

## ROLE OF MICROBIAL Fe(III) REDUCTION AND SOLUTION CHEMISTRY IN AGGREGATION AND SETTLING OF SUSPENDED PARTICLES IN THE MISSISSIPPI RIVER DELTA PLAIN, LOUISIANA, USA

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**Abstract**—River-dominated delta areas are primary sites of active biogeochemical cycling, with productivity enhanced by terrestrial inputs of nutrients. Particle aggregation in these areas primarily controls the deposition of suspended particles, yet factors that control particle aggregation and resulting sedimentation in these environments are poorly understood. This study was designed to investigate the role of microbial Fe(III) reduction and solution chemistry in aggregation of suspended particles in the Mississippi Delta. Three representative sites along the salinity gradient were selected and sediments were collected from the sediment-water interface. Based on quantitative mineralogical analyses 88–89 wt.% of all minerals in the sediments are clays, mainly smectite and illite. Consumption of  $\text{SO}_4^{2-}$  and the formation of  $\text{H}_2\text{S}$  and pyrite during microbial Fe(III) reduction of the non-sterile sediments by *Shewanella putrefaciens* CN32 in artificial pore water (APW) media suggest simultaneous sulfate and Fe(III) reduction activity. The  $\text{pH}_{\text{PZNPC}}$  of the sediments was  $\leq 3.5$  and their zeta potentials at the sediment-water interface pH (6.9–7.3) varied from  $-35$  to  $-45$  mV, suggesting that both edges and faces of clay particles have negative surface charge. Therefore, high concentrations of cations in pore water are expected to be a predominant factor in particle aggregation consistent with the Derjaguin-Landau-Verwey-Overbeek (DLVO) theory. Experiments on aggregation of different types of sediments in the same APW composition revealed that the sediment with low zeta potential had a high rate of aggregation. Similarly, addition of external Fe(II) (*i.e.* not derived from sediments) was normally found to enhance particle aggregation and deposition in all sediments, probably resulting from a decrease in surface potential of particles due to specific Fe(II) sorption. Scanning and transmission electron microscopy (SEM, TEM) images showed predominant face-to-face clay aggregation in native sediments and composite mixtures of biopolymer, bacteria, and clay minerals in the bioreduced sediments. However, a clear need remains for additional information on the conditions, if any, that favor the development of anoxia in deep- and bottom-water bodies supporting Fe(III) reduction and resulting in particle aggregation and sedimentation.

**Key Words**—Aggregation, Fe(III) and sulfate reduction, Mississippi Delta, SEM, *Shewanella putrefaciens* CN32, TEM, XRD.

### INTRODUCTION

Aggregation of suspended particles exerts an important influence on contaminant sorption/desorption (Pignatello and Xing, 1996; Dachs and Bayona, 1997), particle movement and sedimentation (O'Melia, 1980; Forsgren *et al.*, 1996), and sediment structure and stability (Theilen and Pecher, 1991). Particular attention in the study of particle aggregation has been directed towards understanding particle-settling properties such as floc architecture, floc size, and settling velocity in various sedimentary environments and to analyzing mechanisms of aggregation in theoretical and laboratory investigations (Mayer, 1982; Orton and Kineke, 2001; Hill *et al.*, 2001; Brassard and Fish, 2000; Tombacz *et al.*, 2001). In general, particle concentration and charge,

solution chemistry, and type and conformational nature of organic polymers are considered major factors in controlling particle aggregation (Theng, 1979; Chenu *et al.*, 1985; Manfredini *et al.*, 1990; Elimelech *et al.*, 1995; Gates *et al.*, 1998; Kim *et al.*, 2005; Jaisi *et al.*, 2007a). In coastal and estuarine environments, aggregation of river-borne colloids is primarily controlled by seawater cations (Mayer, 1982; Stumm and Morgan, 1996; Forsgren *et al.*, 1996). However, pH, concentration and properties of suspended particles, organic matter, and microorganisms also play important roles in the aggregation process (Stumm and Morgan, 1996; Mayer, 1982; Forsgren *et al.*, 1996).

Clay particles are different from other colloids because of their irregular shape, broad range of particle sizes, and heterogeneity of charge (Lagaly and Zeisler, 2003). Clay minerals impart important chemical properties, in part, by virtue of changes in the redox state of iron in their crystal structures (Anastacio *et al.*, 2005). Therefore, changes in the oxidation state of the

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structural iron in clay minerals associated with chemical and biological Fe(III) reduction (Gates *et al.*, 1993; Kostka *et al.*, 1996; Dong *et al.*, 2003; Kim *et al.*, 2004) have been found to be relevant for several environmental applications (Stucki, 2006; Jaisi *et al.*, 2008). However, few recent studies have focused on how Fe(III) reduction in clay minerals affects the aggregation of clay colloids (Gates *et al.*, 1998; Kim *et al.*, 2005; Jaisi *et al.*, 2007a). Results from these studies, performed on well characterized pure clay minerals, indicate that the biogeochemical changes associated with microbial Fe(III) reduction are dominant factors contributing to clay aggregation. Little attention, however, has been given to extending these studies to deep- and bottom-water bodies of natural estuarine and sea-water environments. One of the main limitations to such extended investigations is that estuaries and marine environments are largely oxic and therefore do not favor Fe(III) reduction. However, development of hypoxia is common in the Mississippi River Delta (Rabalais *et al.*, 1994; Rowe, 2001; Dagg and Breed, 2003; Turner *et al.*, 2007). For example, dissolved O<sub>2</sub> concentration decreases sharply below the surface and reaches <0.5 mg/L at 15 m water depth in summer (Rowe, 2001). Increasing input of N and P to the delta stimulates phytoplankton and supports bacterial growth (Turner *et al.*, 2007) thereby increasing the rate of oxygen consumption in deep and bottom waters (Dagg and Breed, 2003). The sediment-water interface is fully anoxic, supporting the growth of sulfate reducers and the formation of hydrogen sulfides (Hover *et al.*, 2002; Rowe *et al.*, 2002). Therefore, if existing hypoxia in deep- and bottom-water bodies changes to anoxia, it may favor conditions suitable for Fe(III) reduction. Under this circumstance, the Fe(III) reduction process in Fe-containing particles and resulting Fe(II) may contribute significantly to particle aggregation and sedimentation.

The objective of this research was, therefore, to determine how Fe(III) bioreduction and pore-water solution chemistry affect aggregation and settling of naturally suspended sediments. To understand these effects, aggregation experiments were performed on the <2.0 μm size fraction of Mississippi sediments collected from three locations along a salinity gradient. The effects of Fe(II), solution chemistry (cation/anion composition), and surface charge of sediments on particle aggregation were a particular focus of this investigation. The results of this study have important implications for understanding particle aggregation and settling in natural environments where redox condition favor Fe(III) reduction.

