ABSTRACT

MICROBIAL REDUCTION OF FE(III) IN MULTIPLE CLAY MINERALS BY SHEWANELLA PUTREFACIENS AND REACTIVITY OF BIOREDUCED CLAY MINERALS TOWARD TC(VII) IMMOBILIZATION

by Michael E. Bishop

The reactivity of clay minerals toward technetium immobilization utilizing a suite of clay minerals ranging from smectite-illite including montmorillonite, nontronite, rectorite, mixed layered I-S (70:30), and illite, with chlorite (ripidolite), and palygorskite common in nature. The clay minerals were characterized utilizing multiple techniques. Fe-Oxides were removed prior to bioreduction using a modified dithionite-citrate-bicarbonate method. Fe (II) in the bioreduced clay minerals is used to reduce Tc(VII) to Tc (IV) in PIPES buffer. In the S:I series, the smectite end member was most effective in reducing Tc (VII) and the illite member the least effective, parallel to the extent and rate of Fe(III) bioreduction of these minerals. For all the clay minerals, the ratio of oxidized Fe(II) to reduced Tc(VII) was ~3.5±0.5. These kinetic results are important for our understanding of how various clay minerals may be used to immobilize heavy metal Tc at DOE contaminated sites.
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ABSTRACT

\(^{99}\)Technetium (\(^{99}\)Tc) is a fission product of uranium-235 and plutonium-239 and poses a high environmental hazard due to its long half-life (\(t_{1/2} = 2.13 \times 10^5\) y), abundance in nuclear wastes, and environmental mobility under oxidizing conditions [i.e., Tc(VII)]. Under reducing conditions, Tc(VII) can be reduced to insoluble Tc(IV). Ferrous iron [Fe(II)], either in aqueous form or in mineral form, has been used to reduce Tc(VII) to Tc(IV). [However, the reactivity of Fe(II) from clay minerals, other than nontronite, toward immobilization of Tc(VII) and its role in retention of reduced Tc(IV) have not been investigated.] In this study the reactivity of a suite of clay minerals toward Tc(VII) reduction and immobilization was evaluated. The clay minerals chosen for this study included five members in the smectite-illite (S-I) series, (i.e., montmorillonite, nontronite, rectorite, mixed layered I-S, and illite), chlorite, and palygorskite. Fe-oxides were removed from these minerals with a modified dithionite-citrate-bicarbonate (DCB) procedure. The total Fe content of these clay minerals ranged from 0.7 to 30.4\% by weight, and the Fe(III)/Fe(total) ratio ranged from 44.9 to 98.5\%. X-ray diffraction (XRD) and Mössbauer spectroscopy results showed that the clay minerals after Fe oxide removal consisted of pure phyllosilicates only. Scanning electron microscopy (SEM) revealed that little dissolution occurred during the DCB treatment. Bioreduction experiments were performed in bicarbonate buffer (pH-7) with Fe(III) in the clay minerals as the sole electron acceptor, lactate as the sole electron donor, and \textit{Shewanella Putrifaciens} CN32 cells as mediators. In select tubes, anthraquinone-2,6-disulfate (AQDS) was added as electron shuttle to facilitate electron transfer. The extent of Fe(III) bioreduction was the highest for chlorite (~43 wt\%) and the lowest for palygorskite (~4.17 wt\%). In the S-I series, NAu-2 was the most reducible (~31 \%) and illite the least (~0.4 \%). The extent and initial rate of bioreduction were positively correlated with the percent smectite in the S-I series (i.e., layer expandability). Fe(II) in the reduced clay minerals was used to reduce Tc(VII) to Tc(IV) in PIPES buffer. Similar to the trend of bioreduction, in the S-I series, reduced smectite showed the highest reactivity toward Tc(VII), and reduced illite exhibited the least reactivity. The initial rate of Tc(VII) reduction, after normalization to clay and Fe(II) concentration, was positively correlated with the percent smectite in the S-I series. Fe(II) in chlorite and palygorskite was also reactive toward Tc(VII) reduction. These data demonstrate that crystal chemical parameters (layer expandability, Fe and Fe(II) contents, and surface area etc.) play important roles in controlling the extent and rate of bioreduction and the reactivity
toward Tc(VII) reduction. Reduced Tc(IV) resides within clay mineral matrix, and this association could minimize any potential of reoxidation over long term.

**Key Words:** Smectite, montmorillonite, nontronite, mixed layered I-S, rectorite, illite, palygorskite, chlorite (ripidolite), *Shewanella Putrifaciens* CN-32, Fe(III) bioreduction, Fe(II) oxidation, Tc(VII) reduction, surface area, XRD, mössbauer, SEM, TEM
1. INTRODUCTION

$^{99}$Technetium ($^{99}$Tc) is a fission product of uranium-235 and plutonium-239 and poses a high environmental hazard due to its long half-life ($t_{1/2} = 2.13 \times 10^5$ y), abundance in nuclear wastes, and environmental mobility under oxidizing conditions. $^{99}$Tc is an important contaminant at several DOE sites such as Hanford, Washington; Oak Ridge, Tennessee; and Paducah, Kentucky (Riley and Zachara, 1992). The mobility and bioavailability of Tc is highly dependent on the redox condition in environments. Under oxic condition, Tc occurs as a pertechnetate anion [Tc(VII)O$_4^-$] and is highly soluble. The mobility of Tc is significantly reduced under reducing condition when soluble [Tc(VII)O$_4^-$] is reduced to insoluble TcO$_2$ (Wildung et al., 2000; Zachara et al., 2007). Therefore, in-situ reduction of Tc(VII) has been proposed as an alternative remediation technology to immobilize Tc in environments (Istok et al., 2004).

Ferrous iron [Fe(II)] as either aqueous or adsorbed forms can reduce Tc(VII) to Tc(IV) (Cui & Eriksen, 1996a; Fredrickson, et al., 2004; Burke, et al., 2006). Based on the current understanding, the homogeneous redox reaction between aqueous Fe$^{2+}$ and Tc(VII) can be written as follows:

$$
\text{Tc(VII)O}_4^- + 3\text{Fe}^{2+} + (n+7)\text{H}_2\text{O} = \text{Tc(IV)O}_2 \cdot n\text{H}_2\text{O} + 3\text{Fe(OH)}_{3(s)} + 5\text{H}^+
$$

(Rard et al., 1999). Tc(VII) can also be reduced by Fe(II) associated with solid minerals such as magnetite, nontronite, illite, vermiculite, and natural sediments (Cui and Eriksen, 1996b; Fredrickson et al., 2004; Wildung, et al., 2004; Burke et al., 2005; McBeth et al., 2007; Peretyazhko et al., 2008; Jaisi et al., 2008a, b; 2009). In particular, the important role of structural Fe(II) associated with natural sediments in Tc(VII) reduction and immobilization has been demonstrated (Cui & Eriksen, 1996b; Wildung et al., 2004; Fredrickson et al., 2004; McBeth et al., 2007; Peretyazhko, et al., 2008).

Iron is an important constituent in clay minerals, and the structural Fe(III) can be reduced either chemically or biologically (Dong et al., 2009 and references therein). Previous research in our group has demonstrated that biologically reduced nontronite (NAu-2) has the capacity to reduce Tc(VII) to Tc(IV) (Jaisi et al., 2008a, 2009), and by residing within smectite matrix, the reduced Tc(IV) is resistant to reoxidation. However, at many Tc contaminated DOE sites, other clay minerals are also present such as chlorite, illite, and various mixed-layer illite-smectite (I-S).
(Fredrickson et al., 2004; Qafoku et al., 2003; Kukkadapu et al., 2006). Therefore, it is important to extend our early results to other important clay minerals.

The objective of this study was therefore to study: 1) Microbial reduction of structural Fe(III) in multiple clay minerals commonly present at DOE contaminant sites and 2) Abiotic reduction of Tc(VII) using Fe(II) in bioreduced clay minerals. To achieve our objectives a suite of clay minerals were chosen including: montmorillonite, nontronite, rectorite, mixed-layer I-S (70:30), illite, chlorite (ripidolite), and palygorskite. These clay minerals are commonly present in natural environments, but they have not been thoroughly studied in terms of biological reduction and subsequent reactivity of reduced clay minerals toward Tc(VII) reduction. A combination of methods were employed to study microbial reduction of Fe(III) in these minerals and their reaction with Tc(VII). Our results indicate that the selected clay minerals contained different amounts of structural Fe(III), and once reduced, the resulting biogenic Fe(II) was reactive toward Tc(VII) reduction. The rate/extent of bioreduction and the reactivity of the reduced minerals were largely controlled by their physical and chemical properties including structure (layer expandability), chemical composition [total Fe and Fe(II) contents], and surface area.

