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SWELLING AND TEXTURE OF IRON-BEARING SMECTITES REDUCED BY BACTERIA

WILL P. GATES,[1,3] ANNE-MARIE JAUNET,[2] DANIEL TESSIER,[2] MICHAEL A. COLE,[3] HENRY T. WILKINSON[3] AND JOSEPH W. STUCKI[3]

[1] University of Georgia, Savannah River Ecology Lab., Drawer E, Aiken, South Carolina 29802, USA
[2] Science du Sol, Institut National de la Recherche Agronomique, Route de Saint-Cyr, F78026, Versailles, France
[3] Dept. of Natural Resources and Environmental Sciences, University of Illinois, Urbana, Illinois 61801, USA

Abstract—Microbial reduction of clay mineral structural Fe(III) decreases the swelling of nontronite gels, most importantly at intermediate oxidation states (40 to 80 cmol Fe(II) kg^{-1} clay). The purpose of this study was to establish whether microbial reduction of structural Fe(III) decreased the swelling of other Fe-bearing smectites and to discern the influence that organic compounds of microbial origin (bacterial cells, cell fragments and/or exudates) may have on clay swelling and texture. Structural Fe(III) was reduced by incubating smectite suspensions with either a combination of *Pseudomonas* bacteria or a mixture of anaerobic bacteria. The influence of organics on clay swelling was estimated on smectites suspended in either organic or inorganic media in the absence of bacteria. The gravimetric water content of the reduced clay gels equilibrated at various applied pressures was recorded as a function of Fe oxidation state. Transmission electron microscopy (TEM) was employed to determine the influence of bacteria and type of media on the texture of reduced smectite gels. Reduction of structural Fe(III) by bacteria decreased the swelling pressure of all Fe-bearing smectites. Increased clay swelling, due to the presence of organics (organic medium, exudates or cell fragments), was correlated to the total Fe content, the extent of structural Fe reduction, as well as the initial swelling characteristics of the Fe-bearing smectites. High structural Fe(II) contents (>50 cmol Fe(II) kg^{-1}) resulted in increased attractive forces between clay platelets that decreased clay swelling, even in organic medium suspensions. Microbial reduction resulted in increased face–face association of individual clay layers, forming larger and more distinct crystallite subunits than in nonreduced clay gels. But, perhaps more importantly, microbial reduction of structural Fe(III) resulted in an increased association between crystallite subunits and, thus, an overall larger particle size and pore size distribution, due to the interaction of bacteria cells, cell fragments and organic exudates.

Key Words—Bacteria, Biological Reduction, Organo-Clay Interactions, Smectites, Structural Iron, Swelling Pressure, Texture.

INTRODUCTION

The volumetric change of smectite clay minerals upon wetting (swelling) is an important physical property that influences many hydrologic and mechanical properties of soils, for example, the infiltration and percolation of water and the stability of soils and sediments for agriculture or engineering purposes. The swelling behavior of smectite gels is influenced by the ionic strength of the bathing solution, the presence of cementing agents (such as organic matter or hydrous oxides) and the microstructure of the gel. Clay swelling behavior is also affected by both the presence and the oxidation state of Fe within the crystalline structure of smectites (Stucki 1988). Reduction of structural Fe(III) by Na-dithionite (Foster 1953; Egashira and Ohtsubo 1983; Stucki et al. 1984c; Lear and Stucki 1989) and bacteria (Gates et al. 1993) is known to significantly decrease clay swelling pressure, thus impacting the dynamic volumetric nature of smectites.

The oxidation state of structural Fe influences or controls many other physical and chemical properties of smectites as well (Stucki 1988; Gates et al. 1996). Reduction of structural Fe(III) by soil-borne microorganisms (Komadel et al. 1987; Stucki et al. 1987; Wu et al. 1988; Kostka et al. 1996; Ernstsen et al. 1998) alters structural properties of smectites (Gates et al. 1996) and the chemical reactivity of soils (Ernstsen et al. 1998). Stucki and Tessier (1991) showed that the oxidation state of structural Fe influenced the textural properties of smectite gels. Decreased swelling (Stucki et al. 1984c; Lear and Stucki 1989) resulted from partial or total collapse of the interlayer space and an increase in the structural order (texture) of the clay gel (Stucki and Tessier 1991) following reduction of structural Fe. Shen et al. (1992) reported that, depending on treatment history, reduction of structural Fe increased the hydraulic conductivity of smectite gels. Gates et al. (1993) found that incubation of Fe-free smectite gels with bacteria resulted in an increased swelling pressure, but the same microbes significantly decreased clay swelling upon reduction of structural Fe(III) in nontronite suspensions. They suggested that the presence of cells, exudates or cell fragments could have affected the formation of a compact gel structure at low Fe(II) contents. Additionally, at levels of re-

† Present address: CSIRO Land and Water, Private Bag No. 2, Glen Osmond, SA 5064 Australia.

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Table 1. Total structural Fe and Fe(II) content, structural formulas and layer charge characteristics of the smectites studied.

Smectite	Total Fe† (cmol kg⁻¹)	Fe(II) (cmol kg⁻¹)	Layer charge Tetrahedral /$O_{20}(OH)_4$	Octahedral /$O_{20}(OH)_4$	Total (cmol kg⁻¹)
API #34	0.4	0.0	0.02	0.79	105.9
API #25	52.2	1.9	0.08	0.68	101.5
SWy-1	62.6	3.0	0.34	0.41	100.0
SWa-1	354.9	2.3	0.67	0.13	97.4

Smectite	Structural formulas‡
API #34	$M^{+}_{0.81}(Mg_{5.29}Li_{0.63})(Si_{7.98}Al_{0.02})O_{20}(OH)_4$
API #25	$M^{+}_{0.76}(Al_{2.92}Fe^{3+}_{0.40}Fe^{2+}_{0.02}Mg_{0.66})(Si_{7.92}Al_{0.08})O_{20}(OH)_4$
SWy-1	$M^{+}_{0.75}(Al_{3.07}Fe^{3+}_{0.42}Fe^{2+}_{0.02}Mg_{0.54})(Si_{7.66}Al_{0.34})O_{20}(OH)_4$
SWa-1	$M^{+}_{0.80}(Al_{0.91}Fe^{3+}_{2.86}Mg_{0.28})(Si_{7.33}Al_{0.67})O_{20}(OH)_4$

† Total Fe and Fe(II) as measured by colorimetric procedure of Komadel and Stucki (1988).
‡ Structural formulas for API #34 and API #25 after Slade et al. (1991), omitting Ti^{4+} from the calculations, and for SWy-1 and SWa-1 after Madejová et al. (1994). Where appropriate, layer charge was adjusted to incorporate Fe(II) content. Total charge was calculated assuming Na as the interlayer cation.

duction greater than about 40 cmol Fe(II) g^{-1}, interlayer attractive forces (Stucki and Tessier 1991) resulting from increased charge (Khaled and Stucki 1991) might have overcome the opposing, organic-induced increased porosity (Chenu 1989; Chenu and Jaunet 1990), causing interlayer collapse. The objectives of this study were to test how the oxidation state of structural Fe and the presence of bacteria cells, cell fragments or exudates within the clay matrix influenced the texture and swelling behavior in smectite gels reduced by bacteria.

