Fe-CYCLE BACTERIA FROM INDUSTRIAL CLAYS MINED IN GEORGIA, USA

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Abstract—Dark Fe oxides and sulfides are major discoloring impurities in mined commercial white kaolin clay. In order to evaluate the potential influence of Fe-cycle bacteria on Fe cycling during post-depositional clay-weathering alteration, Fe(III)-reducing and/or Fe(II)-oxidizing microorganisms were examined in open-pit, subsurface mine samples from kaolin lenses and smectite formations collected from sites in central Georgia. Samples of varying age were examined, including late Eocene smectite overburden, hard kaolin of Middle Eocene age, soft gray kaolin from the late Paleocene, and soft tan kaolin of late Cretaceous age. These clays contained 0.06−5.33% organic carbon, which included various potential organic electron donors for bacterial metabolism: formate (1.1−30.6 mmol/kg), acetate (0−40.5 mmol/kg), lactate (0−12.1 mmol/kg), pyruvate (0.4−78 mmol/kg), oxalate (0−141.7 mmol/kg), and citrate (0−1.4 mmol/kg). All clay samples studied had small concentrations of 'bio-available' Fe(III) (0.5 M HCl-extractable Fe, 0.5−2.8 mmol/kg) compared to total Fe (HF-extractable, 25−171.9 mmol/kg). The highest Fe(III)/[Fe(II)+Fe(III)] ratio and the lowest organic carbon content were in kaolin samples in which Fe(III) reduction was determined to be the dominant terminal electron accepting process by hydrogen analysis. All clay samples showed greater numbers of Fe(II)-oxidizing bacteria (22−22,000 cells/g) than Fe(III)-reducing bacteria (3−410 cells/g) as determined by MPN analysis. The Fe(III)-reducing activity in clays could be stimulated with the addition of 1 mM of the Fe(III) chelator, nitrilotriacetic acid. The addition of nitrate stimulated anaerobic Fe(II) oxidation. These results suggest that anaerobic bacteria involved in both oxidation and reduction of Fe exist in these subsurface clay formations, and might have had an influence on post-depositional weathering reactions.

Key Words—Bacteria, Clay, Fe Cycle, Kaolin, Smectite, Subsurface.

INTRODUCTION

It has recently been recognized that a large population of microorganisms inhabit diverse subsurface environments including sub-sea floor sediment and basement rock, continental sedimentary rocks, ancient salt deposits, aquifers in igneous terrestrial rocks, and caves (Krumholz, 2000). Clay formations represent a unique subsurface microbial habitat. Due to their very fine particle size, subsurface clays have low hydraulic conductivity and consequently the rate of microbial metabolism is extremely slow. Microorganisms are generally larger than clay mineral particles and it has been suggested that microbial populations present in clays were trapped there during deposition of the clay layers. If this is in fact the case, these bacteria may have survived from the time of original clay sedimentation tens of millions of years ago (Chapelle and Lovley, 1990; Fredrickson *et al.*, 1995; Boivin-Jahns *et al.*, 1996; Lawrence *et al.*, 2000).

Kaolin is an industrially important clay made up predominantly of the clay mineral kaolinite. Subsurface kaolin deposits in the Coastal Plain of central and eastern Georgia in the southeastern United States are at the center of the world kaolin mining industry. Key criteria for the industrial use of Georgia's commercial white kaolin deposits are small contents of discoloring Fe oxide, Ti oxide and Fe sulfide impurities, and small dark organic carbon contents. The Fe is present in cream- to tan-tinted kaolins as Fe(III) oxides and hydroxides (hematite and goethite) and as a structural replacement in kaolinite, smectite and mica. In gray kaolins, which have not yet been oxidized, Fe is present mainly as pyrite. Gray kaolins have a relatively large dark organic carbon content, which causes their distinct gray or dark brown appearance.