2. MATERIALS AND METHODS

2.1. Mineral preparation

2.1.1. Clay minerals

Seven of the nine clay minerals were purchased from the Source Clays Repository of the Clay Minerals Society (West Lafayette, IN). A suite of five members in the S-I series were selected: montmorillonite (SWy-2); nontronite (NAu-2); rectorite (RAr-1); 70:30 mixed-layer illite-smectite (ISCz-1); and illite (IMt-1). The SWy-2 and NAu-2 samples represent the smectite end member with a minimal and maximal Fe content, respectively. The IMt-1 sample is the illite end member. Two additional clay minerals, palygorskite (PFl-1) and chlorite (CCa-2) were selected based on their abundance in nature and for comparison with the smectite-illite minerals. Two additional clay minerals were supplied by Warren Huff, University of Cincinnati, Cincinnati, Ohio. These two additional clay minerals, 35:65 mixed-layer illite-smectite (ARG-6) and 85:15 mixed-layer illite-smectite (ARG-24) were selected to complement the S-I series.
Montmorillonite (SWy-2) is an iron poor variety of smectite from Crooke County, Wyoming, USA. Nontronite (NAu-2) is from the Uley graphite mine, South Australia (Keeling et al., 2000) and is an iron-rich variety of smectite. The NAu-2 sample contains 23.4 wt% Fe(tot) in its structure with almost all (99.4%) iron as Fe(III) (Jaisi, et al., 2005; Jaisi, et al., 2007b). The majority of structural Fe(III) in NAu-2 is contained within the octahedral layers (92%) and the remaining Fe(III) in the tetrahedral layers (8%) (Gates, et al., 2002; Jaisi, et al., 2005). Illite-smectite mixed-layer clay (ARG 6) is an ordered 35:65 illite-smectite mixed-layer clay mineral. Rectorite (RAr-1) was discovered at Jeffrey Quarry, North Little Rock, Pulaski County, Arkansas, USA, by Miser and Milton (1964). Rectorite is a 1:1 regularly interstratified mixed-layer clay mineral. Illite-smectite mixed-layer clay (ISCz-1) was originally found in Czechoslovakia and it is an ordered 70:30 illite-smectite mixed-layer clay mineral. Illite-smectite mixed-layer clay (ARG 24) was provided by Warren Huff, University of Cincinnati, Cincinnati Ohio. It is an ordered 15:85 illite-smectite mixed-layer clay mineral. Illite (IMt-1) is from a Cambrian shale of Silver Hill, Montana, USA (Hower and Mowatt, 1966) and it is an iron rich variety of illite. Palygorskite (PFl-1) is from the Hawthorn Formation in the Meigs-Attapulgus-Quincy district of the Georgia-Florida border. Ripidolite (CCa-2) is from Flagstaff Hill, El Dorado County, CA, USA. According to a proposed structural model for ripidolite (Brandt, et al., 2003), and all of the Fe(III) is located in the TOT layer (Zhang, et al., in press).

The bulk clay minerals were ground to powder and sonicated for 8 hrs in a water bath to disperse aggregated particles. The 0.02-0.5 µm size fraction was separated utilizing repeated centrifugation and re-suspension in 30 mM bicarbonate buffer (2.5 g/L sodium bicarbonate, 0.1 g/L KCl). This size fraction was selected to mimic typical clay particle size in natural environment and to compare the results of this study with those published previously in our group (Jaisi et al., 2005; 2007a; b; 2008a; b; 2009).

2.1.2. Fe oxide removal

X-ray diffraction (XRD) analyses of the 0.02-0.5 µm size fraction indicated that various amounts of iron oxide impurities were present. Thus, a dithionite-citrate-bicarbonate (DCB) procedure as outlined by Stucki et al. (1984) was performed to remove them so that bioreduction of pure clay minerals could be studied. As controls of the DCB treatment the following tests were performed: interlayer stabilization test, and a Fe-clay mineral dissolution test. The
interlayer stabilization test was conducted to ensure that the interlayer of the clay minerals stayed expanded during the DCB treatment. Solutions of calcium chloride (CaCl$_2$) and magnesium chloride (MgCl$_2$) at concentrations of 0, 0.05, 0.1, 0.2, 0.3, and 0.4 M were separately used to homoionize nontronite NAu-2. Both salts expanded the interlayer of NAu-2 to the same extent (see below), and therefore 0.1M MgCl$_2$ was chosen for the expansion of all seven clay minerals during the DCB treatment. The Fe-clay mineral dissolution test was conducted to optimize the DCB treatment time so as to remove all Fe oxides but to minimize chemical reduction of structural Fe(III) in the phyllosilicates and to minimize any clay mineral dissolution. Artificial mixtures of Fe-oxide free NAu-2 and synthetic ferrihydrite (0.02 - 0.5 µm) (Fe(OH)$_3$), which was produced by adding 40g Fe(NO$_3$)$_3$ to 500 ml H$_2$O, then adding 330 ml of 1M KOH to adjust pH to 7.8 (Schwartzman and Cornell, 1991). The mixture of NAu-2 and ferrihydrite was then subjected to the DCB treatment, where solid samples were taken at five minute intervals and analyzed with XRD to determine Fe-oxide removal. XRD results indicated that upon completion of a ten minute DCB reaction, the ferrihydrite was completely removed.

The above control experiment determined that 10 mins reaction time was sufficient for Fe-oxide removal (see below). Therefore, all seven clay mineral suspensions in bicarbonate buffer were reacted with DCB in Erlenmeyer flasks for 10 mins under continuous purge with anoxic N$_2$. After the reaction, the flasks were placed in an ice bath to stop the DCB reaction. The flasks were then passed into an anaerobic glove box (Coy Laboratory Products, Grass Lake, MI) and the clay mineral suspension from each flask was dispensed into 20 ml headspace vials. The vials were sealed with thick rubber stoppers and centrifuged (@ 2,500g, 4°C for 60 mins). The supernatant was discarded, and the pellet was resuspended in an anoxic 1M KCl solution and centrifuged (@ 2,500g for 60 mins) to wash off any excess dithionite from the clay mineral. Discarding the supernatant, the pellet was re-suspended in 10 ml of an anoxic 0.5N HCl solution for 2 hrs to remove any aqueous Fe$^{2+}$ (from reduction of ferric Fe oxides) and centrifuged (@ 2,500g for 60 mins). The pellet was re-suspended sequentially in 1, 0.1, and 0.01 M KCl solution to homoionize the interlayer cation. Each resuspension lasted overnight followed by centrifugation. Excess KCl was removed by repeated washing with anoxic sterile DI water and complete removal was confirmed by the AgNO$_3$ test.

Despite our effort, the DCB treatment could have reduced both oxide-Fe(III) and structural Fe(III) of clay minerals. For this reason, the DCB-treated clay minerals were
reoxidized with an aquarium air pump (for 96 hrs) to restore the original structural Fe(III). The DCB-treated, reoxidized clay minerals will be referred to as clean clay minerals hereafter. The reoxidation time of 96 hrs was determined to be sufficient. The complete removal of iron oxides and any alteration to the phyllosilicates were confirmed by XRD, Mössbauer spectroscopy, and scanning electron microscopy (SEM).

2.1.3. BET surface area analyses

The BET surface area of the clay minerals was analyzed utilizing a Beckman Coulter model SA-3100 BET surface area and pore size analyzer (BET SA-3100). The clay minerals were homoionized with 0.1M NaCl for 24 hr in order to prevent the interlayer from collapsing during the outgassing procedure. The homoionized clay minerals were outgassed at 25°C for 15 minutes under nitrogen atmosphere. The samples were then analyzed with helium injection at varying pressures in a liquid nitrogen bath.

2.2. Bioreduction Experiments

2.2.1. Bacterial culture

*Shewanella putrefaciens* strain CN32 cells were routinely cultured aerobically in tryptic soy broth (TSB) from frozen stock culture, which was kept in 35% glycerol at -80°C. After harvesting in TSB until the mid- to late-log phase, CN32 cells were washed three times in filter-sterilized bicarbonate buffer by repeated centrifugation and resuspension. Final cell suspension was prepared in filter-sterilized bicarbonate buffer, and cell concentration was measured by colony forming units (CFU).

2.2.2. Bioreduction

Bioreduction experiments were performed with Fe(III) in pasteurized (80°C for 3 h, 3 times ea.) clean clay minerals (7 g/L final conc.) as the sole electron acceptor and filter-sterilized lactate (0.2 mM final conc.) as the sole electron donor in filter-sterilized bicarbonate buffer with a final CN32 cell concentration of ~1.92 x 10^7 cells/mL. In select tubes filter-sterilized anthraquinone-2, 6-disulfonate (AQDS) (0.1 mM final conc.) was added as an electron shuttle to facilitate electron transfer from lactate to Fe(III) in the clay minerals. The control tube consisted of an equal volume of bicarbonate buffer in place of CN32 cells.
All components of bioreduction including bicarbonate buffer, AQDS, lactate, and stock clean clay mineral suspensions were added to autoclaved 25-mL Balsch tubes, and sealed with thick butyl rubber stoppers. CN32 cells were washed with anoxic 30 mM bicarbonate buffer prior to addition to the bioreduction tubes with a 1cc syringe, which had been purged 15 times with anoxic N₂:CO₂ gas mix (80:20). The tubes were then incubated at 37°C with shaking at 60 rpm. All treatments and measurements were carried out under strictly sterile and anoxic conditions. All experiments were run in duplicate and an average was obtained for time-course Fe(II) concentration measurements. The Fe(II) concentration was tested at select time points in order to determine the extent of reduction utilizing a modified 1,10 phenanthroline method (Amonette and Templeton, 1998). Once the Fe(II) concentration leveled off, the bioreduction experiment was stopped by means of pasteurization (at 80°C for 1 h, 3 times ea.). The reduced clay minerals were characterized by XRD, Mössbauer spectroscopy, SEM and transmission electron microscopy (TEM).

2.3. Tc(VII) Reduction by Fe(II) Associated with the Clay Minerals

Technetium was acquired from Perkin Elmer as ammonium pertechnetate solid (NH₄⁹⁹TcO₄) with a 99.998% purity with a specific activity of 1.679 µCi/µmol. NH₄⁹⁹TcO₄ was dissolved in 1 ml 0.4 M NH₄OH and sonicated for 4 hrs to ensure complete oxidation of technetium (Sekin and Zakir, 2008). The solution was then centrifuged (@14,000 g for 10 mins) and the supernatant was filtered with 0.01 µm syringe filter to remove any undissolved ⁹⁹Tc precipitates. The aqueous solution of Tc(VII) was analyzed for purity with liquid scintillation analysis (LSA) and diluted to 1 mM with 30 mM PIPES (pH 7). This 1mM Tc(VII) solution was used as stock for all subsequent experiments.

