sodium lactate, and 1 mL 0.1% resazurin (redox indicator). The medium was adjusted to pH 7.0 with 1 N HCl and added into the 25-mL Balch bottles. The bottles were degassed with O₂-free N₂/CO₂ gas mix (80:20) via passing through a hot copper column. After autoclaving, the sterile reductant solution (FeSO₄·7H₂O and ascorbic acid) was added to remove any residual O₂. *Desulfovibrio vulgaris* was incubated at 30 °C.

Bioreduction experiments

To mimic natural conditions, the bioreduction experiments were conducted in a non-growth medium (23.8 mM NaHCO₃, 20 mM KCl, and 1 g L⁻¹ amorphous Al(OH)₃·nH₂O, pH 7.0.), which also contained clay mineral (5 g L⁻¹, electron acceptor) and lactate (20 mm, electron donor). Three control experiments were performed: (i) abiotic control without SRB to assess abiotic Fe(III) reduction; (ii) no sulfate to assess the impact of sulfide-driven clay transformation; and (iii) chemical reduction of Fe(III) in clay minerals by 5 mM sodium sulfide to assess the impact of microbial vs. abiotic reduction on clay transformation.

Because both K and Al are the limiting factors in smectite illitization (Zhang et al., 2007a), the K concentration in bicarbonate buffer was increased to 20 mM to favor illite formation. An external Al source (final concentration 1 g L⁻¹) was provided in the form of amorphous Al(OH)₃·nH₂O (Zhang et al., 2007a). Clay minerals (5 g L⁻¹) were made into suspension with K-enhanced bicarbonate buffer in Balch tubes (total volume, 25 mL). The anoxic condition of the medium was adjusted to pH 7.0 with 0.1 N HCl and added into the 25-mL Balch bottles. The bottles were degassed with O₂-free N₂/CO₂ gas mix (80:20) via passing through a hot copper column. After autoclaving, the sterile reductant solution (FeSO₄·7H₂O and ascorbic acid) was added to remove any residual O₂. *Desulfovibrio vulgaris* was incubated at 30 °C.

Chemical analyses

To monitor the progress of Fe(III) reduction, the total Fe(II) content in the clay minerals was measured at selected time points with the 1,10-phenanthroline assay (Amonette & Templeton, 1998). The 1,10-phenanthroline method is based on total digestion of the mineral with HF-H₂SO₄ and has been demonstrated as an effective approach in measuring total Fe(II) in layer silicates (Amonette & Templeton, 1998; Jaisi et al., 2007; Anastacio et al., 2008). The reduction extent at each time point as calculated as follows:

\[
\text{Reduction extent} = \frac{\text{Fe(II)}_{\text{total}} - \text{Fe(II)}_{\text{initial}}}{\text{Fe(III)}_{\text{initial}}} \times 100\% \quad (1)
\]

The ultimate extent of reduction (i.e. a measure of reduction capacity) was measured after 25-days of incubation, at which point Fe(II) production leveled off. This reduction capacity was correlated with clay mineral properties (i.e. percentage of smectite layers in I-S and surface area etc.). The initial reduction rate was calculated from the amount of Fe(II) produced during the first 24 h normalized to the total amount of Fe(III) present in the starting clay minerals according to the following equation.

\[
\text{Fraction of Fe(III) reduced per hour} = \frac{(\text{Fe(II)}_{\text{total}} - \text{Fe(II)}_{\text{initial}}) \text{ within the first } 24 \text{ h}}{\text{Fe(III)}_{\text{total}} \times 24 \text{ h}} \times 100\% \quad (2)
\]

X-ray diffraction and related modeling

Smear mounts (Moore & Reynolds, 1997) were prepared for all XRD analyses of both unreduced and bioreduced clay suspensions after 97-days of incubation to determine any mineralogical changes. Approximately 0.5-mL suspensions were smeared onto glass slides and dried in an anaerobic chamber. Ethylene glycol (EG) saturation was achieved by exposing the oriented clay slides to EG vapor overnight at 60 °C. Samples were run on a Scintag X1 X-ray powder diffractometer using CuKa wavelength, a fixed slit scintillation detector, and a power of 1400 W (40 kV, 35 mA). The analytical conditions were as follows: a step size of 0.02° 2θ, a counting time of 2 s per step, and a scanning range of 2–35° 2θ. Qualitative identification of mineral phases was
made possible with the JADE 7 program (Materials Data, Inc., Livermore, CA, USA).

