Coupling of Fe(II) oxidation in illite with nitrate reduction and its role in clay mineral transformation

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ABSTRACT

In pedogenic and diagenetic processes, clay minerals transform from pre-existing phases to other clay minerals via intermediate interstratified clays. Temperature, pressure, chemical composition of fluids, and time are traditionally considered to be the important geological variables for clay mineral transformations. Nearly ten years ago, the role of microbes was recognized for the first time, where microbial reduction of structural Fe(III) in smectite resulted in formation of illite under ambient conditions within two weeks. However, the opposite process, the oxidation of structural Fe(II) in illite has not been studied and it remains unclear whether or not this process would result in the back reaction, e.g., from illite to smectite. The overall objective of this study was to investigate biological oxidation of structural Fe(II) in illite coupled with nitrate reduction and the effect of this process on clay mineral transformation. Laboratory incubations were set up, where structural Fe(II) in illite served as electron donor, nitrate as electron acceptor, and *Pseudogulbenkiania* sp. strain 2002 as mediator. Solution chemistry and gas composition were monitored over time. Mineralogical transformation resulting from bioreduction was characterized with X-ray diffraction and scanning and transmission electron microscopy. Our results demonstrated that strain 2002 was able to couple oxidation of structural Fe(II) in illite with reduction of nitrate to N₂ with nitrite as a transient intermediate. This oxidation reaction resulted in transformation of illite to smectite and ultimately to kaolinite (illite→smectite→kaolinite transformations). This study illustrates the importance of Fe redox process in mediating the smectite-illite mineral cycle with important implications for Fe redox cycling and mineral evolution in surficial earth environments.

1. INTRODUCTION

Iron-bearing clay minerals are ubiquitous in natural soils, sediments, and sedimentary rocks (Stucki and Kostka, 2006; Favre et al., 2006; Dong et al., 2009). In pedogenic and diagenetic processes, clays continuously transform from pre-existing minerals to other phases, often through a sequence of interstratified clays (Moore and Reynolds, 1997). Early studies have emphasized temperature, pressure, chemical composition of clays, climatic condition, and time as variables for clay mineral transformations (Bethke and Altaner, 1986; Dong et al., 1997; Huang et al., 1993), whereas the role of microbial processes in clay transformation was not recognized until a decade ago (Kim et al., 2004). In its first report, Kim et al. (2004) suggested that microbial transformation of structural Fe(III) in smectite resulted in partial reductive dissolution of smectite and precipitation of newly formed illite under ambient condition (25°C, 1 atmospheric pressure) within two weeks. Since then extensive research has been conducted on the important role of dissimilatory iron-reducing bacteria and other microorganisms in driving the smectite to illite conversion (Kim et al., 2004; Zhang et al., 2007a,b; Vorhies and Gaines, 2009; Dong et al., 2009; Jaisi et al., 2011; Koo et al., 2014; Liu et al., 2014, 2015). In contrast, the opposite process, the oxidation of structural Fe(II) in illite has never been studied and it remains unclear whether or not this process would result in the back reaction, e.g., from illite to smectite.

Among Fe(II)-oxidizing bacteria that are common in natural environments, anaerobic nitrate-dependent Fe(II)-oxidizing bacteria have received considerable attention in the last decade (Kappler et al., 2005; Weber et al., 2006; Miot et al., 2009; Larese-Casanova et al., 2010; Konhauser et al., 2011; Shelobolina et al., 2012b; Carlson et al., 2012;

Melton et al., 2014), and significant progress has been made toward understanding the biogeochemical mechanisms of this process. Such bacteria have been found in various environments, including lakes, streams, hydrothermal vents, wetlands, and aquifer sediments (Weber et al., 2006). These bacteria can utilize the following reaction (1) to provide energy to support their growth (Weber et al., 2006).

In this reaction, both aqueous Fe^{2+} and structural Fe(II) in oxides can be biologically oxidized (Chaudhuri et al., 2001; Weber et al., 2001; Kappler et al., 2005; Weber et al., 2006; Miot et al., 2009; Larese-Casanova et al., 2010). More recent studies have demonstrated biological oxidation of structural Fe(II) in phyllosilicates, including biotite (Shelobolina et al., 2012b), nontronite (Zhao et al., 2013; 2015), and illite-smectite mixed layers (Shelobolina et al., 2012a). However, other phyllosilicates, such as illite, despite its ubiquitous presence and distinct difference in physical/chemical properties from smectite (Moore and Reynolds, 1997), have not received equivalent attention. The microbe-illite interaction could have important implications for iron biogeochemistry and geological processes in nature. In contrast to smectite (including iron-rich variety nontronite) that is usually present in surface soils and sediments, illite is more abundant in consolidated sedimentary rocks at depth (Moore and Reynolds, 1997) where anaerobic microbial community and nitrate-reducing conditions can co-exist with illite. Therefore, the oxidation of structural Fe(II) in illite could be coupled with nitrate reduction by the indigenous microbial community in such subsurface environments. In addition, previous studies have reported that illite could transform to illite/smectite mixed-layer clay by leaching interlayer K^+ in acidic or circumneutral environments (Rimmer, et al., 1982).