The abiotic Tc(VII) reduction experiments were performed utilizing Fe(II) in the washed and pasteurized bioreduced clay minerals as the sole electron donor. The reduced clay mineral stock suspensions, Tc(VII) stock solution, and 30 mM PIPES buffer at pH 7, were combined in various proportions (Table 1) to achieve a final concentration of Tc(VII) of 50 µM and of Fe(II) at 0.5-1.05 mM. The Fe(II) concentration was in excess of Tc(VII) according to the theoretical ratio of 3:1. Two control experiments were designed. The first control was to determine if 30 mM PIPES could reduce any Tc(VII)O₄⁻ by adding 0.5 ml of 1mM Tc(VII)O₄⁻ to 9.5 ml of 30 mM PIPES in absence of any clay minerals. The second control was to determine if Fe(II) in the
clay mineral underwent any redox cycling without any Tc(VII). This control experiment was accomplished by adding the clay minerals (at the same Fe(II) concentration as that in the experimental tubes, Table 1) to 30 mM PIPES buffer but without any Tc(VII) solution. Change of Fe(II) concentration with time was monitored. All experiments were performed in 20 mL glass bottles inside a glove box with a modified oxygen trap (Jeon et al., 2004). Instead of a regular inlet into the first O₂ removal bottle, a Tetra Whisper 20 gallon aquarium air pump was used to drive the glove box gas into the bottle. The tubing between the air pump and the first reactor had a one-directional filter system to ensure that the solution from the first O₂ removal bottle did not back flow. With this modified oxygen trap, the O₂ level in the glove box was below the detection limit of a Coy gas analyzer (1 ppm) and even below the limit detected by CHEMets1 colorimetric analysis kit R-7540 (2.5 ppb sensitivity). The glass bottles were capped with thick butyl rubber stoppers and crimp-sealed. The experimental bottles were slightly and carefully hand-shaken once a day. The Fe(II) concentration was tested at select time points in order to determine the extent of oxidation utilizing a modified 1,10 phenanthroline method. Residual Tc(VII) concentration in aqueous solution at the same time points was monitored by LSA.

Upon exhaustion of the Tc(VII) as indicated from the LSA results, a spike of 0.5 µM of Tc(VII) was added to the previous Tc reaction bottles. This procedure was repeated until there was no more Tc(VII) reduction, and hence the reduction capacity of the clay mineral was calculated. Upon the conclusion of the Tc(VII) reduction experiment, Tc(VII)-reacted clay minerals were observed by SEM and TEM.

2.4. Analyses
2.4.1. Determination of elemental composition by direct current plasma emission spectroscopy (DCP)

The unreduced, DCB treated clay minerals were heated at 60°C overnight to ensure that the samples were completely dry. Three hundred milli-grams of lithium metaborate (LiBO₂) flux were added to 100 mg clay mineral sample. The flux-sample mixture was fused at 995°C in a carbon crucible for 10 mins. The molten mixture was added to 100 g HNO₃, placed on a shaker table overnight, and analyzed by DCP for elemental composition. The following major elements
were analyzed: SiO$_2$, TiO$_2$, Al$_2$O$_3$, Fe$_2$O$_3$, MnO, MgO, CaO, Na$_2$O, K$_2$O, and P$_2$O$_5$. Based on these analyses, structural formulae of the clay minerals were calculated.

2.4.2. Determination of Fe(II) and Fe(III) by titration and 1,10-phenanthroline methods

The unreduced clean clay minerals were analyzed by chemical extractions (Andradae et al., 2002) and DCP (Katoh et al., 1999) to determine the Fe(II) and total Fe ($Fe_{tot}$) contents, respectively. The Fe(III) concentration was determined by the difference between the $Fe_{tot}$ and Fe(II) concentrations. The Fe(II) and Fe(III) concentrations were also determined by the 1,10-Phenanthroline method (Amonette & Templeton, 1998).

2.4.3. Aqueous concentration measurements

Aqueous concentrations of P, Mg, Si, Mn, Fe, Ti, Al, Ca, K, and Na were measured by DCP to monitor any reductive dissolution after filtration of the abiotic controls and bioreduced clay mineral through a 0.22 μm filter and addition of an equal volume of 1 N HCl (1:1 ratio).

2.4.4. Cell counting

At selected time points over the course of the bioreduction experiments, CN32 cells were counted with CFU. Approximately 0.1 ml of the clay-cell suspension was taken from the experimental tubes with a sterile syringe needle and serially diluted to 8 fold. Select dilutions were smeared on TSB agar plates and CFU were numerated after 24 hrs of incubation in air.

2.4.5. Liquid scintillation analysis (LSA):

The Tc(VII) reduction progress was monitored by measuring time-course decrease of aqueous Tc(VII) concentration because the reduced Tc(IV) was a solid. A phase separation between aqueous solution [i.e. Tc(VII)] and solids [clay mineral and Tc(IV)] was carried out by centrifugation. Seventy micro-liters of the supernatant containing soluble Tc(VII) were pipetted into 10 ml of cocktail (Opti-fluor). After homogenization for 4 hrs, the activity of Tc(VII) was measured by a liquid scintillation counter (Perkin Elmer, Tri-Carb 2800TR LSA, Waltham, Massachusetts). All experiments were performed in duplicate.
2.4.6. XRD

Clay mineral smear mounts (Moore and Reynolds, 1997) were prepared on petrographic slides and air-dried overnight at 30°C. For the bioreduced samples, they were dried at 30°C inside a glove box incubator. XRD patterns were collected on both untreated samples and those solvated with ethylene glycol (EG) vapor @ 60°C overnight (Moore and Reynolds, 1997) to expand smectite interlayers. The same slides were subsequently placed in a desiccator at room temperature for 24 hrs to allow further time for EG vapor to penetrate into the smectite interlayers. XRD patterns were obtained immediately after saturation without significant exposure to air in a humidity-controlled laboratory. Powder XRD patterns were collected utilizing a Scintag X1 x-ray powder diffractometer, using CuKα radiation, a fixed slit scintillation detector, and a power of 1,400 W (40 kV, 35 mA). Scans were collected over a range of 2°-70° 2-Theta. Qualitative identification of mineral phases was made utilizing the Jade 7 program. The Jade 7 program utilizes the International Center for Diffraction Data Powder Diffraction File database (ICDD PDF-2, Sets 1-46, 1996) as a reference source.

2.4.7. Mössbauer spectroscopy

The clay mineral samples before and after the DCB treatments were studied by Mössbauer spectroscopy to confirm complete removal of iron oxides. The details of absorber preparation and instrumentation were identical to those reported by Kukkadapu et al. (2004).

2.4.8. Scanning electron microscopy:

Clay suspensions (0.5 ml) were mounted on a 15 mm cover slip pretreated with poly-L-lysine for 15 min. followed by fixation and sequential dehydration (Dong et al., 2003b). The cover slips were mounted onto a SEM stub via clear double-sided sticky tape and carbon-coated. The samples were analyzed with a Zeiss Supra 35 VP SEM with EDAX Genesis 2000 X-ray energy dispersive spectroscopy (SEM/EDS) using 15 KeV accelerating voltage and 8.5 mm working distance. The EDS spectra provided a primary means for mineral identification. Back scattered electron (BSE) imaging was utilized to further determine the presence or absence of Fe oxides in the clay mineral samples after the DCB treatment.
2.4.9. High resolution transmission electron microscopy

Clay suspension samples, after 8-fold dilutions, were pipetted onto 300 mesh copper grids with a nitrocellulosic membrane and carbon coating. The grids were prepared and allowed to dry overnight in an anaerobic glove box. The grids were placed in anaerobic vials for transportation to the TEM.

TEM imaging was performed with a JEOL JEM-2100 LaB$_6$ transmission electron microscope with a 200 keV accelerating voltage. The bright-field imaging mode (TEM BF) was used to study the morphology of the clay samples. TEM images were recorded using a Gatan 833 Orius camera attached on a Gatan GIF Tridiem Post-Column Energy Filter EELS/EFTEM (Gatan Image Filter). Selected area electron diffraction (SAED) patterns were obtained for mineral identification and micro-structure analyses. Furthermore, scanning TEM (STEM) and EDS were employed to study elemental distribution of the bioreduced samples to determine reduced Tc(IV) in relation to other elements.

3. Results

3.1. XRD of the original clay minerals

XRD analyses showed that the non-DCB treated, unreduced clay mineral size fractions (0.02-0.5 μm) contained varying amounts of Fe-oxides (Figure 1). XRD did not detect any Fe oxides in montmorillonite (SWy-2), nontronite (NAu-2), and illite (IMt-1) (Figure 1A, 1B, 1E). Rectorite (RAr-1) contained a minor amount of goethite (Figure 1C), the illite-smectite mineral (ISCz-1) maghemite ($\gamma$ Fe$_2$O$_3$) (Figure 1D), palygorskite (PFl-1) magnetite (Figure 1F), and ripidolite (CCa-2) maghemite (Figure 1G).

