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## The impact of structural Fe(III) reduction by bacteria on the surface chemistry of smectite clay minerals

JOEL E. KOSTKA,<sup>1,\*</sup> JUN WU,<sup>2</sup> KENNETH H. NEALSON,<sup>3</sup> and JOSEPH W. STUCKI<sup>2</sup><sup>1</sup>Skidaway Institute of Oceanography, Savannah, Georgia 31411 USA<sup>2</sup>Natural Resources and Environmental Sciences Department, University of Illinois, Urbana, Illinois 61801 USA<sup>3</sup>Earth and Space Sciences Division, Engineering and Science Directorate, Jet Propulsion Laboratory, Pasadena, California 91109-8099 USA

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**Abstract**—Although clay mineral reduction is thought to occur primarily as a result of the activity of indigenous microorganisms in soil, most research has focused on chemical mechanisms of Fe reduction within clay minerals. Here we show that bacteria isolated from soils and sediments catalyze the rapid reduction of structural Fe(III) in the smectite clay minerals. The extent of Fe(III) reduction is large, from 46% to >90%. Furthermore, the effects of structural Fe(III) reduction by bacteria on the surface chemistry of smectites are dramatic. Swelling pressure, as measured by water content, was shown to decrease by 40% to 44% in smectites reduced by bacteria as compared to unaltered or reoxidized smectites. Particle surface area decreased by 26% to 46% in response to bacterial reduction, and the surface charge density as measured by the ratio of cation exchange capacity to specific surface area increased over the same scale. Measurements of swelling pressure in smectite saturated with the organic cation trimethylphenylammonium (TMPA) indicated that the hydrophilic character of the clay mineral surface was enhanced upon reduction. The valence state of Fe in the octahedral layer of smectite, as revealed through reflectance spectra, correlated to the amount of Fe(III) reduced in bacterial cultures, providing information on the mechanism of intervalence electron transfer in bacterially reduced clay minerals. The extent of reduction and surface chemical effects catalyzed by bacteria in this study are similar in magnitude to those observed previously for potent inorganic reductants. Given that clay minerals dominate the solid phase of porous media and that Fe(III)-reducing bacteria are abundant in soils and aquatic sediments, these data suggest that bacterial clay mineral reduction may play an important role in soil biogeochemistry, affecting processes such as nutrient cycles and the fate of organic contaminants. Copyright © 1999 Elsevier Science Ltd

### 1. INTRODUCTION

Clay minerals, which are ubiquitous in soils and aquatic sediments, play a significant role in many aspects of life, ranging from their domination of soil/sediment physicochemical properties to their ubiquitous commercial use in many products and industries (Newman, 1984; Odom, 1984; Stucki, 1988). Clay minerals such as smectites have large surface areas which bind a large number of inorganic and organic molecules including nutritive cations and contaminants in soils and sediments (Siantar et al., 1994).

Iron is an abundant component in the crystal structures of virtually all soil clay minerals, and studies over the past few decades have determined that the oxidation state of this structural Fe profoundly alters many physicochemical properties of the clay, which may have important industrial and environmental implications (Stucki, 1988; Stucki et al., 1996). Chemical clay reduction studies (carried out primarily with smectites) have shown effects on swelling (Stucki et al., 1984b), cation exchange and fixation capacity (Stucki et al., 1984a; Lear and Stucki, 1989; Khaled and Stucki, 1991), specific surface area (Lear and Stucki, 1989), color (Anderson and Stucki, 1979), and magnetic exchange interactions (Lear and Stucki, 1987). During reduction, clay layers collapse in response to the reduc-

tion of structural Fe (Stucki et al., 1984b; Lear and Stucki, 1989), thereby trapping cations (such as  $K^+$ ,  $NH_4^+$ ) in the mineral matrix, which may be detrimental to agricultural use of a soil because valuable nutrients could become unavailable for plant growth. In addition, the fate and transport of redox active contaminants in soil, heavy metals and organic pollutants, are likely to be highly dependent on transformations at the clay-mineral surface. If the redox state of clay minerals could be manipulated in soil, these properties could be optimized for increased crop production or for in situ bioremediation of contaminants.

Although reduction of structural Fe in clay minerals within soils and sediments is thought to be mediated primarily by the enzymatically catalyzed activity of indigenous microorganisms (Stucki et al. 1987; Gates et al., 1993), most research has focused on chemical mechanisms of structural Fe reduction in clay minerals. Chemical reduction studies have used potent chemical reductants such as dithionite or hydrazine, which are not likely to play a significant role in clay mineral reduction in natural environments (Stucki, 1988). These inorganic chemical reductants are likely to be minor components of soils; whereas, microorganisms capable of Fe(III) reduction have been observed at up to  $10^5$  cells per gram dry weight of agricultural soil (Acht nich et al., 1995).

Microbial Fe(III) reduction has been directly shown or implicated as an important process mediating organic matter decomposition (Lovley, 1991; Canfield et al., 1993; Roden and Wetzel, 1996; Kostka et al., 1999a), the release of nutrients and

\* Author to whom correspondence should be addressed (jkostka@ocean.fsu.edu).