MATERIALS AND METHODS

Smectites

The <2 μm size fraction of the ferruginous smectite SWa-1, Wyoming montmorillonite SWy-1 (both from Source Clays Repository, Columbia, Missouri), Upton montmorillonite API #25, and Arizona hectorite API #34 (both from Ward's Natural Science Establishment, Rochester, New York) were used. Structural formulas, Fe contents and charge characteristics of each smectite are listed in Table 1. In preparation for their use, the clays were Na-saturated, fractionated, dialyzed and freeze-dried according to Stucki et al. (1984a).

Table 2. Compositions of liquid media used in bacterial cultures of smectite suspensions.

Ingredient	MTM† (g L⁻¹)	NYG‡ (g L⁻¹)
K_2HPO_4	1.0	
$MgSO_4 \cdot 7H_2O$	0.2	
$CaCl_2$	0.1	
NaCl	0.1	
KNO_3	0.5	
Asparagine	0.5	
Mannitol	1.0	
Nutrient broth		2.0
Yeast extract		0.2
Glucose		2.0

† MTM = Modified Thornton's Medium (Thornton 1922).
‡ NYG = Nutrient broth–Yeast extract–Glucose.

Bacteria and Media

Complete descriptions of the bacteria, media and reduction conditions used are described by Gates et al. (1993). The bacteria used were: *Pseudomonas fluorescens* (3 strains) and *P. putida* (1 strain). Swelling data for the ferruginous smectite SWa-1 reported previously by Gates et al. (1993) used mixtures containing *P. aureofaciens*. An inorganic medium (Table 2), modified Thornton's (Thornton 1922) medium (MTM), was used with suspensions inoculated with combinations of *Pseudomonas* (Gates et al. 1993). The original medium was modified by eliminating $FeCl_2$ and using high-purity water (>18 MΩ resistivity-cm).

A mixture of facultative anaerobes (MA) was to inoculate some clay suspensions in an organic medium (Table 2) consisting of nutrient broth, yeast extract and glucose (NYG). The bacterial mixture was isolated from unprocessed ferruginous smectite by combining 20 g SWa-1 with 600 mL filter-sterilized (0.45 μm inline filter) high-purity water, covered and left undisturbed for several weeks. Under N_2 flow, aliquots of the sediment were removed and immediately transferred to the bottom of sterile 50-mL culture tubes containing 30 mL of fresh deoxygenated NYG (2× strength) and incubated for several days at room temperature.

Reduction of Structural Fe by Bacteria

Pseudomonas isolates were handled as in Gates et al. (1993). Combined bacterial suspensions were prepared by inoculating suspensions (either 400 or 800 mg of each smectite in 40 mL MTM) with 1.0 mL of a fresh mixture of *Pseudomonas* bacteria containing about 10^7 CFU mL^{-1}. Following 60–90 d incubation,

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100-mg aliquots of clay were removed from the suspensions and transferred to sterile 50-mL polycarbonate centrifuge tubes, sealed with septum-caps and washed free of excess salts for further analysis. All transfers were accomplished within a glove box (Vacuum Atmosphere, Inc.) to maintain an oxygen-free atmosphere. Suspensions containing media and clay were assayed in a like manner to serve as noninoculated controls and as a check for biological contamination.

Aliquots (1 mL from just above the sediment) of MA culture tubes were used to seed sterile tubes containing 400, 600 or 800 mg of each smectite suspended in 40 mL NYG and incubated for 60–90 d at room temperature. Aliquots (100 mg clay) of biologically reduced smectite suspensions were then transferred to 50-mL polycarbonate centrifuge tubes within a glove box, sealed, washed free of excess salts and assayed for swelling pressure and Fe(II) content. Sterile control suspensions lacking bacteria were prepared as well.

Swelling Pressure Assay

Excess salts (organic and inorganic electrolytes) within the suspensions were removed as described in Stucki et al. (1984a) and Gates et al. (1993). The contents of each sample were divided and transferred to 3 porous-plate cells (9-mm dia.) attached to a pressurized tank containing H_2O-saturated N_2 gas (Stucki et al. 1984c) and equilibrated at either 0.1, 0.3 or 0.5 MPa applied pressure. The porous-plate cells were disassembled following equilibration and the gravimetric water content (m_w/m_c) was determined on 2 of the clay gels. The third clay gel was immediately transferred into a polypropylene tube containing acid digestion solution for Fe(II) analysis following the colorimetric procedure outlined in Komadel and Stucki (1988).

TEM

Based on Fe(II) analysis, selected reduced gels were prepared for TEM observations in a manner similar to that described in Stucki and Tessier (1991). About 100 mg of reduced suspension (both washed and unwashed) was transferred to a single porous plate cell and equilibrated at 0.01 MPa applied pressure. Following equilibration, the gel was cut in half and immediately transferred under N_2 flow into porous glass vials (7 × 7 mm). The vials were then immersed in 1:1 deoxygenated methanol : water (deoxygenated by bubbling N_2 through the solution) within a polypropylene bottle (8 mL) and equilibrated for 4 h at 4 °C to replace interlayer water. Vials were transferred to a second bottle of 100% deoxygenated methanol solution and stored overnight at 4 °C.

