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**Effect of Sb on Precipitation of Biogenic Minerals during
the Reduction of Sb-bearing Ferrihydrites**

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ABSTRACT

Antimony (Sb) is a naturally occurring element; it is enriched in the environment by anthropogenic activities. Like other metalloid species, Sb partitions to mineral phases such as oxyhydroxides. In reducing environments, Fe(III) may serve as a terminal electron acceptor during dissimilatory iron reduction leading to its transformation. Relatively little is known concerning the effect of Sb(V) on the precipitation of biogenic minerals in relation to microbiologically mediated redox reactions. To further our understanding, Sb-bearing ferrihydrites with variable molar ratios [Sb/(Fe + Sb)] were incubated in the presence of *Shewanella oneidensis* MR-1. A combination of wet chemistry and solid analysis techniques (XRD, Mössbauer and Raman spectroscopies) was used to characterize the reactions.

The Sb loading affected the rate and the extent of bio-reduction compared with pure ferrihydrite. Only a minor fraction of the total Sb was released into the solution by the end of the incubation period, suggesting that the metalloid partitioned mainly in a newly formed phase. Furthermore, XPS analyses showed the presence of Sb(V) and Sb(III) species on the biogenic minerals. Magnetite was the main biogenic precipitate in the absence of Sb(V). Increasing of the molar ratios (Sb/(Fe + Sb)) resulted mainly in the precipitation of carbonated green rust and goethite. Abiotic green rust synthesis carried out in the presence of Sb(V) indicated the latter's stabilizing effect on the green rust structure, as for phosphate species. Thus, it is likely that Sb preserves biogenic green rust, hindering its transformation to more thermodynamically stable phases.

1. INTRODUCTION

Natural deposits (e.g. ores, hot springs) and anthropogenic activities (e.g. mining, road traffic emissions) have led to a significant dispersion of inorganic elements, including antimony (Sb), in the lithosphere (Liu et al., 2010; Anderson, 2012; Okkenhaug et al., 2016; Li et al., 2018; He et al., 2019). There is a small but growing body of evidence that demonstrates the deleterious effect of Sb on biota (Gebel, 1997; Oorts et al., 2008; An and Kim, 2009). As a result, both the United States Environmental Protection Agency (U.S. EPA) and the European Union (E.U.) have listed Sb as a “priority pollutant” (Official Journal L 129, 1976; United States Environmental Protection Agency), which implies the need to assess the behavior Sb(V) in natural settings. In the environment, antimonite $[Sb(OH)_3]$ and antimonate $[Sb(OH)_6^-]$ are the dominant chemical species (Filella et al., 2002; Wilson et al., 2010). Antimony has been observed interacting with soil components, in particular, iron oxides (e.g. goethite, lepidocrocite), and it is positively correlated with Fe content (Craw et al., 2004; Mitsunobu et al., 2010a; Takahashi et al., 2010).

Iron oxides display a large surface area and high chemical reactivity given their crystallinity and porosity (Cornell and Schwertmann, 2003). They therefore contribute significantly to the speciation and the fate of elements (e.g. As, Sb) that play a central role in various biogeochemical cycles in soils and sediments (Dixit and Hering, 2003; Borch and Fendorf, 2007; Mitsunobu et al., 2008a; Frierdich and Catalano, 2012a; Ilgen and Trainor, 2012; Okkenhaug et al., 2013; Mallet et al., 2013; Perez et al., 2019). Due to their large specific surface area, iron oxides are considered to be an effective sink for Sb in shooting range soils (Okkenhaug et al., 2016; Okkenhaug et al., 2018). Thus, the properties of these iron oxides have led to the consideration of Fe-based amendments as a potential strategy for the removal and the immobilization of Sb in contaminated areas. In freshwater, Sb is found to

be much more mobile in low Fe systems, suggesting that the adsorption or the co-precipitation with iron oxides is a critical factor controlling Sb dynamics (Mitsunobu et al., 2010a; Hockmann et al., 2014; Burton et al., 2020). Furthermore, X-ray Absorption Fine Structure (XAFS) spectroscopic analyses have demonstrated the incorporation of Sb(V) into the crystallographic structure of iron oxides synthesized in the laboratory, such as goethite and ferrihydrite (Mitsunobu et al., 2013; Karimian et al., 2019b; Burton et al., 2020). This structural incorporation has also been reported to occur in natural soil, suggesting that the substitution of Fe in iron minerals is widespread in the environment (Mitsunobu et al., 2010b).

In subsurface environments, iron oxides are also used as electron acceptors for dissimilatory iron-reducing bacteria (DIRB) (Lovley, 1993; Coates et al., 1996; Maier and Myers, 2001; Nealson et al., 2002; Sung et al., 2012). The bio-reduction of Sb-bearing iron oxide can lead to the potential mobilization into the aqueous phase and (or) the sorption onto biogenic precipitates of the previously retained Sb(V). DIRB such as *Geobacter sp.* and *Shewanella sp.* couple the oxidation of an organic (e.g. formate, pyruvate, lactate) or inorganic (e.g. H₂) electron source with the reduction of Fe(III) during anaerobic respiration (Lovley et al., 1993; Lovley, 2000). Subsequently, the Fe(II) generated by bio-reduction is released into solutions and/or sorbed on mineral surfaces, which leads to the transformation of Fe-bearing minerals, such as magnetite (Fe₃O₄), siderite (FeCO₃), green rust (GR), goethite (α -FeOOH) and lepidocrocite (γ -FeOOH), depending on the biogeochemical conditions (Kukkadapu et al., 2005; Zegeye et al., 2007; O'Loughlin et al., 2007; Ona-Nguema et al., 2009; Eusterhues et al., 2014; Muehe et al., 2016).

Of these biogenic minerals, GRs are Fe(II-III)-layered double-hydroxysalts constituted of positively charged layers $\{\text{FeII}_{(1-x)} \text{FeIII}_x (\text{OH})_2\}^{x+}$, which alternate with negatively charged interlayers that include m water molecules per Aⁿ⁻ anion $\{(x/n)\text{A}^{n-} \cdot (mx/n)\text{H}_2\text{O}\}^{x-}$

(Hansen, 1989; Génin et al., 1996). Two groups of GRs are identified based on X-ray diffraction (XRD) patterns: green rust 1 (GR1) and green rust 2 (GR2) (Bernal, 1959). Their structure and composition depend upon the specific anions they incorporate. GR1 is arranged in rhombohedral geometry and inserts planar or spherical anions (e.g. Cl^- , CO_3^{2-}), whereas GR2 is arranged in a hexagonal geometry and inserts three-dimensional anions (e.g. SO_4^{2-} , SeO_4^{2-}) (Hansen et al., 1994; Abdelmoula et al., 1996; Refait et al., 1998; Refait et al., 2000; Simon et al., 2003). GRs have demonstrated their potential ability to interact with Sb(V) with an inner sphere complex, thus reducing the metalloid's mobility (Mitsunobu et al., 2009).