## MATERIALS AND METHODS

### *Sediment collection and analysis*

*Field sampling and sediment retrieval.* The sediments, originally collected from the Terrebonne Marsh area,

Mississippi Delta plain, were kindly provided by Dr Victoria C. Hover, University of Louisiana, Lafayette, Louisiana. Two sediments (S1 and S2) were collected from the Atchafalaya River estuary (Hover *et al.*, 2002). The S1 sediment (salinity of ~12‰) was collected from a small unnamed lake at the most seaward site while S2 (salinity of ~0‰) was collected from the freshwater portion of the estuary. The S3 sediment was collected from Moss Bay south of Cocodrie and had a salinity of ~3‰ (see figure 1 in Hover *et al.*, 2002).

*Sample processing and analyses.* The pore-water solution was previously extracted from the sediments (Hover *et al.*, 2002). The sediments retrieved were sonicated in an ultrasonic water bath for 10 min and stirred slightly to disperse loosely attached aggregates. The resulting suspension was centrifuged to obtain size fractions of <2.0 μm. All experiments were performed using this size fraction. X-ray diffraction (XRD) was performed to identify mineral phases in both native and bioreduced sediments. Quantitative mineralogical analysis was performed using the *RockJock* program (Środoń *et al.*, 2001; Eberl, 2003) (Table 1) which calculates the proportion of a mineral in a complex mixture by comparing the integrated intensity of a characteristic diffraction peak of that mineral to the intensity of the same peak of an internal standard (Środoń *et al.*, 2001; Eberl, 2003).

The total Fe content in sediments of the <2.0 μm size fraction was determined by a standard addition method (Harris, 1998) after fusing the sediments at 950°C. Because the sediments were rich in organic matter,

Table 1. Quantitative mineralogical analysis (wt.% of total minerals) as determined by XRD and the *RockJock* program (Środoń *et al.*, 2001; Eberl, 2003).

Sample ID Minerals	S1	S2	S3
Non-clay minerals			
Quartz	2.8	4.6	4.3
K-feldspar	2.2	2.2	3.6
Plagioclase	2.2	1.8	1.0
Calcite	0.2	0.0	0.0
Dolomite	0.6	0.3	0.4
Amphibole	1.3	0.5	0.6
Maghemite	1.4	2.7	1.8
Total non-clays	10.7	12.0	11.7
Clays minerals			
Kaolinite	8.5	4.3	8.8
Smectite	31.3	32.1	31.3
Illite	33.3	34.3	31.9
Muscovite (2M <sub>1</sub> )	4.3	5.0	6.0
Chlorite	11.9	12.3	10.3
Total clays	89.3	88.0	88.3
Total	100.0	100.0	100.0

measurement of Fe(II) by the HF dissolution method (Andrade *et al.*, 2002) was inefficient. Therefore, a dilute sediment suspension (0.5g/L) in 12 N HCl was shaken vigorously for a week to extract Fe(II). However, the extraction by 0.5 N HCl and 12 N HCl did not result in significant difference (1.5%) in Fe(II). The extracted Fe(II) was measured by the Ferrozine method (Stookey, 1970).

Pore-water and sediment geochemistry were analyzed at the Service Testing and Research Laboratory (STAR) at Ohio State University. Pore water was analyzed directly for major cations and anions using inductively coupled plasma mass spectrometry (ICP-MS) and high performance liquid chromatography (HPLC), respectively. Organic matter content was determined from loss-on-ignition (LOI).

#### Bioreduction experiments

*Cell preparation.* *Shewanella putrefaciens* CN32 is a facultative anaerobic bacterium originally isolated from a subsurface core sample (Fredrickson *et al.*, 1998). It has been known to exist in different natural environments and has diverse metabolic and physiological

capabilities (Lovley *et al.*, 2004). It was routinely cultured aerobically in tryptic soy broth (TSB) (30 g/L) from a stock culture, which was kept in 40% glycerol at  $-80^{\circ}\text{C}$ . After harvesting in TSB until mid- to late-log phase, CN32 cells were washed three times in deionized water. After the final wash, cells were resuspended in three artificial pore water (APW) media prepared by mimicking the original pore-water composition corresponding to the S1, S2, and S3 sediments (Table 2). The initial cell density in all cultures was measured by the viable cell count method.

*Bacterial Fe(III)-reduction experiments.* Fe(III)-bioreduction experiments were performed in three different APW media. The experimental setup consisted of Fe(III) in sediments (3.0 g of sediment/L) and  $\text{SO}_4^{2-}$  in the solution as electron acceptors, lactate (10 mM) as the sole electron donor, and CN32 cells ( $\sim 1.2 \times 10^7/\text{mL}$ ) as a mediator both in the presence and absence of anthraquinone-2,6-disulfonate (AQDS) (0.1 mM) as an electron shuttle (Jaisi *et al.*, 2005). Experiments were performed in duplicate both with and without autoclaving the sediment stocks and APW media to test the activity of indigenous

Table 2. Pore water and sediment geochemistry.

#### (a) Cation concentration (mg/L) in the sediment pore water.

ID	P	K	Ca	Mg	S	Al	B	Cu	Fe	Mn	Mo	Na	Zn
S1	0.03	32.05	49.01	72.38	194.91	0.58	0.76	0.00	0.05	1.75	0.01	298.30	0.08
S2	<0.01	3.44	43.51	17.13	55.24	<0.01	0.12	<0.01	0.01	<0.01	<0.01	9.17	<0.01
S3	0.01	17.20	23.74	31.73	94.98	<0.01	0.18	<0.01	0.01	<0.01	<0.01	116.83	<0.01
	Ba	Be	Cd	Co	Cr	Li	Ni	Pb	Sb	Se	Si	Sr	V
S1	0.05	<0.01	<0.01	0.04	0.01	0.02	0.05	0.01	0.02	<0.01	7.01	0.57	0.01
S2	0.07	<0.01	<0.01	<0.01	<0.01	0.06	0.01	<0.01	0.01	<0.01	5.93	0.18	<0.01
S3	0.04	<0.01	<0.01	<0.01	<0.01	0.02	0.01	<0.01	0.01	<0.01	3.98	0.19	0.01

#### (b) Major anion composition in the sediment pore water (mg/L, except where otherwise stated).

ID	Alkalinity (mg/L)	Acidity (meq/L)	F	Cl	Br	NO <sub>3</sub> -N	PO <sub>4</sub> -P	SO <sub>4</sub> -S
S1	<0.01	0.96	0.76	405.87	1.92	0.24	0.40	203.58
S2	28.67	0.14	0.59	2.43	<0.01	<0.01	<0.01	44.16
S3	27.89	0.19	0.37	85.16	<0.01	<0.01	<0.01	84.36