However, microbial activity has never been considered as an environmental variable in promoting the illite-to-smectite transformation under anaerobic conditions. Considering the microbially mediated smectite-to-illite reaction that occurs through reduction of structural Fe(III) (Kim et al., 2004; Dong et al., 2009), we hypothesize that bio-oxidation of structural Fe(II) in illite could reverse the process, i.e., the illite-to-smectite transformation. In summary, the nitrate-dependent Fe(II)-oxidizing bacteria may have a potential role in promoting the illite-to-smectite transformation and thus completing the iron redox and mineral transformation cycles as a result of clay mineral-microbe interactions.

The overall objective of the present study was therefore to fill the above knowledge gap through examining biological nitrate-dependent Fe(II) oxidation in illite (IMt-1) and the effect of this reaction on clay mineral transformation. Specifically, the present investigation was designed to determine: (1) the extent and rate of biological Fe(II) oxidation in illite when coupled with nitrate reduction; (2) the mechanisms of biological Fe(II) oxidation in illite; and (3) clay mineral transformation associated with this oxidation reaction.

2. MATERIAL AND METHODS

2.1. Mineral, media, and experimental setup

Illite (IMt-1) was purchased from the Source Clays Repository of the Clay Minerals Society (West Lafayette, IN, USA). The total Fe content in IMt-1 was 12.3%, of which 10% was Fe(II) (Bishop, et al., 2011). The formula of IMt-1 is:

(Ca_{0.01}Na_{0.08}K_{1.58})(Al_{2.78}Fe³⁺_{0.67}Fe²⁺_{0.08}Mg_{0.47})(Si_{6.89}Al_{1.11})O₂₀(OH)₄. The IMt-1 sample was size-fractionated as previously described (Zhang et al., 2007b). The 0.5-2 μm size fraction was separated, dried, suspended in double distilled water (ddH₂O), and autoclaved (Zhao et al., 2013). This size fraction was hereafter designated as original IMt-1.

To study the mechanism of bio-oxidation of structural Fe(II) in illite, experiments were performed for two groups of IMt-1 sample: original IMt-1 and pre-reduced IMt-1. The pre-reduced IMt-1 sample was prepared by biologically reducing it with *Shewanella putrefaciens* CN32, with lactate as electron donor, as previously described (Jaisi et al., 2007). After Fe reduction reached its maximum (Fe(II)/Fe_{total}:15%), lactate and cells were removed from the reduced clay suspension by washing it five times with sterile and anoxic (80:20 N₂:CO₂ purged) sodium bicarbonate buffer (29.76 mM NaHCO₃ and 1.34 KCl, pH 7.0). Pre-reduced IMt-1 was then re-suspended with the same sterile and anoxic bicarbonate buffer with an illite concentration of 13.3 g/L (corresponding to 0.22 mmol/g or 4.01 mM structural Fe(II)). Original IMt-1 (without prior reduction) was also suspended in bicarbonate buffer with an illite concentration of 10 g/L (corresponding to 0.12 mmol/g or 2.14 mM structural Fe(II)). After adjustment of the pH to 7.0 with NaOH or HCl, twenty milliliters of the original and pre-reduced IMt-1 suspensions were transferred to 50 mL serum bottles. Anoxic condition was maintained for pre-reduced IMt-1 with the procedure described above (i.e., washing followed by resuspension and pH adjustment) in an anaerobic chamber (96% N₂ and 4% H₂). The original IMt-1 suspension was purged with N₂/CO₂ (80:20) gas mix to achieve anoxic condition. Both

original and pre-reduced IMt-1 suspensions were pasteurized as previously described (Zhao et al., 2015).

Bio-oxidation experiment was conducted using anaerobic Fe-oxidizing bacterium *Pseudogulbenkiania* sp. strain 2002 (ATCC BAA-1479; DSM 18807) with nitrate as the sole electron acceptor and Fe(II) in IMt-1 as the sole electron donor. Strain 2002 was routinely cultured with acetate (10 mM) and nitrate (10 mM) as electron donor and acceptor, respectively (Zhao et al., 2013). When the culture reached the exponential phase, cells were harvested, washed with sterile and anoxic sodium bicarbonate buffer, and added into both the original and pre-reduced IMt-1 suspensions to achieve a final cell concentration of $\sim 10^8$ cells/mL. The headspace of each serum bottle was purged with CO₂ gas (a carbon source) for 15 minutes using a sterile needle right after strain 2002 inoculation. Clay suspension pH did not decrease after purging. A small volume of KNO₃ solution was added as electron acceptor to the bottles to achieve a nitrate concentration of 6-7 mM. Abiotic controls were the same as the treatments except that they were devoid of strain 2002 cells.

2.2. Analysis

2.2.1. Measurement of Fe(II), nitrate, nitrite, and N₂ concentration.

The reaction progress was monitored over a 10-day time period by collecting samples from incubation bottles and analyzing the concentrations of total Fe(II) in IMt-1 illite and solution, nitrate, nitrite, and N₂. The 1,10-phenanthroline method was used to quantify total Fe(II) concentration (Amonette and Templeton, 1998). Nitrate and nitrite samples were collected from the supernatant following centrifugation of IMt-1-cell suspension

(10,000 g for 10 min) and their concentrations were determined using a Dionex 500X ion chromatograph equipped with an Ion Pac AS22 analytical column. After 10 days of incubation, when nitrate was completely consumed, gas samples were collected from the headspace of the experimental tubes. The amounts of N₂, NO, and N₂O were quantified by a Hewlett Packard 5890 series II Gas Chromatographic (GC) system equipped with a thermal conductivity detector.