3.2. Fe-oxide removal

Both MgCl$_2$ and CaCl$_2$ expanded the interlayer of NAu-2 to the same extent (Figure 2), and therefore 0.1M MgCl$_2$ was chosen for the expansion of all seven clay minerals in the DCB treatment. The ferrihydrite control test determined that 10 mins reaction time was sufficient for Fe-oxide removal (Figure 3). Therefore, all seven clay minerals underwent a 10-min DCB treatment.
SWy-2 did not show a net change of Fe(III) after DCB treatment and Fe(II) reoxidation (Figure 4), consistent with lack of any Fe oxides in this mineral. However, NAu-2 and IMt-1 lost 6 and 11% of Fe(III) upon the DCB treatment, respectively, and reoxidation did not increase any amount of Fe(III), suggesting that a small amount of oxide-Fe(III), non-detectable by bulk XRD, was reduced and removed. There was a minimal amount of alteration to the phyllosilicate-Fe(III). RAr-1, ISCz-1, and PFl-1 lost 26%, 18%, and 12% of Fe(III), respectively, after the DCB treatment, and upon reoxidation, there was no gain of Fe(III) (Figure 4). These data suggest that the Fe(III) loss was due to removal of Fe(III) oxides, not due to dissolution of phyllosilicates. CCa-2 lost 80% of Fe(III) after the DCB treatment and upon reoxidation, gained 36% (Figure 4). The initial loss was possibly due to removal of Fe oxides and reduction of structural Fe(III) of ripidolite. Upon reoxidation, some of structural Fe(II) in ripidolite was likely converted back to Fe(III). The DCB treated, reoxidized clay minerals were also analyzed for total Fe and Fe(II) contents by DCP and the results were consistent with those measured by the 1,10-phenanthroline method.

XRD patterns of the DCB treated, reoxidized clay minerals did not show any Fe oxides, suggesting that the DCB treatment was successful in removing any Fe oxides (Figure 1). Mössbauer data confirmed these results in showing that there were no sextets (Figure 5) indicating absence of any Fe oxides in the DCB-treated, reoxidized clay minerals. However, the RAr-1 clay mineral (Figure 5B) exhibited an unusual spectrum, which should be further studied. SEM observations for two representative clay minerals (Figure 6) were in agreement with the XRD and Mössbauer results. Fe oxides were absent in SWy-2 before (data not shown) and after DCB treatment (Figure 6A). The untreated ISCz-1 contained some Fe-oxides (Figure 6C), but they were removed by the DCB treatment (Figure 6D). No obvious dissolution textures were observed on DCB treated samples.

3.3. Clay mineral characterization

The chemical composition of SWy-2 as determined by DCP in this study is:

\[(\text{Ca}_{0.16}\text{Na}_{0.24})\text{[Al}_{1.45}\text{Fe}^{2+}_{0.01}\text{Fe}^{3+}_{0.12}\text{Mg}_{0.44}]\text{[Si}_{6.73}\text{Al}_{1.27}}\text{O}_{20}(\text{OH})_{4}\]

The measured total iron content was 2.27 wt%, lower than the reported value of 3.73% in literature (Vogt et al., 2002), possibly due to different size fractions used between our study.
(0.02-0.5 μm) and the Vogt et al. study (<2 μm) and sample inhomogeneity. The ferrous Fe content was 2.61% of the total iron (Table 2).

The chemical composition of NAu-2 is:
\[ \text{(Ca}_{0.28}\text{Na}_{4.56}\text{K}_{0.42})\text{(Al}_{0.23}\text{Fe}^{2+}_{0.06}\text{Fe}^{3+}_{3.76}\text{Mg}_{0.10}\text{Ti}_{0.06})\text{(Si}_{7.19}\text{Al}_{0.81}\text{Fe}_{0.33})\text{O}_{20}(\text{OH})_{4} \]
The Fe(tot) content of NAu-2 size fraction was 21.23 wt% with an Fe(II) content of 1.53 % (Fe(II)/Fe(tot)) (Table 2). These contents were consistent with reported values (Jaisi et al., 2005).

The chemical composition of RAr-1 is:
\[ \text{(Ca}_{0.05}\text{Na}_{3.71}\text{K}_{0.42})\text{(Al}_{0.14}\text{Fe}^{2+}_{0.20}\text{Fe}^{3+}_{1.63}\text{Mg}_{0.02}\text{Ti}_{0.06})\text{(Si}_{6.30}\text{Al}_{1.70})\text{O}_{20}(\text{OH})_{4} \]
The total iron content measured by DCP was 4.91 wt%, and 22.61% of the total iron was Fe(II) (Fe(II)/Fe(tot)) (Table 2).

The chemical composition of ISCz-1 is:
\[ \text{(Ca}_{0.06}\text{Na}_{2.47}\text{K}_{0.92})\text{(Al}_{1.18}\text{Fe}^{2+}_{0.03}\text{Fe}^{3+}_{0.18}\text{Mg}_{0.24}\text{Ti}_{0.02})\text{(Si}_{4.44}\text{Al}_{0.66})\text{O}_{20}(\text{OH})_{4} \]
The total iron content (Fe(tot)) has been reported to range from 0.97 wt% (Gailhanou, et al., 2007) to 1.32 wt% (Vogt, et al., 2002). The total Fe content measured in this study was 2.27 wt%, and 2.61% of the total iron was Fe(II) (Fe(II)/Fe(tot)) (Table 2). Our measured total Fe content was higher than published values of 0.97 wt% (Gailhanou et al., 2007), possibly due to the different size fractions used in the published studies (<2 μm) and our study (0.02 - 0.5 μm) and sample inhomogeneity.

The chemical composition of IMt-1 was:
\[ \text{(Ca}_{0.20}\text{Na}_{2.55}\text{K}_{1.08}\text{H}_{2}O)\text{(Al}_{0.07}\text{Fe}^{2+}_{0.23}\text{Fe}^{3+}_{2.24}\text{Mg}_{0.27}\text{Mn}_{0.01}\text{Ti}_{0.01})\text{(Si}_{3.52}\text{Al}_{0.48})\text{O}_{10}(\text{OH})_{2}(\text{H}_{2}O) \]
The total Fe content in this study was 12.29 wt%, higher than published value of 6.35% (Gailhanou et al., 2007), whereas the measured (Fe(II)/Fe(tot)) ratio of 10.00% (Table 2) was lower than published value of 20.47% (Gailhanou, et al., 2007). Again this difference was contributed to different size fractions used between this study (0.02-0.05 μm) and that published study (<2 μm) and/or sample inhomogeneity.

The chemical composition of PFl-1 is:
\[ \text{[(Ca}_{0.36}\text{Na}_{5.04}\text{K}_{0.49})\text{[(Al}_{1.45}\text{Fe}^{2+}_{0.05}\text{Fe}^{3+}_{0.65}\text{Mg}_{1.70}\text{Ti}_{0.08})\text{(Si}_{7.80}\text{Al}_{0.20})\text{O}_{20}(\text{OH})_{2}\cdot8\text{H}_{2}O}] \]
The total Fe content measured in this study was 1.69 wt%, lower than reported value of 3.00 wt% (Vogt et al., 2002). The different size fraction used between the Vogt et al. study (<2 μm) and our study may have accounted for this difference. The Fe(II) (Fe(II)/Fe(tot)) content was 6.84% (Table 2).
The chemical composition of CCa-2 is:

\[
[(\text{Mg}_{0.46}\text{Fe}^{2+}_{1.50}\text{Fe}^{3+}_{3.43}\text{Ti}_{0.44}\text{Mn}_{0.03})(\text{Si}_{5.51}\text{Al}_{2.49})\text{O}_{20}(\text{OH})_4] \cdot [\text{Mg}_4\text{Al}_{1.81}(\text{OH})_{12}]
\]

The total Fe content of 30.42 wt% fell with the reported values of 17.6 (Brandt et al., 2003) and 34.5% (Jaisi et al., 2007). Similarly, the measured (Fe(II)/Fe(tot)) ratio of 55% (Table 2) fell with the range of 54% (Jaisi, et al., 2007), 76% (Brandt, et al., 2003) to 86% (Keeling, et al., 2000).

In summary, the Fe(tot) content ranged from 0.74 to 21.23 wt% in the S-I series with NAu-2 containing the highest amount of Fe(tot), and ISCz-1 having the least (Table 2). Ripidolite (30.42 wt%) contained the most amount of Fe(tot) among the non S-I clay minerals. The Fe(II)/Fe(tot) ratio ranged from 1.53 to 22.61% with NAu-2 having the lowest, and RAr-1 and ISCz-1 the highest. The Fe(II)/Fe(tot) ratio ranged from 6.84 to 55.15% for the non S-I clay minerals.

For the S-I samples, the BET total surface area ranged from 5.13 to 271.30 m\(^2\)/g (Figure 7) and showed a positive correlation with the percent of smectite with smectite exhibiting the highest BET total surface area, and illite the lowest. This type of correlation has been observed for these minerals (Srodon et al., 2009).

### 3.4. Microbial Reduction of Fe(III) in Clay Minerals

*S. putrefaciens* CN32 strain coupled reduction of Fe(III) in the clay minerals with lactate oxidation. The rate of microbial reduction was fast in the first 3-6 days (depending on specific clay mineral) and gradually decreased with time. In general, the bioreduction experiments were complete in two weeks. Abiotic controls did not show any bioreduction.

