American Mineralogist, Volume 92, pages 1411–1419, 2007

Microbial reduction of structural Fe^{3+} in nontronite by a thermophilic bacterium and its role in promoting the smectite to illite reaction

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ABSTRACT

The illitization process of Fe-rich smectite (nontronite NAu-2) promoted by microbial reduction of structural Fe^{3+} was investigated by using a thermophilic metal-reducing bacterium, *Thermoanaerobacter ethanolicus*, isolated from the deep subsurface. *T. ethanolicus* was incubated with lactate as the sole electron donor and structural Fe^{3+} in nontronite as the sole electron acceptor, and anthraquinone-2, 6-disulfonate (AQDS) as an electron shuttle in a growth medium (pH 6.2 and 9.2, 65 °C) with or without an external supply of Al and K sources. With an external supply of Al and K, the extent of reduction of Fe^{3+} in NAu-2 was 43.7 and 40.4% at pH 6.2 and 9.2, respectively. X-ray diffraction and scanning and transmission electron microscopy revealed formation of discrete illite at pH 9.2 with external Al and K sources, while mixed layers of illite/smectite or highly charged smectite were detected under other conditions. The morphology of biogenic illite evolved from lath and flake to pseudo-hexagonal shape. An external supply of Al and K under alkaline conditions enhances the smectite-illite reaction during microbial Fe^{3+} reduction of smectite. Biogenic SiO_2 was observed as a result of bioreduction under all conditions. The microbially promoted smectite-illite reaction proceeds via dissolution of smectite and precipitation of illite. Thermophilic iron reducing bacteria have a significant role in promoting the smectite to illite reaction under conditions common in sedimentary basins.

Keywords: Dissolution, illite, microbial Fe^{3+} reduction, nontronite, precipitation, sedimentary basin, smectite, *Thermoanaerobacter ethanolicus*

INTRODUCTION

Smectite-illite interstratified clay minerals are dioctahedral layer phyllosilicates, which are ubiquitous in soils, sediments, and pelitic rocks. While smectite is common in soils and shallow sediments, illite is a more stable phase under diagenetic conditions. When water-bearing and expandable smectite is buried and subject to increasing temperature and pressure, it tends to transform to illite (Dong and Peacor 1996; Dong et al. 1997; Dong 2005). The resultant illite is fundamentally different from smectite in both structure and composition. The smectite to illite (S-I) reaction is considered to be one of the most important mineral reactions during sediment diagenesis of mudstones and shales (Peacor 1992), as the degree of the smectite to illite reaction, termed "smectite illitization," is linked to the maturation, migration and trapping of hydrocarbons (Burst 1969; Pevear 1999; Weaver 1960), the development of pore pressures (Freed and Peacor 1989), growth faults (Bruce 1984), rock cementation and porosity reduction (Bjorkum and Nadeau 1998; Boles and Franks 1979), and pore water chemistry (Brown et al. 2001).

The smectite to illite reaction proceeds through mixed-layer illite-smectite (I-S) intermediates in which the percentage of illite layers increases with increasing temperature (Hower et al. 1976), time (Pytte and Reynolds 1989), K concentration (Huang et al. 1993), water/rock ratio (Whitney 1990), and pH (Drief et al. 2002; Eberl et al. 1993). Recent studies have shown an effect of microbes in promoting the smectite to illite reaction (Kim et al. 2004; Zhang et al. 2007). The microbially promoted smectite-illite reaction can take place at room temperature and one atmosphere within two weeks (Kim et al. 2004). This reaction typically requires conditions of 300–350 °C, 100 MPa, and 4–5 months in the absence of microbial activity. Our most recent study (Zhang et al. 2007) has demonstrated a strong catalytic effect of organic matter intercalated in the interlayer of the smectite structure.

The S-I reaction can be promoted by reducing structural Fe^{3+} in smectite, either biologically (Kim et al. 2004; Zhang et al. 2007) or chemically (Eslinger et al. 1979; Russell et al. 1979). During the process, smectite may be partially dissolved, and illite precipitated. The extent of microbial dissolution of smectite may depend on several factors, such as the amount of Fe^{3+} in the structure and site occupancy (tetrahedral vs. octahedral), the extent of Fe^{3+} reduction, the type of bacteria, solution chemistry, and other experimental conditions (such as temperature). Iron-rich smectite is fairly common in nature, and the ferric iron content may vary from 0.4 mmol/g for Wyoming Na-Montmorillonite (Swy-1) (Source Clays Repository) to 4.2 mmol/g for nontronite (Keeling et al. 2000). It is also well established that microbes can reduce Fe^{3+} in the smectite structure (Stucki et al. 1987; Wu et al. 1988; Gates et al. 1993; Kostka et al. 1996, 1999a, 1999b; Gates

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0003-004X/07/0809–1411$05.00/DOI: 10.2138/am.2007.2498

REPORT DOCUMENTATION PAGE

Form Approved
OM8 No. 0704-0188

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1. REPORT DATE *(DD-MM-YYYY)*
03042007

2. REPORT TYPE
Journal Article

3. OATES COVEREO *(From - To)*

4. TITLE AND SUBTITLE
Microbial reduction of structural Fe3+ in nontronite by a thermophilic bacterium and its role in promoting the smectite to illite reaction

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Gengxin Zhang, Hailiang Dong, JinWook Kim, D.D. Eberl

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
Naval Research Laboratory
Marine Geoacoustics Division
Stennis Space Center, MS 39529

8. PERFORMING ORGANIZATION REPORT NUMBER
NRL/JA/7430-07-2

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research
800 North Quincy Street
Arlington VA 22217-5000

10. SPONSOR/MONITOR'S ACRONYM(S)
ONR

11. SPONSOR/MONITOR'S REPORT

20090522123

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution is unlimited

13. SUPPLEMENTARY NOTES
American Mineralogist, Volume 92, pages 1411 - 1419, 2007

14. ABSTRACT

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15. SUBJECT TERMS
Dissolution, illite, microbial Fe3+ reduction, nontronite, precipitation, sedimentary basin, smectite, thermoanaerobacter ethanolicus

16. SECURITY CLASSIFICATION OF: a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES
Unclassified	Unclassified	Unclassified	UU	9

19a. NAME OF RESPONSIBLE PERSON
Yoko Furukawa

19b. TELEPHONE NUMBER *(Include area code)*
228-688-5474

et al. 1998; Dong et al. 2003; Kim et al. 2003, 2004; Jaisi et al. 2005, 2007a, 2007b; Zhang et al. 2007), with a varying extent of Fe^{3+} reduction depending on experimental conditions.