To detect the possible formation of illite as a result of microbial reduction and to quantify the relative proportion of smectite, mixed-layer I-S, and illite in the clay samples, XRD was also performed at the United States Geological Survey in Boulder, CO. Approximately 5 mg of Li-saturated clay samples were dispersed in 2 mL distilled water using an ultrasonic water bath, mixed with 100 μL polyvinylpyrrolidone (PVP, molecular weight = 10 000), pipetted onto glass sides and dried for XRD analysis. Samples were X-rayed with a Siemens D500 XRD system using Cu radiation, a monochromator, and were scanned in 0.02° 2θ steps from 2–10° 2θ, with a counting time of 2 s per step. The XRD patterns of the glycolated and PVP-treated samples (unreduced and reduced) were modeled with the Sybilla program (McCarty et al., 2009) to determine the relative proportions of smectite, illite and any mixed-layer I-S phases.

Scanning and transmission electron microscopy

Clay suspensions were prepared for SEM observations. After fixation with 2% paraformaldehyde and 2.5% glutaraldehyde, one droplet of suspension was placed onto the surface of a glass cover slip. The sample on the cover slip was sequentially dehydrated using varying proportions of ethanol followed by critical point drying with a Tousimis Samdro-780A Critical Point Dryer (CPD) (Dong et al., 2003a; Zhang et al., 2007a). The cover slips were mounted onto SEM stubs and Au-coated for observations using a Zeiss Supra 35 VP SEM with Genesis 200 X-ray energy dispersive spectroscopy (Carl Zeiss NTS GmbH, Oberkochen, Germany). The SEM was operated at an accelerating voltage of 10–15 kV. A short working distance (6–10 mm) and low beam current (30–40 μA) were used to achieve the best image resolution.

For TEM observations, diluted clay suspensions were pipetted onto 300 mesh copper grids with a nitrocellulose membrane and carbon coating. The grids were prepared and allowed to dry overnight in an anaerobic chamber. A JEOL JEM-2100 LaB6 TEM (JEOL Ltd., Tokyo, Japan) with a 200 keV accelerating voltage was used for TEM analysis. Energy dispersive X-ray spectroscopy (EDS) fitted in the TEM was employed for mineral identification.

RESULTS

Reduction of Fe(III) in clay minerals by D. vulgaris

The pH of the medium in the abiotic and biotic experimental systems did not change over time, ranging from 6.5 to 6.8. There was a significant difference in Fe(III) reduction between two sets of abiotic control: in the absence of sulfide, there was no appreciable Fe(III) reduction. In the presence of 5 mM sodium sulfide, Fe(II) concentration increased rapidly within the first 8 h and leveled off subsequently (Fig. 1). The extent of Fe(III) reduction by 5 mM sulfide ranged from 0.7% (Imt-1) to 5% (NAu-2).

In contrast to low levels of Fe(II) in the abiotic controls, Fe(II) concentration in the inoculated experimental tubes increased steadily (Fig. 1) with time, indicating that D. vulgaris was able to reduce Fe(III) in the clay minerals. Overall, the patterns of bioreduction were similar for all four clay minerals: rapid Fe(II) production in the first 1–2 days followed by a slow increase over longer time. The final reduction extent (after 25 days of incubation) was significantly higher in the presence of AQDS than in its absence. The effect of sulfate was variable depending on the presence or absence of AQDS.

In the presence of AQDS, 5 mM sulfate exerted a minor effect on the extent of Fe(III) reduction, but enhanced the initial rate of bioreduction (Fig. 1). In contrast, in the absence of

Fig. 1 Production of Fe(II) in mM and the reduction extent vs. elapsed time. All data are averages from duplicate tubes. A, NAu-2; B, RAi-1; C, ISCz-1; D, IMt-1.
AQDS, sulfate greatly increased not only the extent but also the initial rate of Fe(III) reduction.

There was a linear correlation between the bioreduction extent and the percent smectite in the I-S series (Fig. 2A). Because smectite content is typically positively correlated with BET surface area for illite-smectite minerals (Likos & Lu, 2002), the extent of bioreduction was also positively correlated with BET surface area (Fig. 2B). Such a correlation was similar to previous findings for iron oxides (Roden & Zachara, 1996; Roden, 2003) and Fe(III) bioreduction by DIRB S. putrefaciens CN32 (Bishop et al., 2011), suggesting that bioreduction of structural Fe(III) in clay minerals was also largely controlled by surface area. The amount of enhancement of the reduction extent by AQDS (i.e. the difference in the reduction extent with and without AQDS) also displayed a correlation with BET surface area (Fig. 2C). Similar to the correlation between the bioreduction extent and the percent smectite in the I-S minerals, the initial rate of bioreduction was also correlated to percent smectite in the I-S series and surface area (Fig. 3), similar to a previous study of Fe(III) bioreduction by DIRB (Bishop et al., 2011).

