Effects of Phosphate on Secondary Mineral Formation During the Bioreduction of Akaganeite (β-FeOOH): Green Rust Versus Framboidal Magnetite

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Abstract: The activity of microorganisms is a key component of the biogeochemical cycle of Fe in natural systems, where green rusts are often observed as products of microbially driven redox processes. To better define the factors that control green rust formation during microbial Fe(III) reduction, we examined the effects of the presence of an electron shuttle [9,10-anthraquinone-2,6-disulfonate (AQDS)] and phosphate on akaganeite (β-FeOOH) bioreduction by the iron(III)-reducing bacterium (IRB) Shewanella putrefaciens CN32. Framboidal magnetite was the principal secondary mineral formed during akaganeite bioreduction in the absence of phosphate; this is the first time framboidal magnetite has been reported as a product of microbial Fe(III) oxide reduction. Framboidal magnetite was less crystalline when formed in the presence of AQDS than without AQDS and over time was further reduced to chukanovite. Carbonate green rust was the primary secondary mineral observed from akaganeite bioreduction in the presence of phosphate, with and without AQDS; however, siderite was also observed in the presence of AQDS. This first report of green rust as a product of akaganeite bioreduction expands the range of Fe(III) oxides that can be transformed to green rust by IRB, suggesting that the reduction of Fe(III) oxides such as ferricydrite, lepidocrocite, and akaganeite by IRB is a key process leading to the formation of green rusts in aquatic and terrestrial environments.

Keywords: AQDS, akaganeite, bioreduction, chukanovite, electron shuttle, framboidal magnetite, green rust, iron(III) oxide, iron-reducing bacteria, magnetite, phosphate, Shewanella, siderite.

1. INTRODUCTION

Green rusts are a class of Fe(II)-Fe(III) layered double hydroxides with a pyroaurite-type structure. The Fe(III) content of green rusts is highly variable, ranging from 0.33 in stoichiometric green rust—i.e., $(\text{Fe}^{II}_4\text{Fe}^{III}_2\text{(OH)}_{12}\text{)}^{12+}$ $[(\text{A})_{2n}\text{H}_2\text{O}]^{y-}$; where $\text{A}$ is an $n$-valent anion (typically Cl, SO$_4$$^-$, or CO$_3$$^-$) and $y$ denotes varying amounts of interlayer water ($y=2$ to 4 for most green rusts)—to 1.0 in so called “ferric green rusts.” Green rusts are found in a wide range of natural and engineered environments with Fe(II)/Fe(III) transition zones including surface waters [1], groundwater [2-3], soils [4-8], and sediments [9-11], as well as among corrosion products in zero-valent-iron permeable reactive barriers [12-14]. These environments typically exhibit conditions that promote the redox cycling of Fe, and green rust minerals such as fougérite, trebèurdenite, and mössbauerite [15-17] may play a central role in the biogeochemistry of Fe in these environments. Besides their importance in the biogeochemical cycling of Fe, green rusts have been widely studied due to their ability to reduce a range of pollutants, including chlorinated solvents, nitroaromatics, azo dyes, toxic metals, metalloids, and radionuclides [18-29].