† Present address: Oceanography Department, Florida State University, Tallahassee, FL 32306.

trace metals (Ponnamperuma et al., 1967; Roden and Edmonds, 1997), and in the fate of organic contaminants (Lovley et al., 1994; Anderson and Lovley, 1997) in soils and sediments. In freshwater environments such as soils, Fe reduction is likely to be an important terminal electron accepting process in that Fe(III) is second only to oxygen as the most abundant electron acceptor available for microbial growth/activity (Lovley, 1991). A phylogenetically and physiologically diverse group of bacteria has been isolated that is capable of dissimilatory Fe(III) reduction. Often, these organisms have been shown to couple growth and energy production to the respiration of Fe(III) oxyhydroxide minerals or soluble Fe(III) forms (Lovley, 1991; Nealson and Saffarini, 1994; Kostka and Nealson, 1995; Roden and Zachara, 1996). Recently, the list of Fe(III) forms available for use by bacteria as an electron acceptor was expanded to include Fe(III)-containing clay minerals. The dissimilatory Fe(III)-reducing bacterium, *Shewanella putrefaciens*, was observed to rapidly reduce the structural Fe(III) in smectite (29% to 41%) in less than 24 h (Kostka et al., 1996). Clay mineral reduction occurred at temperatures and pH common to soils/sediments, and it was linked to energy generation and carbon metabolism in this Fe(III)-reducer. Respiration of smectite-bound Fe(III) was further shown to be coupled to the rapid oxidation of organic acids to CO<sub>2</sub> by Fe-reducing bacteria, and in the presence of organic ligands found in soils, bacterial reduction catalyzed the dissolution of smectite (Kostka et al., 1999b).

In this study we show that a large extent of smectite-bound Fe(III) reduction may be catalyzed by a range of soil bacteria, and we further document the effects of structural Fe(III) reduction by bacteria on the surface chemistry of phyllosilicates. Bacterial clay mineral reduction is shown to mediate changes to phyllosilicate surface chemistry comparable to potent chemical reductants. These discoveries have important implications for contaminated aquifers and agricultural soils such as rice paddies where Fe(III)-bearing clay minerals are abundant and important to soil biogeochemistry.

## 2. MATERIALS AND METHODS

### 2.1. Bacterial Cultures

Four bacterial cultures representing a range of bacterial phenotypes were used. Pure cultures included *Shewanella putrefaciens* strains MR-1 and MR-4, *Geobacter metallireducens*, and *Pseudomonas putida* strain WB9113. MR-1 and *Geobacter* were isolated from suboxic aquatic sediments and are the most completely characterized of Fe-reducing bacteria cultured to date (Lovley et al., 1993; Nealson and Saffarini, 1994). The marine strain MR-4 was isolated near the anoxic zone in the Black Sea (Nealson et al., 1991). *Pseudomonas putida* strain WB9113 was isolated from aerobic, wheat rhizosphere soils at the Agricultural Experiment Station near Pullman, Washington (Weller and Cook, 1983). WB9113 was shown to catalyze the largest extent of smectite reduction of five *Pseudomonas* strains studied previously under microaerophilic conditions (Komadel et al., 1987; Gates et al., 1993). *Shewanella* and WB9113 are oxygen-tolerant organisms, whereas *Geobacter* is an obligate anaerobe.

Bacterial clay mineral reduction experiments were also carried out with purified enrichment cultures of Fe-reducing bacterial consortia from rice paddy soil (sampled at the Institute of Soil Science, Nanjing, Peoples Republic of China). These enrichment cultures, purified by 15 successive transfers over an 18-month period, were produced using the same culture medium and methods described below. Enrichments selected for respiratory Fe(III)-reducing bacteria as Fe(III) was added as the sole electron acceptor and acetate as the sole carbon source in a

minimal medium throughout successive transfers. Dissimilatory Fe(III) reduction was shown repeatedly in the enrichments as the production of reduced Fe in acid extracts coupled to the depletion of organic acids. Furthermore, Fe-reducing bacteria were observed to be abundant in these soils (10<sup>4</sup> to 10<sup>6</sup> cells/g wet sediment) using a most probable number assay (J. E. Kostka, R. Viehweger, and D. Dalton, unpublished results).

Standard methods for the culture of anaerobic bacteria were used, which have been modified for clay mineral reduction experiments as in Kostka et al. (1996). For experiments with *Shewanella* strains, a minimal culture medium was prepared according to Kostka and Nealson (1998). *Pseudomonas* was cultured using nutrient-rich Luria broth. For *Geobacter* and the rice paddy enrichments, the minimal culture medium was prepared according to Lovley and Phillips (1988) with acetate added as the sole carbon source. Carbon substrates (lactate or acetate) were added at 10 to 20 mmol/L final concentration from sterile, anoxic stock solutions. Clay minerals (see next paragraph) were sterilized and added as the sole electron acceptor in the culture medium at 1 to 2 g/L final concentration. Culture medium was prepared and dispensed into serum bottles that were sealed with butyl rubber stoppers and incubated at 30°C in the dark. All manipulations of culture media and samples were carried out under strict anoxic conditions within a Coy anaerobic chamber (90% N<sub>2</sub>, 10% H<sub>2</sub>). *Geobacter* and rice paddy enrichment inocula were grown with 50 mmol/L Fe(III) citrate as the electron acceptor on the medium described above. *Shewanella* and *Pseudomonas* inocula were grown aerobically to late log phase on the appropriate medium. The initial cell density of all cultures was  $\approx 2 \times 10^8$  mL<sup>-1</sup>, as estimated by the relationship between optical density (absorbance at 500 nm) and direct cell counts.