Our previous studies have focused on the microbially promoted S-I reaction at room temperature and in presence of organic matter (Kim et al. 2004; Zhang et al. 2006b). However, the effect of microbial Fe^{3+} reduction on the S-I reaction at elevated temperatures, that may be common in sedimentary basins, has not been studied. The objectives of this study were (1) to understand if bacteria can promote the S-I reaction under diagenetically relevant conditions by using a thermophilic bacterium, and (2) to understand if the reaction rate is affected by environmental conditions, such as pH and availability of K and Al. We found that the S-I reaction was favored at an alkaline pH with an external supply of Al and K source. The present study, therefore, enhances our fundamental understanding of the S-I reaction and has significant implications for sediment diagenesis.

MATERIALS AND METHODS

Bacterium and clay mineral

CCSD_DF2450_M1_68_isolate1 was isolated from a circulating drilling fluid taken at 2450 m depth in the Chinese Continental Scientific Deep Drilling project (Zhang et al. 2006). 16S rRNA gene analysis identified that it is 98–99% similar to *Thermoanaerobacter ethanolicus* (Zhang et al. 2006). *T. ethanolicus* is an obligately anaerobic, thermophilic, metal-reducing bacterium isolated from the deep subsurface of the Piceance Basin, Colorado (Liu et al. 1997; Roh et al. 2002). CCSD_DF2450_M168_isolate1 was enriched and isolated in M1 medium (Kostka and Nealson 1998) under strictly anaerobic conditions at an incubation temperature of 68 °C. The isolate was able to use lactate, acetate, and H_2 as electron donors and Fe^{3+} in chlorite and nontronite as electron acceptor (Zhang et al. 2006).

Nontronite is a dioctahedral smectite-group mineral and represents the ferric end-member of the nontronite-beidellite series. The nontronite sample (NAu-2) used in this study was purchased from the Source Clays Repository of the Clay Minerals Society. NAu-2 was originally uncovered from veins in deeply weathered granulite facies containing schist, gneiss, and amphibolite from Uley Graphite Mine near Port Lincoln in South Australia (Keeling et al. 2000). Bulk clay was size fractionated and a size fraction of 0.5–2 μm was used in this study. The total Fe content in NAu-2 is 23.4%, of which 0.6% is Fe^{2+} (Jaisi et al. 2005). NAu-2 consists of pure nontronite with no other Fe-bearing minerals. It contains both octahedral (91%) and tetrahedral Fe^{3+} (~9%) (Gates et al. 2002; Keeling et al. 2000).

Bacterial reduction experiments

For nontronite reduction experiments, a modified basal medium was prepared with the Hungate technique (Hungate 1969). The composition of the medium (Prowe and Antranikian 2001) contained (per L of deionized water) Na_2HPO_4, 0.5 g; $(NH_4)_2SO_4$, 1.5 g; $MgSO_4 \cdot 7H_2O$, 0.1 g; $CaCl_2 \cdot 2H_2O$, 0.05 g; vitamin solution 141 (DSMZ), 10 mL; trace element solution 141 (DSMZ), 10 mL; yeast extract, 0.2 g; and resazurin, 0.0001 g. The basal medium was adjusted to pH of 6.2 and 9.2 to test the effect of pH on the S-I reaction. *T. ethanolicus* can grow in a wide pH range (4.4~9.8) (Wiegel and Ljungdahl 1981), and growth curves are similar at both pH 6.2 and 9.2. The medium pH of 6.2 was achieved with addition of 1.68 g of $NaHCO_3$ (per L of the modified basal medium) under a N_2-CO_2 (80:20) gas atmosphere. The medium pH of 9.2 was achieved with addition of 2.2 g of $NaHCO_3$ and 2.2 g of Na_2CO_3 under a N_2 (100%) gas atmosphere. In selected experiments, external K and Al sources were added to the basal medium to promote the S-I reaction. An external K source was added in the form of $KHCO_3$ (to replace $NaHCO_3$) and K_2CO_3 (to replace Na_2CO_3) with an additional amount of KCl to achieve a final K concentration of 50 *mM*. An external Al source (1 g per liter of medium) was added in the form of amorphous $Al(OH)_3 \cdot nH_2O$, which was synthesized according to the method described previously (Sato and Sato 1996). The components in reduction experiments were composed of Fe^{3+} in nontronite (5 mg/mL, final concentration) as the sole electron acceptor, lactate (20 *mM*, final concentration) as the sole electron donor, and CCSD_DF_M168_isolate1 cells as a mediator in a modified basal medium (at pH 6.2 and 9.2) with anthraquinone-2,6-disulfonate (AQDS) (Sigma, St. Louis, Missouri) as an electron shuttle (Table 1). The modified basal medium (without vitamin, lactate, and AQDS) was dispensed into anaerobic culture tubes purged with N_2 or N_2-CO_2 mix gases, and autoclaved. After autoclaving, a minimum amount of reducing agent (cysteine) was added to achieve a fully anaerobic condition. CCSD_DF2450_M1_68_isolate1 cells (final concentration ~1×10^6 cells/mL) were inoculated into autoclaved, anaerobic culture tubes (duplicates for each type of treatment). Controls were identical to the treatment tubes except that anaerobic medium replaced cells. The culture tubes were incubated at 68 °C without shaking.

Analysis of bactcrial Fe^{3+} rcduction

The extent of microbial reduction of nontronite was monitored by measuring Fe^{2+} production with ferrozine assay (Stookey 1970). At selected time points, 0.5 mL of cell-mineral suspension, sampled with a sterile syringe, was added to plastic tubes containing 0.5 mL of 1 *N* HCl (Ultrex grade, Sigma-Aldrich). The cell-mineral suspension was allowed to stand in HCl for 24 h before analyzing for Fe^{2+}. This extraction is termed the 0.5 *N* HCl extractable Fe^{2+}. Other studies have shown 0.5 *N* HCl to be effective for extracting microbially produced Fe^{2+} including both the adsorbed form and Fe^{2+} in biogenic solids (except for magnetite) (Fredrickson et al. 1998; Zachara et al. 1998). However, this treatment may underestimate the degree of reduction in nontronite (Jaisi et al. 2007b). For this reason, the extent of reduction was also measured by using 1, 10-phenanthroline at the conclusion of the experiments (Stucki 1981) (Komadel and Stucki 1988). The concentration of aqueous Fe^{2+} was determined by filtering 0.5 mL of cell-mineral suspension through a 0.2 μm polycarbonate filter into 0.5 mL of 1 *N* Ultrex HCl followed by ferrozine assay. Concentrations of other cations in the filtrate were measured with inductively coupled plasma mass spectrometry (ICP-MS).