The activity of microorganisms is a key driver of the biogeochemical cycle of Fe in natural systems (directly by catalyzing Fe redox reactions and indirectly by creating redox gradients), and green rusts are frequently observed as intermediates or products of microbially driven oxidative and reductive processes. Green rusts have been observed as products of direct microbial or coupled biotic/abiotic oxidation of Fe(II) by denitrifying bacteria under anoxic conditions [30-32]. Conversely, iron-reducing bacteria (IRB) and archaea are diverse groups of microorganisms that can use Fe(III) oxides such as ferricydrite, goethite (α-FeOOH), akaganeite (β-FeOOH), lepidocrocite (γ-FeOOH), hematite (α-Fe$_2$O$_3$), maghemite (γ-Fe$_2$O$_3$), and magnetite (Fe$_3$O$_4$) as terminal electron acceptors (TEAs) for anaerobic respiration, and in the process generate secondary minerals containing Fe(II) such as magnetite (Fe$_3$O$_4$), siderite (FeCO$_3$), vivianite [Fe$_6$(PO$_4$)$_3\cdot$8H$_2$O], chukanovite (ferrous hydroxy carbonate), and green rusts [33-39]. The specific factors controlling the formation of green rusts during the reduction of Fe(III) oxides by IRB are not unambiguously defined; however, the rate and magnitude of Fe(II) production and its reaction with...
residual Fe(III) oxides as well as the presence of ligands such as phosphate, silicate, and organic matter have been cited as primary factors [35, 38, 40-43]. To further our understanding of the factors controlling green rust formation during the reduction of Fe(III) oxides by IRB, we examined the effects of the presence of an electron shuttle [which can increase the rate of microbial reduction of Fe(III) oxides] and phosphate (the presence of which has been implicated in the formation of green rust during microbial reduction of ferrihydrite [38, 40-41] and lepidocrocite [35, 44]) on the reduction of akaganeite by the IRB *Shewanella putrefaciens* CN32.

2. MATERIALS AND METHODS

2.1. Experimental Setup

Akaganeite was prepared as described by Schwertmann and Cornell by dissolving 81 g of FeCl₃·6H₂O in 3 L of water and maintaining the solution [0.1 M Fe(III)] at 60 °C for 8 d [45]. The resulting solids were washed repeatedly by centrifugation and resuspension in water (18 Mohm-cm), dried at 60 °C, and ground to pass a 200-mesh sieve. The solids had a surface area of 35.16 ± 0.16 m²g⁻¹ as determined by Brunauer–Emmett–Teller (BET) analysis of N₂ sorption and were identified as akaganeite on the basis of characterization by powder X-ray diffraction (pXRD) and ⁵⁷Fe Mössbauer spectroscopy (Fig. 1).

The bioreduction of akaganeite was examined in 160-mL serum bottles containing 100 mL of sterile defined mineral medium [46] amended with 80 mM Fe(III) (as akaganeite) and 75 mM formate, with or without the electron shuttle 9,10-anthraquinone-2,6-disulfonate (AQDS, 100 μM) or phosphate (500 μM), adjusted to a pH of 7.5. Oxygen was removed by sparging with sterile Ar. After 24 h, a subsample of suspension from the phosphate-amended systems was removed for determination of the extent of phosphate sorption to akaganeite.

The dissimilatory IRB *S. putrefaciens* strain CN32 (ATCC BAA-543) was cultured and prepared for use as described by O’Loughlin et al. [47]. The bottles were inoculated with 5 × 10⁹ cells mL⁻¹ of washed *S. putrefaciens* CN32; a control for examining the potential for aggregation of akaganeite in the presence of biomass was “inoculated” with an equivalent amount of pasteurized (1 h at 60 °C) biomass. The bottles were incubated in the dark at 30 °C and the suspensions were kept well mixed on a Bellco Glass, Inc. roller drum. At selected times, samples of the suspensions were collected with sterile syringes for monitoring of pH and Fe(II) production and for identification of secondary minerals with pXRD, scanning electron microscopy (SEM), and ⁵⁷Fe Mössbauer spectroscopy. With exceptions as noted, samples were collected and prepared for analysis under anoxic conditions in a glove box containing N₂ with 3-5% H₂. Linear regression was used to calculate Fe(II) production rates over the time of maximum sustained production.

2.2. Analytical Methods

The Fe(II) content of 0.75 M HCl extracts of the suspensions was used to monitor the bioreduction of akaganeite as described in O’Loughlin et al. [35] [referred to hereafter as total Fe(II) or Fe(II)ₜ]. Briefly, samples for Fe(II)ₜ analysis were prepared by adding 0.75 mL of anoxic 1 M HCl to 0.25 mL of suspension. The samples were mixed on an end-over-end shaker for two weeks, then centrifuged at 25,000 × g for 10 min. The concentration of Fe(II) in the supernatants was measured with the ferrozine assay [48] using HEPES-buffered ferrozine reagent [49]. The sorption of phosphate to akaganeite was determined by measuring dissolved P with inductively coupled plasma-optical emission spectroscopy with a PerkinElmer 4300DV instrument.

