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SWELLING PROPERTIES OF MICROBIALLY REDUCED FERRUGINOUS SMECTITE

W. P. GATES, H. T. WILKINSON, AND J. W. STUCKI[1]

Department of Agronomy, University of Illinois, Urbana, Illinois 61801

Abstract—Structural Fe in ferruginous smectite (sample SWa-1, Source Clays Repository of the Clay Minerals Society) was reduced by a mixture of five *Pseudomonas* species of bacteria in a defined Fe-free medium to determine the effect of microbial reduction on clay swelling. Iron(II), total Fe, and gravimetric water content (m_w/m_c) were determined in clay gels equilibrated at applied pressures of 0.1, 0.3, and 0.5 MPa. The water content of microbially reduced SWa-1 decreased at all three applied pressures as the Fe(II) content approached about 0.8 mmol Fe(II)/g-clay. As Fe(II) increased from 0.8 mmol/g-clay, however, further change in m_w/m_c was negligible. Concurrent with microbial reduction of structural Fe was a significant decrease in the swelling pressure (PI) of SWa-1: for example, when m_w/m_c = 1.2 (g/g), PI changed from 0.47 MPa at Fe(II) = 0.2, to 0.19 MPa at Fe(II) = 0.9 mmol/g-clay. Both biologically and chemically reduced smectites displayed lower values of m_w/m_c and a concurrent decrease in II as Fe(II) content increased, but the effect of Fe(II) on m_w/m_c was greater for the microbially reduced smectites at all applied pressures.

Key Words—Dithionite, Microorganism, *Pseudomonas*.

INTRODUCTION

The ability of smectites to retain interlayer and interparticle water profoundly impacts many hydrologic and mechanical properties of soils and sediments. Clay swelling controls permeability and drainage of water through and in soils, and influences the suitability of soils and sediments for agricultural and engineering purposes. The pressure applied by swelling clays can injure crops (Lamb and Grady, 1963; Portz, 1967), and damage engineered structures such as buildings and highways (Madsen and Müller-Vonmoos, 1985). *In situ* modification of the swelling characteristics of smectites may, therefore, have important implications for agricultural and engineering practices.

Chemical reduction of octahedral Fe decreases the swelling of Na-smectite (Foster, 1953; Egashira and Ohtsubo, 1983; Stucki *et al.*, 1984b; Lear and Stucki, 1989) and affects other physico-chemical properties of smectites, such as surface charge density (Stucki, 1988), specific surface area (Stucki and Lear, 1989), cation fixation (Khaled and Stucki, 1991), hydraulic conductivity (Shen *et al.*, 1993), and structural order (Stucki and Tessier, 1991). Until recently, reduction of structural Fe has been achieved with inorganic reducing agents (Stucki, 1988), but indigenous soil microorganisms also have the ability to reduce structural Fe (Komadel *et al.*, 1987; Stucki *et al.*, 1987; Wu *et al.*, 1988). The similar magnitude of structural Fe reduction achieved by either microorganisms or inorganic chemicals (Wu *et al.*, 1988) infers that changes in physico-chemical properties of smectites may be similar, regardless of the agent causing reduction. Indeed, Wu *et al.* (1988) found that the optical reflectance spectra of microbially reduced dioctahedral smectite were comparable to dithionite-reduced smectite.

Inorganic reducing agents such as dithionite are unlikely to occur in sufficient quantities in soil environments to significantly alter Fe oxidation state *in situ*, and their addition to soils or sediments would be neither economically practical nor environmentally prudent. Indigenous soil microorganisms, however, may prove to be useful in controlling the oxidation state of structural Fe in soils and sediments. The purpose of the present study was to investigate the effect of microbial reduction on the swelling pressure of ferruginous smectite.