### 2.2. Iron Mineral Preparation

Amorphous Fe(III) oxyhydroxide (surface area [SA] = 600 m<sup>2</sup>/g), goethite (SA = 20 m<sup>2</sup>/g), and magnetite (SA = 4 m<sup>2</sup>/g) were all prepared according to Schwertmann and Cornell (1991). For all experiments with smectite clay, the 0.5- to 2- $\mu$ m size fraction of the ferruginous smectite SWa-1 from Grant County, Washington (Source Clays Repository, The Clay Minerals Society), was used. The clay was Na<sup>+</sup>-saturated, fractionated, dialyzed, and freeze-dried before use (Stucki et al., 1984a). Lear and Stucki (1989) reported the structural Fe content of the same dialyzed SWa-1 to be 3.549 mmol Fe/g (with <0.1 mmol/g of this Fe present as Fe oxide impurities) and the SA to be 720 m<sup>2</sup>/g. For some SWa-1 preparations, the exchanged counterion was changed, by repeated centrifuge washings, from Na<sup>+</sup> to trimethylphenylammonium (TMPA), prepared as a 0.5 mol/L deoxygenated aqueous solution of its chloride salt. Upton montmorillonite (API #25, Ward's Natural Science Establishment; also classified as a smectite) was prepared using identical methods to those described for SWa-1, and its Fe content (0.522 mmol Fe/g) was determined previously by Gates et al. (1996). All solutions were exchanged under inert atmosphere conditions using an updated, commercially available version of the apparatus described by Stucki et al. (1984a). After structural Fe reduction in bacterial cultures, the suspensions were immediately sterilized before analysis, and some suspensions were reoxidized by bubbling with O<sub>2</sub> gas. All Fe minerals were sterilized by heating using microwave radiation (Keller et al., 1988) before addition to the culture medium.

### 2.3. Clay Mineral Chemistry

The extent of structural Fe(III) reduction in clay minerals was measured as the production of Fe(II) in acid extracts as described by Stucki and Anderson (1981) and modified by Komadel and Stucki (1988). Briefly, samples from bacterial cultures were digested with HF and H<sub>2</sub>SO<sub>4</sub>, in the presence of 1,10-phenanthroline. The digestate was diluted with sodium citrate and the absorbance measured at 510 nm before and after photochemical reduction using a mercury vapor lamp. Extent of reduction was calculated as the percentage of the ratio of [Fe(II)]/[total Fe] extracted. Swelling pressure was determined by measuring  $m_v/m_c$  gravimetrically as described in Stucki et al. (1984b). After reduction and before measurement of swelling pressure, some smectite suspensions were saturated with the organic cation TMPA (see methodology above). Particle surface area was measured using the

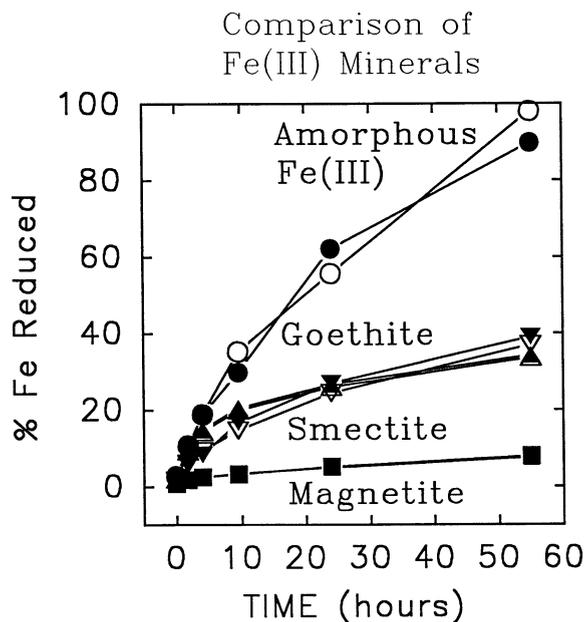


Fig. 1. Reduction of various Fe(III) minerals by the Fe(III)-reducing bacterium, *Shewanella putrefaciens* strain MR-1, in a minimal medium with lactate as the carbon source. Results from duplicate cultures are plotted as circles (amorphous Fe oxide; surface area, S.A. = 600 m<sup>2</sup>/g), triangles pointing down (goethite; S.A. = 20 m<sup>2</sup>/g), triangles pointing up (smectite; S.A. = 720 m<sup>2</sup>/g), and squares (magnetite; S.A. = 4 m<sup>2</sup>/g). Production of Fe(II) was measured in acid extracts as described by Lovley and Phillips (1988).

ethylene glycol-monoethylether method of Carter et al. (1965) as modified by Odom and Low (1978). Cation exchange capacity was determined as in Lear and Stucki (1985). Intervalence electron transfer of Fe(III)–O–Fe(II) was monitored by visible diffuse reflectance spectroscopy of whole cell suspensions at 350 to 800 nm using a Cary 5E UV-Vis-Nir spectrophotometer with pure Mg oxide as the reference material (R index = 1.00 or R = 100%). All preparation and manipulations of clay mineral suspensions were carried out under strictly anoxic conditions.