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The secondary minerals were identified with pXRD by using a Rigaku MiniFlex X-ray diffractometer with Ni-filtered Cu Kα radiation [35]. Samples for pXRD analysis were collected by filtration on 25-mm, 0.22-μm nylon filters, then covered with 8.4-μm-thick Kapton® film under anoxic conditions and placed in the instrument; although the pXRD analysis was conducted under an oxic atmosphere, there was no evidence of sample oxidation when the samples were scanned between 5° and 80° 2θ at a speed of 1.25° 2θ min⁻¹. The pXRD patterns were analyzed with the JADE 7 software package (MDI, Livermore, CA) to remove the background through polynomial fitting and also to remove the Kα₂ components.

Samples for SEM imaging were prepared by depositing 500 μL of suspension on aluminum specimen mounts, allowing the solids to settle, removing the overlying liquid with a pipette, and drying the film of solids in a glove box. Specimens were briefly (< 30 s) exposed to air during transfer to the Hitachi S-4700-II FEG-SEM.

We collected ⁵⁷Fe Mössbauer spectra by using a variable-temperature He-cooled system equipped with a 1024-channel detector [35]. The ⁵⁷Co source used (~50 mCi) was in a Rh matrix at room temperature. All center shifts are reported relative to α-⁵⁷Fe foil analyzed at room temperature. Samples were prepared by filtering an aliquot of suspension (~4 mL) in an anaerobic glove box (<1 ppm O₂; 5% H₂/95% N₂). The filtered samples were then sealed between two pieces of 5-mm Kapton tape. We fitted the spectra using Recoil Software (University of Ottawa, Ottawa, Canada). All samples were fit using a Voigt-based model, holding the Lorentzian linewidth at 0.12 mm s⁻¹; this value was determined experimentally by fitting the α-⁵⁷Fe foil with a Lorentzian model. For sextets, the relative peak areas were held constant at their ideal values (1:1 for doublets, 3:2:1:1:2:3 for sextets).

3. RESULTS

3.1. Bioreduction of Akaganeite in the Absence of Added Phosphate

Akaganeite reduction by S. putrefaciens CN32 in systems not amended with AQDS or phosphate resulted in the accumulation of 23 mM Fe(II)₇ within the first 2 d and within 7 d Fe(II)₇ reached a plateau of ~28 mM (Fig. 2) that was accompanied by the disappearance of akaganeite, as determined by pXRD (Fig. 3). Highly crystalline magnetite was the dominant secondary mineral, with indications of siderite after 80 d. SEM imaging revealed that the magnetite was composed of fine-grained particles (20-50 nm) associated in nominally spherical aggregates (up to 500 nm in diameter), with a framboidal arrangement that remained stable for many months (Fig. 4), as well as cubic crystallites of siderite [39].

The presence of the electron shuttle AQDS greatly enhanced the rate of Fe(II) production during akaganeite bioreduction, having a maximum sustained rate of 59.3±4.3 mM d⁻¹ versus 13.7±0.43 mM d⁻¹ in the absence of AQDS. Within 12 h the Fe(II)₇ concentration reached 26 mM, before plateauing at ~32 mM by 7 d. As with the non-AQDS-amended system, the bioreduction of akaganeite was accompanied by the formation of framboidal magnetite (Figs. 3 and 5); however, the magnetite was less crystalline than the magnetite peaks decreased concomitantly with the appearance and growth of peaks corresponding to chukanovite (Fig. 3). SEM imaging of the secondary minerals over time confirmed that chukanovite formed at the expense of magnetite (Fig. 5). Well-formed framboidal magnetite was evident at 5 d, along with irregularly shaped laths (presumably nascent chukanovite crystals). By 80 d the magnetite framboids were less abundant and platy, micrometer-sized crystallites with well-defined edges typical of the morphology of chukanovite were apparent. After 1 y, large laths of chukanovite dominated, and there was little indication of magnetite. Furthermore, Mössbauer analysis indicated that at 1 y chukanovite accounted for 74% of the Fe in the solids, with the remainder present as magnetite (Fig. 6).