#### (c) Fe and C contents of the sediments (sample $n = 3$ ).

ID	Total Fe in sediments (%)	Total Fe(III) (as Fe%)	Carbon (%)	Inorganic carbon (%)	Conductivity (mmhos/cm)
S1	3.29	94.04	7.64	BD	3.30
S2	3.98	91.34	3.78	0.03	1.02
S3	4.11	91.09	2.75	0.01	0.95

bacteria such as sulfate reducers. After mixing these components in Belco glass tubes, the tubes were purged with a N<sub>2</sub>:CO<sub>2</sub> gas mixture (80:20), sealed with thick, butyl rubber stoppers, then incubated at 30°C with shaking at 60 rpm. The bioreduction experiments were typically run until the extent of Fe(III) reduction had leveled off. Two types of control experiments (*i.e.* without CN32 cells) were performed: one with the sediments only in APW medium and the other with lactate (10 mM) added to the sediments in APW medium. The four different treatments: sediment-only controls; sediment + lactate; sediment + lactate + cells; and sediment + lactate + cells + AQDS are referred to here as S, S+L, S+L+C, and S+L+C+A, respectively. Time-course production of Fe(II) in all bioreduction experiments was measured by 0.5 N HCl extraction (Zachara *et al.*, 1998; Fredrickson *et al.*, 1998) followed by Ferrozine assay (Stookey, 1970).

**Chemical analyses.** The concentrations of lactate, acetate, and other anions remaining in the incubated sediment solutions were measured using HPLC. The soluble sulfate at the start and end of bioreduction experiments was extracted twice in a 1000:3 water-sediment solution in order to leach out >99% of the water-soluble sulfate (Bao, 2005). Similarly, insoluble sulfates (*e.g.* barium sulfate, iron sulfate, see Table 3) were extracted using 50 mM DTPA (diethylene-triamine-pentaacetic acid) in 1 M NaOH solution for 24 h (Bao, 2006). Both forms of sulfates were pooled and analyzed using HPLC.

To measure H<sub>2</sub>S produced from sulfate reduction, the headspace gas from unsampled Fe(III) reduction tubes was carefully transferred to a new 200 mL bottle and reacted with 200 mM of zinc acetate to form zinc sulfide. Zinc sulfide was again reacted with silver nitrate to produce silver sulfide precipitate (modified from Cline, 1969). The mass of the precipitate was quantified by gravimetric analysis after drying at 65°C for 72 h.

#### *Particle aggregation and settling measurements*

**Measurement of particle settling using spectrophotometry.** The Goldberg and Forster (1990) method was used to measure the aggregation and settling of the <2.0 μm size fraction of sediments in APW media. In brief, 1 mL of homogeneous sediment suspension was placed in a quartz cuvette and changes in transmittance as a function of time were measured at 420 nm wavelength. Deionized water was used as the blank to set 100% transmittance. Particle settling was measured for controls (without addition of CN32 cells, both with and without lactate) and sediments with added Fe(II) (see below).

**Sediment-particle aggregation as a result of Fe(II) production.** The green and yellow-green color produced as a result of bioreduction of Fe(III) precluded the use of the Goldberg and Forster (1990) method for the measurement of particle settling (and thus the particle-

aggregation kinetics). This problem was overcome by adding Fe(II) (equal to that produced by bioreduction) to unreduced sediments under identical conditions. The media containing sediments and Fe(II) stock were made fully anoxic by using the method described by Jaisi *et al.* (2008). In brief, the sediments in the APW media were purged repeatedly with N<sub>2</sub> gas after four boiling cycles for at least 8 h to remove dissolved oxygen prior to Fe(II) addition. All treatments were performed inside a glove box to maintain an anoxic atmosphere. The possible presence of any trace contaminant oxygen was checked using a CHEMets<sup>®</sup> colorimetric analysis kit R-7540 (2.5 ppb sensitivity). Only those samples showing negative test results were used for measurement of aggregation and settling.

#### *Surface-charge measurements*

To understand the role of the surface charge of sediments in the aggregation process, the <2.0 μm size fractions of the sediments were purified. For this, the sediments were dialyzed repeatedly in deionized water using a dialysis membrane that had a pore size of 5 nm. Dialyzed solution was replaced with fresh deionized water twice a day until the final conductivity of dialyzed solutions decreased to ≤3 μS. The density of dialyzed sediment suspensions was measured by gravimetric analysis.

**Zeta potential.** To explicitly understand the role of electrical charge at the double layer of the sediments on particle aggregation, the electrophoretic mobility of the dialyzed sediments was measured in deionized water using *ZetaPALS* (Brookhaven Instruments Corp., Holtsville, New York) over a range of pH (3–10). The suitable sediment concentration (0.04 g/L) for electrophoretic mobility measurement was optimized by trial and error methods. Clay particles at this concentration have been found to disperse randomly with negligible interaction among particles (Ramsay and Lindner, 1993). Two to three electrophoretic mobility measurements were taken for each sample at a given pH and the results with residuals of <2% were averaged. The zeta potentials of the sediments were obtained by converting the electrophoretic mobility using the Smoluchowski approximation (Hunter, 1981).

**Point of zero net proton charge.** Electrolyte titration experiments were performed to determine the point of zero net proton charge (pH<sub>PZNPC</sub>) of the sediments (Sposito, 1984; Liu *et al.*, 2007). For this purpose, the sediments (3.0 g/L final concentration) were first equilibrated in 0.01 M NaClO<sub>4</sub> for 4 h and a constant equilibrium pH of 7.0 was attained by adding 0.01M HCl or NaOH. Aliquots of pH-stabilized stock sediment suspensions were then adjusted to a desired pH value (2.2–10.0) using 0.1 or 0.01 N HCl or NaOH. The ionic strength of the solution was then increased to 0.5 M

using  $\text{NaClO}_4$  salt. The stabilized pH after 4 h of equilibration was measured. The change in pH ( $\Delta\text{pH}$ ) as a result of the increase in ionic strength (from 0.01 to 0.5 M) was plotted as a function of initial pH. The  $\text{pH}_{\text{PZNPC}}$  was determined at  $\Delta\text{pH} = 0$ .