### 3. RESULTS

A model system was established with the known Fe(III)-reducing bacterium, *Shewanella putrefaciens* strain MR-1, in a minimal culture medium (resembling groundwater) with Fe-containing smectites added as the sole electron acceptor. We used this system to determine the influence of bacterial reduction on clay mineral chemistry and to further explore the mechanism of Fe(III) reduction in the octahedral layer of smectite.

To place bacterial clay mineral reduction in the context of the reduction of other solid Fe(III) minerals, we compared the reduction of a variety of Fe(III) minerals displaying a range of surface areas. With cell density kept constant, the reduction rate of Fe oxide minerals by strain MR-1 was generally observed to positively correlate with the surface area of the various minerals serving as the sole electron acceptor in culture (Fig. 1). When normalized to the surface area present, rates of smectite reduction (Fig. 1; Kostka et al., 1999b) approached those observed in previous studies of amorphous and crystalline Fe

Table 1. The average extent of reduction observed in a variety of pure cultures of Fe(III)-reducing bacteria and in a highly purified bacterial enrichment from rice paddy soil (Nanjing, China).

Bacterial Culture	% Fe(II) <sup>a</sup>
MR-1	
smectite (SWa-1)	29–46 <sup>b</sup>
Upton montmorillonite	74–90
MR-4	23–43 <sup>b</sup>
<i>Geobacter</i>	20
<i>Pseudomonas</i>	1 <sup>c</sup>
Rice paddy enrichment	55

<sup>a</sup> Calculated from the average of at least duplicate cultures after 14 d of incubation under strictly anaerobic conditions.

<sup>b</sup> Average calculated from at least 10 cultures grown with formate or lactate as the carbon source.

<sup>c</sup> Average calculated from duplicate cultures of *Pseudomonas putida* strain WB9113. However, extent of reduction was monitored for duplicate cultures of five different *Pseudomonas* strains total (including WB9113) isolated from agricultural soils (see Materials and Methods section; Weller and Cook, 1983). None of these strains was shown to reduce more than 2% of the Fe(III) bound in SWa-1.

Values represent the reduction of smectite (SWa-1) unless otherwise indicated.

oxides with a related organism *Shewanella alga* strain BrY (Roden and Zachara, 1996).

#### 3.1. Variety of Soil Bacteria Capable of Smectite Reduction

If clay mineral reduction was only carried out by one or a few strains of soil bacteria, then this process may not be as important as perceived in the literature (Gates et al., 1993; Stucki et al., 1996). However, by testing a variety of organisms in our culture collection, we have found that several anaerobic cultures will mediate the process. Four pure cultures and one enrichment culture (isolated from soils, sediments, and an anoxic marine basin) were tested for the ability to reduce the structural Fe(III) in smectite (SWa-1) under anaerobic conditions (Table 1), and bacterial smectite reduction was quite rapid for most cultures (as shown in Fig. 1 for MR-1). Ranges of clay mineral reduction extent overlapped for the freshwater (MR-1) and marine (MR-4) strains of *Shewanella putrefaciens* in cultures with lactate as a carbon source. *Geobacter* reduced smectite to a lower average extent than other pure cultures of Fe-reducing bacteria. The largest extent of smectite reduction (55%; Table 1) was observed in a highly purified enrichment of Fe-reducing bacteria cultured from rice paddy soil taken from Nanjing, China. This rice paddy culture was successively transferred for 1.5 years in a minimal medium with acetate as the only carbon source and Fe(III) oxyhydroxide as the sole electron acceptor before introduction into the medium containing smectite. The same rice paddy soil enrichment maintained viability after four successive transfers in a minimal medium with smectite as the only electron acceptor. The only cultures observed to reduce Fe(III) in smectite to an insignificant extent under anaerobic conditions were those of the *Pseudomonas* group (Table 1). *Pseudomonas* strains, however, were previously shown to reduce the structural Fe(III) in smectite under microaerophilic conditions (Stucki et al., 1987; Gates et al., 1993). We suggest that *Pseudomonas* is capable of clay mineral

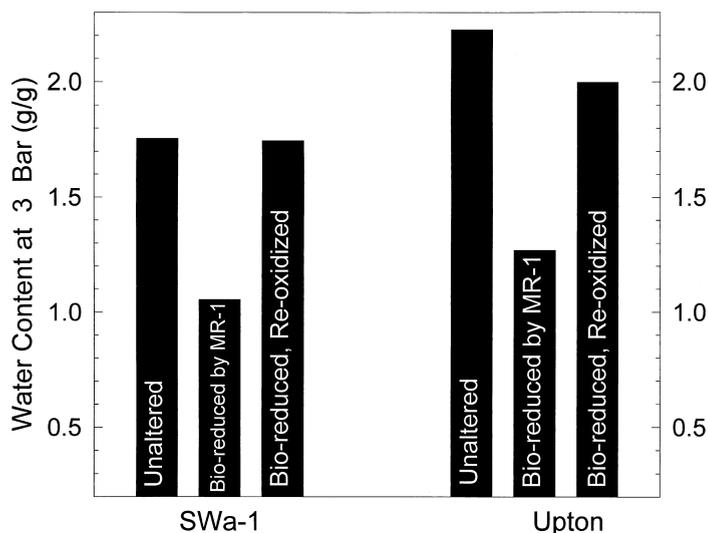


Fig. 2. Effect of structural Fe reduction by MR-1 on the swelling pressure of smectite (SWa-1) and Upton montmorillonite clay minerals. Clay minerals were added as the sole electron acceptor to a final concentration of 1 g/L. Each bar represents the average of 5 swelling pressure determinations; the standard deviation ranged from 1.7 to 6.0% of the average for each determination.

reduction in the presence of trace amounts of oxygen but cannot use clay-bound Fe(III) as a sole electron acceptor.