Kaolin deposits in Georgia are sedimentary, and composed of material that has been transported from the much older Piedmont and Blue Ridge rocks. These weathering products were eroded and deposited in ponds and lagoons near and along the coastline during the late Cretaceous and early Tertiary period (Elzea Kogel *et al.*, 2002). The Georgia kaolins were long thought to have been deposited as unusual white nearly monomineralic clays, but recent evidence indicates that they were deposited as typical dark organic muds in a deltaic sequence of interlayered sand and clay swamp and marsh flood plain clastics. It was suggested that what is now nearly pure kaolin clay has been greatly whitened by weathering over the term of subsurface burial, and that bacterial action has played a critical role in removal of Fe and organic matter (Hurst and Pickering, 1997).

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Smectite clays, in the form of industrial fuller's earth, are another important clay mineral mined in the southeastern US. Sedimentary smectite deposits of late Eocene age are mined in Jefferson County in east-central Georgia, and of mid-Miocene age in southwestern Georgia and adjacent western Florida. These useful and commercially versatile fuller's earths are predominantly calcium montmorillonite and magnesium palygorskite (attapulgite), and are used for their sorbent, thickening and gelling properties.

The aim of this study was to evaluate whether Fe-cycle bacteria, which are able to change the valence state of Fe, are present in Georgia kaolin and smectite deposits, and to determine the extent to which indigenous bacteria may have influenced Fe chemistry in these clays. For this purpose, samples of clay from subsurface kaolin lenses and overlying smectite deposits exposed in a variety of Georgia open pit mines were collected and examined.

MATERIALS AND METHODS

Study sites and sample collection

Clay samples (Table 1) were collected in November 1999 from active kaolin mines in central Georgia using a mine backhoe excavator and/or a geological pickaxe. Samples #2, #3 and #5 were taken from a Dry Branch Kaolin Co. (now Imerys) T.M. Bloodworth mine 1000 m east of the intersection of Georgia Highway 18 and an unpaved mine road 4 miles south of Georgia highway 57, in Wilkinson County. Sample #7 was taken from the former Dry Branch (Imerys) Brooks mine north of Asbury Church road, also located in Wilkinson County. Sample #12 was taken from the Thiele Kaolin Co. Avant mine, 1 km west of Georgia highway 272. Samples #13 and #14 were taken from Thiele's General Refractories mine 3.2 km south of Tabernacle Church. The last two mines are both located in Washington County.

Large pieces of naturally moist kaolin were cut from freshly exposed clay surfaces in active mines and were then wrapped with plastic, sealed, and immediately sent by overnight delivery service to the laboratory. After arrival at the laboratory, all samples were placed in an N_2-filled glove bag. Large pieces of each kaolin sample were cut with sterile knives, and the middle of the samples extracted, homogenized and dispensed into serum bottles for immediate experimentation or into large sterilized Pyrex bottles with thick rubber stoppers for storage and/or later use. After removal from the anaerobic chamber, all bottle headspaces were gassed out with a N_2/CO_2 mixture (95:5) that had been passed over hot copper filings to remove residual oxygen.

Most probable number analysis

Strict anaerobic laboratory techniques (Miller and Wolin, 1974; Balch et al., 1979) were used to quantify anaerobic Fe-cycle bacteria. An anaerobic basal bicarbonate-buffered freshwater (FW) medium was dispensed into 27 mL anaerobic pressure tubes (Bellco Glass, Inc.) under N_2/CO_2 (80:20). The tubes were capped with butyl rubber stoppers and sterilized by autoclaving. The medium for Fe(III)-reducing bacteria contained 100 mM poorly crystalline ferric Fe oxide (PCFO) as a terminal electron acceptor, either H_2 (the headspace in the tube exchanged with $H_2:CO_2$, 80:20%), acetate (20 mM), or lactate (20 mM) as the electron donor, and 1.3 mM $FeCl_2$ as the reducing agent. The medium for Fe(II)-oxidizing bacteria contained 5 mM ferrous sulfate, 1 mM acetate, and 5 mM nitrate. For aerobic acidophilic Fe(II)-oxidizing bacteria, the following medium was used (g/L): KH_2PO_4 (0.4), $MgSO_4x7H_2O$(0.4), $(NH_4)_2SO_4$(0.4), $FeSO_4x7H_2O$(33.3). The pH of the medium was adjusted to 3.5 with sulfuric acid.