MATERIALS AND METHODS

The 0.5 to 2 μm size fraction of the ferruginous smectite SWa-1 (Source Clays Repository of the Clay Minerals Society) from Grant County, Washington, was used. Elemental composition of SWa-1 was reported by Goodman *et al.* (1976). The clay was Na^+-saturated, fractionated, dialyzed, and freeze-dried prior to use (Stucki *et al.*, 1984a). The Fe content of SWa-1 (total Fe = 3.549 mmol Fe/g-SWa-1) was reported previously by Lear and Stucki (1989).

Bacteria used were collected from wheat rhizosphere soils by D. Weller and L. Thomashow, USDA-ARS, Pullman, WA (Weller and Cook, 1983). They were: *Pseudomonas fluorescens* isolates WB919, WB9110, and WB9111; *P. aureofaciens* isolate WB9112; and *P. putida* isolate WB9113. Previous studies indicated these

[1] Graduate Research Assistant, Associate Professor of Soil Ecology, and Professor of Soil Physical Chemistry, respectively, Department of Agronomy, University of Illinois, 1102 South Goodwin Avenue, Urbana, IL 61801 USA.

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bacteria had Fe reducing activity (Komadel *et al.*, 1987). Single-cell colonies from initial cultures were aseptically transferred to King's B (KB) medium (King *et al.*, 1954) and incubated at room temperature for 48 hr. These actively growing cultures were then used to seed 250 ml of freshly prepared KB liquid medium and incubated at room temperature until slightly turbid. Aliquots (1 ml) were transferred to sterile, 1.5 ml cryovials with 1 drop of reagent-grade dimethyl sulfoxide (DMSO) and stored at −80°C. Prior to each use, isolates were thawed and transferred to 250 ml of freshly-prepared, modified Thornton's [Thornton (1922): 1.0 g K_2HPO_4; 0.5 g KNO_3; 0.2 g $MgSO_4 \cdot 7H_2O$; 0.1 g $CaCl_2$; 0.1 g NaCl; 0.002 g $FeCl_3$; 0.5 g asparagine; 1.0 g manitol per liter] liquid medium [MT—modified by eliminating $FeCl_3$ and using purified (18 Mohm-cm resistivity) water] and incubated for 24 hr. Each experiment used 24-hr cultures that had been transferred no more than two times. Colony forming units (CFU) were determined by diluting and plating on KB medium as a check for final recovery of *Pseudomonas* strains.

To identify *Pseudomonas* strains that reduce structural Fe, 30 mg SWa-1 was added to a pre-weighed 50 ml reaction vessel consisting of a polycarbonate centrifuge tube fitted with a polypropylene cap. The samples were sterilized (40 min at 121°C and 0.124 MPa) twice; each sterilization treatment was separated by 24 hr at room temperature. Twenty-five ml of fresh MT and 1 ml of 24-hr culture were transferred to each reaction vessel. The reaction vessel was tightly capped and placed on a variable speed platform shaker (±1.7 cm/s) and incubated at room temperature for 2 weeks. Six to 12 such samples were prepared for each 24 hr culture. Bacteria from the reduced samples were isolated, cultured on KB, and prepared for −80°C storage as described above. Clay samples were analyzed for Fe(II) and total Fe by the method of Komadel and Stucki (1988) with the following changes. The samples were centrifuged (12,000 g) for 15 min, decanted, and the acid digestion solution was added immediately to the sample in the reaction vessel. Iron(II) content as mmol Fe(II)/g-clay and as Fe(II)/total Fe ratio was calculated.

The five *Pseudomonas* isolates previously incubated with SWa-1 were combined to produce an inoculum suspension in fresh MT. Comparable bacterial concentrations (CFU $\approx 2 \times 10^7$/ml) of each isolate were combined with 800 mg SWa-1 and 600 ml MT in a 1 liter flask to produce a combined primary inoculum suspension (PIS). The flask was covered with a rubber stopper and incubated at room temperature for up to 60 days. During incubation, the flask was monitored for blue-green color indicative of Fe reduction (Stucki, 1988). Structural Fe(II) levels were not determined during incubation of the PIS, although samples were assayed from this flask to estimate the microbial population. Secondary inoculum suspensions (SIS) for additional experimentation were prepared using sterile reaction vessels containing 80 ml of sterile SWa-1, 25 ml MT, and 1 ml PIS inoculum. After 60 days, clay samples from both PIS and SIS were assayed for swelling pressure and Fe(II) content.