**X-ray diffraction and related modeling**

The XRD profiles of NAu-2 and ISCz-1 (both abiotic control and bioreduced samples treated with 5 mM sulfate) were selected as representatives to investigate mineralogical changes as a result of bioreduction (Fig. 4). Bioreduction of Fe(III) in nontronite resulted in a decline of intensity and broadening of the (001) peak (Fig. 4A), which suggested a certain extent of structural alteration, consistent with those of Zhang et al. (2007a,b) and Liu et al. (2011). Specifically, for the air-dried samples, the d(001) value shifted from 13.11 Å for the abiotic control to 11.23 and 12.52 Å for bioreduced sample without and with AQDS, respectively (Fig. 4A). A small peak with a d value of 10.05 Å emerged after bioreduction, especially for the bioreduced sample with AQDS. Upon ethylene glycolation, the (001) peak was split into two, one with larger d-spacing and the other with smaller one (Fig. 4A). The peak at 8.80° 2θ (d = 10.05 Å) was better resolved.

The XRD patterns of ISCz-1 did not show any obvious change after bioreduction. A dominant peak occurred at approximately 10.6 Å for both the abiotic control and the bioreduced samples (Fig. 4B). After the treatment with EG, the main peak of the abiotic control was split into two with d values of 12.52 and 9.55 Å, whereas for the bioreduced samples, the main peak was split into 11.85 and 9.85 Å. No peak with a d value of approximately 10 Å was observed. The Sybilla modeling results of the XRD patterns for the glycolated samples showed that there were mixed-layer I-S...
(R2 and R3) and discrete illite formation in the bioreduced NAu-2, whereas in the bioreduced ISCz-1 R2 I-S largely remained with only a small amount of R3 I-S formation (Table 1).

To verify the presence of illite, the bioreduced NAu-2 samples were treated with Li saturation and PVP followed by XRD. These treatments would disperse smectite layers into single unit-cell particles lacking diffraction peaks (Eberl et al., 1998). After these treatments, two main peaks were detected in the range from 7 to 10° 2θ (Fig. 5) for both the unreduced and reduced NAu-2 samples. One had a spacing of 10.05 Å, characteristic of discrete illite or biotite, and the other had a spacing of 9.34 Å, characteristic of talc. The peak with 10.05 Å in the abiotic control was either illite or biotite, but the same peak in the bioreduced sample was likely biogenic illite. Indeed, a trace amount of biotite and talc in NAu-2 was detected in an early study (Keeling et al., 2000). The biogenic illite in the bioreduced NAu-2 was further confirmed by TEM data as described below. The ratio of illite to talc for the bioreduced sample was much higher than the ratio of biotite to talc for the abiotic control (Fig. 5). This increase was interpreted to be due to formation of biogenic illite, because talc with a formula of Mg₃Si₄O₁₀(OH)₂ should be stable during bioreduction. Hence, illite formation as a result of bioreduction was in agreement with the Sybilla modeling results (Table 1) and SEM/TEM data described below.

(Fig. 4) X-ray diffraction patterns of NAu-2 (A) and ISCz-2 (B) samples after 97-days of incubation with Desulfovibrio vulgaris and 5 mM sulfate. Both air-dried (shorten as AD) and ethylene glycolated (EG) samples were run. See text for data description.

<table>
<thead>
<tr>
<th>Samples</th>
<th>Smectite (%)</th>
<th>R0</th>
<th>R1</th>
<th>R2</th>
<th>R3</th>
<th>Discrete illite (%)</th>
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<td>NAu-2</td>
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<td>Abiotic control</td>
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<td>Bioreduced with AQDS</td>
<td>32</td>
<td>–</td>
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<td>ISCz-1</td>
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<td>Bioreduced without AQDS</td>
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<td>87</td>
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<tr>
<td>Bioreduced with AQDS</td>
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<td>–</td>
<td>–</td>
<td>84</td>
<td>16</td>
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AQDS, anthraquione-2,6-disulfonate.
 ratio with no K, possibly a mixture of amorphous Al(OH)$_3$·nH$_2$O and residual NAu-2. Lath-shaped grains D and E displayed the Al/Si ratio and K content characteristic of illite (Kim et al., 2004).