Stimulation experiments

All the stimulation experiments were performed under $N_2:CO_2$ (95:5) in 160 mL serum bottles containing 100 mL of the clay slurry. To stimulate growth of indigenous anaerobic Fe(III)-reducing bacteria, clay

Table 1. Samples collected for study.

Geological age	Formation	Lithology	Sample numbers
Late Eocene	Twiggs Clay Formation Clinchfield Sand Formation	Marine to lagoonal smectite clay Quartz sand with marine fossils	2, 14 (smectite) —
Early to middle Eocene	Jeffersonville Member of Huber Formation	Cross-bedded quartz sands interlayered with lenses of hard, fine-sized kaolin	13* (hard kaolin)
Late Paleocene	Marion Member of Huber Formation	Soft tan and gray kaolin and lignite lenses in sands	3, 5*, 12 (soft gray kaolin)
Latest Cretaceous	Buffalo Creek Formation	Lenses of soft, coarse kaolin interlayered with cross-bedded quartz sands	7* (soft tan kaolin)

* Some of the data for samples #5, #7 and #13 are from Elzea Kogel et al. (2002).

slurries (20 wt.%) were incubated with addition of 1 mM nitriloacetic acid (NTA). To stimulate growth of indigenous anaerobic Fe(II)-oxidizing bacteria, clay slurries (20 wt.%) were incubated with the addition of 4 mM NO_3^- (the electron acceptor) and 1 mM NTA. For controls the slurries were autoclaved and then the same compounds as for the experimental tubes were added. All samples were incubated at 30°C for 30 days.

Analytical techniques

Bio-available Fe was measured with the ferrozine assay after 0.5 M HCl extraction (Lovley and Phillips, 1988). Total Fe was determined by hydrofluoric acid extraction followed by the 1,10-phenanthroline assay as described by Stucki (1981) and modified by Komadel and Stucki (1988). Organic matter content was determined by the modified Mebius wet combustion procedure (Nelson and Sommers, 1982). Organic acids were measured by high-performance liquid chromatography after 1 h of extraction with water (with a clay to water ratio of 1/10 w/v). A Hewlett Packard series 1100 Liquid Chromatograph was equipped with an Aminex HPX-87H column (300 by 7.8 mm; Bio-Rad, Hercules, California) and an SPD-10VP UV detector (Shimadzu, Kyoto, Japan) set at 215 nm. The mobile phase was 8 mM H_2SO_4.

To determine dissolved hydrogen concentration in the porewater within representative clay samples, 30 g of each clay were placed into 58 mL serum bottles under an $N_2:CO_2$ (95:5) atmosphere. Hydrogen concentration in the headspace was monitored over time with a reduction gas analyzer (RGD2, Trace Analytical, Sparks, Maryland) as previously described (Lovley *et al.*, 1994) until stability was reached.

RESULTS AND DISCUSSION

Georgia subsurface clays as a habitat for microorganisms

For this study, clay samples of varying geological age and mineralogy were examined, including smectite overburden from the late Eocene, hard kaolin from the early Eocene, late Paleocene gray kaolin, and soft kaolin of late Cretaceous age (Table 1). Parameters characterizing the studied clays as habitats for microorganisms are summarized in Table 2.

The clays studied contained 0.06−5.33% organic carbon, including various potential electron donors for bacterial metabolism: formate (1.1−30.6 mmol/kg), acetate (0−40.5 mmol/kg), lactate (0−12.1 mmol/kg), pyruvate (0.4−78 mmol/kg), oxalate (0−141.7 mmol/kg), and citrate (0−1.4 mmol/kg). The terminal-electron accepting process (TEAP) in the clays was determined by measuring the steady-state concentration of dissolved molecular hydrogen in porewater (Lovley and Goodwin, 1988; Chapelle *et al.*, 1995). According to the porewater hydrogen concentrations, Fe(III) reduction was the predominant TEAP in kaolin samples #7 and #13, sulfate reduction was prevalent in kaolin sample #12, and methane production predominated in the kaolin samples #3 and #5 as well as in the smectite samples. It is notable that Fe(III) reduction was the dominant TEAP in kaolins with the lowest organic carbon concentrations (Table 2).