We measured Eh and pH within treatment tubes at selected time points. The culture tubes were opened inside a glove box and the pH was measured with a probe. Initial measurements indicated that the Eh value changed dramatically when the anaerobic culture tubes were opened inside a glove box, apparently because the gas composition of headspace in the tubes was different from that in the glove box. Thus, subsequent Eh measurements were made without opening the anaerobic culture tubes. Eh microelectrodes were inserted into a 1 cc syringe with a connected needle. The needle penetrated through the thick rubber stopper of the culture tubes and was able to bring a small amount of liquid sample in contact with the Eh microelectrodes for Eh measurement.

X-ray diffraction (XRD)

Both unreduced and bioreduced NAu-2 samples were studied by XRD to identify mineralogical changes as a result of bioreduction. The samples were dispersed in 2 mL distilled water using an ultrasonic probe and then Ca saturated. An oriented nontronite layer was prepared by repeated pipetting of clay slurry onto a glass slide followed by air-drying. Samples were X-rayed with a Siemens D500 X-ray diffraction system using Cu radiation, a monochromator, and were scanned in 0.02 two-theta steps from 2 to 40 degrees, with a count time of 2 s per step. The proportion of illite and nontronite layers in mixed layered illite/nontronite were determined by using the NEWMOD and the LayerCharge (Christidis and Eberl 2003) programs.

Scanning and transmission electron microscopy (SEM and TEM)

Mineralogical changes were further studied with SEM and TEM. SEM samples were prepared following a previously published procedure (Dong et al. 2003). Briefly, cell-mineral suspensions were fixed in 2.5% glutaraldehyde in a bicarbonate solution and one droplet of fixed suspension was placed on the sur-

TABLE 1. Experimental conditions used for nontronite reduction in bacterial cultures and abiotic controls

Exp.	Amendments				
	pH 1 g/L	$Al(OH)_3 \cdot nH_2O$ 20 mM	Lactate 5 mg/mL	NAu-2 50 *mM*	K
1	6.2	√			
2	6.2	√	√		
3	6.2	√		√	√
4	6.2	√	√	√	√
5	9.2	√		√	√
6	9.2	√	√	√	√
7	9.2	√			
8	9.2	√	√		

face of a glass cover slip that was pre-cleaned with 1 mg/mL polylysine solution. Nontronite particles were allowed to settle onto the cover slip for 15 min. The sample-coated cover slips were sequentially dehydrated using varying proportions of ethanol and distilled water followed by critical point drying. The cover slips were mounted onto SEM stubs and Au coated for observation using a Zeiss low vacuum SEM. The SEM was operated at an accelerating voltage of 10 to 15 kV. A short working distance (6–10 mm) and low beam current (30–40 mA) were used to achieve the best image resolution. A longer working distance (8.0 mm) and higher beam current (50–70 mA) were used for qualitative energy dispersive spectroscopy (EDS) analysis.

XRD data and SEM observations revealed that bioreduced NAu-2 at pH 9.2 with external K and Al sources contained illite. Thus, this sample was selected for high-resolution TEM observation, along with an abiotic control. The sample was imbedded within L.R. White resin (Kiin et al. 1995) and sliced using a microtome for TEM observations (Kim et al. 2003, 2004). The advantage of using L.R. White resin in this study is that smectite layer collapse due to dehydration during conventional TEM observation is avoided and 12–13 Å smectite layers are easily differentiated from 10 Å illite layers (Kim et al. 1995). A JEOL 3010 TEM operating at 300 keV with a LaB_6 filament was used for TEM analysis. A total of 124 packets of bioreduced smectite were measured on TEM lattice fringe images and statistical analysis of layer spacing distribution was performed.

RESULTS

Reduction of Fe^{3+} in nontronite by *T. ethanolicus*

As a result of bioreduction, the pH value decreased in both pH 6.2 and 9.2 experiments by 0.2–0.3 and 0.6–0.7 unit, respectively. This decrease was likely due to release of CO_2 by bacterial respiration of lactate. Microbial oxidization of lactate and Fe^{3+} reduction also resulted in a significant decrease in Eh for both experiments. The pH 9.2 experiments under N_2 atmosphere resulted in a significantly lower Eh value (–414 to –448 mV) than the pH 6.2 experiments under N_2-CO_2 (–251 to –325 mV) atmosphere. The Eh value also decreased in abiotic controls, which might have resulted from abiotic degradation of organic substrates such as yeast extract. However, these decreases were not significant relative to those in biotic experiments (Table 2).

At pH 6.2, the extent of Fe^{3+} bioreduction as measured with 0.5 *N* HCl extraction reached 42.4% (Fig. 1) and 27.1% with and without an external supply of K and Al source, respectively. At pH 9.2 the extent of reduction was 34.1% (Fig. 1) and 30.9% with and without K and Al, respectively. The abiotic (non-inoculated) controls did not show any significant reduction. The extent of reduction of Fe^{3+} at the end of experiments was also measured with 1, 10-phenanthroline. At pH 6.2, it was 43.7 and 32.8% with and without an external supply of K and Al source, respectively. At pH 9.2 the extent of reduction was 40.4 and 31.7% with and without K and Al, respectively. In comparison with the extent of reduction measured by 0.5 *N* HCl extraction, the phenanthroline method extracted more Fe^{2+} because the NAu-2 structure was dissolved (Komadel and Stueki 1988).

TABLE 2. Change in pH and Eh as a result of Fe^{3+} bioreduction

		Without K and Al ($80\%N_2$-$20\%CO_2$)		With K and Al ($100\% N_2$)	
		Initial*	Final†	Initial	Final
pH	Bioreduction	6.2	5.9 to 6.0	9.2	8.6 to 8.5
	Control	6.2	6.2	9.2	8.7
Eh (mV)	Bioreduction	–50	–251 to –325	–49	–414 to –448
	Control	–50	–34 to –97	–49	–83 to –171

* Initial time = before the inoculation of *Thermoanoerobacter ethonolicus*.
† Final time = end of experiments (18 days of incubation).