In preparation for TEM observations, samples thus treated were transferred to a 1:1 (v/v) mixture of deoxygenated methanol : LR White resin (LRW—Ernest F. Fullam, Inc.) for 2 h, to a 1:2 (v/v) mixture for 2 h, to pure LRW for 2 h and to pure LRW overnight. To polymerize the LRW, each reduced clay–gel half was transferred into a gelatin capsule containing LRW and placed in a desiccator. The desiccator was twice evacuated (0.7 MPa tension), refilled with N_2 and placed in an oven at 60 °C for 24 h. Only samples with good LRW resin polymerization were analyzed by TEM, since LRW will only harden in the absence of oxygen. The technique has been shown to minimize reoxidation of structural Fe(II) as well as minimize the occurrence of experimental artifacts (Stucki and Tessier 1991). All samples were treated identically and at the same time, using the same reagents and epoxy mixtures to minimize possible spurious effects associated with resin curing.

Sectioning of samples was conducted at room temperature within a glove bag purged with N_2 to minimize possible reoxidation (Stucki and Tessier 1991). Thin sections were floated onto TEM grids in N_2-saturated water and the grids were desiccated under inert (N_2) atmosphere. Experience in our laboratories has shown that, when dry, the oxidation state of reduced smectites is quite stable (Stucki and Tessier 1991; Gates et al. 1996). Care was taken during sectioning to avoid disrupting the structural organization of the microfabric (Tessier 1984). To observe the physical interaction between bacterial products and the reduced smectite gel, TEM imaging was accomplished with a Philips 420 STEM microscope operating at 120 kV. The magnification ranged from 3000 to 51,000, under conditions to obtain high-resolution lattice-fringe images. Photos were enlarged up to 500,000×. Photomicrographs were prepared to detail representative (several thin sections per grid, several locations per section) physical interactions within the microbial-clay system. Because of difficulties in staining under reducing conditions, no heavy-metal staining was accomplished to corroborate the presence of organics.

RESULTS

Reduction of Smectite Structural Fe by Bacteria

Significant levels of structural Fe(II) were measured following 60–90 d incubation of microbial-smectite suspensions in inorganic or organic medium (Figure 1). The amount of structural Fe reduced by either the *Pseudomonas* combination in MTM (Figure 1A) or the anaerobic mixture (MA) in NYG (Figure 1B) increased with increasing total Fe content of the smectites. However, with increasing total Fe content, the variability of the amount of structural Fe(II) measured increased as well.

Influence of Structural Fe(II) on Clay Swelling

Values of the gravimetric water content (m_w/m_c) for the microbially reduced SWa-1 decreased with increasing Fe(II) content at all applied pressures (Figure 2A).

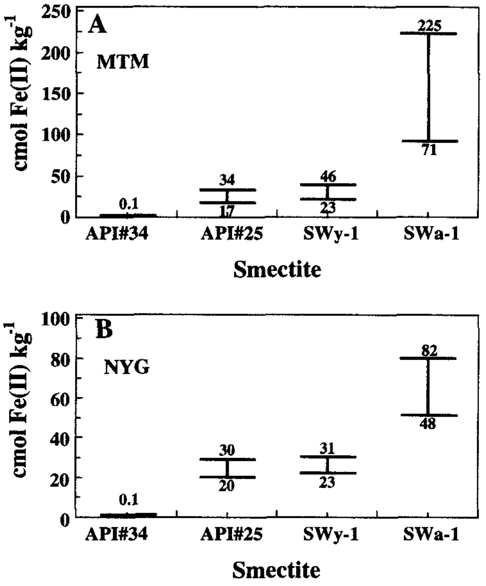

Figure 1. Levels of biologically reduced structural Fe obtained after 60–90 d incubations of smectite suspensions with (A) combination of *Pseudomonas* in inorganic medium (MTM), and (B) mixture of anaerobes in organic medium (NYG). Values indicate minimum and maximum content of structural Fe(II) attained. Total Fe contents of smectites are listed in Table 1.

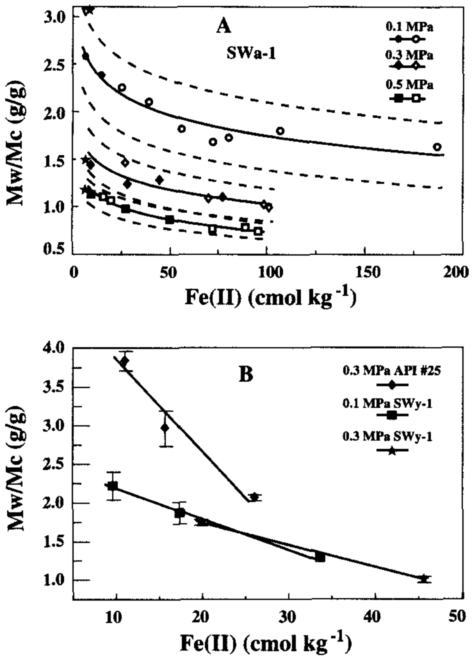

Figure 2. Effect of microbial reduction of structural Fe on the gravimetric water content (m_w/m_c) of (A) ferruginous smectite SWa-1, (B) montmorillonites SWy-1 and API #25 at various applied pressures, inoculated with a combination of *Pseudomonas* bacteria in inorganic medium (MTM). In (A), solid markers are from this study; solid stars represent uninoculated treatments; hollow markers are from Gates et al. (1993). Dashed lines represent 95% confidence intervals of the best least-squares (logarithmic) fit for all samples within an applied pressure (solid lines) that were inoculated with *Pseudomonas* combined cultures. All functions significant $p < 0.05$. In (B), solid lines represent best least-squares fit (where appropriate). Bars represent 1 standard deviation of duplicate samples.

For SWa-1, the decrease in m_w/m_c was greatest for Fe(II) contents up to about 40 cmol kg^{-1} clay (= 0.4 mmol g^{-1} or about 11% total Fe), but beyond about 80 cmol Fe(II) kg^{-1} clay (about 22% total Fe) the change in m_w/m_c with Fe(II) content was negligible (Figure 2A data for 0.1 MPa). The effects of structural Fe(II) on the m_w/m_c values of the montmorillonites SWy-1 and API #25 are shown in Figure 2B. Note that the relationship between m_w/m_c is more linear in character for both SWy-1 and API #25. The maximum level of structural Fe(II) measured for SWy-1 was 46 cmol kg^{-1} (about 70% of total Fe) and for API #25, 34 cmol kg^{-1} (about 65% of total Fe).