Fe-cycle bacteria in Georgia subsurface clays

All of the clay samples had small concentrations of 'bio-available' 0.5 M HCl-extractable Fe (0.5−2.8 mmol/kg), as compared to total HF-extractable Fe (25−171.9 mmol/kg) (Table 3). In the smectite, hard

Table 2. Characterization of samples.

Samples		H₂*(nM)	Dominating TEAP**	Organic carbon (%)	Identified organic acids (mmol/kg)
Smectite	2	14.7	Methane production	0.91±0.09	acetate (17.0±2.8), formate (3.8±1.0), pyruvate (0.6±0.3), lactate (0.2±0.2)
Hard kaolin	14	10.9	Methane production	0.57±0.04	formate (1.1±0.2), pyruvate (1.4±0.1)
	13	0.1	Fe(III) reduction	0.07±0.00	formate (1.6±0.1), pyruvate (0.4±0.0), lactate (0.4±0.1)
Soft gray kaolin	3	5.6	Methane production	2.49±0.13	acetate (15.6±1.8), formate (13.8±2.6), pyruvate (78.0±0.9), oxalate (1.2±0.2)
	5	5.0	Methane production	1.58±0.09	formate (30.6±5.8), pyruvate (15.5±1.0), oxalate (141.7±7.3)
	12	2.7	Sulfate reduction	5.33±0.19	acetate (40.5±1.6), formate (17.3±2.5), pyruvate (8.6±1.0), lactate (12.1±0.3), citrate (1.4±0.2)
Soft tan kaolin	7	0.4	Fe(III) reduction	0.06±0.00	acetate (0.1±0.1), formate (3.6±0.1), pyruvate (1.6±0.2), lactate (1.7±0.3)

* Steady-state concentration of hydrogen in the porewater
** Terminal electron accepting process

Table 3. Total (HF-extractable) and bio-available (0.5 M HCl-extractable) Fe in clay samples.

Samples		Fe (mmol/kg)					
		HF-extractable			0.5 M HCl-extractable		
		Fe(II)	Fe(III)	Fe(III)/[Fe(II)+Fe(III)]	Fe(II)	Fe(III)	Fe(III)/[Fe(II)+Fe(III)]
Smectite	2	65.1±0.7	106.8±0.9	0.62	1.5±0.0	0.3±0.1	0.17
	14	21.1±0.6	43.6±0.8	0.67	0.4±0.0	0.1±0.0	0.20
Hard kaolin	13	6.6±0.1	48.6±0.1	0.88	0.4±0.0	0.3±0.0	0.43
Soft gray kaolin	3	44.9±0.5	15.4±0.6	0.26	1.0±0.0	0.0±0.0	0.00
	5	29.4±0.2	22.2±0.2	0.43	2.3±0.0	0.5±0.1	0.18
	12	25.2±0.2	7.7±0.4	0.23	0.8±0.0	0.2±0.0	0.20
Soft tan kaolin	7	5.3±0.0	19.7±0.1	0.79	0.2±0.0	0.9±0.0	0.82

kaolin and soft tan kaolin samples, HF-extractable Fe was mostly in an oxidized ferric state, whereas in the gray kaolins ferrous Fe predominated. The highest Fe(III)/[Fe(II)+Fe(III)] ratio for both total and bio-available Fe measurements was in kaolin samples #7 and #13, in which Fe(III) reduction was the dominant TEAP (Tables 1, 2).

Both Fe(III)-reducing and Fe(II)-oxidizing bacteria were present in the clay samples (Table 4). According to MPN analysis, the clays contained greater numbers of Fe(II)-oxidizing bacteria (22−22,000 cells/g) than Fe(III)-reducing bacteria (3−410 cells/g). Aerobic acidophilic Fe(II)-oxidizing bacteria were found only in gray kaolin sample #5. The greater number of anaerobic nitrate-reducing Fe(II) oxidizing bacteria may in part be a consequence of the medium used for MPN analysis, which contained acetate. The presence of acetate may have promoted growth of both autotrophic bacteria able to couple Fe(II) oxidation to nitrate reduction, as well as heterotrophic bacteria coupling acetate oxidation to the reduction of nitrate to nitrite, followed by chemical oxidation of Fe(II) by nitrite. The abundance of organic matter in the clay samples suggests that such indirect Fe(II) oxidation by heterotrophic bacteria may be an important process in these clays.