### 3.2. Effects of Bacterial Reduction on the Surface Chemistry of Clay Minerals

On the basis of experiments with chemical reductants, our hypothesis has been that bacterial reduction causes the structure of phyllosilicate clay minerals to collapse, leading to drastic chemical changes on the mineral surface. This was the case because swelling pressure, measured by water content, was shown to decrease by nearly 40% to 44% in smectites reduced

by *Shewanella putrefaciens* strain MR-1 cultures in comparison to unaltered or reoxidized smectites (Fig. 2). The observed changes in swellability upon reduction were supported by measurements of particle surface area and cation exchange capacity. Particle surface area decreased by 170 m<sup>2</sup>/g (26% of unaltered) in SWa-1 and by 366 m<sup>2</sup>/g (46% of unaltered) in Upton montmorillonite upon reduction by MR-1 (Fig. 3). The cation exchange capacity increased (Fig. 4) from 0.81 to 1.05 mEq/g in SWa-1 and from 0.85 to 0.98 mEq/g in Upton montmorillonite upon reduction of structural Fe by bacteria. Consequently, surface charge density (the ratio of cation ex-

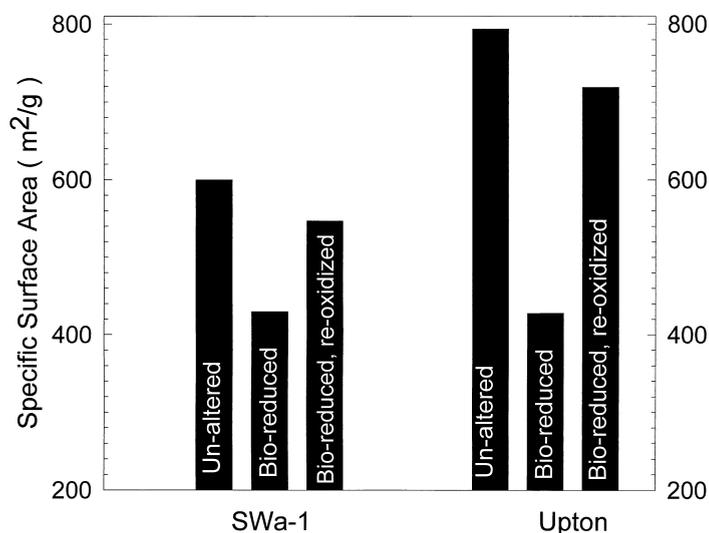


Fig. 3. Effect of structural Fe reduction by MR-1 on the specific surface area of smectite (SWa-1) and Upton montmorillonite clay minerals. Clay minerals were added as the sole electron acceptor to a final concentration of 1 g/L. Each bar represents the average of at least triplicate surface area determinations; the standard deviation ranged from 1.2% to 4.2% of the average for each determination.

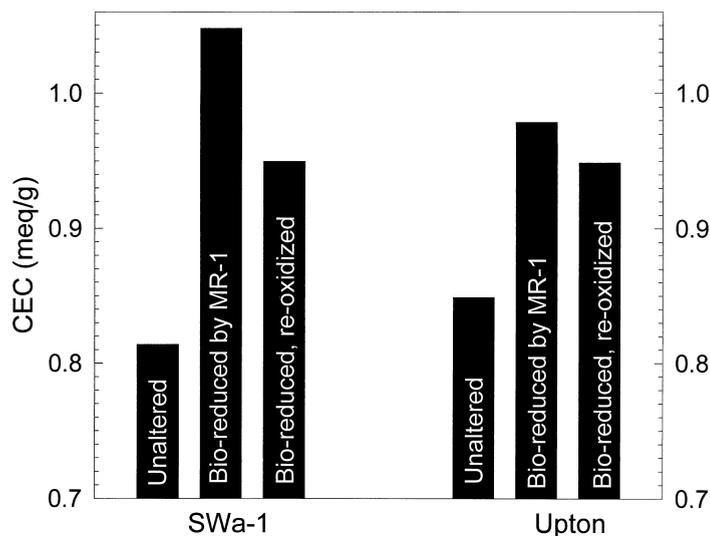


Fig. 4. Effect of structural Fe reduction by MR-1 on the cation exchange capacity (CEC) of smectite (SWa-1) and Upton montmorillonite clay minerals. Clay minerals were added as the sole electron acceptor to a final concentration of 1 g/L. Each bar represents the average of at least triplicate CEC determinations; the standard deviation ranged from 0.6% to 4.7% of the average for each determination.

change capacity to specific surface area) increased substantially. For all surface parameters analyzed, smectites that were chemically reoxidized (by simply bubbling with O<sub>2</sub>) after bacterial reduction would revert to a surface chemistry similar to that observed in the unaltered clay minerals (Figs. 2 to 4), but they never completely returned to their original surface chemistry, indicating some irreversible effects of bacterial Fe(III) reduction consistent with previous chemical reduction studies (Komadel et al., 1990, 1995).