2.2.2. XRD & FT-IR

Mineralogical transformations as a result of bio-oxidation of structural Fe(II) in original IMt-1 illite were studied with X-ray diffraction (XRD) for two time points (3 months and 2 years). The reasons for selecting the original illite (as opposed pre-reduced illit) were two folds: 1) original illite was naturally present; 2) pre-reduction of illite by bacteria can lead to illite structural alterations (Dong et al., 2003; Seabaugh et al., 2006) so that it may be difficult to understand the mechanisms of mineralogical transformations. Powder XRD patterns were collected and analyzed as previously described (Zhao et al., 2015). Any structural changes of the original IMt-1 upon bio-oxidation for 12 months were analyzed with Fourier transform infrared spectroscopy (FT-IR) in the middle-infrared region. Samples were prepared and analyzed as previously described (Zhao et al., 2015).

2.2.3. SEM & TEM

Scanning and transmission electron microscopy (SEM and TEM) was performed to study the bio-oxidation mechanism and time-course transformation of clay minerals. The original IMt-1 sample before and after bio-oxidation (for 0, 6 and 24 months) were

selected for SEM observations. The original IMt-1 before and after bio-oxidation (for 0, 2 weeks, 1, 6, and 24 months) were selected for TEM observations.

For SEM analyses, clay suspensions were mounted onto glass cover slips, which were pretreated with poly-L-lysine for 10 min. After dehydration through a grade series of ethanol and water followed by critical point drying with a Tousimis Samdri-780A Critical Point Dryer (CPD) (Dong et al., 2003), the cover slips with clay particles were mounted onto SEM stubs with clear double-sided sticky tape, and then carbon-coated. The samples were then observed with a Zeiss Supra 35 variable pressure (VP) SEM with EDAX Genesis 2000 X-ray energy dispersive spectroscopy (SEM/EDS). In order to mitigate the charging problem and to increase image resolution, low accelerating voltage (i.e., 3-7 keV) and short working distance (i.e., 4-5 mm) were used for imaging. To obtain the chemical composition of the clay samples, EDS was performed at 7-10 keV accelerating voltage and 8.5 mm working distance.

For TEM analysis, the clay suspensions (0.2 mL) were diluted by anoxic double-distilled water (ddH₂O) with a dilution factor of 200, and pipetted onto 300 mesh copper grids with carbon-coated nitrocellulose membrane. The grids were dried overnight in an anaerobic glove box (96% N₂, 4% H₂). TEM imaging and analysis were performed with a JEOL JEM-2100 LaB₆ TEM with a 200 keV accelerating voltage. TEM bright field images (GatanOrius SC200D camera) and EDS chemical composition (Bruker AXS Microanalysis Quantax200 with 4030 SDD detector) were obtained.

3. RESULTS

3.1. Microbial oxidation of Fe(II) in IMt-1 by strain 2002.

In contrast to minor changes in the abiotic controls, the Fe(II) concentration in the inoculated experimental bottles of both the original and pre-reduced IMt-1 samples decreased with time, indicating that strain 2002 was able to oxidize structural Fe(II) in IMt-1 (Figure 1). Both bio-oxidation extent and rate for the pre-reduced IMt-1 were higher than those for the original IMt-1 (Table 1). Relative to bio-reduced nontronite (NAu-2), the extent and rate of bio-oxidation of Fe(II) in IMt-1 were much lower (Zhao et al., 2015) (Table 1).

In both the original and pre-reduced IMt-1 treatment groups, 6.5 mM nitrate was completely reduced to N₂ gas in 7 days (Figures 1 and 2), but Fe(II) was never fully oxidized, despite the fact that excess nitrate was added relative to Fe(II) (assuming a stoichiometric Fe/NO₃⁻ molar ratio of 5 if the N₂ was the ultimate NO₃⁻ reduction product). Nitrite was produced as a transient intermediate during the nitrate reduction process in both the original and pre-reduced IMt-1 treatment groups, up to 0.38 mM and 0.26 mM, respectively. More nitrite accumulation was observed in the pre-reduced NAu-2 (1.5 mM) than in the original IMt-1 (0.5 mM). Similar to our previous studies (Zhao et al., 2013; 2015), neither NO nor N₂O was detected at any time point, presumably due to their reactive/transient nature. The observed molar ratio of nitrate reduced to Fe(II) oxidized in the original and the pre-reduced IMt-1 was 31.27 and 4.89, respectively. These ratios greatly exceeded the theoretical values of 0.2 according to Equation 1. Similar observations were made in other Fe(II) oxidation studies with the same bacterial strain, and suggest a certain amount of heterotrophic nitrate reduction coupled with oxidation of dead bacterial biomass or energy reserves (i.e., glycogen) stored in cells (Weber, et al., 2001; Zhao et al., 2013; 2015).