Relative to the treatment without AQDS, AQDS stimulated Fe(III) reduction to various extents depending on the nature of each clay mineral (Figure 8). For the smectite end-member (Figure 8A & B), AQDS stimulated the extent of bioreduction by as much as 55%, whereas for the illite end member, AQDS stimulated the extent of bioreduction by ~29% (Figure 8E). AQDS also significantly stimulated the extent of bioreduction of Fe(III) in PFl-1 (Figure 8F), but its effect on CCa-2 bioreduction was negligible (Figure 8G). For the samples in the S-I series, the extent of bioreduction ranged from 0.2 to 18.5 % without AQDS, and from 0.4 to 42.7 % with AQDS (Figure 8A-E). The extent of bioreduction in this series was positively correlated with the percent smectite in the S-I series, with smectite exhibiting the highest extent of reduction,
and illite the lowest (Figure 9A). Likewise, the initial rate of bioreduction (mmol per gram of NAu-2 per hr), calculated from the first two hours, was positively correlated with the percent smectite in the series, with smectite having the highest rate and illite the lowest (Figure 9C).

Because the BET surface area was positively correlated with the percent smectite in the S-I series (Figure 7), the extent of bioreduction was also positively correlated with the BET surface area (data not shown). Likewise, the initial rate, calculated from the first two hours, was positively correlated with the BET total surface area (data not shown). However, when the extent and the initial rate was normalized to the measured BET surface, the correlations no longer held (Figure 9B & D), suggesting that the positive correlation could largely be accounted for by BET surface area.

The extent of bioreduction in NAu-2 was 17.4 % and 38.5 % without and with AQDS, respectively. These values were consistent with previously reported values of 20.26 % and 40.53 % for this mineral (Jaisi et al., 2007a). The initial rate of bioreduction in NAu-2 was 7.86 µmol/g/hr, also consistent with previous results (Jaisi et al., 2007a; Dong et al., 2009). The extent of bioreduction of palygorskite (PFl-1) was low, 2.65 % and 4.17 % without and with AQDS, respectively. The initial rate of bioreduction in PFl-1 was 20.29 µmol/g/hr, much higher than the other clay minerals such as nontronite (7.86 µmol/g/hr) or CCa-2 (14.44 µmol/g/hr). The extent of bioreduction for CCa-2 was 40.37% and 42.95% without and with AQDS, respectively, much higher than previously reported values of 6.12 % and 12.24 % for this mineral (Jaisi et al., 2007a), possibly due to different CCA-2 concentrations used (7 vs. 2 g/L). Another possibility was that the DCB treatment may have partially altered the CCa-2 structure.

In general, the CFU for all experiments decreased from ~1.92 x 10^7 cells/mL at the beginning of the bioreduction experiments to ~1.35 x 10^7 cells/mL at the end (Figure 10). The bioreduced clay minerals were analyzed for aqueous concentrations of P, Mg, Si, Mn, Fe, Ti, Al, Ca, K, and Na by DCP to measure possible clay mineral dissolution. Overall, the bioreduced clay minerals released higher amounts of Al, Fe, K, Mg, and Si than the unreduced controls (Table 3), suggesting that there was a certain amount of reductive dissolution. XRD of the bioreduced clay minerals did not show any discernable changes in the peak patterns, even for ripidolite (CCa-2), which showed the largest amount of bioreduction (Figure 1). Even when SEM and the aqueous DCP results suggested that there was some reductive dissolution for ISCz-1, the corresponding XRD pattern did not show any discernable change. SEM of the bioreduced
clay minerals exhibited some mineralogical changes. In comparison with the unreduced material (Figure 6A), bioreduced SWy-2 (Figure 6B) showed some flake-like crystals. The EDS composition showed Na, K, Ti, Al, Si, and O, suggesting that it is a smectite, possibly newly precipitated, as has been observed in another study (Dong et al., 2003b). ISCz-1 exhibited the most drastic change as a result of bioreduction. Euhedral flakes emerged after bioreduction (Compare Figure 6D and 6E) and the EDS analyses identified these minerals as smectite. These dissolution features were consistent with the DCP results (Table 3) which suggested that ISCz-1 underwent the largest amount of reductive dissolution, but the newly formed and residual ISCz-1 appeared to be the same as evidenced by similar XRD patterns for unreduced and bioreduced samples (Figure 1).

3.5. Abiotic reduction of TcO$_4^-$ by Fe(II) in the clay minerals:

The Tc(VII) concentration in absence of any clay minerals decreased after 800 hrs and this decrease was ascribed to abiotic reduction of Tc(VII) by PIPES buffer (Yang et al., unpublished data). Therefore, all Tc reduction experiments were run for no more than 800 hrs. In case longer time was needed, PIPES buffer was changed at approximately 600 hrs.

The rate and extent of Tc(VII)O$_4^-$ reduction varied depending on clay mineral type and the amount of biogenic Fe(II) within each clay mineral. In the S-I series complete Tc(VII) reduction occurred between ~1 to ~12 days at pH 7 (Figure 11). NAu-2 reduced the Tc(VII) the fastest (Figure 11B) and illite the slowest (Figure 11E). In the non-S-I clays complete Tc(VII) reduction occurred between ~9 to ~15 days with PFl-1 (Figure 11F) reacting faster than CCa-2 (Figure 11G). After exhaustion of the first spike of Tc(VII), additional Tc(VII) was spiked until there was no more Tc(VII) reduction upon further spiking (Figure 11). At this point, the clay mineral was considered non-reactive.

The amount of Tc(VII) reduced vs. the amount of Fe(II) oxidized was stoichiometrically balanced at a ratio of approximately 3.4 ± 0.5 (Tc(VII):Fe(II)) (Figure 11). There was varying amounts of residual Fe(II) remaining at the end of the Tc(VII) reduction experiment. In general, the residual amount of Fe(II) was lower than the initial, non-biogenic amount of Fe(II) with the exception of NAu-2. These data suggest that a certain fraction of initial, non-biogenic Fe(II) originally present in the structure of the clay minerals (Table 2) was Tc(VII) reactive. However, for NAu-2, not all biogenic Fe(II) was reactive toward Tc(VII) reduction. A close examination
revealed that the amount of residual Fe(II) was inversely proportional to the total amount of Tc(VII) reduced [or the amount of Fe(II) oxidized] per unit surface area of the clay mineral (Figure 12). For example, SWy-2 montmorillonite, because of its low Fe(II) content, reduced a small amount of Tc(VII), as a result, nearly all Fe(II) was reactive. In contrast, for nontronite NAu-2, because of its high Fe(II) content, a large amount of Tc(VII) was oxidized, as a result, a large fraction of Fe(II) remained and non-reactive. This correlation suggests that accumulation of the products of the Fe(II) reaction with Tc(VII), i.e., Fe(III) and Tc(IV), possibly passivated the further reaction, and thus, the capacity of Tc(VII) reduction could not be used to assess the relative reactivity of the clay minerals.

One way to assess the relative reactivity of the clay minerals toward Tc(VII) was to use initial rate of Tc(VII) reduction, when the product accumulation was insignificant. To assess the intrinsic reactivity of the clay minerals, the Tc(VII) reduction data from the first spike were normalized by dividing the time-course Tc(VII) concentration (μM) by the product of Fe(II) concentration [mM of Fe(II)] and clay mineral concentration (mg/mL). The normalized kinetic data showed that in the S-I series nontronite (NAu-2) was the most reactive followed by montmorillonite (SWy-2) and rectorite (RAr-1), the illite-smectite mixed-layer phase (ISCz-1), and finally illite (IMt-1) (Figure 12). This sequence of reactivity was again correlated with the percent smectite in this series. The reactivities of palygorskite and ripidolite were between those for the I-S mixed-layer phase and illite.

Selected clay mineral-Tc(IV) mixtures were analyzed by SEM and EDS. Reduced Tc(IV) appeared to be localized in clusters of submicron-sized particles of ripidolite CCa-2 (point a & d of Figure 13A), whereas the adjacent areas of these clusters were free of Tc (points b & c of Figure 13A). Similar clustering of reduced Tc(IV) was also observed for other clay minerals such as ISCz-1 (Figure 13C).

TEM images confirmed the SEM data in showing that reduced Tc(IV) occurred as clusters in close association with residual clay particles (Figure 14). Those areas free of Tc displayed pseudo-hexagonal hkl SAED patterns consisting of single or several crystals, characteristic of ripidolite. Those patterns obtained from Tc-containing areas displayed reflections with some streaking, indicative of superimposition of another set of reflections, likely from reduced Tc(IV). However, the exact nature of this association could not be resolved at present.
4. DISCUSSION

The experimental results revealed four major findings: (1) The rate and extent of bioreduction was proportional to the percent of smectite interlayers in the S-I series. (2) The enhancement of bioreduction by AQDS was different depending on the nature of clay minerals bioreduced; (3) Biogenic Fe(II) in various clay minerals was all reactive toward Tc(II) reduction but with very different activity. (4) The reactivity of bioreduced clay minerals toward Tc(VII) reduction was correlated with the percent of smectite interlayers in the S-I series.

4.1 Mechanism of bioreduction of structural Fe(III) in the S-I clay minerals

The positive correlation between the rate/extent of Fe(III) bioreduction and the proportion of smectite in the mixed-layer I-S may be explained by multiple factors including 1) physical factors such as surface area; 2) chemical factors such as zeta potential (surface charge); 3) crystal-chemical factors such as interlayer expandability, layer charge, interlayer cation composition, and accessibility of electron shuttling compounds and electrons. For phyllosilicates, these physical and chemical factors are related to one another. For example, smectite has larger surface area, lower layer charge, and higher layer expandability (swelling) than illite. For the matter of clarity, these factors are discussed separately.