The TEM lattice fringe images of the bioreduced NAu-2 sample further demonstrated the formation of illite. In contrast to wavy smectite layers, illite fringes were straight and constrained within a well-defined packet (Fig. 9), as consistent with previous observations of illite (Dong et al., 1997; Kim et al., 2004).

**DISCUSSION**

**Reduction of structural Fe(III) in clay minerals by *D. vulgaris***

There have been numerous studies in showing that SRB can reduce oxidized metals, such as Fe(III), Mn(IV), Cr(VI), and U(VI) (Lovley et al., 1993; Lovley & Phillips, 1994; Michel et al., 2001; Li et al., 2006). Some SRB are capable of coupling metal reduction to growth (Tebo & Obraztsova, 1998; Li et al., 2006). Most studies on Fe(III) reduction by SRB have used soluble Fe$_3^+$ such as ferric citrate (Tebo & Obraztsova, 1998) or poorly crystalline iron oxyhydroxides (Lovley et al., 1993). Our results unambiguously showed that SRB could reduce structural Fe(III) in clay minerals. The Fe(III)-reducing ability of *D. vulgaris* was similar to, and even higher than that of other micro-organisms. For example, the Fe(III) reduction extent of 33.6% by *D. vulgaris* in the presence of AQDS was similar to that reported by Jaisi et al. (2005, 2007), in which *S. putrefaciens* CN32 was used to reduce NAu-2 under similar conditions (bicarbonate buffer and non-growth medium). Recently, Liu et al. (2011) observed approximately 33% reduction extent of NAu-2 using a mesophilic methanogen *Methanoarculina barkeri* when methanol was provided as substrate, but only approximately 13% reduction extent was achieved using H$_2$/CO$_2$ as substrate. The extent of reduction observed in this study was higher than the extent of 10–29% for the same mineral (NAu-2) by similar SRB (*Desulfovibrio* strain G-11) (Li et al., 2004). Different SRB strains and variable experimental conditions may have accounted for such differences. Indeed, diverse SRB strains have been shown to exhibit different reduction capacity of Fe(III) and U(VI) (Lovley et al., 1993). In comparison with the study by Li et al. (2004), cell concentration (approximately $1.5 \times 10^8$ cells mL$^{-1}$) utilized in our experiments was nearly 10-fold higher than that used by Li et al. (2004).

Anthraquinone-2,6-disulfonate significantly enhanced the reduction extent and rate in all treatments, consistent with its status as ‘electron shuttle’ (Lovley et al., 1996). The amount of enhancement varied for different clay minerals. These results imply that SRB may play an important role of iron reduction in organic-rich environments, where humic substances could serve as a natural electron shuttle. In the absence of AQDS, sulfide produced during sulfate reduction reacted
with the phyllosilicate Fe(III) (Fig. 3) and significantly enhanced the rate and extent of bioreduction. When both AQDS and sulfate were present, sulfate had little effect, suggesting that the reduced form of AQDS, that is, AH2DS, was a more favorable electron donor in reducing the phyllosilicate Fe(III) than sulfide.

Despite the fact that the Fe(III)-reducing role of SRB has long been recognized, the bioreduction mechanisms are still speculative. At least two strategies have been proposed in literature: an indirect mechanism in which sulfide produced by sulfate reduction can chemically reduce Fe(III) (Li et al., 2006); and the other one is direct enzymatic reduction of Fe(III) by SRB (Lovley et al., 1993; Lovley & Phillips, 1994). The direct enzymatic pathway is supported by the fact that different types of tetraheme cytochromes (c3, c7, b, etc.) have been purified from D. vulgaris (Lovley & Phillips, 1994) and other SRB (Michel et al., 2001), and they have been tested to be metal reductases, similar to cytochromes produced by DIRB (Shi et al., 2007).