## RESULTS

### *Sediment properties: mineralogy and solution chemistry*

The results of the XRD analyses of the  $<2.0 \mu\text{m}$  size fraction of sediments resembled closely those of Hover *et al.* (2002). The fitting of the XRD data for quantitative mineralogical analyses using the *RockJock* program (Środoń *et al.*, 2001; Eberl, 2003) showed that 88–89 wt.% of all the minerals in the sediments are clay minerals (Table 1) consisting mainly of smectite and illites with a subordinate amount of chlorite and mica. The non-clay minerals consisted mainly of quartz and feldspar with some amphibole and maghemite and minor amounts ( $<1\%$ ) of calcite and dolomite. The mineralogy of all three sediments was very similar. However, the interlayer spacing of air-dried smectite obtained from the most saline sediments (S1) was

smaller than that of smectite from the freshwater sediments (S2) ( $\sim 14.1$  and  $15.9 \text{ \AA}$ , respectively).

The Fe content in the  $<2.0 \mu\text{m}$  size fraction of the sediments varied from 3.3 to 4.1 wt.% with 91–94% iron as Fe(III) (Table 2). A significant amount of organic carbon (2.8–7.6%) was present in the sediments. However, the combustion method used in our study may have overestimated the amount of organic carbon, possibly due to the dehydroxylation of clay minerals (Abella and Zimmer, 2007). The high conductivity of the S1 sediment pore water was consistent with its high salt concentration. Alkalinity in the S2 and S3 sediment pore water was very similar (*i.e.* 27.9 and 28.6 mg/L, respectively), but was below detection in the S1 pore-water. The acidity was negligible ( $<1 \text{ meq/L}$ ) in all sediment pore water samples.

### *Bacterial Fe(III) reduction*

*Fe(III) reduction.* The bioreduction of Fe(III) increased sharply in the first 120 h of incubation (Figure 1). The maximum extent of Fe(III) reduction was similar in all sediments with S3 showing the greatest ( $\sim 65\%$ ) extent of reduction. This amount of Fe(III) reduction is signifi-

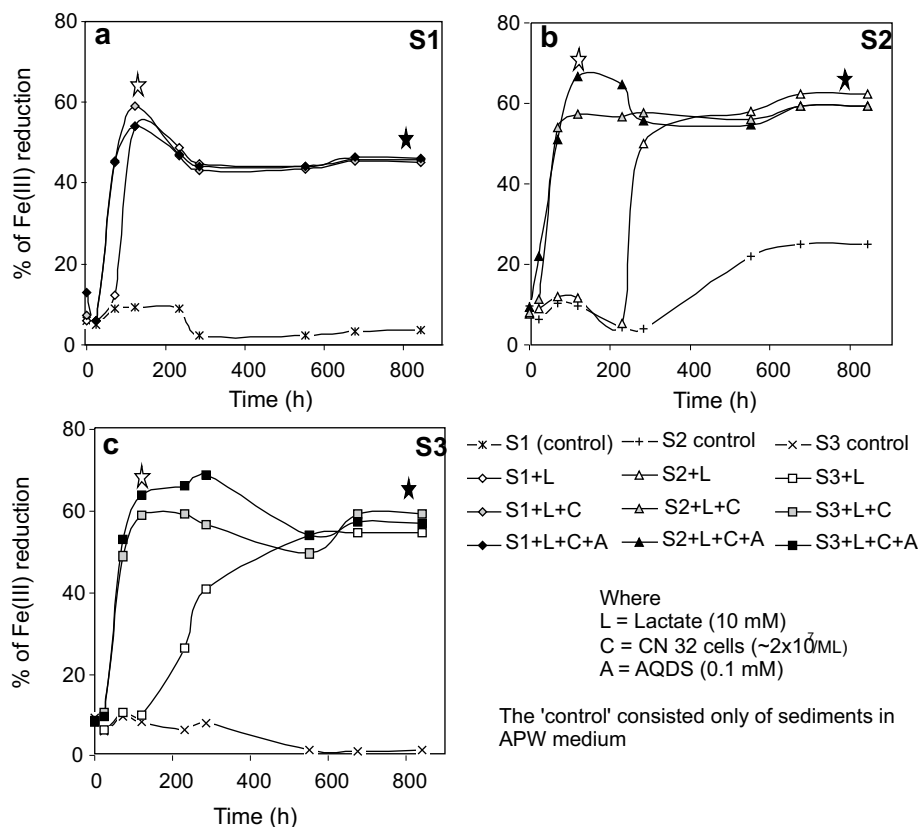


Figure 1. Fe(III) reduction in different treatments as a function of time in S1 (a), S2 (b), and S3 (c) sediments. All experiments were performed in non-sterilized APW medium at a sediment concentration of 3.0 g/L. The open and closed stars (☆, ★) represent the subsamples used for the image in Figure 3 and for sulfate concentration measurement (Table 3), respectively. The sampling time points were the same for all three sediment incubations.

cantly greater than that observed for pure smectite and illite (Dong *et al.*, 2003; Kim *et al.*, 2004; Jaisi *et al.*, 2005) and was still slightly greater than those of Missouri (USA) and China loess sediments, which had initial Fe(III) and Fe(II) concentrations that are similar to Mississippi sediments (Bishop *et al.*, 2006). However, 0.5 N HCl does not extract all the Fe(II) in phyllosilicates (Jaisi *et al.*, 2007b) and the photochemical reduction of Fe(III)-ferrozine complex may overestimate the extent of Fe(III) reduction (Anastácio *et al.*, 2008) e.g. in phen-complex (Stucki, 1981; Amonette and Templeton, 1998). This error varies as a function of Fe(II) and Fe(III) content. For example, the photosensitivity of Fe(II) solution increases when Fe(III) is present (Stucki and Anderson, 1981) and the error due to the partial-extract effect decreases with increase in Fe(II) (assuming other changes are negligible) (Jaisi *et al.*, 2007a). Therefore, avoiding the photochemical and partial-extract effect is necessary for explicit comparison of the Fe(II) and Fe(III) results. Unlike bioreduction experiments with pure clay minerals (Dong *et al.*, 2003; Kim *et al.*, 2004; Jaisi *et al.*, 2005, 2007b), the effect of electron shuttle (AQDS) addition (*i.e.* S+L+C+A treatment on the extent of Fe(III) reduction was negligible. The extent of reduction was found to fluctuate after 120 h of incubation. This might be related to the formation of H<sub>2</sub>S and other changes in solution chemistry (see below).

The control experiments consisting of lactate (10 mM) in APW medium without CN32 cells (S+L), did not show any Fe(III) reduction until 71, 232, and 121 h of incubation in the S1, S2, and S3 sediments, respectively. After this lag time, a significant amount of Fe(III) reduction occurred, comparable to that observed in experiments with CN32 cells (*i.e.* S+L+C and S+L+C+A treatments). The controls without lactate (S) showed no Fe(III) reduction except for S2, which showed a small amount of Fe(III) reduction.