Aqueous concentrations of Fe, Si, Al, and Mg were measured for the samples with an external K and Al supply. Aqueous concentration of Fe^{2+} comprised an insignificant fraction of total biogenic Fe^{2+}. This fraction was pH dependent. At pH 6.2, aqueous concentration of Fe^{2+} reached up 9.5% of total Fe^{2+} by 11 days, and then decreased to 4.1% by 13 days (Fig. 1c). At pH 9.2, aqueous concentration of Fe^{2+} was only 1.8~2.9% of total Fe^{2+} (Fig. 1d). Aqueous concentration of Si and Al did not show any obvious difference between abiotic control and biotic samples at pH 6.2 and 9.2, apparently because of formation of Si and Al precipitates in biotic incubations (see below).

X-ray diffraction

pH 6.2 experiments. Without an external supply of Al and K source, XRD patterns for bioreduced NAu-2 showed some mixed layer of smectite-illite (data not shown). With external Al and K sources, XRD pattern for bioreduced NAu-2 did not show any obvious change with time (from 60 to 120 days), other than a shift of the (001) peak from 12.79 Å ($2\theta = 6.9°$) at 60 days to 12.51 Å ($2\theta = 7.05°$) at 120 day (Fig. 2a). Ethylene glycolation revealed a new 8.78~9.08 Å peak and shifted the nontronite (001) peak to a larger *d*-spacing. For example, for the 120 day sample, the (001) peak at 12.51 Å for the air-dried sample was split into peaks at 9.08 and 16.10 Å after glycolation. Based on the position of the (001)/(002) peak at ~9.72° [a compounded peak from the (001) of illite and (002) of smectite], about 50 to 60% expandable nontronite layers remained in bioreduced NAu-2 after 120 days. These results are consistent with those of Wu et al. (1989) and Lear and Stueki (1989) who either directly or indirectly observed collapsed smectite layers upon chemical reduction of smectites. Nearly 100% nontronite layers remained in abiotic control NAu-2 by 120 days (Fig. 2a).

pH 9.2 experiments. Without an external supply of Al and K source, XRD patterns for bioreduced NAu-2 were similar to those at pH 6.2 with external Al and K source (e.g., Fig. 2a). With external Al and K sources, bioreduction of Fe^{3+} in NAu-2 resulted in appearance of a new peak at 9.94–9.96 Å ($2\theta = 8.86°$) (Fig. 2b), likely the illite (001) peak. To distinguish among nontronite, illite, and mixed-layer illite/smectite phases, bioreduced NAu-2 was subject to ethylene glycolation, Li^+ saturation, and PVP treatment (Eberl et al. 1998). Ethylene glycolation did not change the position of the illite (001) peak (9.94–9.96 Å), but increased the spacing of the nontronite (001) peak. For the 60 day sample, the nontronite (001) peak shifted from 12.94 to 14.78 Å. For the 90 day sample, the 12.90 Å peak shifted to 15.51 Å. After treatments with Li^+ saturation and PVP, the 9.96 Å illite peak was intensified, confirming that it was the (001) peak for discrete illite (Fig. 2b insert). In abiotic control, 100% nontronite layers remained after 90 days.

SEM observations

SEM observations revealed that the abiotic control did not undergo any mineralogical changes (Fig. 3a), but extensive dissolution texture was obvious in the bioreduced samples by the end of the experiments (Fig. 3b). Bioreduction of NAu-2 may have proceeded via close associations between bacterial cells, biofilm and NAu-2 as expected for enzymatic mediation of electron transfer to Fe^{3+} in nontronite (Fig. 3c). In the absence of

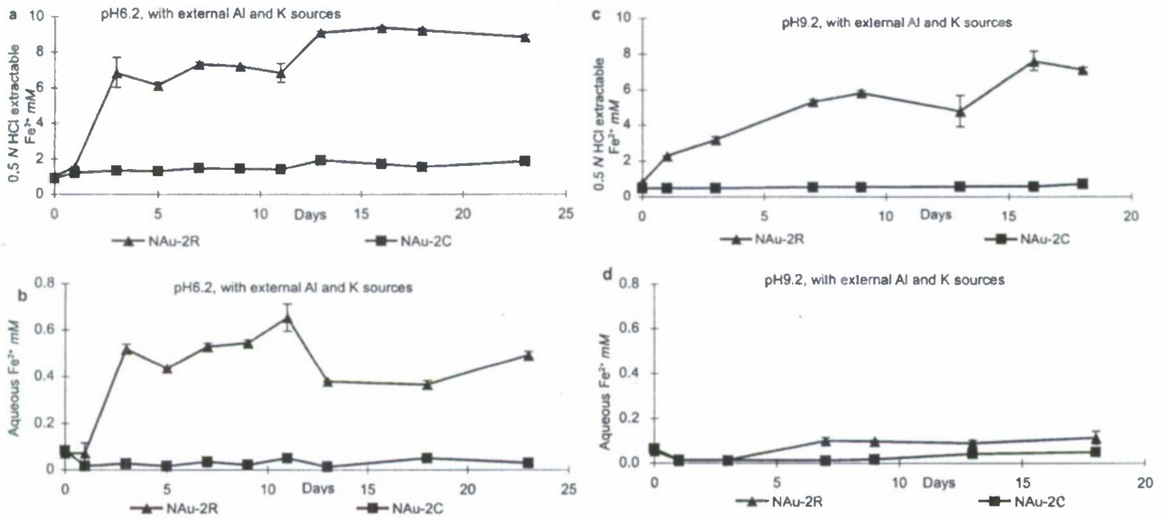

FIGURE 1. (**a**) Production of 0.5 *N* HCl extractable Fe^{2+} with time in bioreduced NAu-2 and abiotic control (no bacterial cells added) at pH 6.2 with an external supply of Al and K. (**b**) Production of aqueous Fe^{2+} with time under the same conditions. (**c**) A similar plot to **a** but at pH 9.2. (**d**) A similar plot to b but at pH 9.2. All results were from duplicate cultures and the error bars represent two-sigma variation. R = bioreduced sample; C = abiotic control.

external K and Al sources, dissolution of NAu-2 in both pH 6.2 and 9.2 experiments resulted in formation of biogenic silica and flaky euhedral crystals of smectite (Fig. 3d). The relatively well-defined crystal morphology of the flakes suggests that these crystals were different from the initial nontronite, and they were likely precipitated from solution as a result of reductive dissolution of nontronite. Precipitation of biogenic smectite, from reductive dissolution of nontronite, has been observed before (Dong et al. 2003). The qualitative SEM-EDS analyses revealed that those flakes had a lower iron content than the initial nontronite. Nanosized biogenic silica particles were associated with biofilms, and with time, these nanoparticles aggregated and transformed