Relatively little is known concerning the effect of Sb(V) on the precipitation of biogenic minerals such as GRs in relation to microbiologically mediated redox reactions. A recent study has shown that Sb(V) favors feroxyhyte formation over goethite during the bio-reduction of Sb(V)-ferrihydrite (Burton et al., 2020). The incorporation of Sb(V) in the iron oxides' structure was also highlighted. To broaden our understanding of Fe and Sb biogeochemical cycles, it is pertinent to investigate how various Sb concentrations impact iron oxide bio-reduction processes and the precipitation of biogenic minerals. Furthermore, the interaction of Sb(V) with GR1(CO_3^{3-}) resulting from the bio-reduction of an Sb(V)-bearing iron oxide has not been reported previously.

Our study aims to investigate the impact of Sb(V) loading on the nature of biogenic minerals, such as GRs precipitated during the bio-reduction of Sb(V) bearing ferrihydrites. To achieve this objective, we incubated Sb bearing ferrihydrites with variable molar ratios (Sb/(Fe + Sb)) with *Shewanella oneidensis* MR-1. We then assessed the following: (i) the influence of Sb content on the bio-reduction of ferrihydrite (i.e. rate and extent of reduction) and (ii) the nature of biogenic minerals. Furthermore, we synthesized abiotic GR1(CO_3^{2-}) in the presence of Sb(V) to examine the Sb(V) effect on this mineral formation and stabilization.

2. METHODS

All reagents and acids were of analytical grade and solutions were prepared using 18.2 MΩ cm⁻¹ deionized water. Anaerobic experiments (bio-reduction, abiotic GR synthesis and aging), sample collection (*i.e.* by centrifugation) and sample drying were conducted under strictly anaerobic conditions in a re-circulating anoxic chamber (Coy Laboratory Products Inc.) in which trace amounts of oxygen were scavenged by palladium catalysts. A 98% N₂ + 2% H₂ atmosphere was maintained by the automatized injection of the gas mixture. When needed, the samples were removed from the anaerobic chamber in an air-tight sample holder for solid analyses.

2.1 Synthesis of Sb(V)-bearing ferrihydrite

Ferrihydrite (Fh hereafter) was synthesized by precipitating 0.4 M FeCl₃ with 4 M NaOH according to the method adapted from Cornell and Schwertmann (2003). When the pH approached the desired endpoint of the hydrolysis, a solution of 0.5 M NaOH was used to adjust the pH to = 7.2 ± 0.1. Antimony co-precipitated Fhs were synthesized in similar fashion, except for the addition of K[Sb(OH)₆] stock solution to the ferric salt before hydrolysis at Sb/(Fe + Sb) molar ratios of 0.04, 0.06 and 0.1 (Fh_Sb0.04, Fh_Sb0.06 and Fh_Sb0.1 hereafter), following a modification of the method used by Mitsunobu et al. (2010b). All suspensions were allowed to equilibrate for 4 hours after the synthesis and the pH was readjusted to pH 7.2 with 0.05 M NaOH if necessary. Thereafter, the co-precipitates were washed six times with Milli-Q water by centrifugation and freeze-dried.

2.2 Bio-reduction of Sb(V)-bearing ferrihydrites

Frozen *Shewanella oneidensis* MR-1 cells from a stock (frozen in 20% glycerol at -80 °C) were revived under aerobic conditions on tryptic soy agar. They were sub-cultured once, and the colonies used to prepare a suspension to inoculate 700 mL of tryptic soy broth. Cells were grown, continuously agitated at 300 rpm and 30°C, harvested after 14 hours of culture by centrifugation (10 000 x g at 20°C for 10 minutes), washed twice with sterile 0.9% NaCl, and concentrated in the same medium. The cells were purged for 30 minutes by bubbling with filter-sterilized N₂ filtration through a membrane of pore size 0.2 µm, and used to inoculate batches with ferrihydrite.

The bio-reduction were performed in a culture medium composed of 22 mM NH₄Cl, 1.5 M NaCl, 1.2 mM KCl, 1.1 mM MgSO₄•7H₂O, 0.71 mM nitrilotriacetic acid (NTA), 0.67 M CaCl₂, 0.27 mM MnSO₄•H₂O, 86 µM ZnCl₂, 38 µM CoSO₄•7H₂O, 32 µM FeSO₄•7 H₂O, 9.3µM Na₂MoO₄ •2 H₂O, 9.1 µM NiCl₂ •6H₂O, 6.8 µM Na₂WO₄•2 H₂O, 3.6 µM CuSO₄•5 H₂O, 1.9 µM AlK(SO₄)₂•12 H₂O, 1.5 µM H₃BO₃. The medium was heat-sterilized and purged with filter-sterilized N₂, and dispensed into sterile 100 mL serum bottles in the presence of 0.5 g of Sb-bearing Fh, and crimp sealed using butyl rubber stoppers. Sodium methanoate, as the sole electron source, and anthraquinone-2,6-disulfonate (AQDS), as an electron shuttle, were sterilized by filtration (filter pore size 0.2 µm), purged with filter-sterilized N₂, and introduced in the culture medium under aseptic conditions in order to achieve 50 mM (i.e. excess of ≈ 55% with respect to Fe(III)) and 100 µM respectively in each 80 mL assay. *S. oneidensis* cell suspensions were added to obtain final concentrations of 1.0×10^8 cell mL⁻¹. The initial pH after mixing all the components was 7.2 ± 0.1 . The assays were incubated in the dark for

25 days, in triplicates. The control experiments were cell-free but otherwise identical to the biotic assays.

2.3 Precipitation of abiotic GR1(CO₃²⁻)

Carbonate green rusts were synthesized according to the method adapted from Bocher et al. (2004). Ferrous sulfate heptahydrate FeSO₄ and ferric sulfate Fe₂(SO₄)₃ were dissolved in 30 mL of ultra-pure water with a Fe(II) / Fe(III) ratio of 2:1 and titrated with 30 mL of an appropriate concentration of a basic solution (NaOH, [OH⁻] / [Fe_t] = 2, and Na₂CO₃, [CO₃²⁻] / [Fe_t] = 7/6). Other sets of GR synthesis were conducted by mixing either (i) phosphate solution (PO₄³⁻ / Fe_t = 0.016) or (ii) Sb^V solution (Sb(V) / Fe_t = 0.09) with the ferric/ferrous solution before the co-precipitation. Phosphate was used to hinder the transformation of GR and to stabilize the mineral. A further synthesis was run with the Sb(V) solution being added directly after GR precipitation (~ 10 min) in order to obtain a Sb(V) / Fe_t ratio of 0.09. The different precipitates were characterized after one week of aging in an anaerobic chamber.

2.4 Analytical procedure

2.4.1 Chemical analyses

The Sb/(Fe + Sb) molar fractions of the initial Fh minerals were measured by Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) after being dissolved in acid, (6 M HCl) (Amstaetter et al., 2012).