Influence of Organics on Swelling

To estimate the influence that soluble organics (medium, bacterial cells, exudates and/or lysis products) had on clay swelling in the absence of reduction, inoculated and noninoculated suspensions of Arizona hectorite (API #34) in both MTM and NYG were studied. Hectorite has a negligible structural Fe content (0.4 cmol kg^{-1}); thus, clay swelling should be a function of the presence or absence of organics and bacterial products. Note that the m_w/m_c values for hectorite suspended in MTM are lower than the corresponding NYG values without bacteria, but no difference occurred following incubation with the bacteria treatments (Figure 3). In both media, values for m_w/m_c were nearly doubled after incubations with bacteria compared to the uninoculated samples at 0.3 MPa applied pressure. These results provide further support of the hypothesis that microbial cells or lysis products (organics) tended to increase clay swelling when little or no Fe reduction occurred. Figure 3 also indicates that bacterial type probably had little influence on clay swelling.

Values of m_w/m_c for reduced nontronite (Figure 4A) and montmorillonites (Figures 4B–4C) suspended in NYG incubated with MA bacteria also decreased with increasing Fe(II) content at 0.3 MPa applied pressure. In both media, values of m_w/m_c for the bacteria-free treatments of SWa-1 (hollow markers, Figure 4A) were not significantly ($p < 0.05$) different from the biologically reduced-reoxidized treatments (solid markers near 10 cmol kg^{-1}, Figure 4A). Generally, at lower structural Fe(II) levels, the smectite gels reduced by MA in NYG retained more water than gels reduced by PC in MTM (Figures 4A–4C).

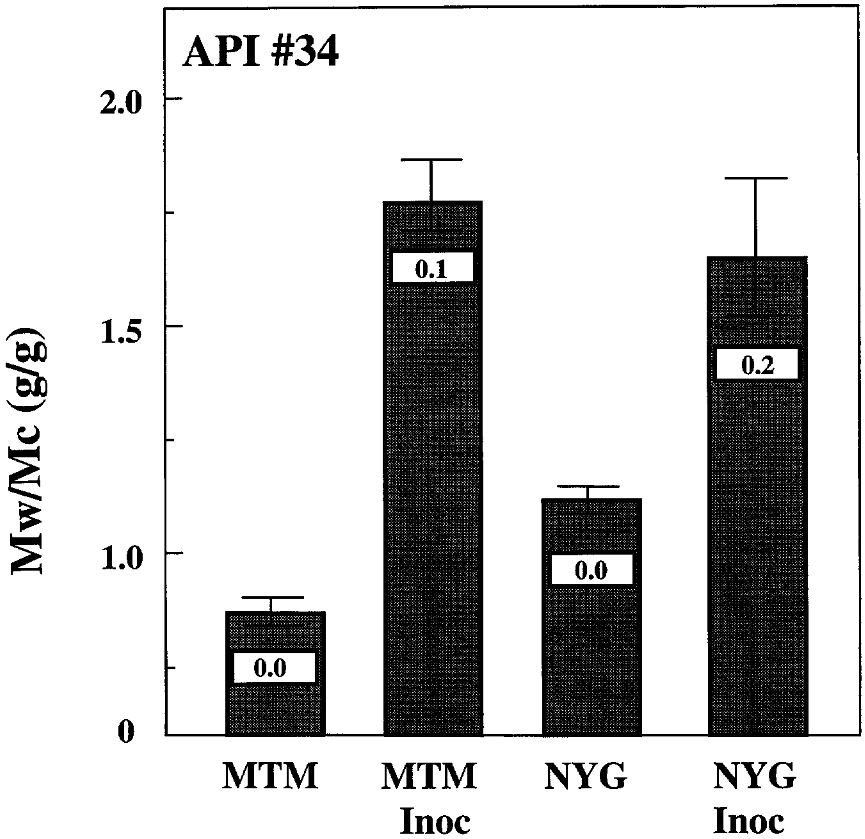

Figure 3. The effect of medium composition and presence of bacteria on the gravimetric water content (m_w/m_c) of Arizona hectorite API #34 suspensions in organic medium (NYG), or inorganic medium (MTM) at 0.3 MPa applied pressure. Suspensions in MTM were inoculated with the combination of *Pseudomonas* bacteria; suspensions in NYG were inoculated with the mixture of anaerobic bacteria (MA). Values within each bar are the Fe(II) contents (cmol kg^{-1}) measured for the particular m_w/m_c value. Bars represent 1 standard deviation of duplicates.

Texture of Biologically Reduced Ferruginous Smectite

Typical transmission electron micrographs of biologically reduced gels of SWa-1 indicate dramatic differences in clay structural order, or texture, relative to both untreated and dithionite-reduced SWa-1 gels (Figures 5–8). Different magnifications (from 21,000 to 510,000×) of the untreated SWa-1 revealed a highly dispersed and disorganized system (Figure 5A). The *a-b* dimensions of associated particles and layers in the untreated SWa-1 were not extensive, as previously noted (Stucki and Tessier 1991). Particles consisted of several crystallite subunits made up of 1 to 6 layers stacked in disordered face–face and edge–edge orientation (Figure 5B), and the overall sizes of pores were smaller than that described by Stucki and Tessier (1991) for the same smectite. The untreated SWa-1 contained some goethite (Stucki and Tessier 1991) and other impurities (Figure 5A).

Representative thin sections of the dithionite-reduced sample (about 50% of total, or 175 cmol Fe(II) kg^{-1}) revealed a more extensive, homogeneous network of particles (Figure 6A) of greater lateral extent (0.3–1.5 μm, Figure 6B) than the oxidized sample, each made up of discrete crystallite subunits composed of 5–10 layers (Figure 6C). The layer stacking and subsequent organization of the crystallite subunits composing the particles of the reduced sample was more ordered than that of the untreated sample, in agreement with Stucki and Tessier (1991).

In the microbially reduced, unwashed system, most crystallites were composed of subunits of 5 to 10 individual layers interconnected predominantly by overlapping face–face and face–face associations with adjoining subunits forming undulating filaments of extensive length (Figure 7). The level of Fe(III) reduction in this system was about 30% of total, or 100 cmol Fe(II) kg^{-1}. Pores between crystallite subunits interrupted extensive face–face crystallite formation (Figure 7B) compared with dithionite-reduced smectite and are presumed to be associated with microbial lysis or exudate residues (Chenu and Jaunet 1990).