3.2. Mineralogical changes associated with microbial oxidation of Fe(II) in IMt-1

3.2.1. XRD results

XRD patterns showed that illite was the dominant mineral throughout the bio-oxidation experiment of the original IMt-1 sample with four broad peaks corresponding to d_{001} , d_{002} , d_{003} and d_{005} reflections of illite (Figure 3). XRD patterns revealed that the air-dried, original IMt-1 sample after 3 months and 24 months of bio-oxidation contained 7-Å and 3.5-Å peaks that corresponded to those of kaolinite (Figure 3). A peak at 14Å, which could correspond to the d_{001} peak of smectite, was only found in the air-dried, 3-month sample, and disappeared in the 24-month sample. Unexpectedly, this 14-Å peak disappeared upon ethylene glycol treatment, probably because the newly formed smectite was poorly crystalline and its periodicity was destroyed upon intercalation by ethylene glycol molecule (Figure 4).

3.2.2. FT-IR results

FT-IR spectra (Figure 5) for the original IMt-1 displayed distinct difference in the position of OH-stretching (ν_{OH}) vibration bands upon exposure to strain 2002 for 12 months. In the OH-stretching region, the position of the peak at 3644 cm^{-1} shifted to 3628 cm^{-1} upon bio-oxidation. In addition, bio-oxidation resulted in a peak shift of the absorption band from 3670 cm^{-1} to 3656 cm^{-1} . In addition, a shoulder at 3699 cm^{-1} emerged upon bio-oxidation. All three peaks (3628 cm^{-1} , 3656 cm^{-1} , and 3699 cm^{-1}) correspond to the OH groups between the octahedral and tetrahedral sheets in different 1:1 units of the kaolinite structure (Madejová, 2003). In summary, the observed band

shifts indicated that the crystal structure of IMt-1 gradually transformed into a kaolinite-like structure upon bio-oxidation.

3.2.3. SEM observations

Biogenic smectite and kaolinite in bio-oxidized IMt-1 sample were further confirmed by SEM images and energy-dispersive X-ray spectroscopy (EDS) analyses (Figures 6 and 7). After 3 months of incubation of the original IMt-1 sample, two types of clay particles were observed (Figure 6) that exhibited different morphologies from the original illite (Figure 6A): platy particle aggregates (Figures 6B and 6C) and particles with a wavy texture (Figures 6B and 6D). Qualitative SEM-EDS analyses revealed that these particles had a kaolinite-like and smectite-like composition, with relatively higher and lower Al/Si atomic ratio (0.32 and 0.04), respectively, than that of the original illite (0.19) (Figure 6E). The platy particles (Figures 6B and 6C) were tentatively identified as a growing biogenic kaolinite, considering its diagnostic morphology and a high Al/Si ratio. Similarly, the observed wavy particles (Figures 6B and 6D) were tentatively identified as growing biogenic smectite. However, the high carbon peak observed for the wavy particles (Figure 6D) may suggest some contribution from biological products (such as extracellular polysaccharides).

After 24 months of incubation, stacks of platy particles with a hexagonal shape emerged in the bio-oxidized IMt-1 sample (Figures 7A and 7B). This shape was consistent with the morphology of well-crystallized natural kaolinite. Furthermore, SEM-EDS analysis indicated that these particles were mainly composed of O, Si, and Al, with an Al: Si atomic ratio of 0.8 (Figure 7C), which was close to the stoichiometric ratio for

kaolinite. Peaks of Na and K may have been derived from adjacent illite particles (e.g., other particles in Figure 7A) or cations adsorbed on the surface of kaolinite. These lines of evidence strongly suggest that biogenic kaolinite formed as a result of bio-oxidation of structural Fe(II) in IMt-1. No smectite-like particles were observed in the 24-month sample. These SEM observations are in good agreement with XRD results described earlier.

3.2.4. TEM observations

TEM observations further confirmed XRD and SEM results. TEM lattice fringe images of the original IMt-1 sample showed illite particles in well-defined packets, and TEM-EDS analysis revealed a typical composition of illite, with relatively abundant Fe and K, and a high Al/Si ratio (Figures 8A and 8F). These data suggest that illite was the only clay mineral present in the original IMt-1 sample. However, two types of clay minerals were observed in the bio-oxidized IMt-1 sample after 2 weeks and 6 months of incubation: (1) platy particles with similar morphology and composition to the original IMt-1 sample (Figures 8B and 8C); (2) wavy fringes with 1.4-nm spacing, which are typical of smectite (Figures 8D and 8E). These wavy fringes were more susceptible to beam damage than typical illite, presumably due to its poorly crystalline nature. More smectite-like fringes were observed in the 6-month sample than in 2-week sample (compare Figures 8B and 8C). Wavy smectite-type fringes showed decreased K and Fe contents and Al/Si ratio relative to the original illite (Figure 8F).

HRTEM images of bio-oxidized IMt-1 samples after 24 months of incubation showed a pseudo-hexagonal crystal (Fig. 9A) with 0.7 and 1.0 nm lattice fringe spacing (Fig. 9B),

which matched the dominant peaks in XRD pattern (Fig. 3C), and corresponded to the d_{001} reflections of kaolinite and illite, respectively. Furthermore, fringes with 1.7 nm d-spacing were observed, and they suggest a regularly interstratified illite-kaolinite mixed-layer. This mixed-layer phase was not observed in XRD pattern, possibly due to its low abundance or a small coherent domain size. Both 0.7 and 1.0 nm fringes were commonly observed in other areas of this sample (Figure 9D). TEM-EDS analyses revealed higher Al:Si ratios of 0.58 & 0.52 for the bio-oxidized IMt-1 (Figures 9C and 9E, for the white squared area in Figure 9A and the black squared area in Figure 9D, respectively) than a ratio of 0.4 for the original IMt-1 sample (Figure 8A). The increased Al:Si ratio in the bio-oxidized IMt-1 sample suggests a chemical composition that reflects a mixture of newly formed kaolinite and residual illite. Cations Mg and K in the spectrum may have been derived from adjacent illite particles or illite-kaolinite mixed-layer in the sample.