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Fig. (2). Production of Fe(II)₇ [0.75 M HCl-extractable Fe(II)] (A) during the first 4 h of akaganeite bioreduction and (B) over 80 d in the presence and absence of AQDS and phosphate.

3.2. Bioreduction of Akaganeite in Phosphate Amended Systems

The addition of 500 μM phosphate significantly affected akaganeite bioreduction (Fig. 2) in both the presence and absence of AQDS. In the absence of AQDS, total Fe(II) production was initially inhibited, with accumulation of only 3 mM Fe(II) within the first 2 d versus 23 mM in the same period in the system without AQDS and phosphate. The kinetic profile of total Fe(II) accumulation was relatively complex, with production rates of 0.38±0.03 mM d⁻¹ at 4–33 d, 1.31±0.22 mM d⁻¹ at 33–47 d, and 0.35±0.01 mM d⁻¹ at 47–68 d. At 80 d the Fe(II)₇ concentration plateaued at 41 mM—significantly higher than 29 mM at 80 d in the non-phosphate-amended system without AQDS. The bioreduction of akaganeite in phosphate amended systems was accompanied by carbonate green rust formation (Fig. 7); other secondary minerals such as magnetite, siderite, or...
chukanovite were not evident. The green rust crystallites were platy and nominally hexagonal but lacked the discrete well-defined edges often observed for biogenic green rust (Fig. 8) [36-38, 44, 50].

The inhibitory effect of 500 μM phosphate on akaganeite bioreduction was largely overcome by the addition of the electron shuttle AQDS (Fig. 2). No initial lag period was seen (as in the absence of AQDS), and the maximum sustained total Fe(II) production rate of 22.9±1.4 mM d⁻¹ was faster than for both the non-phosphate-amended system without AQDS (13.7±0.43 mM d⁻¹) and the 500-μM-phosphate-amended system without AQDS (1.31±0.22 mM d⁻¹). Within 12 d, the Fe(II)ₜ concentration reached ~50 mM and then remained stable through the end of the experiment. As in the absence of AQDS, the primary secondary mineral was carbonate green rust (~79% of Fe in the solids as determined by Mössbauer spectroscopy (Fig. 6)], although the platy hexagonal crystals were better formed and had more defined edges (Fig. 8). In addition, unlike the non-AQDS-amended system, other secondary minerals were observed. Mössbauer analysis of the solids indicated ~21 % of the Fe was present as siderite, and cubic crystallites consistent with siderite were observed with SEM (Fig. 8). Although there was no clear indication of chukanovite in the pXRD pattern (Fig. 7) and Mössbauer spectrum, occasional bladed crystals of chukanovite were observed by SEM (Fig. 8).

4. DISCUSSION

4.1. Framboidal Magnetite

The transformation of akaganeite to magnetite in aqueous systems can occur by means of multiple abiotic and biotic processes. Akaganeite was transformed to magnetite by reduction with hydrazine at 100 ºC and alkaline pH [51] and by reaction with Fe²⁺ over a wide range of temperatures (25–200 ºC) and pH values (3–13) [52]. The formation of magnetite as a secondary mineral during the reduction of akaganeite has been reported for phylogenetically diverse IRB including mesophilic Geobacter sulfurreducens [53], multiple mesophilic or psychrotolerant species of Shewanella [34, 54-57], and thermophilic Thermoanaerobacter ethanolicus TOR-39 [54, 58-59]. The magnetites formed by these biotic and abiotic processes have typically been fine grained (< 100 nm) crystallites present as dispersed particles or amorphous aggregates. The magnetite formed in our experimental system was similarly fine grained (20–50 nm), but the crystallites formed well-defined frambooids up to 500 nm in diameter.
Fig. (5). SEM images of the solids formed during the bioreduction of akaganeite with AQDS but no added phosphate. (A) Framboidal magnetite and nascent chukanovite crystals at 5 d; (B) chukanovite and framboidal magnetite at 130 d; and (C) chukanovite with residual magnetite at 364 d.