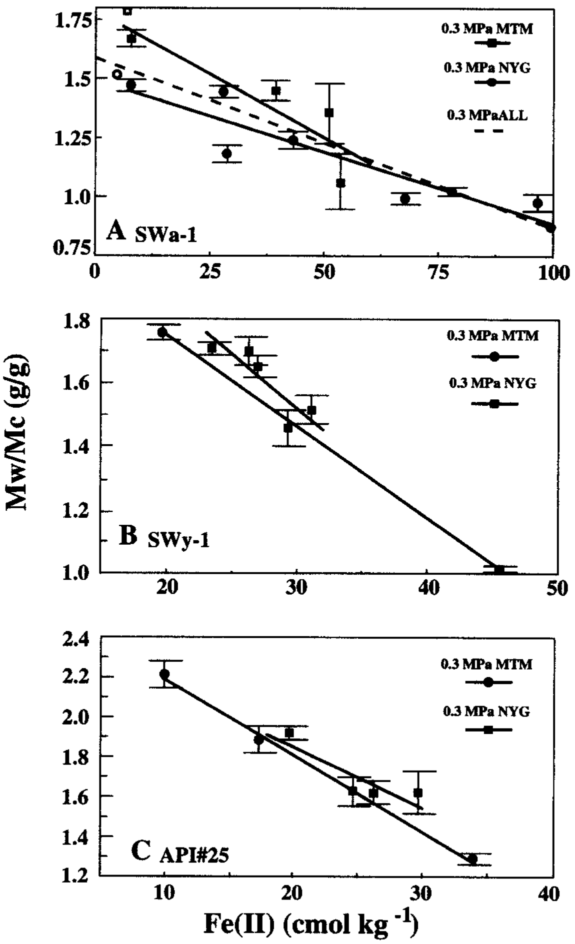

Figure 4. The effect of medium composition and microbial reduction of structural Fe(III) on the gravimetric water content (m_w/m_c) at 0.3 MPa applied pressure, of smectite suspensions inoculated with bacteria in inorganic (MTM) or organic medium (NYG): (A) ferruginous smectite SWa-1; (B) Wyoming montmorillonite SWy-1; and (C) Upton montmorillonite API #25. Hollow markers in (A) represent uninoculated treatment. Solid lines represent best least-squares fit (where appropriate). Bars represent 1 standard deviation of duplicates.

Although no staining of organics was attempted, it is striking how similar the overall texture of the microbial–clay system shown in Figures 7A–7C was with the clay–scleroglucan system studied by Chenu (1989) and Chenu and Jaunet (1990). The presence of organics within the interstices of the unwashed sample was probably responsible for the increased lateral (*a-b*) extension of particles (Permien and Lagaly 1994).

While the presence of bacteria cells, cell remnants and organic exudates affected the reduction-induced textural changes noted in Figure 7C, their removal by washing after reduction dramatically changed the overall fabric of the gels. Most obvious was the increased thickness, lateral extension and parallel alignment of the particles (Figure 8A), indicative of increased layer charge associated with structural Fe reduction (combination of *Pseudomonas* bacteria, about 30% of total or 100 cmol Fe(II) kg^{-1}). The particles within the microbially reduced, washed system were made up of discrete crystallite subunits aligned face–face and overlapping face–face (Figures 8B and 8C) such that an increased pore size was promoted (Figure 8B) relative to the untreated (Figure 5B) or the dithionite-reduced (Figure 6B) samples. The overall number of layers (Figure 8C) composing crystallite subunits increased (8–15) relative to the untreated sample (Figure 5C), and substantially more subunits were face–face associated, thus increasing the total number of layers per particle (>40) over that of even the dithionite-reduced sample (Figure 6C).

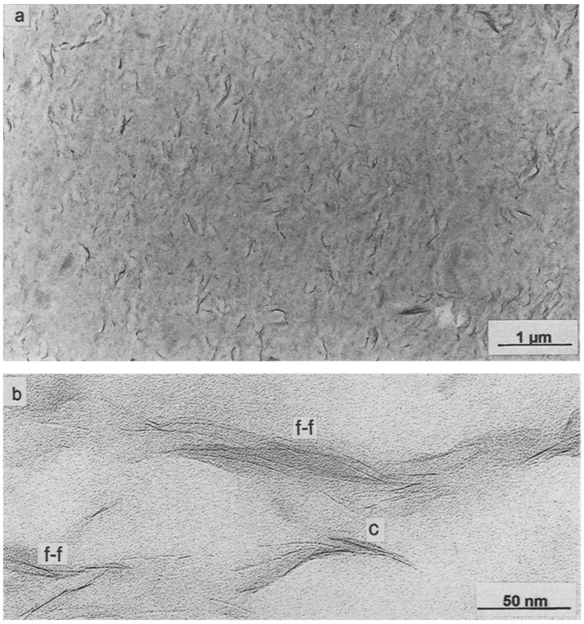

Figure 5. The textural organization of the untreated ferruginous smectite SWa-1. (A) Low-resolution image showing the highly dispersed nature of the reference smectite. Magnification 21,000×. (B) High-resolution lattice fringe image of a clay particle showing the predominantly face–face (f–f) association of individual layers and crystallite subunits (c) forming the particles. Some edge–face associations are discernable. Magnification 510,000×.

DISCUSSION

Reduction of Smectite Structural Fe by Bacteria

The present results indicate that the amount of total structural Fe(III) available for reduction by microorganisms limited the amount of reduction achieved. Stucki et al. (1987) found a similar level of Fe(II) (29 cmol kg^{-1}) in each of the 2 clays, SWa-1 and API #25, when using medium MTM, whereas in the present study increased levels of Fe(II) and a greater dispersion about the mean were obtained with greater total structural Fe contents. One possible cause of this dif-