To prepare samples for swelling pressure determination, approximately 30-ml of reduced clay suspension (≈80 mg SWa-1) was transferred to each of 16 sterile reaction vessels and sealed with an aluminum cap and septum (Stucki *et al.*, 1984a). The samples were washed free of excess salts as described by Stucki *et al.* (1984a) and the final wash solution was retained for Na analysis by atomic emission spectroscopy.

At this time, some of the samples were re-oxidized by bubbling O_2 gas through the suspensions to provide as wide a range of Fe(II)/total Fe as possible. The contents of each reaction vessel were divided and transferred using an air tight syringe to porous-plate cells on a swelling pressure apparatus (Stucki *et al.*, 1984b). Primary inoculum suspension samples were transferred to two cells and SIS samples to three cells on the pressure apparatus. Following equilibration at 0.1, 0.3, and 0.5 MPa applied pressure, m_w/m_c was determined gravimetrically on half of each PIS clay cake and on two of the three SIS clay cakes. The remaining clay cake (or portion) was transferred into a tube containing acid digestion solution for Fe(II) analysis.

RESULTS AND DISCUSSION

Levels of structural Fe reduction by each of the five *Pseudomonas* strains and both PIS and SIS treatments of the combined inoculum suspensions are shown in Table 1. The average levels of Fe reduction by the combined inoculum suspensions (both PIS and SIS) were greater than Fe(II) contents produced by any individual *Pseudomonas* strain. In the absence of bacteria, no structural Fe reduction was observed (data not shown). Color changes occurred during incubation of all treatments and were indicative of the Fe(II) contents measured (Stucki, 1988). For example, clay suspensions reduced by single *Pseudomonas* isolates turned from yellow to green, whereas clay suspensions reduced by combined inoculum suspensions, PIS and SIS, turned blue-green to dark-blue and dark green to blue-green, respectively, indicating an increase in Fe(II). Upon reoxidation, all samples returned to a yellow color typical of the oxidized clay.

Values of m_w/m_c for the microbially reduced clay decreased with increasing Fe(II) content at all applied pressures (Figure 1). A decrease in m_w/m_c was apparent up to Fe(II) contents of 0.8 mmol/g-clay (about 22% total Fe); beyond 0.8 mmol Fe(II)/g-clay, the change in m_w/m_c with Fe(II) content was less evident (see Figure 1a).

To show the effects of microbial reduction on the swelling pressure, PI, m_w/m_c values at Fe(II) contents

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Table 1. Levels of microbial reduction of structural Fe in SWa-1 and the final population levels for individual *Pseudomonas* species and both the primary inoculation suspension (PIS) and secondary inoculation suspension (SIS) of the combined inoculum suspensions.

Micro-organism	Fe(II) (/total Fe)	Fe(II) (mmol/g)*	Populations (CFU/mL)†
WB919	0.162	0.575	2.2×10^7
WB9110	0.044	0.156	n.d.
WB9111	0.038	0.135	n.d.
WB9112	0.027	0.096	n.d.
WB9113	0.206	0.731	2.1×10^7
Combined culture‡			
PIS	0.350	1.242	2.4×10^7
SIS	0.295	1.047	2.0×10^7

* Total Fe = 3.549 mmol g^{-1}-clay (Lear and Stucki, 1989).
† n.d., not determined.
‡ Average Fe(II) content from non-reoxidized samples.

of 0.2, 0.4, and 0.9 mmol/g-clay were determined directly from Figure 1 and plotted with PI (Figure 2). The swelling pressure of the clay decreased with increasing microbial reduction of structural Fe. For example, at m_w/m_c = 1.2 g/g, increasing Fe(II) from 0.2 to 0.4 and 0.9 mmol/g-clay caused II to decrease from 0.47 to 0.32 and 0.19 MPa, respectively.