At each time point, samples (0.5 mL) were removed from the bio-reduction assays and extracted with 4 M HCl in an anaerobic chamber in order to quantify total Fe (Fe_{tot}) and total

Fe(II) ($\text{Fe}^{\text{II}}_{\text{tot}}$) using the ferrozine method (Viollier et al., 2000). The apparent initial reduction rates were computed from the first derivative of a nonlinear curve fit (1)

$$\%(\text{Fe}^{\text{II}}_{\text{tot}}/\text{Fe}_{\text{tot}})_t = \%(\text{Fe}^{\text{II}}_{\text{tot}}/\text{Fe}_{\text{tot}})_{\text{max}} [1 - \exp(-k_{\text{obs}}t)] \quad (1)$$

where $\%(\text{Fe}^{\text{II}}_{\text{tot}}/\text{Fe}_{\text{tot}})_t$ is the $(\text{Fe}^{\text{II}}_{\text{tot}}/\text{Fe}_{\text{tot}})$ (%) produced at time t , $\%(\text{Fe}^{\text{II}}_{\text{tot}}/\text{Fe}_{\text{tot}})_{\text{max}}$ is the maximum $\%(\text{Fe}^{\text{II}}_{\text{tot}}/\text{Fe}_{\text{tot}})$ observed at the end of the reduction period (i.e. 25 days), and k_{obs} is the pseudo first-order constant.

At the end of the incubation period, 1 mL was withdrawn from the assays, filtered through 0.22 μm pore filters, and placed in 2 M HCl in an anaerobic chamber to measure the concentration of Sb in solution (Sb_{sol}) by Hydride Generation - Atomic Fluorescence Spectroscopy (HG-AFS). Stibnite was generated in a continuous flow system (PS Analytical's Millennium ExcaliburTM) using 0.7%, m/v NaBH_4 solution (stabilized with 0.1 M NaOH) and 25% v/v HCl as carrier solution. The sample solution (flow rate: 9 mL min⁻¹) and carrier solution (NaBH_4 flow rate: 4.5 mL min⁻¹; HCl flow rate: 9 mL min⁻¹) were pumped simultaneously into the sample valve for mixing. The standard and the sample solution were prepared in a 30% v/v HCl medium and 1% m/v KI / 0.2% m/v ascorbic acid mix to achieve the pre-reduction of the Sb(V). The mixture was transferred into the gas-liquid separator, and the generated gaseous hydrides were transported to the atomizer flame of the AFS. A boosted discharge hollow cathode lamp (BDHCL) for Sb was used throughout the experiments.

2.4.2 Solid analyses

2.4.2.1 X-Ray Diffraction (XRD)

The X-Ray Diffraction (XRD) data of the Fh and Fh_Sb series and the biogenic minerals were collected using a Bruker D8 diffractometer equipped with a monochromator and position-sensitive detector. The X-ray source was a Co anode ($\lambda = 0.17902$ nm). The diffractogram was recorded in the $3 - 84^\circ 2\theta$ range, with a 0.0359° step size and collection time of 3 seconds per point. Crystalline size was calculated using the Scherrer equation (2)

$$D = K\lambda/\beta\cos\theta \quad (2)$$

where D is the mean size of the crystalline domains, K is a dimensionless shape factor (0.94), λ is the wavelength of the X-ray radiation, β is the line broadening at the full width at half maximum (FWHM) and θ is the Bragg angle of the particular peak of interest.

2.4.2.2 Transmission Mössbauer Spectroscopy (TMS)

Transmission Mössbauer Spectroscopy (TMS) was performed using a ^{57}Co source to identify and quantify iron phases and magnetic ordering formed after the bio-reduction of Fh, Fh_Sb(0.04), Fh_Sb(0.06) and Fh_Sb(0.1). ^{57}Fe Mössbauer spectra were collected using a conventional spectrometer in transmission geometry coupled with an Advances Research Systems (USA) cold head cryostat, equipped with in-house made vibration isolation stand. The 50 mCi ^{57}Co in Rh matrix radioactive source was mounted in a constant acceleration velocity transducer. Solid samples were prepared under an N_2 atmosphere in an anoxic glove box (Jacomex) using appropriate amounts (10 mg of powder per cm^2). Measurements were taken at the wide range of velocity $\pm 11 \text{ mm s}^{-1}$ or $\pm 4 \text{ mm s}^{-1}$ for experiments at room temperature (RT). This approach is useful for the unambiguous identification of iron oxides at

room temperature and low-temperature studies by comparing the hyperfine split obtained at low temperature with the spectra measured at room temperature. The hyperfine interaction parameters were determined by fitting the experimental spectra by a least-squares method using Recoil software (Lagarec and Rancourt, 1998) with Voigt-based fitting analysis or the Lorentzian model, except in the case of the appearance of a magnetic order Fe(II) phases (siderite), whereas the full static Hamiltonian site model was required at very low temperature (15 K) to solve the mixed hyperfine interactions. Center shifts were reported with respect to that of 25 μm -thick $\alpha\text{-Fe}$ foil at room temperature.

2.4.2.3 Raman Spectroscopy

Raman spectra were collected on a Renishaw inVia™ Qontor® microspectrometer equipped with a confocal microscope and an Olympus X50 objective (numerical aperture = 0.55). The samples were prepared in an anaerobic chamber and enclosed in an airtight cell with sapphire windows. 785 nm exciting radiation was used with a laser power below 0.1 mW for all samples, in order to prevent their degradation. The spot area was a few μm^2 . Several locations were probed on each sample. The spectral resolution was about 4 cm^{-1} and the precision on the wavenumber was below than 1 cm^{-1} .

2.4.2.4 X-ray Photoelectron Spectroscopy (XPS)

The surface physicochemical properties of Fh_Sb(0.1) were characterized before and after bio-reduction using X-ray Photoelectron Spectroscopy (XPS). Spectra were recorded on a Kratos Axis Ultra X-ray photoelectron spectrometer equipped with a monochromatic Al $K\alpha$ source (1486.6 eV) operated at 120 W. Powder samples were deposited onto a copper

adhesive tape in an anaerobic chamber and kept in sealed aluminum pouches that were re-opened in an anaerobic glove box connected to the UHV preparation chamber at 10^{-7} mb. The analysis area was equal to $300 \times 700 \mu\text{m}^2$. The spectra were collected at a take-off angle of 90° and the pressure in the analysis chamber was approximately 10^{-9} mbar. A pass energy of 20 eV was used for the high-resolution spectra. Charge compensation was applied by flooding low-energy electrons. Binding energies were calibrated by assigning the adventitious carbon C 1s peak to 284.6 eV. Curve fitting was performed using a Gaussian/Lorentzian (70/30) peak shape after Shirley's background subtraction and using X-vision 2.2.11 software.

2.4.2.5 Magnetism

A Quantum Design Superconducting Quantum Interference Device (SQUID) was used to perform Zero field cooled (ZFC) and field cooled (FC) measurements. The ZFC procedure consisted of demagnetizing the samples at 300 K using an oscillatory decaying field from 1 T to zero field (resp. magnetized under 7.5 mT), and then cooling them to 2 K at a rate of 1 K/min. ZFC magnetic moment was then measured while the temperature was ramped back up from 2 K to 300 K at a rate of 1 K/min under 7.5 mT constant field. The FC procedure consisted of cooling down the sample from 300K under 7.5 mT and then measuring moment while heating it from 2 K back up to 300 K.