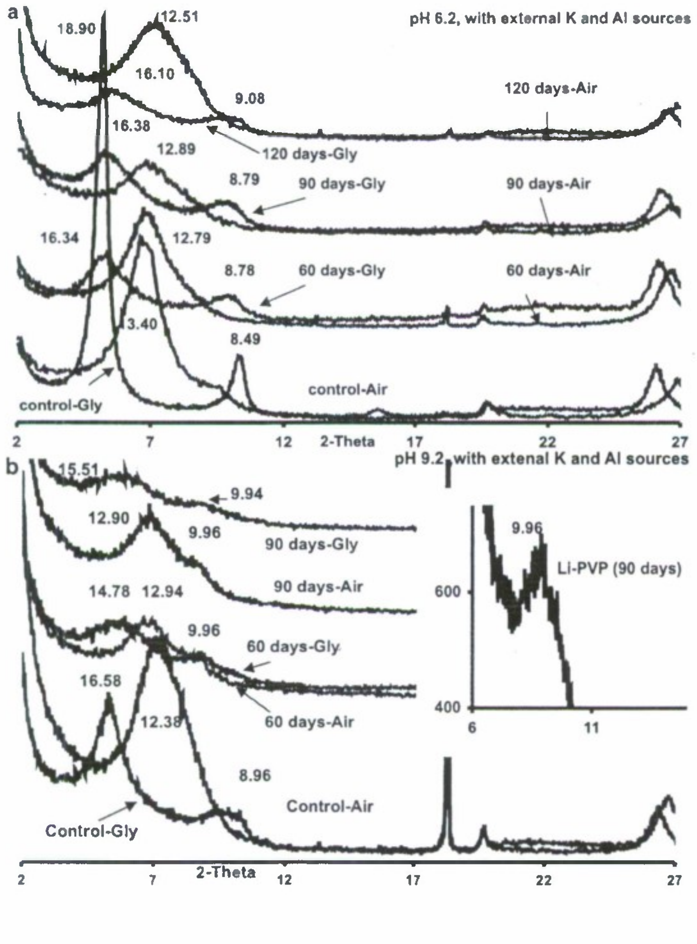

FIGURE 2. (**a**) XRD patterns for oriented bioreduced NAu-2 at pH 6.2 with an external supply of Al and K source. Both air-dried and ethylene glycolated samples were run. Three time points (and one abiotic control) were selected to show time-course changes in the patterns as bioreduction continued. (**b**) XRD patterns for oriented bioreduced NAu-2 at pH 9.2 with an external supply of Al and K source. Two time points (along with one abiotic control) were selected to show changes as bioreduction continued. 60 days-Air = 60 day sample air-dried; 60 days-Gly = 60 day sample solvated with ethylene glycol vapor at 65 °C. The inset pattern was for a 90 day sample (pH 9.2) that was saturated wit Li^+ and then intercalated with PVP. This method intensifies the illite (001) peak. See text for description of the differences between patterns.

to well-crystalline quartz (Figs. 3d and 3e). With an external K and Al supply at pH 6.2, bioreduced NAu-2 displayed similar features as without external K and Al supplies.

Discrete illite formation was only observed in bioreduced NAu-2 at pH 9.2 with an external supply of Al and K source. SEM observations revealed a progressive change in illite morphology. Lath-shaped aggregates were observed in the 23 and 60 day samples (Figs. 4a and 4b). By 90 days, distinct euhedral plates formed (Fig. 4c). SEM-EDS analyses identified all these crystals as illite (higher Al and K, and lower Fe than nontronite). The crystals not only changed the morphology from laths to plates, but also slightly increased in size (Figs. 4a, 4b, and 4c). The 90 day sample contained a significant number of euhedral plates and elongated laths (Fig. 4d). In the abiotic controls, no mineral transformations were observed. The unaltered nontronite remained at the end of the incubations.

TEM observations

Discrete illite packets (14–20 layers) with 1.0 nm spacings were the dominant phase in the bioreduced NAu-2 sample (pH 9.2 containing external K and Al sources) (Fig. 5a), compared with the variable layer spacings (1.2–1.3 nm) in the nonreduced abiotic control (Fig. 5b). Newly formed illite layers associated with microbial Fe^{3+} reduction were differentiated from the smectite layers based on layer spacing (10 Å vs. 12–13 Å). The inset selected area electron diffraction (SAED) pattern of illite showed discrete Brag reflections, d_{001} = 1.0 nm (Fig. 5a), as opposed to diffuse reflections characteristic of unreduced nontronite.