Framboidal morphology (named for its raspberry-like shape) is not typical for magnetite; however, it has been observed in diverse terrestrial and extraterrestrial materials, including hydrocarbon deposits [60-61], sedimentary carbonate rocks [62-63], and carbonaceous meteorites [64-65]. Several processes leading to the formation of frambooidal magnetite have been proposed. Framboidal magnetite bears a strong resemblance to frambooidal pyrite, which is commonly observed in many sedimentary environments [66-70], and there are indications that frambooidal magnetite can form by oxidative transformation of frambooidal pyrite [62]. This pathway to frambooidal magnetite is not relevant to our experimental system, as conditions were unfavorable for pyrite formation (sulfide would not be produced, as S. putrefaciens CN32 does not respire with sulfate as a terminal electron acceptor); furthermore, pyrite was not observed at any point in the bioreduction of akaganeite to magnetite (Fig. 3). Other possibilities include formation by a route analogous to that proposed by Sweeney and Kaplan [71] for frambooidal pyrite but with an undefined spherical Fe oxide precursor [65], or by direct precipitation from solution [64].

The formation of frambooidal magnetite from a framboidal akaganeite precursor seems unlikely in our system, as we saw no indication of spherical or frambooidal akaganeite aggregates in our control system containing pasteurized biomass (Fig. 9); the biomass was pasteurized at 60 °C for 1 h to render the cells inactive, thus preventing bioreduction of akaganeite and presumably maintaining the presence of cells and extracellular polymeric substances that might have played a role in the aggregation of akaganeite (or magnetite in the presence of live cells) and subsequent development of frambooids. In both the experimental system and the control, the solids were kept suspended with gentle mixing on a roller drum. Framboidal magnetite was not observed in the static control, suggesting that the mixing action was necessary for its development. Moreover, something specific about akaga-
ganite apparently allows for framboidal magnetite formation, as it was not observed in identical experimental systems that contained lepidocrocite in place of akaganeite [35].

4.2. Electron Shuttle Effects

Soluble TEAs such as oxygen, sulfate, and nitrate are easily transported into the cell. However, Fe(III) oxides are poorly soluble and their use as TEAs by IRB requires specialized approaches. One approach used by IRB transfer electrons to Fe(III) oxides involves direct physical contact of outer membrane reductases [72] or conductive appendages [73-74] with the oxide surface. However, the need for physical contact with the Fe(III) oxide can be circumvented by the use of soluble electron shuttles, i.e., compounds that can cycle between reduced and oxidized forms. The oxidized form of the electron shuttle can be reduced by the organism and then, in reduced form, can transfer electrons to the Fe(III) oxide at a distance and thus become reoxidized. Since, in principle, electron shuttles can be cycled repeatedly, the presence of even relatively low concentrations of electron shuttles can facilitate the bioreduction of Fe(III) oxides.

A variety of organic and inorganic compounds can be used as electron shuttles by IRB respiring on Fe(III) oxides, including humic substances (HS)—a class of naturally occurring, chemically heterogeneous organic oligoelectrolytes derived primarily from the decomposition of bacterial, algal, and higher plant material that are key components of the organic C pool in aquatic and terrestrial environments [75-80]. The ability of HS to act as electron shuttles is primarily due to the presence of quinone groups in their structure, [81-83] and model quinones with well-defined redox characteristics (e.g., reduction potentials), like AQDS, have been widely used as analogs for the quinone groups in HS [38, 46, 75, 84].

Fig. (7). pXRD analysis of the solids formed during the bioreduction of akaganeite, with and without AQDS, in the presence of phosphate.

Fig. (8). SEM images of the solids formed during the bioreduction of akaganeite in the presence of 500 μM phosphate. (A) Poorly formed green rust crystals with irregular edges formed in the absence of AQDS. (B) Hexagonal, platy green rust crystals with well-defined edges and occasional cubic siderite and bladed chukanovite crystals.