The relationships of m_w/m_c and PI to Fe(II) content of microbially reduced SWa-1 are consistent with results of Stucki *et al.* (1984b) and Lear and Stucki (1989) for chemically reduced dioctahedral smectites, and they support the hypothesis that microbial and chemical reduction invoke similar changes in the physico-chemical properties of smectites. Comparison of these results with those of Lear and Stucki (1989) for dithionite-reduced SWa-1 (Figure 1) revealed that both methods decreased m_w/m_c, but the magnitude was greater for the microbially reduced smectite.

Differences in the relationship between m_w/m_c and Fe(II) of the microbially reduced SWa-1 as compared to dithionite-reduced SWa-1 may be due to: 1) particle size differences of the samples used; 2) the addition of growth medium; and 3) the presence of microbial cells and/or lysis products. In the present study, the 0.5 to 2 μm fraction was used, whereas in the chemical studies the entire <2-μm particle-size fraction was used. However, the similar trends between the two systems may indicate that the presence of Fe(II) affects the entire <2-μm particle-size distribution uniformly. The medium used contained K, Ca, and Mg. Significant amounts of these cations may have been retained on the exchange complex following reduction (Khaled and Stucki, 1991), creating a more collapsed gel structure (Stucki and Tessier, 1991) and lowering PI.

In an attempt to measure the effects of bacterial cells and/or lysis products on clay swelling, inoculated and non-inoculated suspensions of Na-hectorite (API #34: total Fe = 0.006 mmol Fe/g) were studied using the same methods as for SWa-1. Values for m_w/m_c were greater in the inoculated than in the non-inoculated samples at all applied pressures (data not shown), indicating that microbial cells or lysis products tended to increase swelling when no Fe reduction occurred.