In contrast, MPN analysis of Fe(III)-reducing bacteria selected only dissimilatory Fe(III)-reducing microorganisms, which conserve energy to support growth by oxidizing the electron donor provided (hydrogen, acetate or lactate, in our experiments), with the reduction of Fe(III). Therefore, the number of Fe(III)-reducing bacteria might have been underestimated. It is known that microorganisms of other physiological groups, such as fermenting (Lovley, 1991), sulfate-reducing (Lovley et al., 1993; Li et al., 2004), and methanogenic (Bond and Lovley, 2002) bacteria can contribute to Fe(III) reduction as well and all these groups of microorganisms have been found in subsurface kaolin deposits (Turova et al., 1996; Shelobolina et al., 1999).

Fe transformations by indigenous Fe-cycle bacteria

In order to evaluate the extent to which the indigenous bacteria can influence Fe chemistry in Georgia subsurface clays, several model experiments

Table 4. Most probable number analysis for abundance of Fe-cycle bacteria.

Samples		Fe(II)-oxidizing bacteria		Fe(III)-reducing bacteria; Electron donors added		
		Anaerobic, NO$_3^-$-reducing	Aerobic acidophilic	H$_2$	Acetate	Lactate
Smectite	2	$9.3 \times 10^3 \pm 4.2 \times 10^3$	<0.03	<0.03	<0.03	66±66
	14	$2.2 \times 10^2 \pm 8.1 \times 10^1$	<0.03	<0.03	<0.03	5±5
Hard kaolin	13	$2.2 \times 10^3 \pm 8.1 \times 10^2$	<0.03	<0.03	<0.03	11±8
Soft gray kaolin	3	46±17	<0.03	6±3	<0.03	4±2
	5	$4.6 \times 10^3 \pm 1.7 \times 10^3$	$2.2 \times 10^4 \pm 0.8 \times 10^4$	36±21	36±21	410±230
	12	$9.3 \times 10^3 \pm 4.2 \times 10^3$	<0.03	<0.03	13±9	<0.03
Soft tan kaolin	7	22±8	<0.03	3.2±1.8	2.7±1.5	3.2±1.8

were performed. In a first set of experiments, growth of anaerobic Fe(III)-reducing bacteria was stimulated by the addition of the Fe chelator, nitriloacetic acid (NTA) which was used to increase the bioavailability of Fe in the clays and to intensify natural processes. The Fe(III)-reducing activity in the clays was clearly stimulated with the addition of 1 mM NTA, as proven by a decrease in the Fe(III)/[Fe(II)+Fe(III)] ratio (Figure 1). No additional electron donor was required in these experiments because all the clays had sufficient organic matter to support growth (Table 2). In another series of experiments, indigenous anaerobic nitrate-reducing Fe(II)-oxidizing bacteria were stimulated by the addition of both 1 mM NTA and 4 mM nitrate. Addition of nitrate resulted in an increase in the Fe(III)/[Fe(II)+Fe(III)] ratio, indicating stimulation of anaerobic Fe(II) oxidation (Figure 1). There was no valence change in the Fe in sterile controls (data not shown).

The extent to which indigenous microorganisms change the valency of Fe depends on the initial Fe(III)/[Fe(II)+Fe(III)] ratio in clay. The clay samples (#2, #7, #13 and #14) with the highest initial Fe(III)/[Fe(II)+Fe(III)] ratios exhibited maximal Fe(III) reduction and only modest Fe(II) oxidation (Tables 2 and 4). The clay samples with small initial Fe(III)/[Fe(II)+Fe(III)] ratios, on the other hand, exhibited large Fe(II) oxidizing activities and almost no Fe(III) reduction. Kaolin sample #5 with an intermediate Fe(III)/[Fe(II)+Fe(III)] initial ratio showed high levels of both Fe(III)-reducing and Fe(II)-oxidizing activities (Figure 1).