Swelling pressure was further studied in smectite that was saturated with the organic cation TMPA, which normally adds some hydrophobic character to the unaltered clay mineral surface (Stucki et al., 2000). Unlike Na<sup>+</sup>-SWa-1 without added organic cation, swelling pressure increased after structural Fe(III) reduction, and approximately equal effects were observed whether SWa-1 was reduced by bacteria (Fig. 5A) or dithionite (Fig. 5B; Stucki et al., 2000). Apparently, bacterial or chemical Fe(III) reduction actually increased the hydrophilic character of the smectite surface (Fig. 5).

Intervalence electron transfer was studied using reflectance spectra in conjunction with wet chemical measurements of Fe oxidation state in growing MR-1 cultures with SWa-1 added as the sole electron acceptor. The bacterially reduced, chemically reoxidized spectrum closely resembled that of the oxidized, unaltered smectite (Fig. 6), and changes in reflectance spectra correlated to the extent of Fe(III) reduction (Table 2). The extent of reduction was similar to that observed previously in MR-1 cultures (Kostka et al., 1996).

The UV-vis reflectance spectra reveal similar behavior to that reported by Anderson and Stucki (1979) and Lear and Stucki (1987) for dithionite-reduced smectites wherein an increased absorbance (decreased reflectance; Fig. 6) occurs at the position of the Fe(III)-O-Fe(II) intervalence electron transition (700 to 800 nm), with a concomitant decrease in absorbance (increase in reflectance) in the higher energy region (350 to 550 nm) of the spectrum. The latter occurs because of the loss of

intensity in the very strong O-Fe(III) charge transfer transition commonly observed at 265 nm (Karickhoff and Bailey, 1973) as Fe(III) is converted to Fe(II). The reflectance spectra shown in Figure 6A agree with previous studies of chemical clay mineral reduction (Anderson and Stucki, 1979; Lear and Stucki, 1987; Komadel et al., 1990), and the variation in reflectance at 750 nm (due to intervalence electron transfer) with time of exposure to bacterial reduction (Fig. 6B), appears to be a direct quantitative indicator of structural Fe(II) content. The decrease in reflectance (Fig. 6B) showed a highly negative correlation ( $r^2 = 0.951$ ; slope =  $-59.4$ ) to the level of Fe reduction by the bacteria (Table 2).

#### 4. DISCUSSION

##### 4.1. Biological and Chemical Reduction of Structural Fe(III) in Clay Minerals

More than a decade has elapsed since researchers first determined that microorganisms were capable of structural Fe(III) reduction in clay minerals (Stucki et al., 1987; Wu et al., 1988). In early studies, the bacterial cultures used were not well characterized and the mechanism by which clay mineral reduction was catalyzed was not identified. In addition, the extent of bacterial reduction observed in these studies was lower and the reaction times much longer than in our study which used well-characterized bacterial cultures (Table 3). Here we have determined that a number of anaerobic soil bacteria are capable of reducing a large percentage of the Fe(III) bound in the structure of clay minerals (Table 1). This drastic change in the oxidation state of Fe(III) was further shown to result in dramatic changes in the physicochemical characteristics of the clay mineral surface (Figs 2 to 6).

Although the surface chemistry of bacterially reduced layered silicates was not comprehensively analyzed in previous work, some effects were elucidated. Wu et al. (1988) inferred

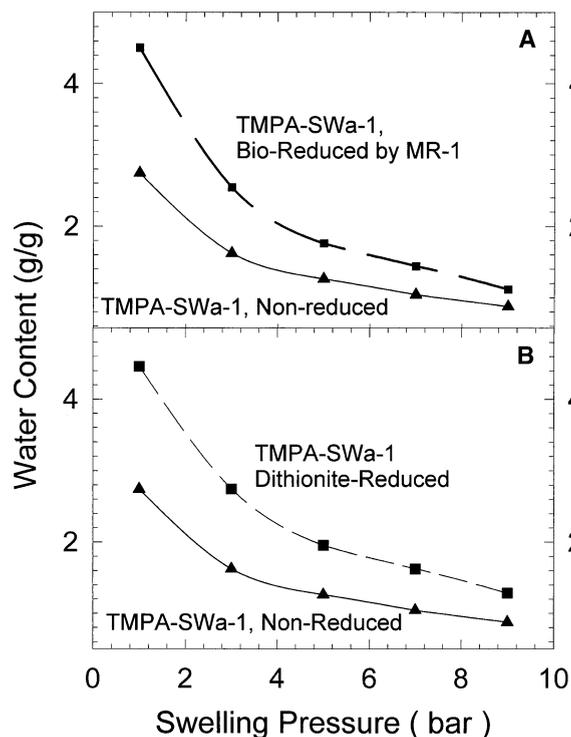


Fig. 5. Effect of structural Fe reduction on the swelling pressure of smectite (SWa-1) clay saturated with the organic cation, trimethylphenylammonium (TMPA). (A) SWa-1 was reduced by MR-1 using the same culture conditions as described for Fig. 2. (B) SWa-1 was reduced by the inorganic chemical reductant, dithionite, under strictly anoxic conditions according to Stucki et al. (1984b). Each point represents the average of five swelling pressure determinations; the standard deviation ranged from 1.7% to 6.0% of the average for each determination.