3. RESULTS

3.1 Sb-bearing ferrihydrite characterization

The Fe and Sb contents of the co-precipitates were measured by ICP-OES; the concentrations obtained were approximately equal to the initial molar ratio of Sb/(Sb + Fe) (*i.e.* 0.04, 0.06 and 0.1) used to precipitate the solids. X-ray diffraction was used to characterize the co-precipitated phases (Fig. 1), and the signal displayed two broad humps (at $2\theta \sim 41^\circ$ and 75°) with d-spacing of ~ 0.258 nm and ~ 0.149 nm indicative of Two-line ferrihydrite. No other Fe(III) oxides were detected. We observed a broadening of the XRD peaks as the Sb content of the Fh increased; this was due to either a decrease in crystallinity as a result the incorporation of Sb in the structure of the Fe oxyhydroxide as revealed by Mitsunobu et al. (2010b) or a decrease in crystallite size as hypothesized in the case of arsenic substitution (Waychunas et al., 1996).

3.2 Microbial bio-reduction of Sb-bearing ferrihydrite

We quantitatively monitored the bio-reduction of Sb-bearing ferrihydrite series over time. Bio-reduction started without a lag phase and reached a plateau after 25 days (Fig. 2). No measurable Fe(III) reduction took place in the non-inoculated treatments (data not shown). The apparent rate of reduction was $16.40 \pm 0.07\%$ ($\text{Fe}^{\text{II}}_{\text{tot}}/\text{Fe}_{\text{tot}}$) day^{-1} for Fh, while the rate was less than 50% slower for Fh_Sb (Table 1). Additionally, the extent of reduction reached $\sim 35\%$ for pure Fh, and $\sim 30\%$ for Fh_Sb(0.04) and Fh_Sb(0.06), whereas it plateaued at $\sim 50\%$ for Fh_Sb(0.1) (Table 1).

After 25 days of incubation, only a minor fraction - less than 0.5% - of the Sb ions were present in the aqueous phase irrespective of the molar ratio $\text{Sb}/(\text{Sb} + \text{Fe})$ of the initial Fhs.

3.3 Biogenic minerals

The mineralogical transformation of Fh and Fh_Sb series was monitored after the incubation period using a suite of spectroscopic techniques.

3.3.1 Biogenic minerals formed by Sb free Fh bio-reduction

The biogenic minerals displayed X-ray diffraction peaks with d-spacing characteristic of magnetite and siderite (Fig. 3). Based on the FWHM of the strongest peaks at $d = 0.251$ (hkl: 311) and 0.294 nm (hkl: 220), the estimated crystalline size of the magnetite was 9.4 nm which is in the same range as the sizes observed in bio-reduction experiments described in the literature (Kukkadapu et al., 2005; Byrne et al., 2011).

We performed Mössbauer temperature profiles at room temperature (RT), 143 K, 78 K and 15 K (Fig. 4) to identify and quantify the biogenic minerals precipitated during Fh bio-reduction. At RT and 143 K, Mössbauer spectra are complex, with the superposition of a paramagnetic doublet as well as the significant broadening of a magnetic component. At RT the quadrupole doublet is characterized by large center shifts (CS) and a quadrupole splitting (Δ) values of 1.14 mm s^{-1} and 1.92 mm s^{-1} respectively, which are similar to those published for siderite at room temperature, i.e. $\text{CS} = 1.14 \text{ mm s}^{-1}$ and $\Delta = 1.98 \text{ mm s}^{-1}$ (Génin et al., 2002). The magnetic component was fitted using two distributions of the hyperfine magnetic field with widened lines, possibly due to magnetite observed using XRD. We fitted this component with two broad overlapping sextets with two weak mean magnetic fields at 40.8 T

and 13.2 T respectively. However, the two sextets are usually clearly defined and fitted with a Lorentzian model when dealing with large-particle magnetite. This discrepancy in the line shape of the Mössbauer spectrum at RT is due to the small particle size (less than 20 nm), which may induce a greater distribution in the magnetic behavior (Silva et al., 2013; Alves et al., 2016).

During cooling, the spectral features of the magnetic component change considerably. At 77 K, the ferrous doublet of the siderite phase observed at RT remains and the CS and the Δ increase as expected for the hyperfine parameters of the Fe(II), and adopt a high spin configuration at the octahedral site. The magnetic contribution of the spectrum exhibits five sextets that were deconvoluted using a Lorentzian model; they correspond to the five iron sites observed for magnetite below the Verwey temperature (Srivastava et al., 1981; Vandenberghe et al., 2000). At 15 K, the spectrum becomes more complex in its central part. This indicates that all the carbonate-containing Fe(II) sites are now magnetically ordered and represented by an octet versus paramagnetic doublet at 77 K (Maitte et al., 2015). Modelling of this spectrum component, using the software package Recoil (Lagarec and Rancourt, 1998), is intricate, owing to the mixing of states, particularly given the fact that, for Fe(II) compounds below magnetic transition, the electric quadrupole and magnetic hyperfine interactions are generally of the same order of magnitude. Thus, it is not likely that this component can be analyzed in terms of six-line patterns (sextets) as is the case for the magnetism of ferric compounds (Duboscq et al., 2020). The octet comprises eight lines that have to be computed fully by solving the eigenvalues of the full Hamiltonian. The octet is characterized by hyperfine parameters (Table 2), a CS of 1.76 mm s⁻¹, electric quadrupole interaction ($e^2qQ/2$) of 2.72 mm s⁻¹, and a hyperfine field H of 18.7 T typical of Fe(II) in siderite (Maitte et al., 2015). The relative abundance of siderite was ~ 9 %.

The second interesting feature in the spectrum at 15 K is the appearance of resonance lines on the high energies which correspond, after spectral deconvolution, to the five sextets S1, S2, S3, S4 and S5. Their hyperfine parameters, listed in Table 2, are consistent with those generally reported for magnetite of five-sextets as observed in the literature (Srivastava et al., 1981) at low temperature. Moreover, the particle size constraints generate the phenomenon of superparamagnetism and influence the magnetic properties, which could explain the gradual evolution with regards to temperature from a broad sextet component to sharp sextets as the temperature decreases. This behavior is typical of superparamagnetic particles (Goya et al., 2003), and may be related to the widening of the diffraction lines assigned to magnetite by XRD (Fig. 3). The relative abundance of magnetite was ~ 91 %.

The Raman spectra realized at different locations on the sample (analyzed spot size of a few μm) exhibited a strong band at 680 cm^{-1} and a weaker one in the region of 310 cm^{-1} , indicating the precipitation of magnetite (Fig. A1) (Shebanova and Lazor, 2003; Jubb and Allen, 2010). Additional bands at 280, 725 and 1084 cm^{-1} were attributed to siderite (Fig. A1) (Hanesch, 2009; Maitte et al., 2015). The fact that different locations display a different mineral indicates that the system is heterogeneous, and that various mineral precipitation pathways are involved.