Both MPN analysis of Fe-cycle bacteria and model experiments demonstrate that Georgia subsurface clays contain viable populations of bacteria able to change the oxidation state of Fe in these clays, and suggest that it is likely that bacteria involved in Fe cycling have played a significant role in removal of Fe and organic matter in Georgia subsurface clays.

Another important group of microorganisms that was not a subject of this study but could potentially influence the Fe chemistry of clays is aerobic heterotrophic microorganisms producing Fe(III)-specific chelating agents called siderophores. Iron is an essential micronutrient for most organisms. In oxic neutrophilic environments, in the absence of organic or inorganic chelators, Fe availability is limited by the solubility of Fe hydroxides (Boukhalfa and Crumbliss, 2002). In response to low Fe availability, aerobic microorganisms excrete siderophores to mobilize this metal (Briat, 1992; Kraemer, 2004). One siderophore-producing bacterium, *Pseudomonas mendocina*, was documented to acquire micromolar concentrations of Fe from kaolinite and to grow above the levels of non-kaolin-containing controls (Maurice *et al.*, 2001). In an additional study, bacterial siderophore desferrioxamine B enhanced the release of Fe, Si and Al from kaolinite (Rosenberg and Maurice, 2003). Aerobic heterotrophic microorganisms have been found in large numbers in several kaolin samples
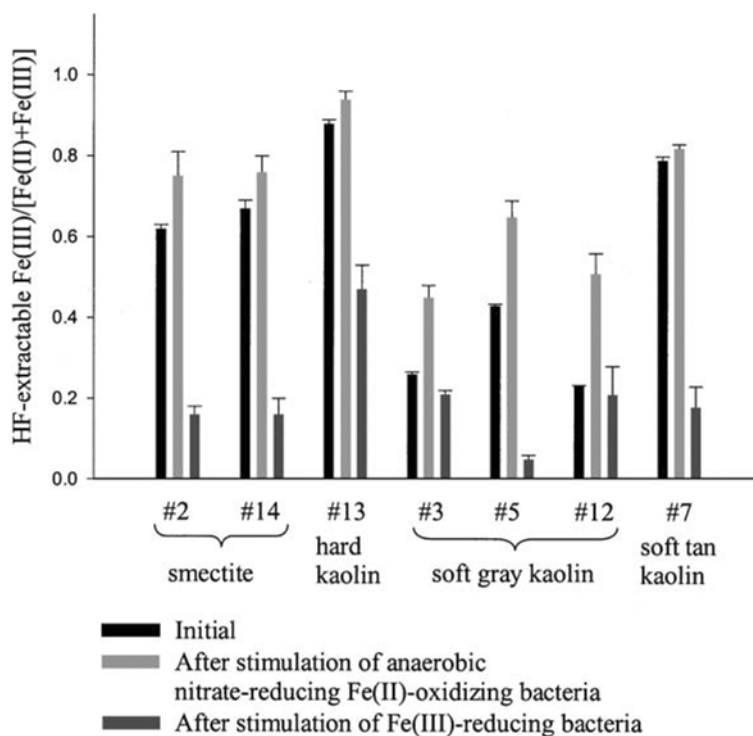

Figure 1. Results of stimulation of indigenous bacteria in clay samples representing the means for duplicate model experiments.

(Turova *et al.*, 1996; Shelobolina *et al.*, 1999) and therefore may be important agents of subsurface clay weathering when conditions are favorable for their growth.

Potential for industrial biotechnology to remove Fe from kaolin

After more than 100 y of intensive kaolin mining in Georgia, most of the high-quality updip clay deposits have already been mined. These naturally whiter clays contained the more easily removable hematite and/or goethite, and most of their dark organic matter had already been oxidized. The industry is now forced to mine in a more downdip direction, where the as-yet unoxidized kaolin is grayer, with more organic matter and pyrite.