Various extents of bioreduction with Fe(III) in clay minerals as the sole electron acceptor in absence of AQDS and sulfate demonstrated that Fe(III) reduction by D. vulgaris was enzymatic. In the treatments without AQDS, the enhancement of Fe(III) bioreduction by sulfate relative to the no-sulfate treatment (Fig. 1) suggested a two-step process: (i) production of sulfide by sulfate reduction; (ii) abiotic reduction of structural Fe(III) in clay minerals by sulfide. Such redox reactions can be written as follows:

\[
2\text{CH}_3\text{CHOHCOO}^- + \text{SO}_4^{2-} \xrightarrow{\text{SRB}} 2\text{CH}_3\text{COO}^- + \text{HS}^- + 2\text{HCO}_3^- + \text{H}^+ \quad (3)
\]

\[
\text{HS}^- + 2\text{Fe}^{3+} \rightarrow 2\text{Fe}^{2+} + \text{S}^0 + \text{H}^+ \quad (4)
\]

The rate-limiting factor in this process appeared to be the reducible fraction of structural Fe(III), because sulfide should have been in excess. Indeed, our abiotic experiment with
sulfide showed that the amount of produced Fe(II) by 5 mM sulfide was much lower than an stoichiometric amount if all sulfide was exhausted (Fig. 1). Similarly, Li et al. (2004) observed that Fe(II) produced from reduction of structural Fe(III) in nontronite by 50 mM sulfide never exceeded 0.32 mmol g$^{-1}$ (2.3 mM). These results were in general agreement with previous observations in showing that only a small fraction of structural Fe(III) in clay minerals is bioreducible depending on exact experimental conditions (Dong et al., 2009).

The positive correlation between the extent of bioreduction and the percentage of smectite/surface area (Fig. 2) suggested that bacterial reduction of Fe(III) in smectite occurred at the edges of clay mineral layers rather than at the basal surface, as consistent with a previous study (Ribeiro et al., 2009). In this case, much of the Fe(III) at the center of the clay particle would be inaccessible to the reductant and bacteria. This inaccessibility would partially account for incomplete Fe(III) bioreduction as well. In addition, *D. vulgaris* may have partially dissolved NAu-2 and reductive dissolution could release other ions, such as Al, Si, etc. (Li et al., 2004). Released Al$^{3+}$ can directly inhibit SRB activity (Wong et al., 2004), possibly accounting for why bioreduction ceased before all Fe(III) in NAu-2 was exhausted. Bioproduced Fe(II) could also inhibit further bioreduction through surface adsorption (Jaisi et al., 2007).

Although the exact mechanisms of Fe(III) bioreduction by SRB could not be determined, the fact that the Fe(III) bioreduction extent was much higher than that reduced by sulfide strongly suggested that enzymatic interaction was a predominant pathway responsible for the bioreduction of Fe(III) in the clay minerals by *D. vulgaris*.

**Influence of mineralogical properties on the bioreduction of Fe(III) in clay minerals**

Several lines of evidence suggest that mineralogical parameters of iron oxides can play a crucial role in dissimilatory Fe(III) reduction, including surface area, mineral solubility, and particle size (Roden & Zachara, 1996; Roden, 2003, 2006; Bose et al., 2009; Bosch et al., 2010). In comparison with iron...
oxides, clay minerals, such as the ones used in this study, display distinctly different mineral structure, chemical composition, and various physical and chemical properties. Smectite and illite have the same basic layer structure (2:1 phyllosilicates) (Dong et al., 2003b), but there exist many differences between them owing to the different extents of tetrahedral and octahedral substitutions in the structure (Jaisi et al., 2007). In general, smectite has higher layer expandability, larger surface area, and lower layer charge than illite. All these differences could be the important factors controlling the extent of reduction (Fig. 2). In absence of any external electron shuttles, direct contact between cells and solid minerals is believed to be a major mode of electron transfer from cell membrane to the Fe(III) in solid minerals (Dong et al., 2009; Bosch et al., 2010). The unique properties of smectite can favor such electron transfer via different pathways.

First, higher layer expandability of smectite than illite, also related to larger surface area and lower layer charger, provides more exposed Fe(III) sites for microbial attachment, possible dissolution, and transfer of electrons to Fe(III) centers in the structures, resulting a higher reduction extent than illite. Recent studies (Jaisi et al., 2007; Bishop et al., 2011) demonstrated that microbial reducibility of more expandable nontronite NAu-2 was much higher than non-expandable chlorite and illite.

Second, crystal chemistry of clay minerals could be another important factor. Clay minerals usually carry a net negative surface charge (Sposito et al., 1999), and microbial surface is also negatively charged (partly arising from carboxylic acids, amino and other groups), resulting in a repulsive force between clay minerals and microbes. However, the presence of a proton dissociation-association mechanism on the edges of clay structure can result in positive charges (Frisssel & Bolt, 1962). Hence the edges of clay minerals are expected to attach to microorganisms. Among the S-I series, the surface charge of smectite is less negative than illite, thus the edges of smectite particles might be preferably sorbed onto microbial surfaces (Bishop et al., 2011). Such smectite-microbe association would result in electron transfer from the edge sites to the interior of the smectite structure, as consistent with a recent suggestion based on Mössbauer data of bioreduced smectite (Ribeiro et al., 2009), in contrast to smectite reduction along the basal surfaces using chemical reluctant (Komadel et al., 2006).