Fig. (9). SEM image of akaganeite with pasteurized S. putrefaciens CN32.

Enhancement of the rate and extent of akaganeite reduction by S. putrefaciens CN32 in the presence of AQDS is in agreement with other studies demonstrating greater
bioreduction of Fe(III) oxides when AQDS is present [38, 46, 53, 75-76, 85-91, among others]. Although enhanced reduction of Fe(III) oxides by IRB is commonly observed in the presence of electron shuttles, a consistent effect of electron shuttles on the formation of secondary minerals is not evident. Zegeye et al. [88] and Coker et al. [89] observed that the presence or absence of AQDS had no effect on secondary mineral formation during the bioreduction of lepidocrocite and ferrihydrite, respectively. Conversely, Fredrickson et al. [38] and Zachara et al. [85] reported that AQDS affects both the types of secondary minerals and their relative crystallinity. These differences are likely due to differences in experimental conditions other than the presence/absence of AQDS.

In our experimental systems, framboidal magnetite was observed with and without AQDS; however, the magnetite was significantly more crystalline when formed in the absence of AQDS than in its presence. Moreover, most of the magnetite formed in the AQDS-amended system was ultimately transformed to chukanovite, but only a minor amount of the magnetite in the AQDS-free system was converted to siderite. In our phosphate-amended systems, carbonate green rust formed in both the presence and absence of AQDS, but the crystallites were better formed in the presence of AQDS (i.e., the crystallites had more well-defined edges and overall were more representative of the hexagonal platy morphology typically observed for green rusts), and a significant amount of siderite formed, along with traces of chukanovite. Chukanovite has been observed as a tertiary mineral in several studies of the bioreduction of ferrihydrite, lepidocrocite, and maghemite when magnetite [34, 35, 43, 44, 92] or green rust [44, 93] was the secondary mineral—that is, magnetite or green rust formed first, and subsequent chukanovite precipitation was at their expense—and in all cases, AQDS was present. As the role of AQDS in chukanovite formation as a tertiary mineral in Fe(III) oxide bioreduction has not been studied explicitly, it is not clear if chukanovite can form in the absence of AQDS. Given that electron shuttles like AQDS can enhance both the rate and extent of Fe(III) oxide bioreduction, they might well have a similar effect on the bioreduction of magnetite and green rust, thus enhancing formation of tertiary minerals like chukanovite.

4.3. Role of Phosphate in Controlling the Formation of Secondary Minerals

Secondary minerals formed during Fe(III) oxide reduction can include magnetite, green rust, siderite, chukanovite, and vivianite; however, the specific factors controlling which minerals form are often not readily apparent. The formation of green rust as a secondary mineral has been reported for the bioreduction of ferrihydrite [37, 38, 40, 41, 94-99], lepidocrocite [35, 36, 43, 44, 46, 47, 88, 90, 92, 93, 100, 101], mixtures of ferrihydrite and lepidocrocite [42, 102], and ferric green rust [44, 50]. Our results are the first showing that green rust can also form during the bioreduction of akaganeite.

The multitude of factors, some interrelated, that have been proposed as contributing to green rust formation include the rate and extent of Fe(II) production, electron shuttle utilization, the availability of electron donors, the cell density and species of IRB, the type of Fe(III) oxide present, Fe(III) oxide particle aggregation, and the presence of ligands including oxyanions (phosphate, silicate, arsenate, etc.) and dissolved organic carbon (e.g., HS and microbial exudates) [35-38, 40-44, 88, 91-92, 95-96, 100]. In our study of the bioreduction of akaganeite, we observed a clear differentiation of secondary mineral formation based on the absence (magnetite) or presence (green rust) of phosphate, consistent with previous studies identifying phosphate (as well as oxyanions such as silicate, arsenate, molybdate, and tungstate) as the key determinant in controlling green rust versus magnetite formation during reduction of ferrihydrite, lepidocrocite, and ferric green rust by IRB [35, 38, 40-41, 43-44]. Sorption of phosphate as inner-sphere complexes on Fe(III) oxides [103] can greatly affect the chemical and biological reduction of Fe(III) oxides [41, 104-105]. In our experimental system, 96% of the phosphate was sorbed to akaganeite at the time of inoculation, corresponding to a phosphate loading of 68 μmol g⁻¹ of akaganeite. For a surface area of 35.2 m² g⁻¹, an akaganeite loading of 0.71 g in 100 mL, and a phosphate sorption capacity of 4.82 μmol m⁻² [106], we estimate that ~40% of the phosphate sorption sites on akaganeite were occupied.