The results of the present study may serve as a starting point for development of flexible biotechnology for kaolin beneficiation, where either microbial Fe(III) reduction or Fe(II) oxidation would be stimulated, depending on the Fe mineralogy in the kaolin. For commercial quality kaolin from Georgia, Fe(III) reduction of hematitic and goethitic Fe would enhance the quality of the soft tan and hard kaolins. Although using indigenous Fe(III)-reducing bacteria for kaolin whitening has been proposed in a number of papers (Turova *et al.*, 1996; Avakyan *et al.*, 1997; Lee *et al.*, 1999; Lee *et al.*, 2002), the utilization of indigenous Fe(II)-oxidizing nitrate-reducing bacteria for this purpose has not yet been studied. Our results demonstrate that stimulation of microbial Fe(II) oxidation has the potential to improve the quality of Georgia gray kaolin.

CONCLUSIONS

The results demonstrate that both Fe(III)-reducing and Fe(II)-oxidizing bacteria are present in subsurface clays of differing origin and mineralogy and that they are capable of changing the oxidation state of Fe in the clays. Thus, it is likely that bacteria involved in Fe cycling have played a significant role in the removal of Fe and organic matter in the Georgia subsurface clays. The results of this study show potential for development of new biotechnological methods for improving whiteness of commercially mined kaolin clays by Fe removal.

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REFERENCES

Avakyan, Z.A., Platov, Y.T., Khaliullova, R.A., Turova (Shelobolina), E.S., Karavaiko, G.I., Maslennikova, G.N. and Vodyanitskii, Y.N. (1997) *Method of bleaching of argillaceous ceramic materials*, Patent no. 2083527, Russian Federation.

Balch, W.E., Fox, G.E., Magrum, L.J., Woese, C.R. and Wolfe, R.S. (1979) Methanogens: reevaluation of a unique biological group. *Microbiological Reviews*, **43**, 260–296.

Boivin-Jahns, V., Ruimy, R., Bianchi, A., Daumas, S. and Christen, R. (1996) Bacterial diversity in a deep-subsurface clay environment. *Applied and Environmental Microbiology*, **62**, 3405–3412.

Bond, D.R. and Lovley, D.R. (2002) Reduction of Fe(III) oxide by methanogens in the presence and absence of extracellular quinones. *Environmental Microbiology*, **4**, 115–124.

Boukhalfa, H. and Crumbliss, A.L. (2002) Chemical aspects of siderophore mediated iron transport. *BioMetals*, **15**, 325–339.

Briat, J.-F. (1992) Iron assimilation and storage in prokaryotes. *Journal of General Microbiology*, **138**, 2475–2483.

Chapelle, F.H. and Lovley, D.R. (1990) Rates of microbial metabolism in deep coastal plain aquifers. *Applied and Environmental Microbiology*, **56**, 1865–1874.

Chapelle, F.H., McMahon, P.B., Dubrovsky, N.M., Fujii, R.F., Oaksford, E.T. and Vroblesky, D.A. (1995) Deducing the distribution of terminal electron-accepting processes in hydrologically diverse groundwater systems. *Water Resources Research*, **31**, 359–371.

Elzea Kogel, J., Pickering, S.M., Shelobolina, E.S., Chowns, T.M., Yuan, J. and Avant, D.M. (2002) *The Georgia Kaolins: Geology and Utilization*. Society for Mining, Metallurgy, and Exploration, USA, 96 pp.

Fredrickson, J.K., McKinley, J.P., Nierzwickibauer, S.A., White, D.C., Ringelberg, D.B., Rawson, S.A., Li, S.M., Brockman, F.J. and Bjornstad, B.N. (1995) Microbial community structure and biogeochemistry of Miocene subsurface sediments – implications for long-term microbial survival. *Molecular Ecology*, **4**, 619–626.

Hurst, V.J. and Pickering, S.M. (1997) Origin and classification of Coastal Plain kaolins, southeastern USA, and the role of groundwater and microbial action. *Clays and Clay Minerals*, **45**, 274–285.

Komadel, P. and Stucki, J.W. (1988) Quantitative assay of minerals for Fe^{2+} and Fe^{3+} using 1,10-phenanthroline: III. A rapid photochemical method. *Clays and Clay Minerals*, **36**, 379–381.

Kraemer, S. (2004) Iron oxide dissolution and solubility in the presence of siderophores. *Aquatic Sciences*, **66**, 3–18.