from X-ray diffraction patterns and reflectance spectra that the physicochemical properties of nontronite changed after bacterial treatment, and they further concluded that the effects of reduction were similar regardless of the reducing agent (biological or chemical). Some evidence to the contrary was provided by Gates et al. (1993), who observed that microbially reduced smectite showed a more dramatic decrease in swelling pressure relative to smectite treated with the potent chemical reductant dithionite. Gates and co-workers (1993) hypothesized that the increased impact of bacteria on smectite swelling was attributable to the interaction with bacterial cells or cell exudates that altered the water retention characteristics of the mineral. They observed that mixed cultures containing a number of strains reduced greater amounts of smectite-bound Fe(III) than individual *Pseudomonas* strains.

We have quantified the effects of bacterial Fe(III) reduction on smectite surface chemistry for the model ferruginous smectite (SWa-1) and Upton montmorillonite. The magnitude of changes to clay mineral chemistry appears comparable to or greater than that observed during chemical reduction studies. For example, Lear and Stucki (1989) observed an 11% decrease in the specific surface area and up to a 27% decrease in swelling pressure upon chemical reduction of 1 to 1.2 mmol Fe per gram of smectite (SWa-1). At similar reduction levels and using the same methods of characterization, we observed a 28%

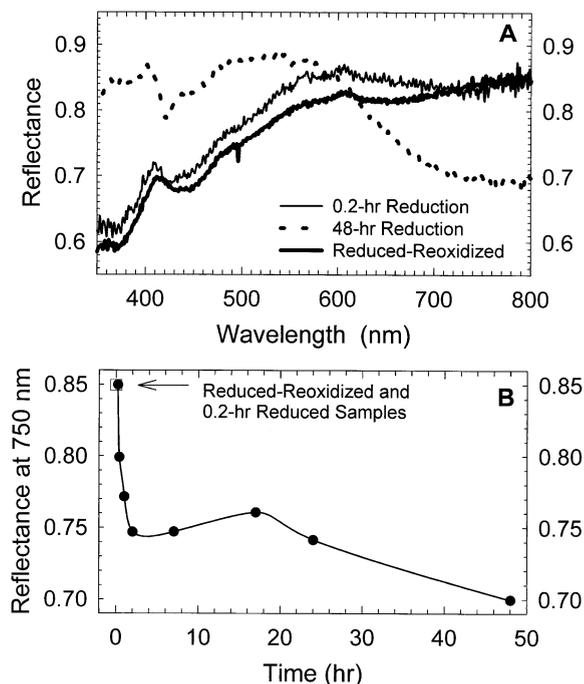


Fig. 6. (A) Visible reflectance spectra of whole MR-1 cell suspensions at 350 to 800 nm with smectite (SWa-1) added as the sole electron acceptor to a final concentration of 1 g/L. (B) Variation in reflectance at 750 nm plotted over time in the same MR-1 cultures. Reflectance was measured while stirring continuously under strictly anoxic conditions in a specially designed cuvet.

decrease in specific surface area (Fig. 3) and a 40% decrease in the swelling pressure (Fig. 2) of bacterially reduced SWa-1. Bacterial Fe reduction in Upton montmorillonite resulted in equal or larger decreases in surface area and swelling pressure. Our results concur with the study by Gates et al. (1993), which observed that swelling pressure in smectites reduced by *Pseudomonas* sp. was nearly two times lower than in smectite reduced by dithionite. Studies to date indicate that above changes to clay mineral surface properties are two to three times more pronounced in response to bacterial Fe reduction as opposed to chemical Fe reduction by dithionite.

We observed opposing effects on the swellability of smectite (SWa-1) after structural Fe(III) reduction (regardless of the reducing agent) that depended on whether the clay mineral surface was saturated with an inorganic cation ( $\text{Na}^+$ ; Fig. 2) or an organic cation ( $\text{TMPA}^+$ ; Fig. 5). Reduction of Fe(III) to Fe(II) in the octahedral sheets increased the layer charge and

Table 2. Average extent of smectite (SWa-1) reduction observed over time in the same duplicate MR-1 cultures for which reflectance spectra were generated in Fig. 6.

Time of Sampling (h)	% Fe(II)
0.10	4.8
0.33	8.9
1.0	10.1
24	10.3
48	14.5

Table 3. Summary comparisons of the average extents of clay mineral reduction and reaction times observed in studies to date with soil/sediment bacteria.

Study	Maximum Extent of Reduction [% Fe(II)]	Time to Half Maximum (d)
Stucki et al., 1987	9	7
Wu et al., 1988	39	7
Gates et al., 1993	35	7
This study	46	0.5 <sup>a</sup>

<sup>a</sup> Average extent of SWa-1 reduction for MR-1 cultures represented in Fig. 1.