3.3.2 *Biogenic minerals formed by Fh_Sb bio-reduction*

Neither magnetite nor siderite was detected as a result of the transformation of Sb-bearing minerals at the level discernible by XRD (Fig. 3). Instead, the d-spacing displayed the sharp diffraction peaks of green rust 1 (GR1) and goethite as biogenic minerals irrespective of the Sb/(Fe + Sb) molar ratio. Peaks of an unidentified mineral (indicated by *) were also present on the diffractograms independently of the Sb/(Sb + Fe) molar ratio of the initial Fhs. No evidence for the formation of other Sb-hosting minerals such as hydroxyferroromeite or

Fe- and Sb-bearing minerals such as tripuhyite was detected. Based on the experimental setup, the reduction medium composition and the electron source used in our study (i.e. methanoate), the precipitated GR1 is likely a carbonated GR ($\text{GR1}(\text{CO}_3^{2-})$) as described elsewhere (Ona-Nguema et al., 2004; Zegeye et al., 2007; O'Loughlin et al., 2007).

We used the FWHM of the strongest peaks at $d = 0.755 \text{ nm}$ ($hkl : 003$) and $d = 0.378 \text{ nm}$ ($hkl : 006$) to estimate the average GR1 crystal size. Our goal was not to determine the exact crystal size but to monitor any variation due to the loading of Sb(V). The estimated crystal sizes of the $\text{GR1}(\text{CO}_3^{2-})$ bio-precipitated from the Fh_Sb(0.04), Fh_Sb(0.06) and Fh_Sb(0.1) bio-reductions were 77 nm, 40 nm and 66 nm for respectively.

At room temperature, the Mössbauer spectra Fh_Sb(0.04), Fh_Sb(0.06), and Fh_Sb(0.1) were computer fitted with 5, 3 and 5 quadrupole doublets respectively (Fig. A2). Their hyperfine parameters are typical of ferrous and ferric cations (Table A1), but their mineralogical identification remains difficult. Nevertheless, the presence of $\text{GR1}(\text{CO}_3^{2-})$ and superparamagnetic goethite phases cannot be ruled out on the basis of the XRD characterization.

Iron oxides are often sensitive to effects such as small particle size in the nanometer range, which complicates their identification and characterization. These constraints require Mössbauer spectra to be recorded at a lower temperature. At 15 K, all the Fh_Sb(0.04), Fh_Sb(0.06) and Fh_Sb(0.1) samples displayed magnetically ordered components on which paramagnetic doublets were superimposed (Fig. 5). The magnetic components exhibit broad and asymmetric lines with a distribution of the hyperfine magnetic fields centered on three components at 45 T, 47.5 T and 49.4 T. The quadrupole shift obtained (-0.22 mm s^{-1}) is characteristic of goethite, also identified by XRD but with widened lines. The broadening of magnetic lines is the result of relaxation effects of the smallest particles and/or a poor

crystallinity. This could explain the spectral behavior observed at RT, with a significant doublet suspected as being superparamagnetic goethite (Kone et al., 2009).

The paramagnetic components were fitted with two quadrupole doublets (D1 and D2). Doublet D1 with values of $CS = 1.21 \text{ mm s}^{-1}$ and $\Delta = 2.9 \text{ mm s}^{-1}$ differs from doublet D2 with smaller values for $CS (0.38 \text{ mm s}^{-1})$ and $\Delta (0.74 \text{ mm s}^{-1})$, which are ascribed to $\text{GR1}(\text{CO}_3^{2-})$ (Génin et al., 2006). It is also possible to detect another magnetic component appearing as an octet characterized by hyperfine parameters (Table 2) with a CS of 1.52 mm s^{-1} , electric quadrupole interaction ($e^2qQ/2$) of 2.47 mm s^{-1} and a hyperfine field H of 11 T, which is typical of Fe(II) in probable association with Sb. This component unambiguously distinguishes it from both siderite, which has a much higher magnetic field, and $\text{GR1}(\text{CO}_3^{2-})$, which is a mixed-valence (ferrous-ferric) compound leading to two magnetic ordering temperatures, 5.2 and 7 K (Rusch et al., 2008). The relative abundance of $\text{GR1}(\text{CO}_3^{2-})$ and of unspecified Fe(II) species increases with the increase of Sb(V) loading (Table 2).

We performed field cooled (FC) and zero-field cooled (ZFC) moment measurement (Fig. A3). FC and ZFC curves overlap from 300 K down to approximately 15 K. At temperatures lower than 15 K, there are two maxima in the ZFC curve. In a system including particles, such maxima are usually associated with the blocking process of particles, thus determining the onset of superparamagnetic behavior (Néel, 1949; Brown, 1963). The blocking temperatures estimated for the maximum magnetization of ZFC curves were 10.5 K and 4.9 K. We expect that these two blocking temperatures are relative to the two main minerals detected by Mössbauer spectroscopy and XRD, namely goethite and green rust.

The characterization by Raman spectroscopy of different locations on the biogenic minerals precipitated from the Fh_Sb series samples revealed different biogenic minerals (Fig. 6A). Thus, the results exhibited strong bands at 435 and 510 cm^{-1} and a weak band at 220 cm^{-1} , characteristics of $\text{GR1}(\text{CO}_3^{2-})$ (Legrand et al., 2001b; Refait et al., 2011). We also

observed bands related to the precipitation of magnetite and siderite that were not detected by XRD, implying that these phases were present as minor species.

XPS analysis was carried out to investigate the chemical composition of the surface of biogenic solid, with special emphasis on determining the chemical speciation of Fe, Sb and C. The Fe 2p, Sb 3d and C 1s spectra of unreacted and bio-reacted Fh_Sb(0.1) samples are compared in Fig. 7. The Fe 2p high-resolution spectrum of unreacted ferrihydrite Fh_Sb(0.1) displays a maximum at ~ 711.0 eV and a broad satellite peak at ~ 719 eV, as is commonly observed for Fe(III) species in iron (oxyhydr)oxides (Mallet et al., 2013; Fan et al., 2016). Four multiplet peaks at 710.2, 711.3, 712.4 and 713.5 (FWHM = 1.3 eV) and peak area ratios relative to the first Fe(III)-O component of 1.1, 0.69 and 0.38 respectively were used to fit the $2p_{3/2}$ spectrum in agreement with Gupta and Sen, 1974; Gupta and Sen, 1975 predictions (Fig. 7A, top). An additional surface peak at 714.7 eV is required to fit the data (Grosvenor et al., 2004).