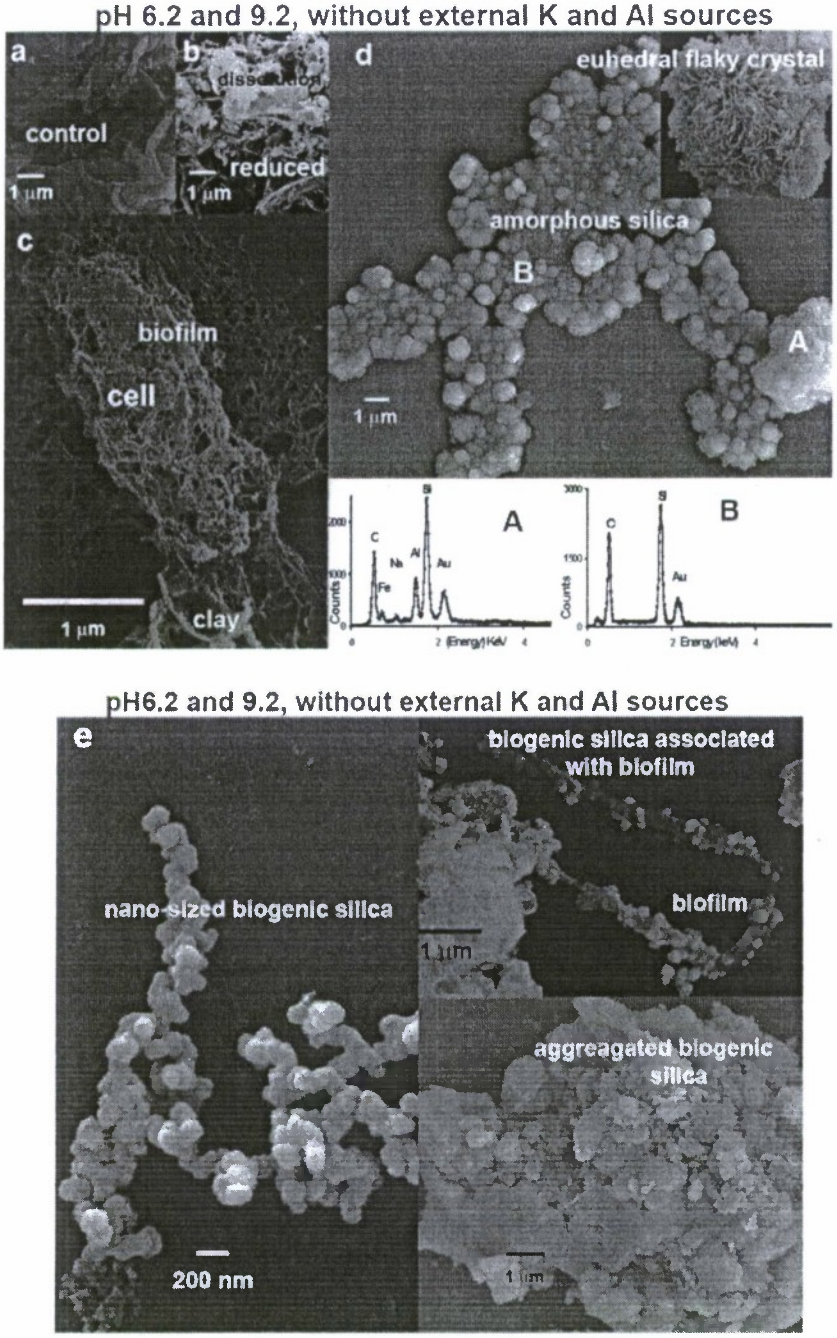

Figure 3. Secondary electron images showing various changes in NAu-2 as a result of bioreduction at pH 6.2 and 9.2 without external K and Al sources. (**a**) Abiotic control after 120 days of incubation showing no changes. (**b**) Dissolution features (pits and etches) of bioreduced NAu-2 after 120 days of incubation. (**c**) Biogenic silica and euhedral smectite in bioreduced NAu-2 after 23 days of incubation. The bottom panel shows SEM-EDS composition of grain A and B on c corresponding to euhedral smectite and silica, respectively. The right insert shows neoformed euhedral flaky smectite in bioreduced NAu-2 after 23 days of incubation. The composition of the euhedral smectite flakes is different from the initial nontronite NAu-2 in that it has higher Al and lower Fe content. (**d**) Biofilm formation in bioreduced NAu-2 after 23 days of incubation. (**e**) Secondary electron image showing nano-sized and aggregated biogenic silica in bioreduced NAu-2 after 23 and 120 days of incubation. The left subfigure shows nano-sized silica after 23 days of incubation; the top right subfigure shows nano-sized silica associated with biofilm after 23 days of incubation and the low right subfigure shows aggregated biogenic silica after 120 days of incubation.

A statistical measurement of 132 packets in bioreduced NAu-2 showed that about 42% of the measured layers had layer spacings of 0.9–1.1 nm, most likely illite layers (Fig. 6).

DISCUSSION

Microbially mediated S-I reaction at diagenetically relevant temperature

To the best of our knowledge, this is the first study to investigate microbial Fe^{3+} reduction in nontronite using a thermophilic iron-reducing bacterium. In general, the extent of reduction by thermophilic bacteria is higher than that achieved by mesophilic bacteria. For example, mesophilic iron-reducing or sulfate-reducing bacteria can reduce 21~32% of Fe^{3+} in NAu-2 (Li et al. 2004; O'Reilly et al. 2005; Jaisi et al. 2005, 2007a, 2007b; Zhang et al. 2007). In comparison, the extent of reduction by a thermophilic iron-reducing bacterium in this study can reach as high as 32–44%, depending on solution chemistry. Apparently, temperature enhances the extent and rate of bioreduction. Our data on abiotic controls did not show any evidence for smectite dissolution, suggesting that our experimental temperature was not high enough to dissolve smectite. Therefore, this enhanced bioreduction was likely a result of enhanced rate of electron transfer at elevated temperature. This enhanced rate may also be related to differences in microbial metabolic activities between mesophilic and thermophilic bacteria.

This study demonstrates that thermophilic bacterium can promote the S-I reaction. Optimum conditions (pure isolate, rich carbon sources, and pure nontronite) were used in our study to accelerate the rate of the S-I reaction. We recognize that these conditions may not predominate or exist in natural environments. However, the lack of these optimal conditions in nature may be compensated for by long geological time scales beyond those of our study. It is certainly possible that one or more of these conditions may occur in certain geological environments. For instance, several thermophilic, dissimilatory iron-reducing bacteria have been isolated from sedimentary basins, such as *T. ethanolicus* from the Piceance Basin, Colorado (Liu et al. 1997; Roh 2003; Roh et al. 2002), *Bacillus infernus* from the Taylorsville Basin (Boone et al. 1995), *Deferribacter thermophilus*, *Tepidimicrobium ferriphilum*, *Thermoterrabacterium ferrireducens*, *Thermoanaerobacter siderophilus* from oil reser-