HRTEM with proper defocus and orientation revealed one-dimensional lattice images at nearly atomic resolution of the bio-oxidized original IMt-1 sample after 1 month of incubation (Fig. 10A). These images were compared with calculated models (Guthrie et al., 1989) to identify individual components of a repeating unit (layer) of phyllosilicates (e.g., 2:1 for illite and 1:1 for kaolinite). In the original IMt-1 sample, the interlayer region of illite 2:1 unit corresponded to the brightest lattice line, as indicated by the white bar on the right (Figure 10B), and the tetrahedral-octahedral-tetrahedral (TOT) structure corresponded to three dark lattice lines labeled with the black bars (Figure 10B). The original IMt-1 samples showed a typical tetrahedral-octahedral-tetrahedral-cation (TOTC) structure with 1 nm spacing (Figures 10A and 10B). All layers were homogeneously arranged. The bio-oxidized IMt-1 sample after 1 month of incubation

showed clay particles with a TOTC structure and fringes with 1 nm d-spacing (Fig. 10C). However, a decreased Al/Si ratio (relative to the original IMt-1 sample, Fig. D) and some changes to the amount of the interlayer cation were observed along the layers (Figures 10C, 10E, and 10F). Interlayer cations were observed on one side of the illite packet (Figure 10E), but disappeared approximately 10 nm away from it (Figure 10F). These observations suggest an onset of structural change of illite after a month of incubation.

4. DISCUSSION

4.1. Mechanisms of Fe(II) oxidation in illite

Our data demonstrated that *Pseudogulbenkiania* sp. strain 2002 was able to couple oxidation of structural Fe(II) in IMt-1 with reduction of nitrate to N₂ with nitrite as a transient intermediate (Figures 1 and 2). The observed non-stoichiometry of oxidized Fe(II) to reduced nitrate, as well as nitrite accumulation, is consistent with our previous investigation (Zhao et al., 2013), and may be explained by heterotrophic nitrate reduction coupled with the oxidation of stored energy reserves (i.e., glycogen) in cells or biomass of inactive cells. However, the stored energy in cells would outcompete Fe(II) as an electron donor in nitrate reduction, due to its generally more negative redox potential (Kappler, et al., 2005), thus leading to a non-stoichiometrically high nitrate removal. For example, the reduction potential of CO₂/acetate is -290 mV (Thauer, et al., 1977) but that of Fe(OH)³⁺/Fe²⁺ is 0 mV (Thamdrup, et al., 2000) . The amount of structural Fe(II) in IMt-1 was lower than in NAu-2, but the amount of nitrate removal by these two minerals was at a similar level. Thus, more organic matter oxidation must have occurred in IMt-1

than in N Au-2. This inference is consistent with the lower accumulation of intermediate product (i.e. nitrite) in IMt-1 oxidation in comparison to N Au-2 oxidation (e.g., 1 mM nitrite in IMt-1 vs. 2-3 mM nitrite in N Au-2 as reported Zhao et al., 2015), because the electron transfer from more negative CO₂/organic matter to nitrate could expedite the rate of nitrate reduction directly to the final product, nitrogen gas (Zhao et al., 2013). In this case, the intermediate product nitrite may have been either bypassed or consumed quickly as it was generated.

The mechanism of bio-oxidation of structural Fe(II) in phyllosilicates is poorly understood. Previous studies on nontronite bio-oxidation suggest that the electron transfer between strain 2002 and nontronite occurs via clay edges through a direct contact (Ribeiro, et al., 2009; Zhao, et al., 2015). In comparison to nontronite, the extent of bio-oxidation of Fe(II) in IMt-1 illite is much lower (Table 1). This type of difference between illite and nontronite has been observed for bioreduction as well (Bishop et al., 2014). In order for bio-oxidation to take place, there should be a direct contact between illite and strain 2002 because 2002 cells are not known to produce any electron shuttles or nanowires. Illite possesses a higher negative layer charge but lower surface area than smectite (Moore and Reynolds, 1997). As a result, smaller areal contact is expected to occur between strain 2002 cells and illite particles relative to nontronite, which may have led to the observed lower Fe(II) bio-oxidation extent and rate of illite than those for nontronite.

Because the electron transfer between strain 2002 and illite is expected to occur via clay edges through direct contact, the interlayer may not be involved. If so, the non-expandable nature of illite interlayer should not be the reason for the lower bio-oxidation

extent of illite relative to smectite. Two additional lines of evidence support that electron transfer in bio-oxidation occurred via clay edges rather than interlayer: 1) Neither IMt-1 nor NAu-2 showed a complete oxidation of structural Fe(II) (Figure 1), presumably because much of the Fe(II) domain within the clay structure could not be reached by the oxidizing bacteria; 2) Not all biogenic Fe(II) in pre-reduced illite was oxidized by strain 2002 (Figure 1). Because biogenic Fe(II), when produced in the presence of electron shuttle, is distributed in both structural and edge sites (Dong et al., 2009; Zhao et al., 2015), its incomplete oxidation by strain 2002 suggests that only the edge-Fe(II) was oxidized.