Krumholz, L.R. (2000) Microbial communities in the deep subsurface. *Hydrogeology Journal*, **8**, 4–10.

Lawrence, J.R., Hendry, M.J., Wassenaar, L.I., Germida, J.J., Wolfaardt, G.M., Fortin, N. and Greer, C.W. (2000) Distribution and biogeochemical importance of bacterial populations in a thick clay-rich aquitard system. *Microbial Ecology*, **40**, 273–291.

Lee, E.-Y., Cho, K.-S., Ryu, H.W. and Chang, Y.K. (1999) Microbial removal of Fe(III) impurities from clay using dissimilatory iron reducers. *Journal of Bioscience and Bioengineering*, **87**, 397–399.

Lee, E.-Y., Cho, K.-S. and Ryu, H.W. (2002) Microbial refinement of kaolin by iron-reducing bacteria. *Applied Clay Science*, **22**, 47–53.

Li, Y.-L., Vali, H., Sears, S.K., Yang, J., Deng, B. and Zhang, C.L. (2004) Iron reduction and alteration of nontronite NAu-2 by a sulfate-reducing bacterium. *Geochimica et Cosmochimica Acta*, **68**, 3251–3260.

Lovley, D.R. (1991) Dissimilatory Fe(III) and Mn(IV) reduction. *Microbiological Reviews*, **55**, 259–287.

Lovley, D.R. and Goodwin, S. (1988) Hydrogen concentrations as an indicator of the predominant terminal electron-accepting reactions in aquatic sediments. *Geochimica et*

Cosmochimica Acta, **52**, 2993−3003.

Lovley, D.R. and Phillips, E.J.P. (1988) Novel mode of microbial energy metabolism: organic carbon oxidation coupled to dissimilatory reduction of iron or manganese. *Applied and Environmental Microbiology*, **54**, 1472−1480.

Lovley, D.R., Roden, E.E., Phillips, E.J.P. and Woodward, J.C. (1993) Enzymatic iron and uranium reduction by sulfate-reducing bacteria. *Marine Geology*, **113**, 41−53.

Lovley, D.R., Chapelle, F.H. and Woodward, J.C. (1994) Use of dissolved H_2 concentrations to determine the distribution of microbially catalyzed redox reactions in anoxic ground water. *Environmental Science and Technology*, **28**, 1205−1210.

Maurice, P.A., Vierkorn, M.A., Hersman, L.E. and Fulghum, J.E. (2001) Dissolution of well and poorly ordered kaolinites by an aerobic bacterium. *Chemical Geology*, **180**, 81−97.

Miller, T.L. and Wolin, M.J. (1974) A serum bottle modification of the Hungate technique for cultivating obligate anaerobes. *Appied Microbiology*, **27**, 985−987.

Nelson, D.W. and Sommers, L.E. (1982) Total carbon, organic carbon, and organic matter. Pp. 539-580 in: *Methods of Soil Analysis* (A.L. Page *et al.*, editors). American Society of Agronomy and Soil Science Society of America, Madison, Wisconsin.

Rosenberg, D.R. and Maurice, P.A. (2003) Siderophore adsorption to and dissolution of kaolinite at pH 3 to 7 and 22ºC. *Geochimica et Cosmochimica Acta*, **67**, 223−229.

Shelobolina, E.S., Parfenova, E.Y. and Avakyan, Z.A. (1999) Microorganisms of kaolins and their role in the processes of iron solubilization and transformation. Pp. 559−568 in: *Biohydrometallurgy and the Environment Toward the Mining of the 21st Century* (R. Amils and A. Ballester, editors). Elsevier, Amsterdam.

Stucki, J.W. (1981) The quantitative assay of minerals for Fe^{2+} and Fe^{3+} using 1,10-phenanthroline: II. A photochemical method. *Soil Science Society of America Journal*, **45**, 638−641.

Turova (Shelobolina), E.S., Avakyan, Z.A. and Karavaiko, G.I. (1996) The role of a bacterial community in transformation of iron minerals in kaolin. *Microbiology*, **65**, 837−843.