In general, the large reactive surface area would expose more Fe(III) centers to allow for higher rate and extent of Fe(III) bioreduction. When the surface area was accounted for by normalizing the extent and rate of Fe(III) bioreduction relative to the measured surface area, the correlation became insignificant (Figure 9 B and D). This result strongly suggests that surface area is a dominant factor in controlling the extent and rate of Fe(III) bioreduction in phyllosilicates. The effect of surface area on the extent and rate of Fe(III) bioreduction has been observed for Fe oxides (Roden and Zachara, 1996) and for smectites (Kostka et al., 1999) and this study confirms this effect for a suite of phyllosilicates. In comparison with Fe oxides, the surface area effect observed in this study may be underestimated because the BET method only measures external surface area, but internal surface area can be significant for phyllosilicates (Kennedy et al., 2002). However, it is unknown if the internal surface area (largely from the interlayer region) is accessible to microorganisms.
In addition to the surface area, zeta potential (surface charge density) of the clay minerals could be important in controlling microbial attachment to the minerals. Overall, the clay minerals are negatively charged at neutral pH (Jaisi et al., 2007c; Sposito, 1982) and would exert a repulsive force to negatively charged CN32 cells (Sokolov et al., 2001), unless there is a bridging effect by positively charged cations released during reductive dissolution (Jaisi et al., 2007c). However, localized positive charges could possibly exist at clay mineral edges (Sposito, 1982), and these edges would facilitate sorption of fine-grained clay particles onto microbial cell surfaces. Although *Shewanella putrefaciens* are capable of producing its own electron shuttles and does not require a direct contact with the clay minerals, attachment of the clay mineral to microbial cell surface would certainly favor bioreduction. Among the S-I series, the overall charge of smectite is less negative (or more positively charged edges) than illite (Jeong et al., 1996), thus more smectite particles would be sorbed onto cell surface on a per cell basis. This preferential sorption of smectite particles onto cell surface would certainly increase the rate and extent of bioreduction of Fe(III) in smectite.

Lastly, the crystal chemistry of phyllosilicates may be important in controlling the Fe(III) bioreduction as well. Smectite and illite have the same basic structure (Peacor, 1992; Dong, 2005), but owing to the different extents of tetrahedral and octahedral substitutions in the structure, there are major differences in cation exchange capacity, layer charge, layer expandability, and layer cation composition and all these factors are related to each other. Smectite is usually characterized by low cation exchange capacity, low layer charge, high layer expandability, and Na/Ca in the interlayer; whereas illite exhibits high cation exchange capacity, high layer charge, low layer expandability, and K as the dominant interlayer cation. The results of this study demonstrated that layer expandability and layer charge were important factors in controlling the rate and extent of bioreduction. The smectites with low layer charge and high interlayer expansion, regardless of Fe(III) contents, both exhibited a high rate and extent of Fe(III) bioreduction; whereas illite or illite-rich I-S phases exhibited an opposite behavior. These data expanded our early observation on the effect of layer charge of illites on bioreduction (Seabaugh et al., 2006) and suggest that the interlayer region of these clay minerals is involved during the electron transfer process. The importance of the interlayer region is further supported by the effect of AQDS, an electron shuttle, on the rate and extent of bioreduction. The amount of enhancement by AQDS was much higher for smectites than for illite or illite-rich I-S minerals.
(Figure 8), suggesting that AQDS was able to enter the expandable interlayer of smectite, but not into non-expandable interlayer of illite.

The important effect of the interlayer expandability on the bioreduction kinetics indicates that for *Shewanella putrefaciens* CN32, electron transfer took place not only parallel to the clay (001) layers, but also perpendicular to them, as suggested by Dong et al. (2009). This model appeared to be reasonable for this bacterial strain, as its own produced (Nevin and Lovley, 2002) and externally added electron shuttles would take advantage of the expandable smectite interlayer to facilitate electron transfer. In contrast, the non-expandable illite interlayer would have little utility to electron transfer. Whereas this electron transfer model (both parallel and perpendicular to the clay layers) is consistent with some published studies in showing reductive dissolution of clay minerals by *Shewanella putrefaciens* strain CN32 (Dong et al., 2003a; 2003b; Furukawa and O’Reilly 2007; Dong et al., 2009 and references therein; Vorhies and Gaines 2009) and significant effects of electron shuttling compounds on promoting the Fe(III) bioreduction, it is inconsistent with the results of other studies (Komadel et al., 2006; Riberio et al., 2009). UV-visible and Mossbauer spectroscopy data (Lear and Stucki, 1987; 1990; Komadel et al., 2006; Riberio et al., 2009) revealed separate domains of Fe(II) and Fe(III) within biotically reduced nontronite by *Shewanella oneidensis* MR-1. The only plausible mechanism for the presence of these domains within the same structure of bioreduced nontronite is through electron transfer from bacteria at the clay edges to the Fe(III) centers in the direction parallel to the (001) layer (Riberio et al., 2009). The edge-contact model requires the presence of a reduction front, which moves into the center of clay particles as bioreduction continues.

There are a couple of possible scenarios to account for the discrepancy in the electron transfer model, i.e., both parallel and perpendicular to the (001) layers vs. parallel to the 001 layers only. First, different bacteria may produce different amounts and types of electron shuttling compounds and therefore differentially influence the electron transfer pathway. When abundant shuttling compounds are available, these molecules may take an advantage of the expandable smectite interlayer, thus resulting in electron transfer both parallel and perpendicular to the (001) layers. When shuttling compounds are insufficient or absent (such as *Geobacter metallireducens*), a physical contact between the solid mineral and the cell may be necessary, resulting in electron transfer parallel to the (001) layers only. Although a recent study has shown that nearly all *Shewanella* species can secrete flavins as electron shuttling compounds, different
species and strains may produce different types of flavins and amounts depending on specific growth conditions (von Canstein et al., 2008) which would in turn influence the electron transfer pathway. Second, clay mineral particles, by nature, are heterogeneous in both chemical composition and structure. Measured properties such as layer charge, layer expandability, interlayer cation composition, and crystallinity only represent bulk averages. In reality, there may be a distribution of all these properties over a broad range (Peacor; 1992; Seabaugh et al., 2006; Czimerova et al., 2006). Even though electron transfer takes place in both parallel and perpendicular to the (001) layers, it is still possible to create separate domains of Fe(II) and Fe(III) as those clay particles with lowest layer charge, most expandable interlayer, and poorest crystallinity would be preferentially reduced before bioreduction of Fe(III) in more crystalline and less expandable clay particles with higher layer charge can be initiated.

The importance of the expandable smectite interlayer in influencing bioreduction can be further illustrated by contrasting the rate and extent of Fe(III) bioreduction in palygorskite and ripidolite. The former has a modulated structure and there is no interlayer. In this case, the electron transfer pathway should be similar regardless if it is parallel or perpendicular to the (001) layers. As a result, the rate and reduction were observed to be low, even negligible (Figure 8). For ripidolite, the presence of the interlayer, despite the brucite layer in the middle, provides a pathway for electrons to move through, hence resulting in a high rate and extent of Fe(III) bioreduction. High levels of initial Fe(II) in the brucite layer did not appear to impede efficiency of electron transfer.

4.2. Relative reactivity of bioreduced clay minerals toward Tc(VII) reduction

This study conclusively demonstrated that Fe(II) in various phyllosilicates was able to reduce Tc(VII) to Tc(IV) with different reactivity. These results confirmed and expanded previous studies showing the reactivity of Fe(II) in nontronite (Jaisi et al., 2008; 2009), chlorite (Cui and Eriksen, 1996), and illite/vermiculite (Peretyazhko et al. 2008) toward Tc(VII) reduction. Because the amount of Fe(II) oxidized by Tc(VII) was much higher than the amount of aqueous Fe$^{2+}$ released from a small amount of reductive dissolution, the reactive Fe(II) species must have been largely structural, consistent with previous studies in showing the reactivity of structural Fe(II) of smectite in reducing nitroaromatic compounds (Hoffsteter et al., 2003; 2006; Neuman et al., 2008) and Tc(VII) (Jaisi et al., 2008; 2009).
The exact reactivity of Fe(II) in the bioreduced clay minerals should be manifested in the capacity and rate of each clay mineral in reducing Tc(VII). If all Fe(II) in a given clay mineral is fully reactive, it should be exhausted if Tc(VII) is in excess (i.e., by continuous spiking). However, various amounts of Fe(II) remained by the end of Tc(VII) reduction experiments, where no more Tc(VII) was reduced upon further spiking. This fraction of non-reactive Fe(II) was lower than the initial amount of Fe(II) present in unreduced RAr-1, ISCz-1, IMt-1, PF1-1, and CCa-2, suggesting that a fraction of the original structural Fe(II) was reactive toward Tc(VII). The inverse correlation between the amount of residual Fe(II) and the amount of Tc(VII) reduced/Fe(II) oxidized, relative to the amount of clay minerals present, strongly suggests that the accumulation of TcO$_2\cdot$nH$_2$O and Fe(III), two important products from the abiotic reaction between Fe(II) and Tc(VII), passivated the reactive surfaces of Fe(II)-containing clay minerals (Brusic, 1972; Morrison, 1980) and eventually resulted in cessation of Tc(VII) reduction. Potentially, the reactivity of these clay minerals could be rejuvenated by removing either Fe(III) or Tc(IV). Because of the above passivation reasons, the true extent of Tc(VII) reduction by Fe(II) in phyllosilicates is difficult to measure, and therefore, this parameter cannot be used to assess the relative reactivity of Fe(II) in various clay minerals. However, the initial rate of Tc(VII) reduction, when there is little accumulation of Tc(IV) and Fe(III), should be a meaningful indicator of the “intrinsic reactivity”. After accounting for the Fe(II) and clay mineral concentration effects, the “intrinsic reactivity” of Fe(II) in the bioreduced clay minerals followed the order of bioreducibility of the clay minerals in the S-I series with smectite exhibiting the highest rate and illite the lowest (Figure 12). Again, this positive correlation between the Tc(VII) reduction rate and the layer expandability suggests that the interlayer region was also involved in the electron transfer from Fe(II) to Tc(VII). It was possible that Tc(VII), as an aqueous ion, could have more readily entered the smectite interlayer relative to the illite interlayer, thus facilitating electron transfer from structural Fe(II) in phyllosilicates to Tc(VII).