Reaction times represent the time over which half of the maximum extent of reduction is reached.

decreased the swelling of Na-saturated smectites by causing the collapse of previously swelling layers. The surfaces of uncollapsed layers hydrated more strongly than in the oxidized state. In contrast, if the interlayer cation was changed from Na<sup>+</sup> to an organocation after reduction, but before swelling pressure measurements, the swelling of the reduced clay mineral increased. Both a greater surface hydration energy and the ability of organocations to disrupt the mechanism that causes phyllosilicate layers to collapse (and become nonswelling) contributed to a higher water content in reduced than in unaltered organoclays. Therefore, the response of (smectite) clay swelling to structural Fe(III) reduction is related to surface hydrophilicity. We propose that the TMPA ion may increase the swelling of reduced clay minerals thereby preventing clay layer collapse due to a steric effect.

Reflectance spectra were analyzed in living cultures of Fe-reducing bacteria to provide information on intervalence electron transfer so that the progression of structural Fe reduction could be compared in the octahedral layer of bacterially reduced versus chemically reduced smectites. The fact that the Fe(III)–O–Fe(II) intervalence electron transfer band increases with progression of bacterial reduction indicates that structural Fe(III) is reduced in a modified random pattern (Lear and Stucki, 1987) where the two valence states are intermingled rather than in separate domains. This supports the hypothesis that reduction occurs at basal surfaces rather than at the clay edge, as has been proposed previously (Lear and Stucki, 1987). The mechanism and pathway by which electron transfer occurs from a donor on the smectite surface to structural Fe in the octahedral layer of the phyllosilicate remains to be completely elucidated. However, our data suggest that the mechanism of electron transfer to structural Fe(III) in smectite is similar, regardless of whether the reductant is biological or chemical.

#### 4.2. Potential Role of Bacterial Clay Mineral Reduction in Soil Biogeochemistry

Iron-bearing clay minerals become reduced in soils and sediments when subjected to reducing conditions (Egashira and Ohtsubo, 1983), such as during flooding, and indigenous microorganisms are thought to be the primary reducing agents (Lovley, 1991; Stucki et al., 1996). The potential importance of microorganisms in clay mineral reduction is further supported by the fact that Fe-reducing bacteria have been shown to be abundant in agricultural soils (Achnich et al., 1995). Our data

suggest that such reduction processes potentially have great impact on the chemical and physical properties of the soil, particularly in terms of swelling and cation exchange capacity, which, in turn, influence soil structure (Stucki, 1988), permeability (Gates et al., 1993), and fertility (Lamb and Grady, 1963). For example, cation exchange capacity, which was observed to increase with bacterial reduction (Fig. 4), is vital in agricultural soils because it determines whether valuable nutrients, such as K<sup>+</sup> and Ca<sup>2+</sup>, reach plant roots. In a landfill, this attribute would determine whether the surrounding soils, sediments, and clay barriers would trap hazardous cations and thereby retard their movement.

Biogeochemical evidence exists to support the potential importance of crystalline Fe minerals as an electron acceptor for Fe-reducing bacteria in soils and subsurface sediments. Several studies have now shown that Fe reducers are capable of coupling the reduction of crystalline Fe minerals, including Fe oxides (Kostka and Nealson, 1995; Roden and Zachara, 1996) and clay minerals (Kostka et al., 1996) to energy generation. Studies of soils and subsurface aquifer sediments have shown that the crystalline Fe fraction, containing a mixture of Fe(III) oxides and layered silicates, is available for reduction to seeded cultures and natural populations of Fe-reducing bacteria (Munch and Ottow, 1980; Lovley and Phillips, 1986a, 1986b; Roden and Zachara, 1996). Crystalline Fe minerals including phyllosilicates have been shown to be abundant in agricultural soils (Munch and Ottow, 1980; Chao and Zhou, 1983) and contaminated aquifers (Heron et al., 1994), but reactivity of the clay mineral fraction under near environmental conditions has so far not been characterized in detail. On the basis of its reactivity in wet chemical extractions and rapid reduction in bacterial cultures, ferruginous smectite was recently defined as part of the crystalline Fe fraction that may undergo reduction–oxidation over short time scales (Kostka et al., 1999b). In this study we have shown that clay minerals not only are reduced rapidly by a variety of soil bacteria, but that bacterial reduction leads to large changes in clay mineral surface chemistry. Given the predominance of clay minerals and bacteria, this process may result in important physicochemical alterations in natural soils.

## 5. CONCLUSIONS

A variety of soil bacteria are capable of catalyzing the reduction of structural Fe(III) in smectite clay minerals, causing up to 90% of the Fe bound in the phyllosilicates to be reduced. One mechanism by which clay minerals are reduced by bacteria is the anaerobic respiration of structural Fe coupled to carbon oxidation. Similar in magnitude to previous studies with chemical reductants, bacterial clay mineral reduction is shown to have a drastic effect on mineral surface chemistry. Bacterial reduction decreases clay swelling, causes the phyllosilicate structure to collapse, decreases surface area, and increases surface charge density as a function of cation exchange capacity. Examination of smectite oxidation state and Fe(II)–Fe(III) adsorption bands indicate that electron transfer follows a similar pathway in bacterial reduction as compared to chemical reduction, but the change to octahedral Fe is less complete. These processes are likely to be important to the biogeochem-

istry of soils, which are often rich in phyllosilicate minerals and where Fe-reducing bacteria have been shown to be abundant.

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