Additional sets of experiments were performed after sterilizing (by autoclaving) both the sediments and APW media and inoculating with fresh CN32 cells to understand if the Fe(III) reduction observed in non-sterilized sediments with lactate (*i.e.* S+L treatment in Figure 1) was due to the presence of other electron-transfer pathways such as sulfate reduction. The results showed that the Fe(III) reduction did not occur in the absence of CN32 in the autoclaved sediments incubated with lactate (Figure 2). Additionally, the maximum extent of Fe(III) bioreduction was limited to 45%, and unlike the non-sterilized sediments, no fluctuation was observed in the extent of Fe(III) reduction in the sterilized sediments.

*Change in solution chemistry due to Fe(III) reduction.* A significant change in solution chemistry due to the Fe(III)-reduction activity occurred. A perceptible odor of H<sub>2</sub>S was detected during sampling after one day of Fe(III) reduction. The measurement of H<sub>2</sub>S by the silver-sulfide

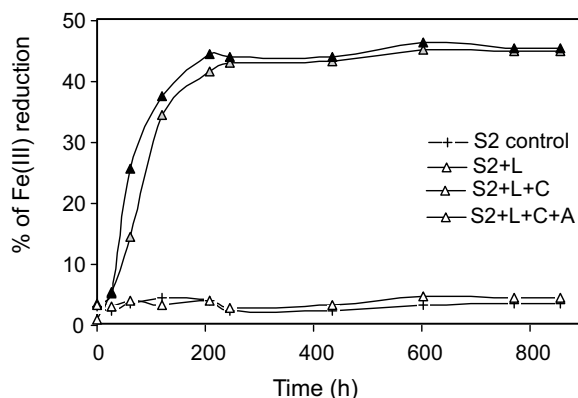


Figure 2. Fe(III) reduction in different treatments as a function of time in S2 in autoclaved sediments. The symbol L refers to lactate, C to cell, and A to AQDS. The nature of reduction in S1 and S3 was very similar to that in S2 (therefore the S1 and S3 results are not shown).

conversion method (Bao, 2005) showed that 6.43, 1.01, and 3.42 mmol/L (*i.e.* 2.12, 0.33, and 1.13 mmole/g) H<sub>2</sub>S was produced in the S1, S2, and S3 sediments, respectively, after 775 h of incubation (Table 3). The XRD results of the bioreduced sediments showed the enhancement of the major (200) peak of pyrite suggesting the formation of pyrite. The amount of H<sub>2</sub>S produced and

Table 3. Sulfate, lactate, and acetate concentrations at the start and the end of Fe(III) reduction experiments. The non-stoichiometric relationship between acetate and lactate may be due to consumption of acetate by sulfate-reducing bacteria to form CO<sub>2</sub>. The sulfate reduction resulted in the formation of hydrogen sulfide and precipitation of pyrite.

Samples	Sulfate mM	Lactate mM	Acetate mM	H <sub>2</sub> S mM
S1 (at the start of experiment)				
	12.70	10.00	0.00	0.00
Results after 775 h of incubation				
S1(control)	12.85	9.87	0.27	
S1+L	2.56	0.25	10.16	
S1+L+C	0.31	0.16	10.32	
S1+L+C+A	0.22	0.08	9.51	6.43
S2 (at the start of experiment)				
	2.75	10.00	0.00	0.00
Results after 775 h of incubation				
S2(control)	2.31	9.45	0.11	
S2+L	0.02	<0.001	0.07	
S2+L+C	0.03	<0.001	2.71	
S2+L+C+A	0.02	<0.001	2.15	1.01
S3 (at the start of experiment)				
	5.29	10.00	0.00	0.00
Results after 775 h of incubation				
S3(control)	5.45	9.87	0.56	
S3+L	2.84	1.07	8.17	
S3+L+C	0.07	0.87	1.65	
S3+L+C+A	0.13	0.75	3.06	3.42

L = lactate; C = CN 32 cells; A = AQDS

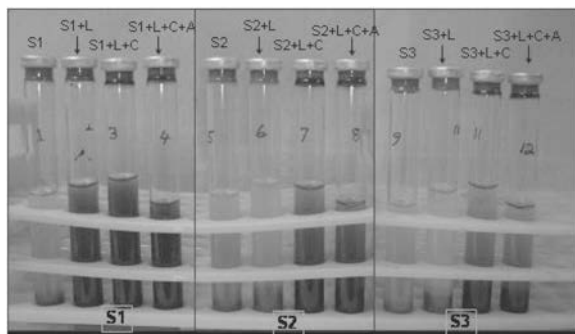


Figure 3. Photographs showing differences in particle settling as a result of different treatments for the three sediments (after 120 h, white star in Figure 1). The samples were first shaken gently and allowed to settle. The picture was taken 20 min after manual shaking.

the enhancement of the (200) peak as a result of pyrite formation were consistent with the amount of sulfate consumed during Fe(III) reduction.

Lactate was consumed in all incubations including control experiments containing lactate (Table 3).

Although the lag time for Fe(III) reduction in the S+L treatments was different in different sediments, the lactate consumption was greater than stoichiometric demand for the Fe(III) reduction (*i.e.* 1:4, Kostka *et al.*, 1996) in all sediments. However, the amount of lactate consumed was nearly stoichiometric to Fe(II) produced in the autoclaved sediments. This result strongly suggests that the presence of additional electron acceptors (*e.g.* sulfate) contributed to lactate consumption. No Fe(III) reduction, sulfate consumption, or H<sub>2</sub>S formation occurred in the control experiments (both with and without lactate) with the autoclaved sediments.

#### Particle aggregation and settling

Particle aggregation and settling of the unreduced and bio-reduced sediments (in the APW media) after 120 h of incubation (Figure 3) shows that the presence of lactate (*i.e.* S+L treatment) enhanced the particle aggregation compared to the sediments only. Although the green color hampered clear distinction, bio-reduction clearly resulted in thicker accumulation of sediment particles at the bottoms of tubes (S+L+C and S+L+C+A treatments) compared to unreduced sediments (*i.e.* S and S + L).