A slight shift of the Fe $2p_{3/2}$ maximum component towards lower binding energy is observed, and the satellite peak characteristic of Fe(III) species is strongly attenuated (Fig. 7A, bottom). In addition to the four Fe(III) multiplet peaks, three multiplet peaks located at 708.6, 709.6 and 710.7 eV that characterize the presence of Fe(II) are required to fit the spectra of the Fh_Sb(0.1) bio-reduced sample (Fig. 7A, bottom). The peak area ratio of the multiplet components relative to the first Fe(II)-O component is 1.1 and 0.5, and the FWHM is 1.6 eV. The presence of Fe(II) in the bio-reduced sample agrees well with the formation of GR1 (and minor amounts of magnetite and siderite) as observed in the XRD and TMS characterizations. The binding energy of Sb(III) and Sb(V) compounds bonded to oxygen species are too close to allow individual quantification (Fig. 7B). Previous studies have reported that the binding energy of Sb $3d_{3/2}$ core for Sb(III) and Sb(V) within antimonite oxides were usually 538.8-539.4 and 539.8-541.7 eV respectively (Huang and Ruiz, 2006; Mittal et al., 2011). The

binding energy of Sb 3d_{5/2} peak position of unreacted Fh_Sb(0.1) at 540.0 eV agrees well with that reported for Sb(V)-O species (Fig 7B, top). Interestingly, the Sb 3d_{3/2} peak of the bio-reduced sample shifted to a slightly lower binding energy of 539.6 eV which may cautiously suggest that some reduction of Sb(V) into Sb(III) occurred at the surface of the sample (Fig 7B, bottom). In particular, Xu et al. (2011) reported Sb 3d_{3/2} binding energy of 539.7 eV for Sb(III) sorption onto goethite.

Lastly, the C 1s peak of unreacted Fh_Sb(0.1) is usually fitted with the components at 284.6 eV, 286.1 eV and 288.7 attributed to C-C, H, C-O and C=O, -O environments (Fig. 7C, top). The presence of carbon reflects some surface contamination often observed in XPS experiments. Bio-reduced samples show a significant increase in the carbonate components located at 288.0 eV (Fig. 7C, bottom), in agreement with the formation of GR1(CO₃²⁻) as observed by XRD and TMS.

3.3.3 Stability of abiotic GR1(CO₃²⁻)

GR1(CO₃²⁻) was synthesized as described by Bocher et al. (2004) and aged for one week before being analyzed by Raman spectroscopy (Fig. 6B). The GR1(CO₃²⁻) synthesized without any additive (i.e. PO₄³⁻ or Sb(V)) had transformed by the end of the aging time (Fig. 6B a). The Raman spectrum displayed a strong band at 670 cm⁻¹, which is assigned to magnetite. Similarly, the study carried out by Bocher et al. (2004) demonstrated the transformation of GR1(CO₃²⁻) toward magnetite (Fe₃O₄) and ferrous hydroxide (Fe(OH)₂). Moreover, Guilbaud et al. (2013) used the same procedure to synthesize GR1(CO₃²⁻) and observed its transformation towards magnetite after 69 hours of aging. The Raman spectrum of the GR1(CO₃²⁻) synthesized in the presence of phosphate with an PO₄³⁻ / Fe_t ratio of 0.016, exhibited two strong bands at 435 and 510 cm⁻¹ and a weak band at 220 cm⁻¹, indicating the

occurrence of $\text{GR1}(\text{CO}_3^{2-})$ (Fig. 6B b). No specific band of either magnetite or of siderite was detected. This result agrees with the data produced by Benali et al. (2001) and Bocher et al. (2004), where the presence of phosphate stabilized the mixed-valence mineral and hindered mineralogic transformation. When the $\text{GR1}(\text{CO}_3^{2-})$ precipitation occurred in the presence of Sb(V), the Raman spectrum exhibited the two strong bands at 435, and 510 cm^{-1} characteristics of $\text{GR1}(\text{CO}_3^{2-})$ plus an additional unassigned phase, probably an iron-bearing solid doped with Sb (Fig. 6B d, e). However, when the Sb(V) was added after the precipitation (~ 10 min) the characteristic Raman bands of $\text{GR1}(\text{CO}_3^{2-})$ and magnetite were observed (Fig. 6B c).

4. DISCUSSION

4.1 *Rate and extent of bio-reduction and Sb partitioning*

The bio-reduction of Fh was not inhibited by Sb(V), which is in line with recent work by Burton et al. (2019). It was expected that Sb(V) loading, like other elements substitutions, would alter the physicochemical properties of the Fh (Tsapin et al., 1996; Fredrickson et al., 2001; Jentsch and Penn, 2006; Ekstrom et al., 2010; Frierdich and Catalano, 2012b; Massey et al., 2014), and consequently affects the rate and the extent of bio-reduction. In this study, the rate of reduction decreased by ~ 50% in the presence of Sb(V) independently of the Sb/(Sb+Fe) molar ratio (Table 1, Fig. 2). The observed decrease in the rate of reduction may be related to the decline of a significant number of reactive sites on the Fh surface due to Sb(V) loading (Fredrickson et al., 2001; Ekstrom et al., 2010; Voegelin et al., 2019).

Fh is a metastable mineral that transforms promptly to more thermodynamically stable phases such as magnetite and goethite when reacted with Fe(II) (Hansel et al., 2005; Pedersen et al., 2005) under anoxic and non-sulfidic conditions. Electron transfer between surface-associated Fe(II) and structural Fe(III) has been shown to initiate the dissolution of Fh and the precipitation of a thermodynamically stable secondary minerals (Williams and Scherer, 2004; Yang et al., 2010; Boland et al., 2014). The bio-generation and sorption on the Fh surface of the minimum amount of Fe(II) necessary to trigger Fh transformation would hamper the bio-reduction process. In the present study, the assays run with an Sb/(Sb+Fe) molar ratio inferior to 0.06 displayed the same extent of reduction as that of pure Fh (Table 1, Fig. 2). The transformation of the host mineral was induced by ~ 35% of bio-generated Fe(II), which subsequently halted the bio-reduction process. This result indicates that Sb(V) loading with an

Sb/(Sb+Fe) molar ratio of up to 0.06 has a minimal effect on Fh_Sb lability or reducibility compared with pure Fh.

The extent of reduction of Fh_Sb(0.1) reached ~ 50%, indicating that a higher Fe(II) loading was required to induce Fh_Sb(0.1) transformation (Table 1, Fig. 2). It may be considered that this Sb loading probably reduced the lability or reducibility of the Fh_Sb(0.1) by likely decreasing the transfer of electrons between the bio-generated Fe(II) and the Fh_Sb(0.1). A higher Fe(II) concentration was thus needed to trigger Fh_Sb(0.1) transformation (Williams and Scherer, 2004; Masue-Slowey et al., 2011; Friedrich et al., 2012). The inhibitory effect of a high Sb(V) concentration (0.25 mmol of Sb(V) per gram of goethite) was also observed during the Fe(II)-catalyzed recrystallization of goethite (Burton et al., 2020). The amount of Sb(V) in the Fh_Sb(0.1) probably increased the extent of reduction by likely retarding the mineralogical transformation of the Fh_Sb(0.1) and preserving the Fh_Sb(0.1) surface area for further reduction (Masue-Slowey et al., 2011).