Unlike our earlier studies where reduced Tc(IV) and clay matrix association was inferred (Jaisi et al., 2008; 2009), in this study, our SEM and TEM results clearly showed that reduced Tc(IV) particles were closely associated with clay mineral matrix. This entrainment may minimize any potential of Tc(IV) reoxidation by intrusion of oxidants over a long term. Even if there is any intrusion of oxidants, the residual bioreduced clay minerals may provide some buffering capacity because various amounts of Fe(II) remained after the Tc(VII) reduction.
ceased. However, the stability of such immobilized Tc(IV) in the bioreduced clay minerals against various oxidants needs to be tested in future studies.

4.3. Implications for Tc immobilization by clay minerals:

Clay minerals are natural constituents of many subsurface contaminated environments, including the Department of Energy's Field Research Center (FRC) site (Fredrickson et al., 2004). The Fe(III) associated with these clay minerals can be biologically or chemically reduced. The results presented in this study suggest that specific clay minerals need not be transported over vast distances for in-situ immobilization of Tc(VII), and that local clay minerals can be used for this purpose so long as they contain Fe(III). The most significant finding is that all the clay minerals tested were capable of Tc immobilization once biologically reduced. However, clay minerals have different reactivity toward Tc immobilization and based on the knowledge of crystal chemistry, it should be possible to predict the relative order of reactivity, if highly reactive clay mineral is desired in practical applications. Once reduced, Tc(IV) resides within clay matrix, potentially minimizing any Tc remobilization over a long term. These results have substantial implications for the development of a long-term, in-situ immobilization technology of technetium.

5. CONCLUSION:

This comprehensive study has demonstrated that most common clay minerals in nature, the smectite-illite series and chlorite, palygorskite, were all reducible by a common iron-reducing bacterium. The rate and extent of bioreduction was proportional to the percent of smectite interlayers in the S-I series. This correlation suggests that electron transfer from bacteria to structural Fe(III) was both parallel and perpendicular to the clay (001) layers. The bioreduced clay minerals were all reactive toward Tc(VII) reduction but with different reactivity. Various fractions of Fe(II) remained and non-reactive by the end of Tc(VII) reduction, despite excess Tc(VII) was added. Surface passivation by Fe(III) and Tc(IV) minerals may have accounted for the limited capacity of the bioreduced clay minerals toward Tc(VII) reduction. The initial rate of Tc(VII) reduction, when normalized to Fe(II) and clay concentrations, was more meaningful in revealing the relative reactivity of bioreduced clay minerals. This “intrinsic reactivity” was
correlated with the percent of smectite in the S-I series, suggesting a role of the clay interlayer in electron transfer from clay-Fe(II) to Tc(VII).
6. BIBLIOGRAPHY


Favre, Fabienne, Christian Bogdal, Sophie Gavillet, and Joseph W. Stucki. (2006), Changes in the CEC of a soil smectite-kaolinite clay fraction as induced by structural iron reduction and iron coatings dissolution. *Applied Clay Science* (34), (1-4), Pages 95-104


Fe(III) oxides and Fe(II)/Fe(III) phyllosilicates. *Geochimica et Cosmochimica Acta*, 70, 3662-3676.


Table 1: Bioreduced Clay Minerals and Tc(VII) Reduction Set-up

<table>
<thead>
<tr>
<th>Clay Mineral</th>
<th>SWy-2</th>
<th>NAu-2</th>
<th>RAr-1</th>
<th>ISCz-1</th>
<th>IMt-1</th>
<th>PFl-1</th>
<th>CCa-2</th>
<th>Tc</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe(II) conc. in stock (mM)</td>
<td>1.74</td>
<td>2.68</td>
<td>1.35</td>
<td>0.75</td>
<td>2.21</td>
<td>1.11</td>
<td>15.09</td>
<td>0.0</td>
</tr>
<tr>
<td>Final Fe(II) conc. desired (mM)</td>
<td>0.5</td>
<td>1.05</td>
<td>1.05</td>
<td>0.5</td>
<td>1.05</td>
<td>0.5</td>
<td>1.05</td>
<td>0.0</td>
</tr>
<tr>
<td>Clay stock needed (ml)</td>
<td>4.13</td>
<td>3.96</td>
<td>7.78</td>
<td>6.67</td>
<td>4.75</td>
<td>4.52</td>
<td>0.70</td>
<td>0.0</td>
</tr>
<tr>
<td>Tc(VII) conc. in stock (mM)</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Final Tc(VII) conc. desired (mM)</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
</tr>
<tr>
<td>Tc(VII) stock added (ml)</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Pipes (ml)</td>
<td>6.62</td>
<td>5.54</td>
<td>1.72</td>
<td>2.83</td>
<td>4.75</td>
<td>4.98</td>
<td>8.80</td>
<td>9.50</td>
</tr>
<tr>
<td>Total volume (mL)</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Note: The stock clay mineral suspension was 40 g/L. In order to achieve a similar level of final Fe(II) concentration for the Tc(VII) reduction experiment, the following stock clay mineral suspensions were concentrated: SWy-2 by 2 X; ISCz-1 7 X; IMt-1 2 X; and PFl-1 3.5 X. Despite these concentrations, these minerals still had a lower final concentration of Fe(II) for the Tc(VII) reduction experiment.
Table 2: Wet chemistry of clay minerals based on DCP and 1, 10 Phenanthroline method.

<table>
<thead>
<tr>
<th>Clay Mineral</th>
<th>Concentration (wt.%)</th>
<th>DCP / Titration Measurements</th>
<th>1,10 Phenanthroline</th>
<th>BET Surface Area (m²/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SWy-2</td>
<td>NAu-2</td>
<td>ARG-6</td>
<td>RAr-6</td>
</tr>
<tr>
<td>Fe(tot)</td>
<td>2.27</td>
<td>21.23</td>
<td>4.92</td>
<td>0.74</td>
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<tr>
<td>Fe(II)</td>
<td>0.06</td>
<td>0.33</td>
<td>1.11</td>
<td>0.19</td>
</tr>
<tr>
<td>Fe(tot) wt%</td>
<td>2.3</td>
<td>20.0</td>
<td>1.99</td>
<td>3.4</td>
</tr>
<tr>
<td>Fe(II) wt%</td>
<td>0.05</td>
<td>0.34</td>
<td>0.38</td>
<td>0.56</td>
</tr>
</tbody>
</table>

Table 3: Wet chemistry of aqueous bioreduced clay minerals based on DCP.

<table>
<thead>
<tr>
<th>Clay Mineral</th>
<th>Concentration in (mmol/L)</th>
<th>Si</th>
<th>Al</th>
<th>Fe</th>
<th>Mg</th>
<th>Ca</th>
<th>Na</th>
<th>K</th>
</tr>
</thead>
<tbody>
<tr>
<td>SWy-2 Control</td>
<td></td>
<td>0.985</td>
<td>0.436</td>
<td>0.050</td>
<td>0.194</td>
<td>0.378</td>
<td>1.944</td>
<td>0.269</td>
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<tr>
<td>SWy-2 Bioreduced</td>
<td></td>
<td>1.148</td>
<td>0.522</td>
<td>0.058</td>
<td>0.225</td>
<td>0.398</td>
<td>1.915</td>
<td>0.300</td>
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<tr>
<td>NAu-2 Control</td>
<td></td>
<td>1.054</td>
<td>0.047</td>
<td>0.204</td>
<td>0.014</td>
<td>0.019</td>
<td>1.618</td>
<td>0.025</td>
</tr>
<tr>
<td>NAu-2 Bioreduced</td>
<td></td>
<td>1.158</td>
<td>0.139</td>
<td>0.207</td>
<td>0.144</td>
<td>0.017</td>
<td>1.669</td>
<td>2.100</td>
</tr>
<tr>
<td>RAr-1 Control</td>
<td></td>
<td>1.641</td>
<td>1.003</td>
<td>0.144</td>
<td>0.014</td>
<td>0.191</td>
<td>1.643</td>
<td>1.121</td>
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<tr>
<td>RAr-1 Bioreduced</td>
<td></td>
<td>2.009</td>
<td>1.207</td>
<td>0.166</td>
<td>0.015</td>
<td>0.181</td>
<td>1.588</td>
<td>1.285</td>
</tr>
<tr>
<td>ISCz-1 Control</td>
<td></td>
<td>0.276</td>
<td>0.061</td>
<td>0.007</td>
<td>0.016</td>
<td>0.164</td>
<td>1.704</td>
<td>1.260</td>
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<tr>
<td>ISCz-1 Bioreduced</td>
<td></td>
<td>0.295</td>
<td>0.086</td>
<td>0.018</td>
<td>0.023</td>
<td>0.166</td>
<td>1.711</td>
<td>1.140</td>
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<tr>
<td>IMt-1 Control</td>
<td></td>
<td>1.713</td>
<td>0.980</td>
<td>0.033</td>
<td>0.140</td>
<td>0.169</td>
<td>1.643</td>
<td>1.407</td>
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<tr>
<td>IMt-1 Bioreduced</td>
<td></td>
<td>5.819</td>
<td>1.162</td>
<td>0.195</td>
<td>0.907</td>
<td>0.155</td>
<td>1.474</td>
<td>1.940</td>
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<tr>
<td>PFl-1 Control</td>
<td></td>
<td>0.558</td>
<td>0.024</td>
<td>0.002</td>
<td>0.055</td>
<td>0.211</td>
<td>1.695</td>
<td>1.151</td>
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<tr>
<td>PFl-1 Bioreduced</td>
<td></td>
<td>0.620</td>
<td>0.024</td>
<td>0.002</td>
<td>0.061</td>
<td>0.200</td>
<td>1.639</td>
<td>1.298</td>
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<tr>
<td>CCa-2 Control</td>
<td></td>
<td>0.304</td>
<td>0.033</td>
<td>0.045</td>
<td>0.055</td>
<td>0.169</td>
<td>1.627</td>
<td>1.109</td>
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<tr>
<td>CCa-2 Bioreduced</td>
<td></td>
<td>0.366</td>
<td>0.113</td>
<td>0.061</td>
<td>0.132</td>
<td>0.172</td>
<td>1.551</td>
<td>1.214</td>
</tr>
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</table>