The role of phosphate in the formation of green rust accompanying the microbial reduction of Fe(III) oxides is unclear; does phosphate explicitly prevent formation of magnetite or does it favor green rust? Reduction and transformation of akaganeite to magnetite or green rust can proceed by dissolution-reprecipitation (reconstruction) or structural rearrangement; evidence (sometimes contradictory) supports each of these processes. Solid-state transformation has been invoked to explain magnetite formation resulting from the interaction of Fe²⁺ with ferrihydrite at circumneutral to mildly alkaline pH [107-109], which is inhibited by sorption of phosphate [108]. As the addition of dissolved Fe(II) to aqueous suspensions of ferrihydrite can be considered analogous to Fe(II) formation during bioreduction of ferrihydrite, solid-state transformation has been proposed as the mechanism of magnetite formation during ferrihydrite bioreduction [38, 97].

In contrast to ferrihydrite, the mechanism(s) by which magnetite forms as a product of the reduction of akaganeite has not been well studied. Akaganeite transformation to magnetite by reduction with hydrazine [51] and by reaction with Fe²⁺ is attributed to a dissolution-reprecipitation process [51-52]. Our results are also suggestive of magnetite formation by dissolution-reprecipitation, as the marked differences in the morphology the 100-300-nm somatoidal akaganeite crystals (Fig. 1) and the fine-grained (20-50 nm) magnetite crystals (Fig. 3) are not consistent with a purely topotactic or pseudomorphic transformation. Similarly, the specific mechanism of formation of green rust from Fe(III) oxide reduction is uncertain.

Mann et al. [108] and Hansen et al. [110] proposed that green rust formation occurs via solid-state rearrangement of ferrihydrite following sorption of Fe²⁺. Solid-state transformation was also invoked for the formation of green rust during the bioreduction of ferrihydrite in the presence of phosphate, for which sorption of phosphate to ferrihydrite was proposed to inhibit magnetite formation [38, 40]. However, the dissimilarity between nanoparticulate ferrihydrite and micron-sized green rust crystals seems to preclude a purely
solid-state process for ferrihydrite transformation to green rust, and a similar argument can be made for akaganeite. During the bioreduction of lepidocrocite in the presence of phosphate, O’Loughlin et al. [44] reported changes in particle shape during the transformation of lepidocrocite to green rust that are suggestive of a structural reorganization. Specifically, a transition from tabular lepidocrocite crystals to proto green rust particles that ultimately ripen to hexagonal, platy green rust crystals. Unfortunately, in this study we do not have time-resolved imaging of the transformation of akaganeite to green rust to assess whether green rust is forming by a similar process.

CONCLUSION

Our study is the first to report on the formation of frambooidal magnetite as a product of the reduction of Fe(III) oxide by IRB. In addition, we are the first to show that green rust can form as a secondary mineral during akaganeite bioreduction in the presence of phosphate. The factors controlling the formation of frambooidal magnetite and the specific mechanism(s) by which phosphate directs green rust formation at the expense of magnetite remain elusive. However, our results indicate that the presence of electron shuttles (e.g., AQDS) does not play a role in overall secondary mineral formation (green rust versus magnetite), but it does appear to promote subsequent secondary mineral transformation to tertiary minerals such as chukanovite. The presence of the green rust mineral fougerite in redoximorphic soils correlates well with the activity of native IRB populations [11], suggesting that the bioreduction of Fe(III) oxides such as ferrihydrite, lepidocrocite, and akaganeite by IRB is a key process leading to the presence of green rusts in aquatic and terrestrial environments.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

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