At the end of the bio-reduction period, a minor fraction of Sb(V) was measured in the solution. Our results indicate that a substantial amount of Sb(V) was probably associated with secondary minerals, in line with the data observed elsewhere under biotic and abiotic conditions (Mitsunobu et al., 2010b; Burton et al., 2019; Karimian et al., 2019a). We hypothesize that a significant proportion of Sb(V) was likely adsorbed on the newly formed Fe(II) minerals or otherwise occluded within aggregates of biogenic minerals. Based on earlier reported data for the incorporation of Sb(V) into iron-bearing minerals (Mitsunobu et al., 2013b; Karimian et al., 2017; Burton et al., 2019; Burton et al., 2020), we assume that Sb(V) may structurally substitute for Fe(III) in the secondary minerals due to the similarity in ion size (Fe: 0.65 Å; Sb: 0.60 Å). The fact that Fe-O and Sb-O bond distances are in the same range (Fe-O: 1.97-2 Å; Sb-O: 1.91 Å) in the Fe^{III}O₆ and Sb^VO₆ octahedra (Mitsunobu et al., 2010b; Mitsunobu et al., 2013b) further supports this assumption. Mössbauer analyses

indicated the precipitation of an unidentified Fe(II)-bearing solid. Given our experimental conditions, the composition of the reduction medium, and hyperfine parameters, this non-identified solid could plausibly be constituted of Sb, which could be an additional reason for the low amount of Sb present in the aqueous phase.

A slight reduction of the solid associated Sb(V) to Sb(III) was observed by the XPS analyses (Fig. 7B); this may have been triggered by electron transfer from Fe(II). Karimian et al. (2019b) noted that humic acids (HA) and an Fe(II)-rich environment induced a substantial reduction of Sb(V) by the potential formation of a ternary complex composed of Fe(II), HA and Sb(V), thereby facilitating the electron transfer. Fe(II)-(III) minerals such as magnetite are also able to reduce Sb(V) to Sb(III), as reported by Kirsch et al. (2008). The spiking of Sb(V) during the precipitation of $\text{GR}_2(\text{SO}_4^{2-})$ triggered its adsorption on the newly formed $\text{GR}_2(\text{SO}_4^{2-})$ and its reduction to Sb(III). However, no reduction of Sb(V) was observed when the latter was supplemented in the medium after the precipitation of $\text{GR}_2(\text{SO}_4^{2-})$ (Mitsunobu et al., 2008b; Mitsunobu et al., 2009). Although there is no data in the literature describing the interaction of $\text{GR}_1(\text{CO}_3^{2-})$ and Sb(V), the slight reduction observed in our study was probably induced by the Fe(II) / Fe(III) complex during the precipitation of $\text{GR}_1(\text{CO}_3^{2-})$.

4.2 Precipitation of biogenic minerals

No detectable biogenic mineral was observed in the control assays (without bacteria), whatever the Sb loading. This observation is in line with previous studies showing that the transformation of Fh to a thermodynamically more stable mineral necessitates a long period (months or years) in our experimental conditions (i.e. near-neutral pH and room temperature) (Schwertmann et al., 2004).

4.2.1 Biogenic minerals formed by bio-reduction of Sb-free Fh

The ratio of electron donor to electron acceptor is a significant parameter, as it impacts the Fe(II) to Fe(III) fraction that controls the nature of the biogenic minerals in the reduction media. Indeed, a lower Fe(II) / Fe(III) ratio (< 1.0 mmol Fe(II)/g Fh) promotes the precipitation of ferric phases, such as lepidocrocite and goethite. At the same time, a higher ratio leads to the precipitation of ferrous and ferrous/ferric phases such as siderite, magnetite and GR (Hansel et al., 2003; Yang et al., 2010). In this study, we conducted the Fh bio-reduction experiments with an excess of electron source, and the resulting biogenic minerals were magnetite (91%) and siderite (9%) (Fig. 8). Iron oxides particles have a high affinity to bacteria cells walls and associated extracellular polymeric substances (Fein et al., 1997; Lower et al., 2001; Luef et al., 2013), leading to the formation of aggregated structures. Furthermore, the Fh drying procedure used in this study entails the aggregation of Fh particles (Gilbert et al., 2009). Such bio-inorganic structures are likely to lead to the formation of micro-environments establishing distinct chemical and redox zones relative to the bulk solution (Zegeye et al., 2010; Luef et al., 2013). Similar conditions may induce the precipitation of various biogenic minerals due to the occurrence of a differential Fe(II) gradient. In the literature, the precipitation of siderite is commonly observed in the presence of an HCO_3^- buffered medium (Fredrickson et al., 1998) or in an atmosphere containing CO_2 (Roh et al., 2003), which was not the case in our experimental setup. As siderite precipitation requires high local bicarbonate concentration, we hypothesized that its formation occurs in the micro-environments of aggregates where the mass transfer is probably limited or slowed down, thereby allowing the simultaneous accumulation of both Fe(II) and bicarbonate (Pallud et al., 2010).

On the other hand, magnetite precipitation is thought to involve a topotactic transformation process (Tronc et al., 1992; Pedersen et al., 2005; Usman et al., 2018). The sorption of Fe(II) generated by bacterial activity induces interfacial electron transfer to the structural Fe(III) and catalyzes the subsequent precipitation of magnetite as the thermodynamically more stable phase.

4.2.2 Biogenic minerals formed by Fh_Sb bio-reduction

The bio-reduction of Sb-bearing Fh led mainly to the precipitation of $\text{GR1}(\text{CO}_3^{2-})$, goethite and an unidentified Fe(II)-bearing solid irrespective of the Sb/Fe ratio. However, the relative abundance of this unidentified Fe(II) bearing solid rises with the increase of Sb loading (Fig. 8). Its relative abundance was $\sim 7\%$ and reached $\sim 17\%$ for the assays run with Fh_Sb(0.04) and Fh_Sb(0.1) respectively (Fig. 8). While which secondary minerals form depends on the partitioning of the Fe(II) between aqueous and solid associated forms, the presence of Sb also seems to orient the nature of the biogenic minerals. The precipitation of magnetite as a very minor phase (Fig. 6A) may be a consequence of the presence of Sb(V). Fe(II) was generated almost to its maximum concentration just after four days of incubation in the absence of Sb(V). The generation of a high Fe(II) concentration in a very short time shifted the precipitation toward magnetite, as witnessed elsewhere in the literature (Ona-Nguema et al., 2002). The presence of Sb(V) reduced the rate of reduction by 50%, resulting in slower production of Fe(II) over time and consequently hindering the formation of magnetite.

In a recent study focusing on the same topic (*i.e.* the bio-reduction of Sb bearing ferrihydrite), feroxyhyte (δ' -FeOOH) and goethite were the main biogenic minerals (Burton et al., 2019). The main difference between the two studies is that in Burton et al. (2019) the

electron source was in deficit relative to ferrihydrite concentration. As a result, the Fe(II) concentration produced in their research was lower, thus providing the chemical condition for the precipitation of Fe(III)-bearing biogenic minerals. In the current study, however, the excess of electron source permitted a more substantial input of Fe(II), allowing the precipitation of both GR1(CO₃²⁻) and goethite. These observations are in agreement with Hansel et al. (2003), who showed a threshold for segregating the formation of magnetite and goethite based on the Fe(II) / Fe(III) ratio in a biotic and abiotic systems.