Note: Error for DCP measurements are ±0.03 mmol/L.
The DCP results for the Bioreduced clay minerals are with AQDS.
Figure Captions

Figure 1. XRD patterns of the unaltered (the bottom pattern); DCB treated (second from the bottom); DCB-treated, reoxidized (third from the bottom); and DCB-treated, reoxidized, and bioreduced (the top pattern) clay minerals. The ethylene glycolation (EG) did not shift the patterns or produce any new peaks. For clarity, only those patterns for ethylene glycolated samples are shown. A) montmorillonite (SWy-2) with no presence of any iron oxides in the unaltered material and DCB treatment did not alter the phyllosilicates; B) nontronite (NAu-2) with no Fe oxides. C) rectorite (RAr-1) with a small amount of goethite (inset) and this amount was removed after the DCB treatment; D) 70:30 illite:smectite (ISCz-1) showing a small amount of maghemite (inset) which was removed by the DCB treatment; E) illite (IMt-1) with no Fe-oxides and the DCB did not alter the phyllosilicates; F) palygorskite (PF1-1) with a small amount of magnetite which was removed by the DCB treatment; and G) ripidolite (RAr-1) with a small amount of maghemite which was removed by the DCB treatment. RAr-1 and IMt-1 exhibited a predominant hump in the XRD pattern, which is indicative of amorphic material.

Figure 2. XRD patterns of various nontronite samples with its interlayer expanded utilizing A: MgCl$_2$ and B: CaCl$_2$. The results illustrated that 0.1 M, 0.2 M, and 0.4 M MgCl$_2$ or CaCl$_2$ expanded d(001) to 17.5 Å.

Figure 3. XRD patterns of NAu-2-ferrihydrite mixture showing that 10 min DCB reaction was sufficient to remove the synthetic ferrhydrite.

Figure 4. A) Change of Fe(III) content as a result of DCB-treatment and reoxidation for various amounts of time. The Fe(III) content was measured by the 1, 10 phenanthroline method.

Figure 5. Room-temperature Mössbauer spectra of DCB-treated, reoxidized (72 hrs) clay minerals showing absence of sextets (Fe-oxides).

Figure 6: SEM images of A) DCB-treated, reoxidized SWy-2 showing typical platy morphology. The EDS spectrum showed a typical composition of low-Fe smectite.; B) DCB-treated, bioreduced SWy-2 exhibiting a new phase with platy morphology. The EDS
composition identified it as smectite; C) unaltered ISCz-1 exhibiting a 2-µm size Fe-oxide particle (bright particle in the middle) and the EDS spectrum shows a mixture of Fe oxides and ISCz-1. D) DCB-treated, reoxidized ISCz-1 exhibiting absence of Fe oxides but presence of fibrous particles. The small bright particles are quartz and salt particles. E) DCB-treated, reoxidized, bioreduced ISCz-1 showing presence of biogenic euhedral platy particles. The EDS analysis identified it as smectite.

Figure 7: Linear relationship between BET total surface area and % smectite in the S-I series.

Figure 8: Time-course change of Fe(II) concentration as measured by 1,10 phenanthroline for A) SWy-2, B) NAu-2, C) ARG-6, D) RAr-1, E) ISCz-1, F) ARG-24, G) IMt-1, H) PFl-1, and I) CCa-2. For each sub-figure, three curves, corresponding to abiotic control, treatment with and without AQDS, are shown. Error bars are smaller than the sizes of the symbols.

Figure 9: A) Positive correlation of the extent of bioreduction (%) with %smectite in the S-I series; B) Relation between the normalized extent of Fe(III) bioreduction, in terms of mmol Fe(II) per gram of clay mineral to % smectite in the S-I series, showing no more correlation; C) Positive correlation between the initial rate of bioreduction in terms of mmol/g/h and % smectite in the S-I series; D) the same plot but with the rate normalized to the measured BET surface area showing no more correlation.

Figure 10: Time-course change of colony forming units over the course of the clay mineral bioreduction.

Figure 11: Tc(VII) reduction kinetics by Fe(II) in various clay minerals and corresponding changes of Fe(II) concentration and Fe(II)/Tc(VII) ratio A) SWy-2; B) NAu-2; C) RAr-1; D) ISCz-1; E) IMt-1; F) PFl-1; and G) CCa-2. The experiments were performed at pH 7 in PIPES buffer. The starting Fe(II) and Tc(VII) concentrations are given in Table 1. Upon complete reduction of the first spike of Tc(VII), additional spikes were added to determine the capacity of each clay mineral in reducing Tc(VII).
Figure 12: A positive correlation between residual Fe(II) (µmol per gram of clay mineral) and the amount of reacted Tc(VII) (µmol per measured BET surface area) suggesting that the accumulation of Fe(III) and Tc(IV) passivated the further abiotic reaction between Fe(II) and Tc(VII).

Figure 13: A) A comparison of Tc(VII) reduction rate among the various bioreduced clay minerals from the data of the first spike of Tc(VII). B) Normalized Tc(VII) reduction relative to Fe(II) and clay mineral concentration [µM of Tc / (µM of Fe(II) in clay minerals) / (mg ml⁻¹ of clay mineral)] in order to reveal the intrinsic reactivity of the clay minerals.

Figure 14: A) SEM image showing CCa-2 particles in association with Tc-rich materials. The EDS spot analyses at points a and d showed the presence of Tc (at 2.424 keV, Lα₁ for Tc). The EDS spot analyses at points b & c showed typical composition of ripidolite. B) SEM image and EDS composition showing CCa-2 particles in association with Tc-rich materials. A bright cluster at the center of the image is rich in Tc as shown by the EDS; whereas the other areas have typical composition of ripidolite. C) SEM image of ISCz-1 where the EDS spot analysis at point a showed the presence of Tc, while point b exhibited a smectite composition. S and Na peaks are from the PIPES buffer.

Figure 15: A) TEM image of CCa-2 clay particles in association with Tc-rich materials. The EDS spot analyses at points 1 & 2 showed typical composition of ripidolite. The EDS spot analyses at points 3 & 4 exhibited the presence of Tc superimposed on the ripidolite composition suggesting a close association of Tc with ripidolite. The SAED patterns obtained from these four areas all exhibited a characteristic pseudo-hexagonal symmetry. The reflections were indexed to be hk0 with d-spacings consistent with ripidolite. In the absence of Tc, the SAED patterns corresponded to single (point 2) or several crystals of ripidolite, whereas in the presence of Tc, all reflections displayed some streaking, likely due to superimposition of reduced Tc(IV) onto ripidolite particles. However, at this level of resolution, the exact nature of Tc(IV)-ripidolite could not be resolved. B) The elemental mapping of Tc(IV)-ripidolite associations exhibited clustering of Tc (magenta) which showed a positive correlation with Fe (red), again confirming
the spatial association between reduced Tc and ripidolite. The first image is a composite map of Fe, Tc, Si and Na, and the second is a bright field image. The rest are individual elemental maps.
FIGURES

Figure 1:
Figure 2:

A) B)

Counts Per Second (CPS)

2-Theta

Figure 3:

Counts Per Second (CPS)

2-Theta

Post DCB N/Au-2 Ferricydride mixture

Pre DCB N/Au-2 Ferricydride mixture
Figure 4:

- Pre DCB
- Post Fe(aq) removal
- reox 24h
- reox 48h
- reox 72h

Fe(III) % vs. Clay Minerals

Clay Minerals: SWy-2, NAu-2, RA-1, ISCz-1, IMt-1, PFI-1, CCA-2
Figure 8:
Figure 9:

(a) Extent of bioreduction (%) vs. % Smectite in the S-I Series

- Extent w/ AQDS
- Extent w/o AQDS

Linear (Extent w/ AQDS) ———— Linear (Extent w/o AQDS)

(b) Normalized Extent of Bioreduction (mmol/sq m/hr) vs. % Smectite in the S-I series

- Normalized Extent w/ AQDS
- Normalized Extent w/o AQDS
Figure 10:
Figure 11:
Figure 12: 

[Graph showing data points and lines representing various conditions and reactions. The graph includes axes labeled as follows: x-axis: Reacted Tc(VII) normalized to BET surface area (umol/sq. m), y-axis: Residual Fe(II) umol/g.]
Figure 13

A

Soluble Tc, μM

Time, (d)

B

Normalized Tc reduction (μM of Tc/(μM of Fe(II)))/(mg/mg of clay)

Time, (d)