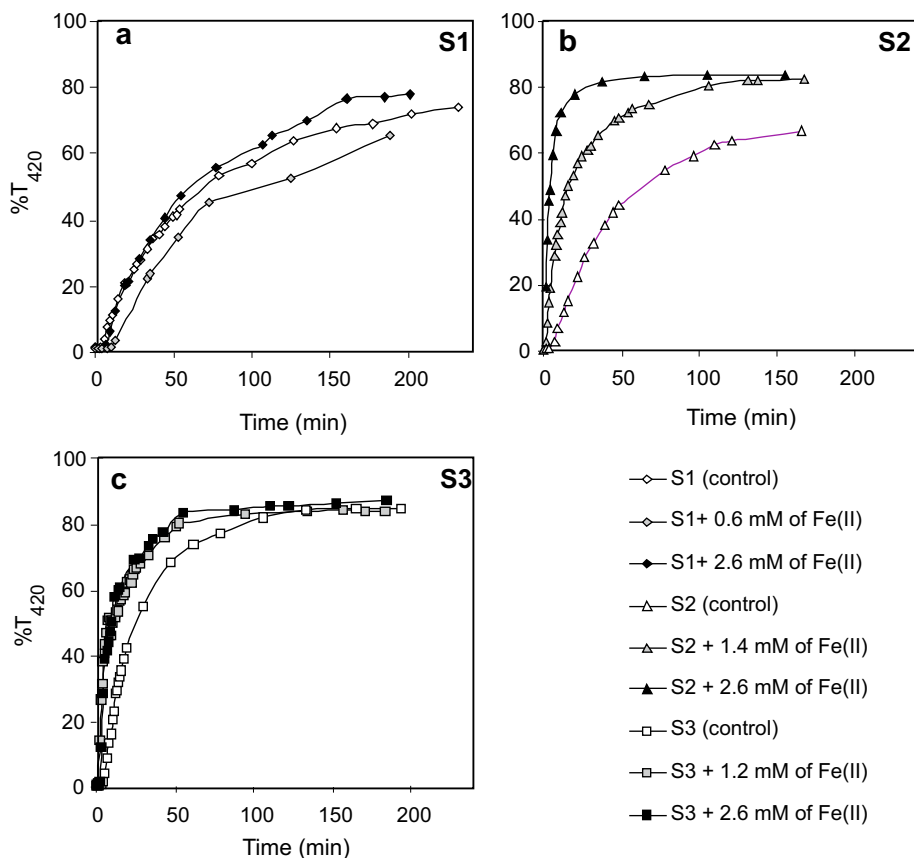


Figure 4. Particle-settling measurement as a percentage of transmittance (%T, at 420 nm) in the different sediments (in their respective APW media) with and without externally added Fe(II). Percent transmittance for deionized water was set as 100% for this measurement. The transmittance of APW media alone for S1, S2, and S3 was 21, 14, and 11%, respectively. Therefore, the net transmittance is the difference between the measured value and that for the APW media only.

The presence of Fe(II) normally promoted the particle-settling rate (Figure 4), despite the fact that each APW medium already had a significant amount of other cations. For example, the percentage of transmittance in the S2 sediments after 50 h of settling was 44, 71, and 82% in the presence of 0, 1.4 mM, and 2.6 mM Fe(II), respectively. For the S3 sediments (after the same settling time), the transmittance was 69, 80, and 84% in the presence of 0, 1.2 mM, and 2.6 mM Fe(II), respectively. However, particle settling in the presence of 0.6 mM Fe(II) in the S1 sediments was even less than that in the Fe(II)-free (control) experiment, where the settling rate increased slightly with 2.6 mM Fe(II). In summary, these results consistently showed that the presence of even a small amount of Fe(II) enhanced the rate of particle settling, despite the fact that the APW solution was rich in other cations.

To compare the role of sediment properties alone in aggregation and settling, the dialyzed sediments (S1, S2, and S3) were dispersed in the same APW medium (corresponding to S1 sediments) and the relative rates of particle aggregation and settling were measured (Figure 5). The S1 sediments showed the slowest rate of settling and S3 the highest. This relative order of settling was consistent with the zeta potential of these sediments (see below).

The SEM images of suspended particles (bioreduced sediments in S3+L+C+A treatment) showed the formation of particle clusters (Figure 6) from originally homogenous suspension. The cluster morphology in different sediments was very similar. The bioreduced S1 sediments prepared by critical point drying showed CN32 cells attached to mineral particles (Figure 7) and formed clusters. High-magnification SEM and TEM images of clusters showed that face-to-face (F-F) association was the primary mode of clay-particle

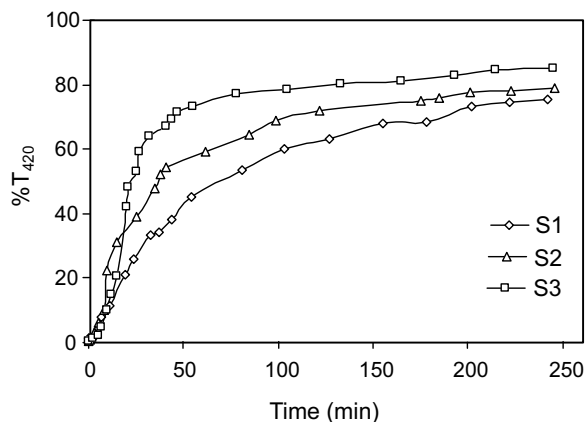


Figure 5. Particle-settling measurement as a percentage of transmittance (%T<sub>420</sub>) in the same APW medium (for the sediments S1) in order to assess the effect of sediment zeta potential on particle aggregation. The size-fractionated sediments (<2.0 μm) were dialyzed until the conductivity of medium decreased to ≤3 μS before measurement.

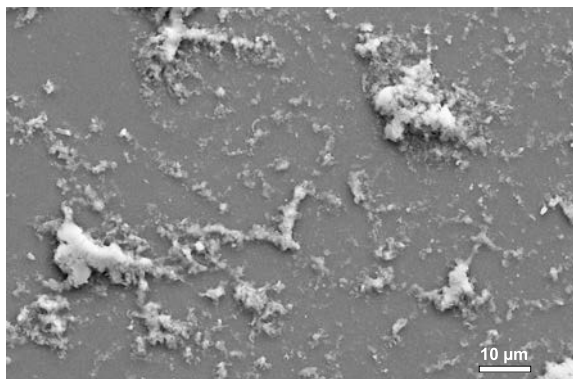


Figure 6. SEM image showing loosely bound clusters of particles forming aggregates in bioreduced sediments (S3+L+C+A treatment). The salt precipitate in each cluster has light tone (EDS spectra not shown).

attachment in all three sediments. However, bioreduced sediments in TEM images showed mixtures of clay, cells, and biopolymers, creating globule-shaped clusters 300–400 nm in diameter (images not shown). The degree of F-F orientation was greater in sediments without externally added Fe(II).

#### Surface charge of the sediments

**Zeta potentials.** The measured conductivity of all three sediments at neutral pH was ~3 μS. Similarly, the conductivity of these sediments at different pHs (3–10) chosen for zeta potential measurement was similar (±4 μS). This result suggests that the role of any residual cations in influencing zeta potential was negligible, and therefore reflected true surface-charge properties of minerals in the sediments.