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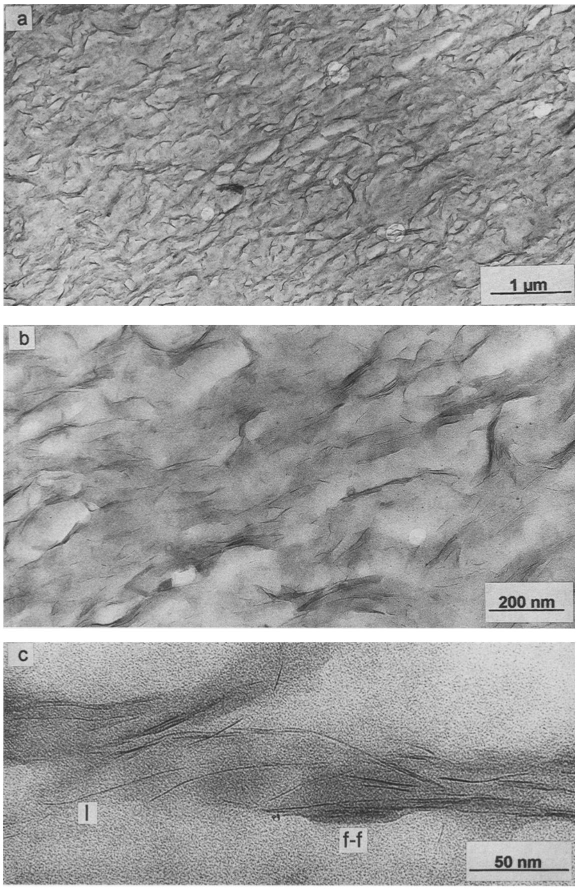

Figure 6. The textural organization of the dithionite-reduced (≈50% or 175 cmol Fe (II) kg^{-1}) ferruginous smectite SWa-1. (A) Low-resolution image showing the semi-parallel alignment of the particles. Magnification 21,000×. (B) Intermediate-resolution image showing the overall texture, composed of face–face alignment of the crystallite subunits and particles. Note the overall increase in both the lateral (*a-b*) and (*c-*) dimensions. Magnification 102,000×. (C) High-resolution lattice fringe image of a clay particle. Note that the association of individual layers (1) and crystallite subunits (c) forming particles is predominantly face–face (f–f). Magnification 510,000×.

ference may be the solids concentration used, which affected the total amount of structural Fe present within the closed system. In this study, 1–2% (wt%) suspensions of each smectite in MTM were used, whereas in prior studies (Stucki et al. 1987; Wu et al. 1988; Gates et al. 1993; Kostka et al. 1996) suspensions generally contained less smectite (0.2–0.5 wt%). An increase in solids concentration would increase the amount of total structural Fe available for reduction by bacteria, independent of the clay. However, this cannot be the sole explanation for the discrepancy, at least for the ferruginous smectite SWa-1, because Gates et al. (1993) showed that high levels of structural Fe(II) can also be obtained in suspensions containing low (0.2 wt%) solids concentration.

The increased variability in the estimate of structural Fe(II) in SWa-1 over that of the montmorillonites and the hectorite is not fully understood. Attempts to find correlations between structural Fe reduction in SWa-1 with individual and total bacterial populations *for the microorganisms used in this study* were unsuccessful (Gates et al. 1993), although other studies (Komadel et al. 1987; Stucki et al. 1987; Kostka et al. 1995) generally revealed that greater microbial populations resulted in increased levels of reduction.

Influence of Structural Fe(II) on Clay Swelling

The results for SWa-1 (Figure 2A) and the montmorillonites (Figure 2B) compare favorably with those reported by Stucki et al. (1984c), for dithionite-reduced smectites, and support the hypothesis that microbial reduction of structural Fe decreases the water content of all Fe-bearing smectites. Stucki et al. (1984c) reported that, for montmorillonites (Fe-diluted smectites), a linear relationship existed between the gravimetric water content and Fe(II) content up to about 40 cmol kg^{-1} Fe(II), but that a curvilinear relationship existed at low Fe(II) values for smectites in which greater than 80 cmol Fe(II) kg^{-1} could be achieved. Least-squares fitting of the data for SWa-1 (Figure 2A) resulted in logarithmic functions that were statistically significant ($p < 0.05$).

With increasing levels of structural Fe(II), we can expect that the layer charge of the clay should increase (Stucki et al. 1984b; Khaled and Stucki 1991). The data presented here (Figure 2, Table 1) are in general agreement with the study of Tessier and Pédro (1987) in terms of how layer charge influences hydration and the cation exchange capacity (CEC) of smectites. They found that the water content of clay gels equilibrated at 0.03 MPa decreased with increasing layer charge above 0.9 per unit cell for a series of 11 smectites, and attributed this response to interlayer collapse. In addition, both studies provided evidence that the CEC decreased and levels of cation fixation increased as the layer charge increased above 0.9 per unit cell (Tessier and Pédro 1987; Khaled and Stucki 1991), providing further evidence of interlayer collapse.

Influence of Organics on Swelling

Interestingly, the organic (NYG) medium influenced clay swelling differently than the inorganic (MTM) medium for the Fe-bearing smectites. Bacteria-free and biologically reduced–reoxidized SWa-1 samples in NYG retained more water following equilibration than corresponding samples in MTM (Figure 4A), suggesting that suspensions in the organic medium may have a more dispersed texture and greater microporosity. With increasing Fe(II) contents, the m_w/m_c values from the 2 systems converged near 50 cmol Fe(II) kg^{-1} clay, indicating that reduction of structural Fe suppressed the swelling of suspensions in NYG to a greater extent than suspensions in MTM (Figure 4A).

The results obtained for SWy-1 (Figure 4B) were similar, although the differences between the m_w/m_c values obtained at 0.3 MPa between the suspensions treated with the *Pseudomonas* combination in MTM or with MA in NYG were not as obvious. No differences were found for API #25 (Figure 4C). Two possibilities may explain the convergence of the swelling curves of the 2 systems: 1) an increased attraction between clay platelets with increasing levels of Fe(II) (Stucki et al. 1984b, 1984c; Tessier and Pédro 1987; Khaled and Stucki 1991; Stucki and Tessier 1991); or 2) repulsion between solution-borne organics and the clay surfaces with increasing reduction of structural Fe (Laird et al. 1992; Permien and Lagaly 1994). In either possibility, the net result would be a system with domains of greater clay–clay interaction. Gates et al. (1993) speculated that interlayer collapse due to increased attractive forces upon reduction of structural Fe overcame the opposing effect of increased pore size distribution caused by the presence of bacterial cells and/or lysis products within the clay matrix at intermediate Fe(II) contents. This hypothesis is supported by the convergence of the m_w/m_c values near 50 cmol kg^{-1} clay for SWa-1 incubated with MTM and NYG when equilibrated at 0.3 MPa (Figure 4A).