4.2. Microbially mediated clay mineral transformation

In our experiments, Fe(II)-oxidizing bacteria facilitated the illite→kaolinite (Figures 3, 5, 6, 7, and 9), the illite→smectite (Figures 3, 4, and 8), and possibly smectite → kaolinite transformations. The biogenic smectite is a transient phase (Figures 3, 6, & 8) that was observed after 2 weeks, 3 and 6 months of bio-oxidation, but it disappeared after extended incubation for 24 months. The disappearance of the biogenic smectite in the 24-month sample suggests that with prolonged bacterial incubation the smectite either transformed into kaolinite or dissolved. Our aqueous chemistry data for the 24-month sample revealed no significant increases of Si, Al, and Fe in solution (data not shown), and TEM and XRD data indicated no precipitates of biogenic silica and/or Fe/Al (oxy)hydroxides. All these lines of evidence support the possibility of smectite→kaolinite conversion. A similar clay transformation sequence of illite→smectite→ kaolinite was observed in nature (red earth sediments) (Robert et al., 1991; Dudek et al., 2007; Hong et al., 2015). It is believed that this clay transformation

starts with an exchange of K^+ in illite by hydrated cations (such as Mg^{2+}), which leads to illite swelling, followed by a local dissolution of a smectite layer, and eventually forms kaolinite. This mechanism is supported by our HRTEM observations, e.g., the illite interlayer cations were removed in the 1-month sample (Fig. 10), a large amount of smectite emerged in the 3- and 6-month samples (Fig. 8), and eventually a mixed-layer illite/kaolinite formed in the 24-months sample (Fig. 9). However, the newly formed platy kaolinite crystals observed in the 24-month sample (Figure 7) suggest a dissolution/recrystallization mechanism. Therefore, the clay transformation sequence illite→smectite→kaolinite may have involved both solid state and dissolution/crystallization mechanisms.

Based on multiple lines of evidence, we postulate that upon microbial nitrate-dependent Fe(II) oxidation, illite transformation to kaolinite can proceed through the following steps: 1) During the initial phase of illite transformation stimulated by Fe(II)-oxidizing bacteria, the bio-oxidation of structural Fe(II) would increase the positive charge in the octahedral sheet, thus leading to expulsion of interlayer cations to balance the charge. The reactant illite could go through partial dissolution due to microbial activity, losing Al in structure, leading to a decreased Al:Si ratio. As a result, with a decreased attractive force between adjacent TOT units (due to a decreased number of interlayer cations), the original illite structure would become expandable and wavy, which are characteristics of smectite. 2) Upon the nitrate-dependent Fe(II) oxidation reaction, smectite would dissolve and release Si and Al (Jaisi et al., 2008; Carroll et al., 1971), the major elements of kaolinite, to solution (Churchman and Jackson 1976). Once the solution reached supersaturation, kaolinite would precipitate around pre-existing

minerals (Figure 7), because these minerals may have served as nucleation sites. In addition, the tetrahedral layers of neoformed smectite could become more readily solubilized because fluid should be more easily able to move through the expandable interlayers, and if one tetrahedral layer dissolved, the smectite could transform to kaolinite via mixed layer K/S as intermediates (Amouric et al., 1998). 3) Alternatively, illite could have transformed to kaolinite directly via intermediate I/K mixed layer phases. Our TEM observations (Figs. 9B & 9D) support this mechanism, where illite and kaolinite layers were observed together in the same region. A previous study has reported an illite→ kaolinite reaction in nature, which usually happens in acidic Al- and Si-rich environments (Sand 1956). The illite→kaolinite reaction requires dissolution of one tetrahedral sheet of illite, and production of domains of layers with only one octahedral and one tetrahedral sheet. The d(001) layer spacing would not collapse to 7 Å kaolinite layers in large amounts and be detected by XRD until those domains are sufficiently large, thus large domains of I/K mixed layers are more likely to be found in samples after long times of incubation. This is supported by our TEM observation, in which I/K mixed layers were only found in 24-month sample (Figure 9).

Given the small amount of Fe(II) oxidation measured, the observation of smectite and kaolinite appeared to be unusual. However, the Fe(II) concentration was measured on bulk samples, it was likely that small illite particles may be preferentially oxidized and local Fe(II) oxidation extent could be much higher, which would trigger the illite-smectite-kaolinite transformation at a local scale. Furthermore, small Fe(II) concentration change may not necessarily stop kaolinite formation. In our postulated illite →kaolinite conversion mechanism (described step 3 above) bacteria may have accelerated this

dissolution process (such as via local pH decrease and metabolic byproducts) without necessarily oxidizing structural Fe(II) in illite.

4.3. Geological implication and future study

The roles of microbial processes in mediating clay mineral transformation are reinforced in this study, showing the conversion of illite to kaolinite with smectite as an intermediate product, which is stimulated by nitrate-dependent iron-oxidizing bacteria. This discovery, coupled with the previous finding of the microbial role in promoting the smectite to illite reaction through Fe-reducing bacteria (Kim et al., 2004), completes the iron redox and mineral transformation cycles. However, because of secondary mineralization, such as biogenic silica and siderite formation during microbial conversion of smectite to illite, and kaolinite formation during microbial conversion of illite to smectite, these cycles may not be completely reversible.