As expected, the zeta potential increased consistently (*i.e.* became more negative) with increase in pH (Figure 8a). The rate of decrease was high at pH from 4 to 5 and was relatively insignificant both at lower and

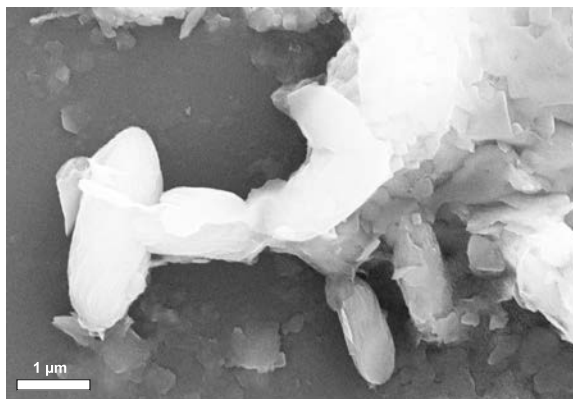


Figure 7. SEM image showing CN32 cells bound to each other as well as to small sediment particles in bioreduced sediments (S1+L+C treatment).



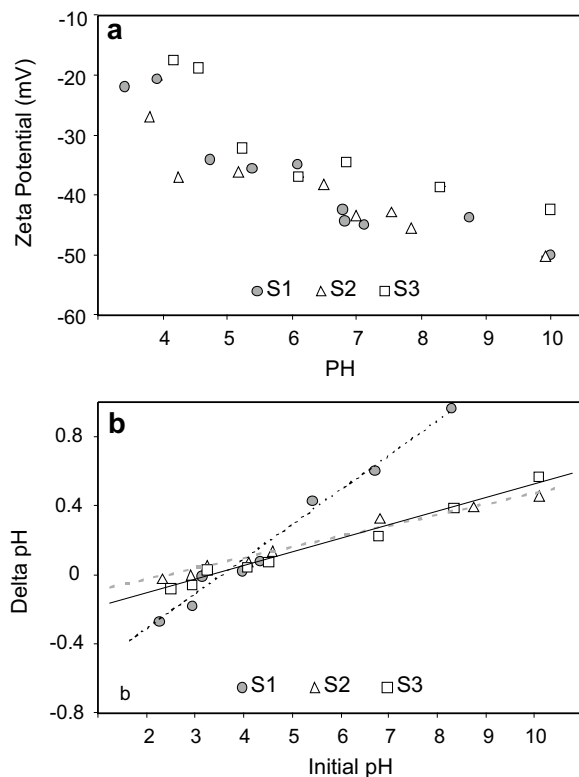


Figure 8. (a) Zeta potential of the native sediments as a function of pH; (b) point of zero net proton charge of sediments. The size-fractionated sediments ( $<2.0 \mu\text{m}$ ) were dialyzed until the conductivity of medium decreased to  $\leq 3 \mu\text{S}$ .

higher pH ranges. The zeta potential of the sediments at the sediment-water interface pH in the Mississippi Delta (*i.e.* 6.9–7.3; Hover *et al.*, 2002) varied from  $-35$  to  $-45$  mV with the lowest potential for the S3 sediments and the highest for S1. The zeta potential of the S1 and S2 sediments was, however, similar.

**Point of zero net proton charge.** The  $\text{pH}_{\text{PZNPC}}$ , where net proton charge [ $\sigma_{\text{H}} = (q_{\text{H}} - q_{\text{OH}})$ ] at the mineral surface is zero, determined from electrolyte titration, was found to be very low (Figure 8b). For example,  $\text{pH}_{\text{PZNPC}}$  was almost the same, with values of 3.6 and 3.3, in the S1 and S3 sediments, respectively, while it was still low (2.5) in the S2 sediments. These values were smaller than literature  $\text{pH}_{\text{PZNPC}}$  values in pure smectite and nontronite (Kriaa *et al.*, 2007; Jaisi *et al.*, unpublished results). This result, together with zeta potential measurement, suggests that all sediments had negatively charged edge and face sites at sediment-water interface pH.

## DISCUSSION

### Sulfate-reduction pathway during Fe(III) reduction

The presence of sulfate reduction was detected during Fe(III) reduction in non-sterilized sediments incubated in the APW media. The decline of sulfate concentration,

production of  $\text{H}_2\text{S}$ , formation of pyrite, and excess consumption of lactate (relative to the stoichiometric demand) during Fe(III) reduction suggest that the sulfate reduction activity originally present in these sediments (Hover *et al.*, 2002) was enhanced by addition of lactate. In experiments with autoclaved sediments and APW media, sulfate-reduction activity was not detected and the extent of Fe(III) reduction decreased from  $\sim 65$  to  $\sim 45\%$  (Figure 1 and 2). This extent of Fe(III) reduction was very close to that obtained in bioreduction of pure clay minerals (Kostka *et al.*, 1996; Gates *et al.*, 1998; Kim *et al.*, 2004; Jaisi *et al.*, 2007b). These results strongly suggest that the significant extent of Fe(III) reduction in the non-sterilized sediments was due to simultaneous Fe(III) and sulfate reduction. In fact, the capability of sulfate-reducing bacteria (SRB) to reduce Fe(III) has been demonstrated in salt-marsh sediment (Coleman *et al.*, 1993), and coupled sulfate and Fe(III) reduction enhancing the reduction of Fe(III) in clay minerals under sulfate-reducing conditions has also been documented (Li *et al.*, 2004). Extensive sulfate consumption in Fe(III) reduction experiments (both with and without CN32 cells, Table 3) is consistent with the explanation of Li *et al.* (2004) that sulfate-reducing bacteria could utilize structural Fe(III) as the sole electron acceptor for growth when sulfate is not available. This order, however, is opposite to normal electron utilization pathways (Lovley *et al.*, 2004) of soluble electron acceptors.

### Role of mineral composition in particle aggregation

Quantitative XRD results revealed that 88–89% of the mineralogical components of Mississippi sediments ( $<2.0 \mu\text{m}$  size fraction) consisted of clay minerals. This result is consistent with previous results (Potter *et al.*, 1975; Weaver, 1989) which suggested that  $>76\%$  of sediments and suspended particle load in the Mississippi Delta plain is composed of clay minerals (smectites, illite, and kaolinite) with  $\sim 10\%$  more clay minerals in the  $<2.0 \mu\text{m}$  size fraction (Hover *et al.*, 2002) than in bulk sediments. In addition, the mineralogy of suspended and deposited sediments was found to be very similar (Hover *et al.*, 2002). Therefore, our choice of the  $<2.0 \mu\text{m}$  size fraction of freshly settled sediments (0–2 cm) is expected to resemble the natural sediments, and particle aggregation and settling measured in this study is expected to depict the natural process of aggregation and settling of suspended sediments if the redox conditions favor Fe(III) reduction.