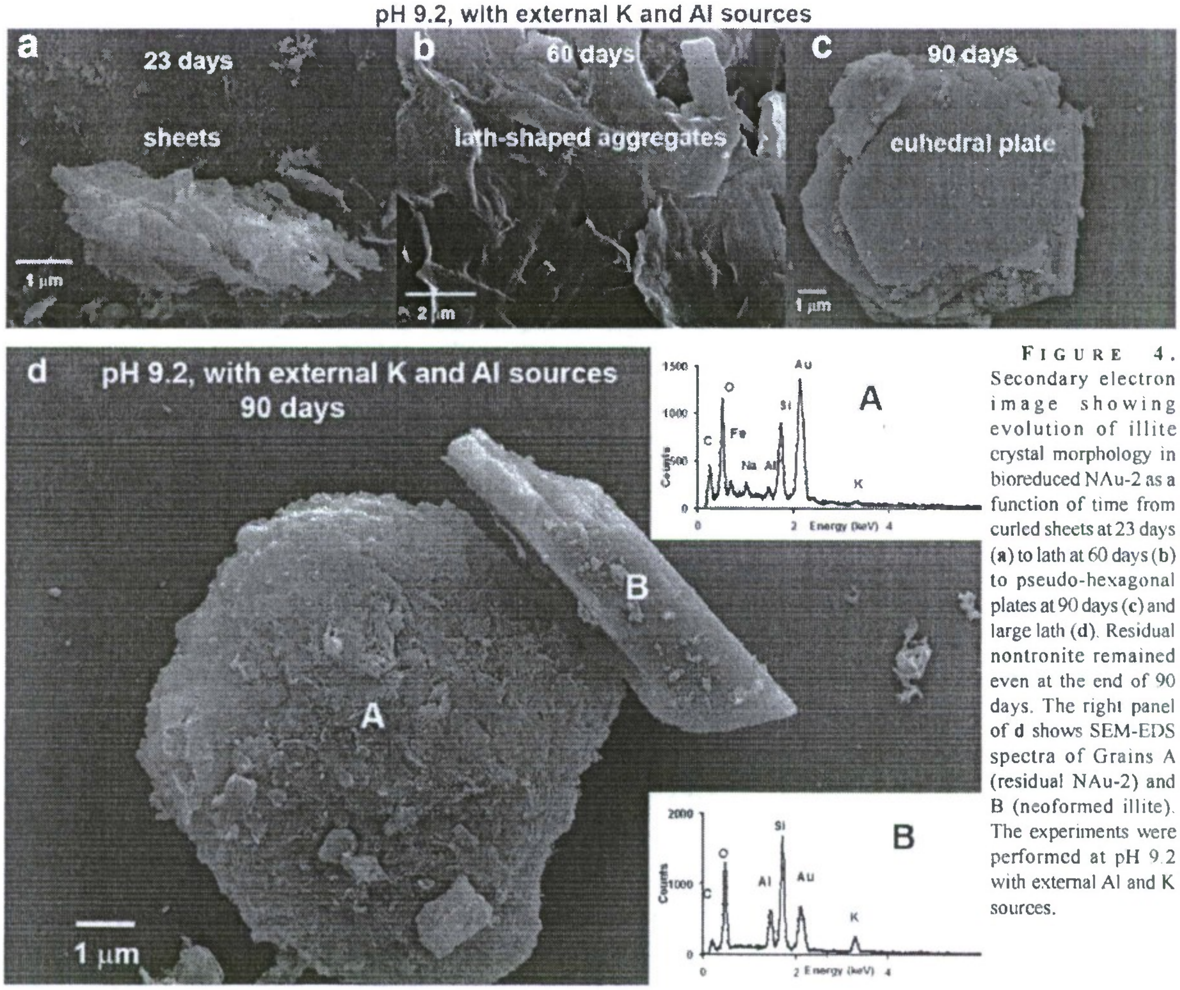

FIGURE 4. Secondary electron image showing evolution of illite crystal morphology in bioreduced NAu-2 as a function of time from curled sheets at 23 days (**a**) to lath at 60 days (**b**) to pseudo-hexagonal plates at 90 days (**c**) and large lath (**d**). Residual nontronite remained even at the end of 90 days. The right panel of **d** shows SEM-EDS spectra of Grains A (residual NAu-2) and B (neoformed illite). The experiments were performed at pH 9.2 with external Al and K sources.

voirs or hot springs (Greene et al. 1997; Slobodkin et al. 1999, 2006). Thus, iron-reducing bacteria may be widely distributed in clay-rich subsurface sedimentary basins. Previous studies have shown that sulfate reducing and fermenting bacteria also reduce Fe^{3+} in the nontronite structure (Li et al. 2004) and in iron oxides (Bond and Lovley 2002; Boone et al. 1995). These studies further expand the diversity of bacteria capable of reducing Fe^{3+} in the subsurface.

In clay-rich rocks, iron-bearing smectite can be abundant, such as in Gulf Coast mudstones (Freed and Peacor 1992) and in shales from Nankai Trough (Masuda et al. 2001). In addition to bacteria and iron-rich smectite, organic acids with concentrations higher than 20 *mM* have been reported to be present in continental shelf formation waters derived from petroleum source rocks (Barth 1991; Barth et al. 1990). Acetate is usually abundant, but benzoate, butyrate, formate, and propionate are also commonly detected (Magot et al. 2000). Thus, in such natural systems, there exist electron donor, acceptor, and bacteria, and these are major components necessary for promoting the smectite-illite reaction. The rate of microbially mediated S-I reactions depends on several factors including temperature, pH and activity/concentration of K and Al.

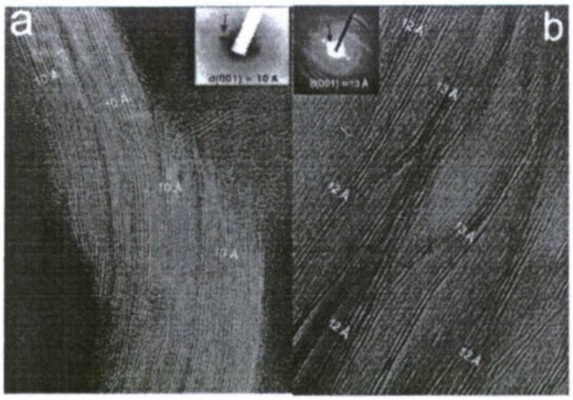

FIGURE 5. TEM micrographs for bioreduced and nonreduced NAu-2 from the pH 9.2 experiments with external K and Al sources. (**a**) Bioreduced NAu-2 with newly formed illite precipitates having 10 Å lattice fringes confirmed with SAED pattern. (**b**) NAu-2 lattice fringes in the abiotic control. 12 Å layer spacings were dominant for this sample.

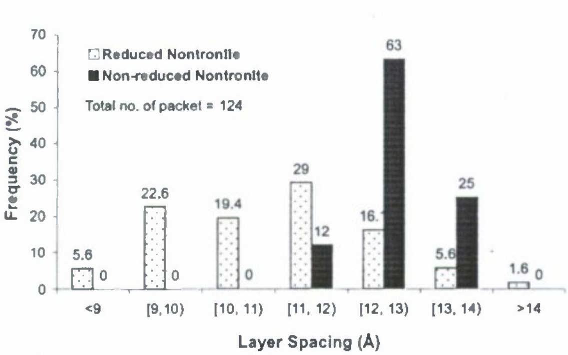

FIGURE 6. Histogram showing the distribution of layer spacings in bioreduced NAu-2 (pH 9.2, with K and Al) in comparison with the abiotic control (no cells added). The *x*-axis should be read as follows with (9, 10) as an example: layer spacing between 9 Å (including 9) and 10 Å (excluding 10).