These results underline the importance of the initial swelling characteristics of the smectite, which are related to both compositional and layer charge factors. Nontronites inherently have lower swelling potential than montmorillonites due to greater total Fe content (Foster 1953; Stucki et al. 1984c; Stucki 1988). Hectorites, although having essentially no structural Fe, are characterized by nearly total octahedral layer charge (whereas nontronites, and to some extent montmorillonites, can have substantial tetrahedral layer charge), and thus higher swelling (Tessier and Pédro 1987). At 0.3 MPa applied pressure, the m_w/m_c values indicate that the swelling pressures for the 4 smectites in this study decreased in the order: montmorillonite API #25 > montmorillonite SWy-1 > smectite SWa-1 ≫ hectorite API #34 (Figures 3 and 4). It may be that any ameliorating effect on texture, such as the presence of organics, is more related to the initial charge characteristics of clays under the conditions of this study.

Texture of Biologically Reduced Ferruginous Smectite

The layer stacking, and subsequent organization of the crystallite subunits composing the particles of both the dithionite-reduced sample and the microbially reduced samples, as observed on representative thin sections, was more ordered than that of the untreated sample, in agreement with Stucki and Tessier (1991). The reorganization of the layers into more extensive crystallite subunits and particles observed here is associated with increased charge of individual layers due to reduction of structural Fe (Figures 5 and 6C). This observation is in agreement with the results of Tessier and Pédro (1987), Malla et al. (1993) and Hetzel et al. (1994), who found that smectite total layer charge profoundly influenced textural organization.

Because different levels of textural organization were affected by microbial reduction, the overall order of the layer and subunit stacking was inferior to that of the dithionite reduced SWa-1 (Stucki and Tessier 1991) as measured by selected area electron diffraction. Thus, the microbial-clay system, whether washed (Figure 8) or unwashed (Figure 7), had greater mesoporosity than the dithionite system (Figure 6), as is also revealed in the swelling behavior described above. We attribute this result as most likely due to the composition of the saturating cations and sorption of organic materials of microbial origin onto the external surfaces of the crystallite subunits or their intermixing within the clay matrix. The type of exchange cation present in the suspensions of smectite could have influenced layer stacking and textural arrangement of the gel (Ben Rhaïem et al. 1987), and we cannot unequivocally assume total Na exchange during washing of the microbial-clay system. The MTM medium contained Ca, Mg and K as well as Na, and interlayer collapse may have resulted in retention of some of these cations (Khaled and Stucki 1991), even after washing (Gates et al. 1993). Various polysaccharides and proteins are present in the NYG medium, and may also have inhibited complete Na exchange.

Washing removed all visible traces of bacterial cells (Figure 7). While we did not stain to observe any interlayer organics, washing apparently did not remove all organics sorbed to particle surfaces or entrapped by the particles. Past studies have shown that polysaccha-

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Figure 7. The textural organization of the ferruginous smectite SWa-1 following reduction (≈30% or 100 cmol Fe(II) kg^{-1}) by the mixture of anaerobes (MA) in the organic medium (NYG). The sample was not washed prior to gel preparation. (A) Low-resolution image showing individual bacterial cells (bc) and cell remnants dispersed within a dense and organized clay matrix. Magnification = 21,000×. (B) Intermediate-resolution image showing the overall texture of the biologically reduced, unwashed clay gel fabric. Note the increased lateral (*a-b* dimension) extension of the particles and the presence of pores (ps) within the particles, indicative of disruption of face–face associations by organics. Magnification = 102,000×. (C) High-resolution lattice fringe image of a biologically reduced, unwashed particle. Note the predominance of overlapping face–face (of–f) associations of crystallite subunits (c) into elongated particles.

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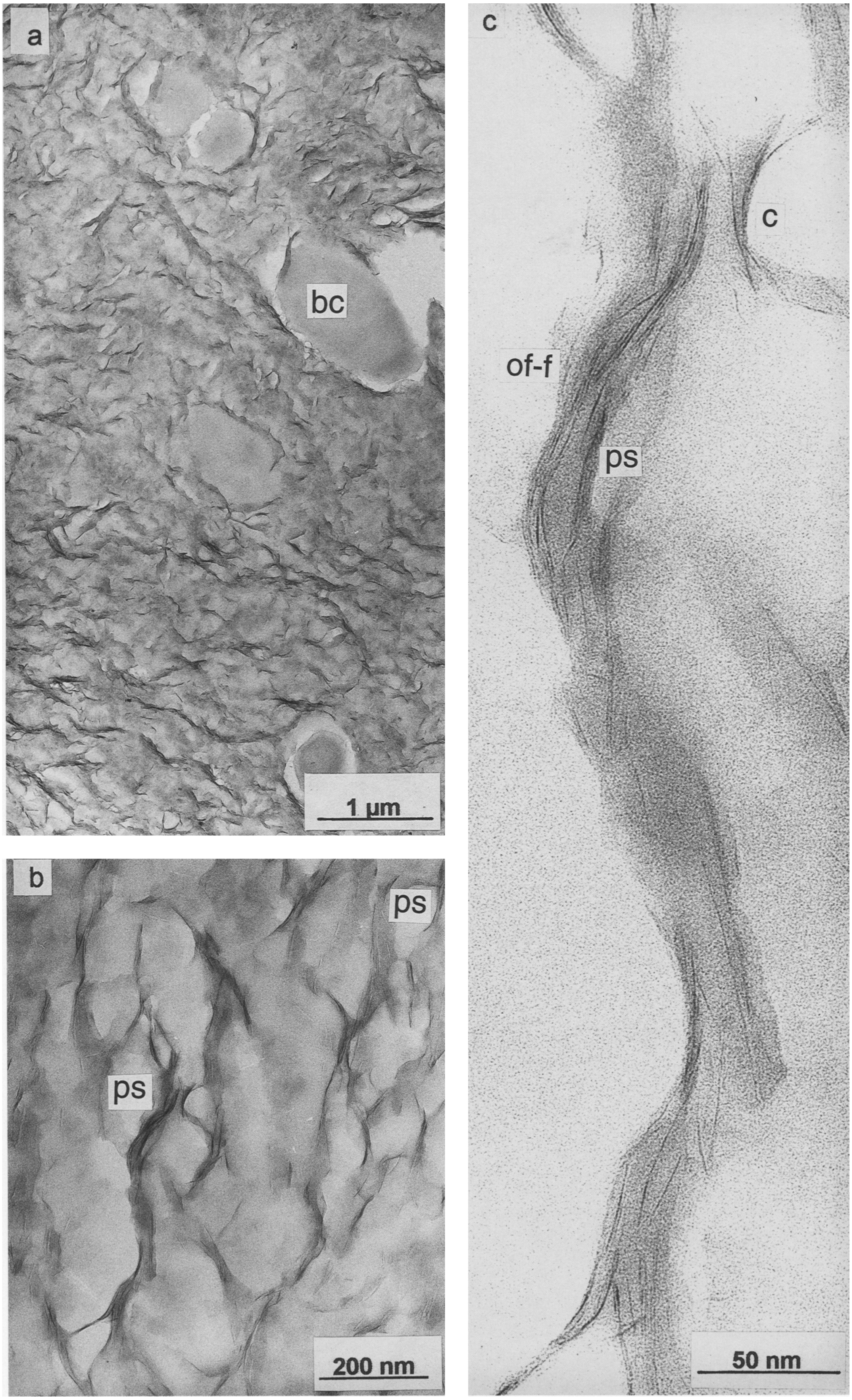