An early study demonstrated that microbial reduction of structural Fe(III) in smectite accelerated the smectite-illite reaction at room temperature in two weeks (Kim et al., 2004). This reaction typically requires 300-350°C, 100 megapascals, and 4 to 5 months in the absence of microbial activity. The Kim et al. study and all other follow-up studies (reviewed in Dong et al., 2009; Dong 2012) are important because this smectite-to-illite reaction is typically used as an index to infer paleo-temperature, based on the fact that smectite is a low temperature mineral, and with increased sediment burial and temperature, smectite should gradually transform to illite. However, with microbial activity, the inferred paleo-temperature will be inaccurate and may be much higher than actual temperature. In this study, we have demonstrated that microbial oxidation of

structural Fe(II) in illite can reverse the reaction, e.g., illite to smectite transformation. In this case, the inferred paleo-temperature is again inaccurate, but in opposite way, i.e., the inferred temperature may be lower than actual temperature.

The discovery of a microbial role in promoting clay mineral transformation through Fe redox cycling may have provided a mechanism to explain seemingly diverse clay studies in the literature. For example, chlorite to vermiculite transformation has been reported to be a consequence of oxidation of structural Fe(II) in chlorite (Gilkes and Little, 1972; Ross and Kodama, 1976). The reduction of Fe(III) to Fe(II) can drive the smectite to illite reaction, such as Cretaceous bentonites (Eslinger et al., 1979). Likewise, smectite illitization has been shown to be catalyzed by organic matter that is released from smectite interlayer upon sediment burial (Li et al., 2016), but this catalytic effect is likely achieved through microbial reduction of structural Fe(III) in smectite (Zhang et al., 2007a). The valence change of structural Fe in clay minerals can alter the stability of clay layers (Stucki J.W., 2011) and cause a transition between trioctahedral and dioctahedral structure types (Manceau et al., 2000a, b). Although these early studies did not explicitly invoke microorganisms as mediating agents, it is certainly possible that these clay reactions were catalyzed by various microbes.

Although it is well-recognized that clay mineral transformation is promoted through microbially mediated Fe redox cycling (Dong et al., 2012), it remains unclear if reduction/oxidation extent or rate is correlated with such transformation and secondary mineralization. It is also poorly understood if these microbially catalyzed biomineralization can readily be recognized from abiotic pathways of clay mineral transformation. In this context, characteristic biogenic minerals (Dong et al., 2012; Zhao

et al., 2015; Liu et al., 2015) are important because they may serve as biosignatures. Recognition of these mineral assemblages in rock records will likely provide clues for involvement of microorganisms in ancient geological processes (Vorhies and Gaines, 2009). Future work should focus on the relative importance of biotic versus abiotic pathways in promoting clay mineral transformation with a combined investigation of model systems and natural samples.

5. CONCLUSIONS

Nitrate-dependent, Fe(II)-oxidizing bacterium *Pseudogulbenkiania* sp. strain 2002 was capable of coupling oxidation of structural Fe(II) in illite with reduction of nitrate to nitrogen gas via nitrite as an intermediate. This microbial process promoted the illite→smectite→kaolinite transformation. This study, when coupled with our early finding on the role of microbial Fe(III) reduction in promoting the smectite to illite reaction, demonstrates that Fe redox process and its effects on clay mineral transformation are reversible.

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648 **TABLE CAPTION**

649 **Table 1.** Fe(II) concentration before and after bio-oxidation, and bio-oxidation extent and rate

		Initial Fe(II) conc.		Final Fe(II) conc. after 10 days		Bio-oxidation extent		Bio-oxidation rate (5 days)	
		mmol/g	Error	mmol/g	Error	%	Error	mmol/g/day	Error
Illite (IMt- 1)	Original	0.202	0.007	0.183	0.009	9.406	1.389	0.004	0.001
	Pre-reduced	0.307	0.020	0.246	0.001	19.870	5.640	0.011	0.003
Nontronite (NAu-2)	Original								
	Pre-reduced	1.105	0.049	0.563	0.043	49.050	1.625	0.109	0.001

650 NOTE: (1). Bio-oxidation extent was calculated according to the equation $Oxidation\ extent = \frac{Fe(II)_{initial} - Fe(II)_{10\ days}}{Fe(II)_{initial}}$

651 (2). Bio-oxidation rate was calculated according to the equation $Bio - oxidation\ rate = \frac{|Fe(II)_{5\ days} - Fe(II)_{initial}|}{\Delta time}$

652 (3). Bio-oxidation data of pre-reduced nontronite were from a previous study (Zhao et al., 2013).

FIGURE CAPTION

Figure 1. Time-course change of concentrations of Fe(II) in illite (IMt-1) and nontronite (NAu-2), nitrate, and nitrite produced from the nitrate-dependent Fe(II) oxidation experiments using strain 2002. The two figures on the left (A & C) represent the Fe(II) concentration, and the two figures on the right (B & D) represent the nitrate and nitrite concentrations. For a comparison purpose, the bio-oxidation data for reduced nontronite NAu-2 from our previous research were included (Zhao et al., 2013). For NAu-2, there was no “original clay”. In other words, NAu-2 samples were pre-reduced first before the bio-oxidation experiment. Nitrate concentration decreases over time, but nitrite concentration started at zero initially, accumulated in a few days, and then decreased to zero near the end of the experiment.