Factors controlling the microbially mediated S-I reaction

This study demonstrates that iron-reducing bacteria, when growing on short-chain fatty acids as an energy source and under varied geochemical conditions, have the ability to promote the S-I reaction. The major factors controlling the S-I reaction are the composition and concentration of cations in aqueous solution, reaction temperature, pH, and time. Other factors such as type of bacteria, energy source for microbial metabolism, and Eh also would have important effects, but these were not examined in this study.

During microbial Fe^{3+} reduction of nontronite, the S-I reaction rate was enhanced when excess K and Al was present. This finding is consistent with previous studies for the chemically mediated S-I reaction (Whitney and Northrop 1988; Huang et al. 1993; Drief et al. 2002). The microbially mediated S-I reaction can qualitatively be written as Ca-smectite + Al^{3+} + K^+ $\rightarrow$ K-illite + SiO_2 + Fe^{2+} + Ca^{2+}. This reaction is based on the fact that smectite usually has a much lower amount of K and Al, but a higher amount of iron than illite (Peacor 1992). Thus, the S-I reaction requires external K and Al sources (Eberl et al. 1986, 1993; Drief et al. 2002). The possible source in natural environments may be from feldspar and/or mica dissolution (Wilkinson and Haszeldine 1996). Smectites with K as the dominant interlayer cation have been reported to occur in many rocks and sediments (Freed and Peacor 1992; Drief and Nieto 2000; Hover et al. 2002). In such cases, an external supply of K may not be necessary for the development of illite, because an intrinsic supply is available.

In nontronite NAu-2, structural Al is present in small amounts. Even if all of this Al were available to build illite layers, the number of such layers would be so few that it would be difficult to observe. At pH 9.2, there was no discrete illite formation without external K and Al sources, and some components from dissolved nontronite formed quartz or recombined to form biogenic smectite (Fig. 3). When external Al and K sources were added to the system, discrete illite formed.

The microbially mediated S-I reaction was enhanced with an alkaline pH. Discrete illite formed in experiments conducted at pH 9.2 but not in those conducted at pH 6.2, despite the fact that the extent of Fe^{3+} bioreduction was similar between pH 6.2 and 9.2. Previous studies have shown the important effects of pH on the S-I reaction. In general, increasing pH can enhance smectite dissolution (Bauer and Velde 1999; Claret et al. 2002) and illite formation (Eberl et al. 1986, 1993; Drief et al. 2002). Our study showed a similar pH effect when microbes (*T. ethanolicus*) were involved in promoting the S-I reaction.

Mechanisms of microbial reduction of Fe^{3+} in nontronite and the S-I reaction

There are two proposed mechanisms for microbial reduction of Fe^{3+} in smectites: solid-state and dissolution. Lee et al. (2006), solely based on infrared spectroscopy, observed that bacterial reduction of structural Fe^{3+} in ferruginous smectite

SWa-1 and Upton montorillonite changes the clay structure, but these changes are reversible upon reoxidation of biogenic Fe^{2+}. Stucki (personal communication) performed Mössbauer analysis of reduced and reoxidized smectites and reached similar conclusion. These studies imply that bacterial Fe^{3+} reduction takes place largely in solid solid without dissolution of the clay structure. In contrast, multiple studies have presented evidence for microbial dissolution of nontronite (Dong et al. 2003; Li et al. 2004; Kim et al. 2004; O'Reilly et al. 2005, 2006; Furukawa and O'Reilly 2007; Jaisi et al. 2007b; Zhang et al. 2007). These studies have largely used SEM and TEM, along with XRD, to directly observe dissolution textures of smectites and formation of biogenic products (Si, siderite, and illite). Kostka et al. (1999b) reported irreversible changes of cation exchange capacity and specific surface area upon reduction and re-oxidation of SWa-1 and Upton montorillonite, implying a certain degree of dissolution. We acknowledge this apparent inconsistency in the mechanisms of microbial reduction of Fe^{3+} in smectites and offer some plausible explanations below.

First, the apparent inconsistency (dissolution vs. solid state) may be related to the nature of smectite samples used in different studies. The authors of the dissolution mechanism (Li et al. 2004; Kim et al. 2004; O'Reilly et al. 2005, 2006; Furukawa and O'Reilly 2007; Jaisi et al. 2007b) have used nontronite samples (NAu-1 and NAu-2), whereas Stucki and his colleagues (Lee et al. 2006; Stucki and Kostka 2006) have largely used the Garfield and SWa-1 samples. Clearly, there are differences in total Fe content, interlayer charge, and other physical/chemical properties between these two sets of samples, as reported by Gates et al. (2002). NAu-2 contains a significant amount of tetrahedral Fe^{3+} (9%, Gates et al. 2002), and microbial reduction of this Fe^{3+} has been shown to result in reductive dissolution (Jaisi et al. 2005). Second, the inconsistency may be related to the extent of dissolution. If the extent of dissolution is small, electron microscopy may readily observe dissolution products, but other methods (such as infrared) may not detect them. Even if there is dissolution of nontronite and formation of biogenic Si (a major dissolution product), it may be difficult to detect it by infrared spectroscopy, because the Si-O vibration bands from biogenic Si (quartz) may be similar to those from residual nontronite. Third, the inconsistency may also be related to the type of bacteria, medium and other solution conditions used for bioreduction experiments (such as pH, temperature, presence or absence of organic matter, etc.). Available data in the literature suggest that a growth medium (such as M1 for *Shewanella* species) promotes reductive dissolution (Dong et al. 2003). The presence of organic matter, high temperature, and pH all favor smectite dissolution (Kostka et al. 1999; Zhang et al. 2007, Stucki et al., personal communication). Fourth, numerous studies (Dong 2005 and references therein) have demonstrated that abiotic smectite to illite reaction occurs via the dissolution-precipitation mechanism, because solid-state diffusion rate is too slow for significant reorganization of the smectite strueture to form illite (such as Al increase in the octahedral site). The difference between solid-state (or sometimes called layer-by-layer replacement) and dissolution-precipitation is only a matter of scale (dissolution at atomic scale vs. bulk scale).

ACKNOWLEDGMENTS

This research was also supported by a grant from National Science Foundation (EAR-0345307). Some part of this research was supported by a student grant from the Clay Minerals Society (Student Research Grant 2004) to G.Z. J.W.K. publishes with NRL contribution number NRL/JA/7430-07-02. We are grateful to Joseph Stucki and an anonymous reviewer for their constructive comments.

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MANUSCRIPT RECEIVED NOVEMBER 5, 2006
MANUSCRIPT ACCEPTED APRIL 3, 2007
MANUSCRIPT HANDLED BY WARREN HUFF.