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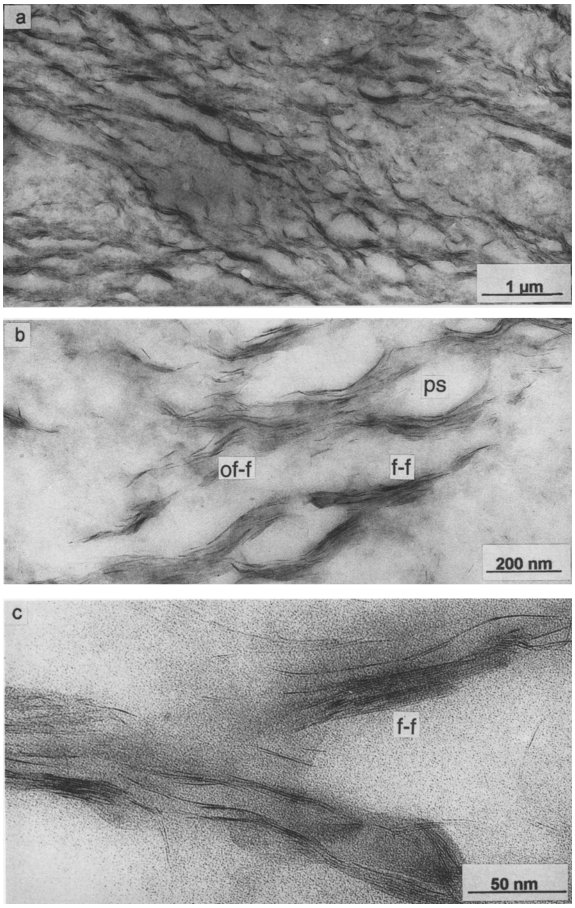

Figure 8. The textural organization of the ferruginous smectite SWa-1 following reduction (≈30% or 100 cmol Fe(II) kg^{-1}) by the combination of *Pseudomonas* bacteria in inorganic medium (MTM). The sample was washed prior to gel preparation. (A) Low-resolution image showing a highly organized clay matrix. Note the absence of bacterial cells and cell remnants. Magnification = 21,000×. (B) Intermediate-resolution image showing the overall texture of the biologically reduced, washed clay gel fabric. Note the increased lateral (*a-b* dimension) extension of the particles due to overlapping face–face (of–f) associations of crystallite subunits, the increased thickness (*c*-dimension) of crystallite subunits due to face–face (f–f) associations of individual layers, and the presence of pores (ps) within the particles, indicative of disruption of face–face associations by organics, but countered by interlayer collapse due to structural Fe reduction and washing. Magnification = 102,000×. (C) High-resolution lattice fringe image of a biologically reduced, washed particle. Note the predominance of face–face (f–f) interactions increasing the crystallite subunit (c) thickness. Overlapping associations of crystallite subunits result in lateral *a-b* extension of particles. Washing did not completely remove evidence of organic-clay interactions, as indicated by the characteristic disruption of the face–face associations by pores.

ride (Chenu 1989; Chenu and Jaunet 1990) and organocation (Malla et al. 1993) sorption onto smectites resulted in similar disruption of the overall gel organization as observed here. In these previous studies, however, *diminished* extent of lateral (*a-b*) and stacking (*c*-) dimensions resulted from the increased disorder. To our knowledge, the present study is the first to show significant *increases* in association at the layer, crystallite subunit and particle level of organization, resulting in increased particle size and pore size distribution following microbial reduction of structural Fe.

Clearly, biological Fe reduction was important in determining the overall texture of the gel and caused an increased interaction between clay particles, most notably at the crystallite *subunit* level, whereas in the dithionite–smectite system, Fe reduction influenced the interaction of individual *layers* into larger crystallite subunits (Stucki and Tessier 1991). One would expect that this textural difference would affect differentially other important bulk properties, such as hydrology (Shen et al. 1992) and rheology (Permien and Lagaly 1994; Stucki et al. 1996) of clays as well.

CONCLUSIONS

Reduction of structural Fe by *Pseudomonas* bacteria in inorganic medium and anaerobic bacteria in organic medium decreased the gravimetric water content of 3 Fe-bearing smectites. As noted in previous work (Gates et al. 1993), the depression of clay swelling by microbial reduction of structural Fe was greater than in chemically reduced smectites in an intermediate range of Fe(II) contents. The hypothesis that this behavior may be due to an interaction of bacterial cells and/or lysis products with the clay surfaces, thereby altering the water retention characteristics of microbially reduced smectites relative to chemically reduced smectites, was confirmed by both gravimetric analysis and TEM.

Smectite gels reduced by bacteria in an organic medium exhibited increased swelling at very low Fe(II) contents in Fe-rich smectite, but the effect of organic interactions diminished rapidly with increasing structural Fe reduction, presumably because the increased presence of Fe(II) resulted in interlayer collapse. Structural Fe(II) probably affects the textural arrangement of the clay matrix by increasing organo-clay repulsion and, concomitantly, increasing clay–clay attraction, thereby allowing for increased interaction between associated clay surfaces.

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