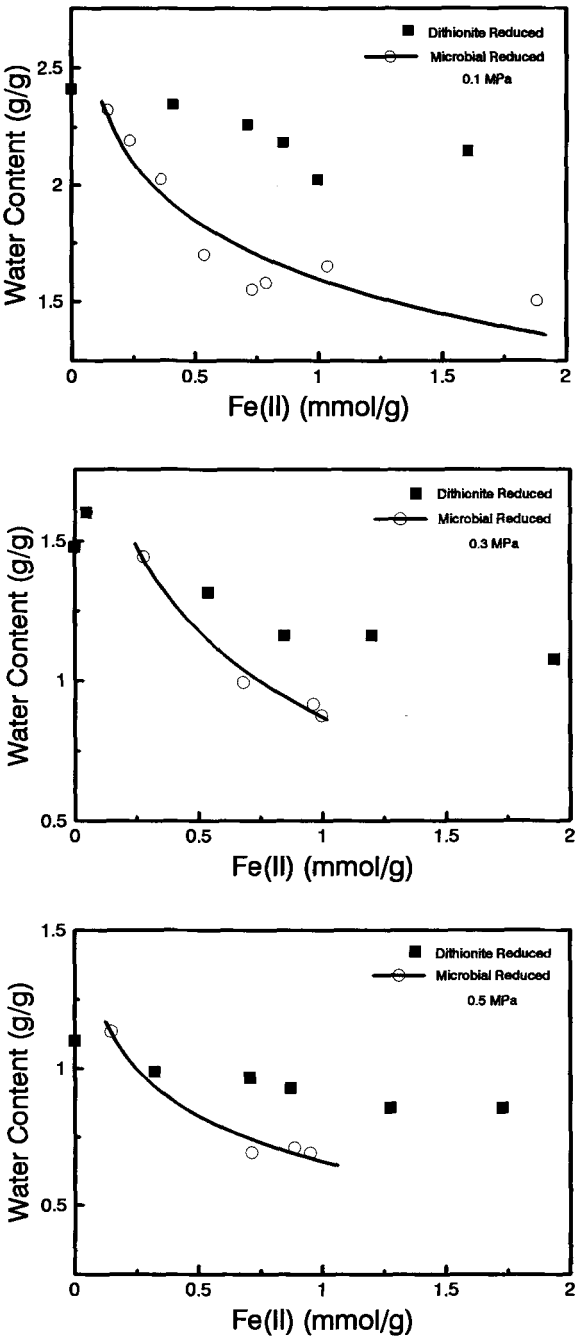


Figure 1. Effect of structural Fe(II) on the water content (m_w/m_c) of SWa-1 reduced by combined *Pseudomonas* inoculum suspensions (PIS and SIS) and dithionite at a) 0.1 MPa, b) 0.3 MPa, and c) 0.5 MPa applied pressure. Solid lines represent best fits; data for dithionite from Lear and Stucki, 1989.

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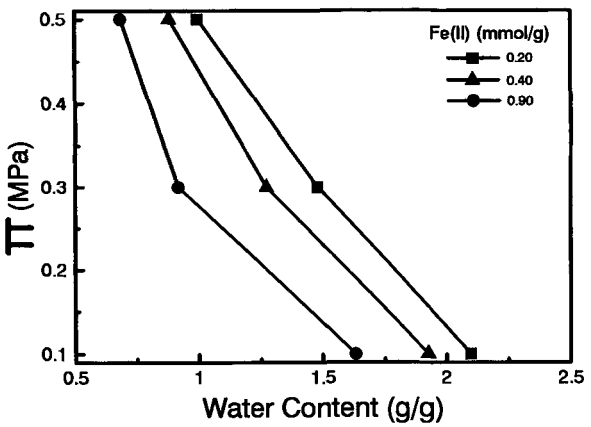


Figure 2. The relationship between the swelling pressure, PI, and the gravimetric water content, m_w/m_c, of microbially reduced SWa-1 at Fe(II) contents of 0.2, 0.4 and 0.9 mmol/g-clay.

Chenu (1989) showed that fungal polysaccharides (sclero-glucan) increased the water content and void ratios of Ca-montmorillonite, especially at lower applied pressures. In addition, Chenu and Jaunet (1990) reported that scleroglucan inhibits inter-layer collapse of Ca-montmorillonite. Their results indicate that, in the present study, bacterial cells and/or lysis products dispersed with clay may have interfered with the formation of a compact gel structure. But such interference, if present, evidently was overcome by the interlayer attractive forces imposed by Fe(II), even at low Fe(II) contents (<0.8 mmol Fe(II)/g-clay).

SUMMARY AND CONCLUSIONS

Reduction of structural Fe by mixed *Pseudomonas* cultures decreased the swelling pressure of ferruginous smectite SWa-1. The magnitude of the decrease in swelling, as revealed by a decrease in the water content (m_w/m_c) of the clay equilibrated at various applied pressures, was greatest at levels of Fe(II) < 0.8 mmol/g-clay. At levels of Fe(II) > 0.8 mmol/g-clay, the changes in swelling were negligible at 0.1 MPa applied pressure. The depression of m_w/m_c by Fe(II) was greater than in chemically-reduced smectites even though the trends were similar. This may be explained by the interaction of bacterial cells and lysis products with the clay surfaces, thereby altering the water retention characteristics of the clay relative to chemically reduced smectites.

Combined cultures of *Pseudomonas* species reduced greater amounts of structural Fe than individual *Pseudomonas* strains despite similar final population levels. More work is necessary to substantiate microbial population trends within the combined cultures and their relation to reduction of structural Fe.

This study shows that reduction of structural Fe in smectites by soil microorganisms can dramatically affect clay-water relationships. Microbial reduction is likely an important soil process and should be considered in studies of the effects of reduction on the physico-chemical properties of soils and sediments.

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