Figure 2. Nitrogen gas production in abiotic controls and bio-oxidized samples (10 day incubation) of the original IMt-1 and pre-reduced-IMt-1.

Figure 3. X-ray diffraction patterns of A) air-dried original IMt-1 sample showing illite (I) only with four peaks corresponding to d001, d002, d003 and d005 reflections of standard illite, B) air-dried bio-oxidized IMt-1 samples after 3 months of incubation showing a 14 Å d-spacing peak that corresponds to d001 reflection of smectite (S), and 7 Å and 3.5 Å d-spacing peaks that correspond to d001 and d002 reflections of kaolinite (K); C) air-dried bio-oxidized IMt-1 samples after 24 months of incubation (C), showing 7 Å and 3.5 Å d-spacing peaks only.

Figure 4. X-ray diffraction patterns of air-dried (A) and ethylene glycolated (B) bio-oxidized IMt-1 sample after 3 months of incubation, showing that 14 Å d-spacing peak was present in air-dried sample, but absent in ethylene glycolated sample.

Figure 5. Second derivative FTIR spectra of OH-stretching bands for abiotic (black) and bio-oxidized (gray, 12 month incubation), original IMt-1 samples.

Figure 6. SEM images showing neoformed biogenic kaolinite and smectite as a result of bio-oxidation of structural Fe(II) in the original IMt-1 after 3 months; (A) Original IMt-1 sample; (B) bio-oxidized IMt-1 sample after 3 months of incubation, showing platy neoformed kaolinite (black arrow) and smectite particles with wavy texture (white arrow); (C) An enlarged image showing biogenic kaolinite; (D) An enlarged image of biogenic smectite showing a wavy texture (grey arrows); (E) SEM-EDS compositions of the above particles. Lack of Fe peak and low K peak for illite are likely due to a low voltage used to take this images (7 KeV). The high C peak for the particle on Figure D may have come from uneven carbon coating on the top (white open arrow) or cell products (such as extracellular polysaccharides).

Figure 7. SEM images of neoformed biogenic kaolinite from the bio-oxidized IMt-1 sample after 24 months of incubation. (A) SEM images of biogenic kaolinite particle with a pseudo-hexagonal shape (white arrow) next to illite particles (black arrow). (B) A magnified image of the biogenic kaolinite particle from Figure A. (C) The corresponding qualitative SEM-EDS compositions for the particles in image A that are characteristic of illite and kaolinite, respectively.

Figure 8. HRTEM images of the neoformed biogenic smectite produced in bio-oxidized IMt-1 sample. (A) TEM image of the original IMt-1 sample, showing well-defined platy particles. (B) TEM image of the bio-oxidized IMt-1 sample after 2 weeks of incubation, showing that a packet of wavy smectite-like layers occurred in the illite matrix (black arrow). (C) TEM images of the bio-oxidized IMt-1 sample after 6 months of incubation, displaying more abundant wavy smectite layers than the 2-week sample (the square areas). (D & E) High resolution TEM lattice fringe images from the enlarged white and black square areas in (C), respectively, showing straight 1.0 nm and wavy fringes (D) and wavy 1.4 nm fringes (E). (F) Qualitative TEM-EDS compositions for illite in (A), the smectite packet in (B), and smectite packets in (C).

Figure 9. HRTEM images of bio-oxidized IMt-1 samples after 24 months of incubation, showing the neoformed biogenic kaolinite particles. (A) TEM image showing a stack of pseudo-hexagonal crystals with a diameter of 0.2 μm . (B) High resolution TEM lattice fringe image from the square area in (A), showing 0.7, 1.0, and 1.7 nm layer spacings corresponding to kaolinite, illite, and mixed-layer illite-kaolinite, respectively. (C) The qualitative TEM-EDS composition for the platy crystal in Figure (A) showing a kaolinite-like composition. (D) High resolution TEM lattice fringe images showing the co-existence of 0.7 and 1.0 nm layer spacings, corresponding to kaolinite and illite, respectively. (E) The corresponding qualitative TEM-EDS composition for the crystals in (D) showing a kaolinite-like composition.

Figure 10. HRTEM images showing structural changes of IMt-1 illite as a result of bio-oxidation for 1 month. (A). High resolution TEM lattice fringe image of the original IMt-1 sample showing 1 nm layer spacing. (B). High resolution TEM lattice fringe image

from the enlarged square area in (A) displaying the individual tetrahedral-octahedral-tetrahedral-cation sheets in the illite structure. (C). High resolution TEM lattice fringe image of the bio-oxidized IMt-1 sample after 1 month of incubation, showing a packet of illite (black square at the lower left corner of the image) showing 1 nm layer spacing and structural change along the layers (grey square in the upper region of the image). (D). The corresponding TEM-EDS composition for (A) and (C). (E) and (F). High resolution TEM lattice fringe images of the enlarged black and grey square areas in (C) showing along-layer structural change of an illite packet from standard illite to altered illite (without the interlayer cations).

	Abiotic Control		Biotic Treatments	
	Fe(II) or Nitrate	Nitrite	Fe(II) or Nitrate	Nitrite
Original Clay	●	★	○	☆
Reduced Clay	■	◆	□	◇

Figure 1

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Figure 2

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Figure 9

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Figure 10