Because the conductivity of different sediments prepared for zeta potential measurement was very close at a given pH, direct comparison of zeta potential was meaningful. The smallest zeta potential value for the S3 sediments, among the three (S1, S2, and S3) (Figure 8a), was consistent with the observation that S3 had the smallest percentage of smectite and illite (Table 1). Because the rate of aggregation of colloidal particles

increases with decrease in zeta potential (Elimelech *et al.*, 1995; Lagaly, 2006 and references therein), the rate of aggregation and settling is expected to be high at low zeta potential. Results from this study consistently show that the sediments with the smallest zeta potential (sediment S3) have the greatest rate of aggregation and settling (Figure 5). In summary, these results suggest that the small differences in clay content played a reasonably significant role in particle aggregation, despite the fact that relative proportions of clay and non-clay minerals were very similar in different sediments.

#### *Role of pore-water chemistry in aggregation and settling of the sediments*

*Role of cations in particle aggregation.* The aggregation behavior of fine-grained soils, especially those with greater specific surface areas such as clay-rich sediments, is intimately related to the pore-fluid properties (Stumm and Morgan, 1996). The increase in electrolyte concentration (those cations which do not specifically sorb to mineral surfaces, *e.g.* Na, Mg, Ca, *etc.*) results in compression of the double layer and therefore promotes particle aggregation (van Olphen, 1977). Hence, the extent of particle aggregation is directly correlated with salt concentration (Lagaly, 2006 and references therein). This explanation, therefore, suggests that the aggregation and settling of suspended sediments in the Mississippi Delta was controlled predominantly by salt concentration (in the absence of significant biological activity) (Tipping and Cooke, 1982; Mitchell, 1993; Forsgren *et al.*, 1996).

*Role of anions in particle aggregation.* Sulfates and phosphates are strong stabilizing anions for clay particles (Penner and Lagaly, 2001; Lagaly and Ziesmer, 2003). For example, the presence of sulfate increases the critical coagulation concentration (CCC) to at least three times that of chloride (Penner and Lagaly, 2001). Similarly, the influence of phosphates has been reported to stabilize particles because they replace structural OH by ligand exchange, thereby increasing edge charge density and double layer thickness (CCC can increase by 82–220 times in Na-phosphate compared to that of NaCl; Muljadi *et al.*, 1966; Lagaly, 1993; Lagaly and Ziesmer, 2003). Therefore, phosphates have been used as deflocculating agents to stabilize clay suspensions (Lagaly, 1989; Manfredini *et al.*, 1990; Penner and Lagaly, 2001; Jaisi *et al.*, 2007a). Calculations using the method of Frey and Lagaly (1979) indicated that the presence of 0.02 mmol/L phosphate in the S1 sediments (Table 2) might increase the CCC by as much as 3.5 times. This effect is clearly seen in the present experimental results where the rate of aggregation was small in the sediment with pore water containing a small amount of sulfate and phosphate despite high salt concentration (*i.e.* the S1 sediments; Table 2, Figure 4).

*Role of changing solution chemistry in particle aggregation.* The greater rate of aggregation observed in the bioreduced sediments compared to those without Fe(II) might be, in part, controlled by the consumption of sulfates during incubation. Although the system pH decreased slightly after Fe(III) reduction (6.9 to 6.5), such a small decrease in pH was not expected to significantly change the edge-surface charge of clay particles because of very low values of  $pH_{PZNPC}$  and small changes ( $\sim 3$ – $5$  mV) in zeta potential (Figure 8b) in all sediments.

The role of Fe(II) in particle aggregation is particularly important because Fe(II) has the greatest preference for surface complexation sites (Jaisi *et al.*, unpublished results) and forms inner-sphere complexes (Stumm and Sulzberger, 1992). Such complexes formed by specifically bound cations (unlike other simple cations such as Ca, Mg, Na *etc.* which contribute only to the double layer) reduce the diffuse double-layer potential and therefore may favor particle aggregation. At high concentration, such cations may cause charge reversal (Stumm and Morgan, 1996). Therefore, the overall edge charge of Fe(II)-sorbed sediments was expected to be less than that without Fe(II) sorption. The low degree of F-F orientation of aggregates in experiments with added Fe(II) supports this speculation. In addition, a decrease in zeta potential in the system containing Fe(II) was also observed (Figure 4, Jaisi *et al.*, 2007a). The greater rate of particle aggregation in the presence of Fe(II) is consistent with this explanation (Figure 3).

#### *Mode of clay-particle attachment*

Since the zeta potential of the sediments was negative and the  $pH_{PZNPC}$  was very small ( $\leq 3.5$ ), both edge and face sites should be negatively charged at neutral and circum-neutral pH, suggesting overall double-layer repulsion. However, large cation concentrations in aqueous solutions would compress the double layer thickness, resulting in closer contact of particles (van Olphen, 1977; Elimelech *et al.*, 1995) and therefore promoting their aggregation. Since clay particles in this study showed predominantly face-to-face (F-F) orientation, cations should have played a role in bridging similarly charged clay plates (Figure 8), probably by sharing hydrated counter-ions between two adjacent layers, similar to that of the previously observed mode of particle attachment in smectites (Gates *et al.*, 1998; Jaisi *et al.*, 2007a).

#### *Aggregation and settling by biopolymers and organic matter*

Although the amount of biopolymers produced by CN32 cells during Fe(III) reduction was not measured in this study, their role in aggregation of nontronite particles was quantified in a previous study (Jaisi *et al.*, 2007a). Since the same bacterium was used in this study, a similar

role of biopolymers in aggregation and settling may be expected. The organic matter associated with the sediments is expected to play a certain role in particle aggregation depending on its hydrophobicity and the nature of its interactions with sediments (specifically vs. non-specifically bound). Since the types and nature of organic compounds and their surface functional groups were not quantified, their specific contributions to particle aggregation cannot be determined.

## CONCLUSIONS

Structural Fe(III) reduction in smectites and illites in unsterilized Mississippi sediments promoted simultaneous sulfate reduction and produced hydrogen sulfide and pyrite. The data presented show clearly that aggregation and settling of clay particles, with negatively charged face and edge sites, occurred primarily as a result of the high concentration of cations in the pore water. In situations where enhanced Fe(II) concentrations occur, enhanced rates of aggregation, possibly due to specific Fe(II) sorption to mineral surfaces, may occur. Additionally, cells and cell exudates along with a decrease in sulfate concentration are expected to enhance the rate of particle aggregation. Although deep-water bodies in the Mississippi Delta region are normally hypoxic and do not support Fe(III) reduction, if conditions favorable for Fe(III) reduction were to develop, additional increases in the rate of particle aggregation and settling would be expected.

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