In the presence of AQDS, *D. vulgaris* cells may reduce AQDS to AH2DS which would subsequently reduce Fe(III) chemically (Jaisi et al., 2007). The positive correlation between the amount of enhancement by AQDS and smectite content in the S-I series suggested that the smectite structure allows easier access of AQDS and AH2DS to facilitate Fe(III) bioreduction.

**Microbial role in smectite illitization**

Smectite illitization, one of the most important reactions in clay mineralogy and geochemistry, is linked to hydrocarbon maturation, geopressing of shales, the formation of growth faults, and changes in pore water chemistry (Kim et al., 2004; Dong et al., 2009). In the last several decades, extensive research has been carried out to understand the mechanism of smectite illitization (Dong, 2005; Dong et al., 2009). Most of these models believe that this reaction is accomplished by increasing temperature, time, K concentration, water/rock ratio, and pH (Dong et al., 2009 and references therein). It is known that the S-I reaction can proceed via either a solid-state transformation producing various mixed-layer intermediates, or by a dissolution-precipitation mechanism (Meunier & Velde, 2004; Dong, 2005). In the solid-state transformation model, structural Fe(III) reduction can be an important driving force for illitization as layer charge created by the Fe(III) reduction attracts cations such as K⁺ into the interlayer (Eslinger et al., 1979), a requirement for smectite illitization. Repeated periodic phyllosilicate-Fe(III) reduction and oxidation could result in a gradual increase in the amount of K fixed in the interlayer of the smectite structure, thus leading to gradual conversion of smectite to a more illitic form, i.e. to a mixed-layer smectite-illite (Shen & Stucki, 1994).
In the dissolution-precipitation mechanism, reactant smectite dissolves and soluble constituents recombine to precipitate as illite in solutions containing K and Al (Meunier & Velde, 2004; Dong, 2005). This mechanism has been demonstrated to be catalyzed by DIRB through reduction of Fe(III) in the smectite structure. Subsequent studies (Zhang et al., 2007a,b; Jaisi et al., 2011) verified that both mesophilic and thermophilic DIRB can catalyze smectite illitization. This study suggested that SRB can also promote this reaction through the same mechanism, that is, dissolution of smectite and precipitation of illite.

The results of this study are not consistent with a previous one showing that no illite was observed after reduction of Fe(III) in nontronite by Desulfovibrio strain G-11 (Li et al., 2004). In addition to different bacterial strains used, there were other factors that may have accounted for this inconsistency, including K concentration, the presence or absence of external Al source, and time duration. Numerous studies have established that for the smectite-illite reaction to proceed, external K and Al sources are required (Huang et al., 2007a). In addition, a long time (6 months) may be needed to induce this reaction by microbial activity (Gaines et al., 2009), especially in the case when non-growth medium was used. In contrast to a high K concentration (20 mM), external Al source and 97-days of incubation used in this study, low amounts of K (approximately 1.3 mM), no external Al, and short time duration were used in the Li et al. (2004) study. Their experimental conditions may not be adequate to promote illite formation.

The microbially catalyzed smectite to illite reaction has been mostly demonstrated in laboratory experiments, where high bacterial and K concentrations and end-member nontronite were typically used. It is important to recognize that these conditions may not be fully realistic in natural sedimentary environments. Therefore, the microbially catalyzed dissolution-precipitation mechanism offers one possibility for the smectite-to-illite reaction and it may be only operative in certain geological environments. For example, smectite illitization has been observed in young sediments and wetland soils, which did not undergo thermal maturation (Turner & Fishman, 1991; Joeckel & Clement, 2005; Vorhies & Gaines, 2009), implying that in-situ smectite illitization in these settings can be enhanced by the activities of both DIRB and SRB (Dong et al., 2009).

CONCLUSIONS

The sulfate-reducing bacterium Desulfovibrio vulgaris could reduce structural Fe(III) in smectite-illite clay minerals. The order of the reducibility was as follows: nontronite > mixed-layer I/S > illite. This order was correlated not only with the smectite proportion in these clay minerals but also with BET surface area. A combination of XRD, Sybilla stimulation, and SEM and TEM characterization demonstrated illite formation after bioreduction of Fe(III) in nontronite.

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