It is commonly accepted that the precipitation of ferrous hydroxides, Fe(III) oxides and oxyhydroxides is the result of the condensation phenomena of iron cations initially present in aqueous solution. Jolivet et al. (2004) described the different steps involved in these precipitation processes and revealed how the structure of polymeric precursors determines the nucleation and growth of the solid phases in a Fe-H₂O system. These authors posited that growth of goethite by the dissolution/crystallization process might be interpreted as taking place on the basis of the planar tetramer [M₄(OH)₁₂ (OH₂)₄]₀. In our study, the superparamagnetic goethite, identified by Mössbauer analysis, appears to be in line with these mechanisms based on the precipitation of nanosized solid phases. We assumed that the ZFC maxima at 10.5 K (Fig A3) originates from goethite (Guyodo et al., 2003; Barrero et al., 2006), and considering an anisotropy constant of 103 J m⁻³, we calculated the average goethite volume, corresponding to a sphere of about 20 nm diameter. This calculated particle size agrees with the intriguing line shapes of the Mössbauer spectrum obtained at RT. Indeed, as was expected for a poorly crystallized goethite, we observed a total absence of any magnetic signal (sextet) which would suggest the presence of nano-crystalline goethite in a superparamagnetic state (Janot et al., 1968; Olowe et al., 1990).

As GR1(CO₃²⁻) double-layered structure bears Fe(II), Fe(III) and CO₃²⁻, we hypothesized that its formation was governed by a dissolution/precipitation process. The intriguing feature is

656 that $\text{GR1}(\text{CO}_3^{2-})$ is a metastable mineral that transforms relatively quickly (> 24 h) into a
657 thermodynamically more stable mineral such as magnetite or goethite, depending on the
658 geochemical conditions (Benali et al., 2001; Guilbaud et al., 2013). However, the increased
659 Sb(V) loading in our system tended to favor the precipitation of $\text{GR1}(\text{CO}_3^{2-})$ rather than
660 goethite (Fig. 8): thus, the relative abundance of goethite declined by 25% when the Sb/(Fe +
661 Sb) molar ratios were raised from 0.06 to 0.1, even though goethite has a much lower
662 standard-state Gibbs energy (-489.8 ± 1.2 kJ/mol) (Hiemstra, 2015) than of $\text{GR1}(\text{CO}_3^{2-})$ ($-$
663 4044.24 kJ/mol) (Drissi et al., 1995). Given the higher thermodynamic stability of goethite
664 compared with $\text{GR1}(\text{CO}_3^{2-})$, it was reasonable to assume that the system would move to
665 goethite. In addition, $\text{GR1}(\text{CO}_3^{2-})$ needs to be in equilibrium with aqueous Fe(II) in order to
666 circumvent any mineralogical transformation. But this aqueous Fe(II) would be depleted by
667 sorption on the goethite surface, thereby inducing the dissolution of $\text{GR1}(\text{CO}_3^{2-})$. For
668 example, Ona-Nguema et al. (2002) demonstrated the depletion of aqueous Fe(II) which was
669 in equilibrium with $\text{GR1}(\text{CO}_3^{2-})$ by sorption on the remaining lepidocrocite surface, thereby
670 inducing the dissolution of $\text{GR1}(\text{CO}_3^{2-})$ and the precipitation of magnetite in a biotic system.
671 Similarly, under anoxic conditions, $\text{GR1}(\text{CO}_3^{2-})$ transforms into magnetite, $\text{Fe}(\text{OH})_2$ or
672 siderite (Benali et al., 2001), but in the presence of phosphate it is likely that the adsorption of
673 the latter species upon the lateral faces of abiotic $\text{GR1}(\text{CO}_3^{2-})$ crystals likely hindered this
674 transformation, by acting as a physical barrier (Bocher et al., 2004). Mitsunobu et al. (2009)
675 also observed the stabilization of abiotic $\text{GR2}(\text{SO}_4^{2-})$ when precipitated in the presence of
676 Sb(V). The adsorption of Sb(V) on the edge sites of $\text{GR2}(\text{SO}_4^{2-})$ with an inner sphere complex
677 was the main parameter hampering its transformation toward magnetite. To the authors'
678 knowledge, no comparable information regarding Sb(V) and $\text{GR1}(\text{CO}_3^{2-})$ is available. We
679 therefore hypothesized that the adsorption of Sb(V) species on the $\text{GR1}(\text{CO}_3^{2-})$ crystals
680 hampered its dissolution by acting as a physical barrier. The observation, after the ageing

period, of magnetite and $\text{GR1}(\text{CO}_3^{2-})$ when Sb(V) was added after $\text{GR1}(\text{CO}_3^{2-})$ precipitation implies that the stabilizing effect of Sb(V) is probably more marked when Sb(V) and iron are co-precipitated together. Stabilization efficiency seems to be related to the interaction of Sb(V) and iron during the $\text{GR1}(\text{CO}_3^{2-})$ crystallite formation rather than to Sb(V) adsorption after the crystal growth (Fig 6B).

4.3 Geochemical significance

While this study addresses the effect of Sb(V) on the bio-reduction of Fh and the resulting secondary minerals, our results may also be of interest in improving our understanding of the mobility of Sb in the presence of iron-reducing bacteria. In natural soils, antimony has been shown to be closely associated with iron oxides. Adsorption and precipitation reactions with Fe(III) oxides play a crucial role in controlling the mobility and the fate of Sb(V) in natural settings.

In subsurface environments, seasonal water table oscillation caused by rainfall and irrigation establishes oxic-anoxic cycles. Under reducing conditions, the iron oxides may undergo dissolution and mineralogy transformation upon reaction with iron-reducing bacteria that can potentially affect the fate and the mobility of sorbed Sb(V) . During Sb(V) -bearing bio-reduction of Fh, goethite and ferrihydrite formation is observed in carbon-deficient setups (Burton et al., 2019). In contrast, goethite and $\text{GR1}(\text{CO}_3^{2-})$ is precipitated in carbon-rich systems (this study). In both cases, Sb(V) partitioned mainly to secondary minerals, suggesting that labile organic carbon's availability does not affect Sb(V) 's sorption into the structure of secondary minerals. Furthermore, even a partial formation of Fe(II) can trigger the mineralogical transformation of iron oxide and lead to Sb(V) sorption in a crystallized secondary mineral. At redox transitions zones, iron oxides undergo numerous redox cycling

that will likely lead to the precipitation of various secondary minerals displaying different affinity to Sb(V) depending on their properties. Ultimately, Sb(V) repartitioning and speciation will vary among environments but should be controlled by the flux of Fe(II) which dictates the secondary minerals' nature.

Our study also demonstrates that Sb(V)-loading affects the mineralogy of secondary minerals indicating that Sb(V) concentration controls the Fe(II) flux. The increase of Sb(V) led to an substantial Fe(II) formation ($\text{Fe(II)/Fe}_{\text{tot}} \sim 0.5$) leading to the simultaneous rise of metastable $\text{GR1(CO}_3^{2-})$ and a decrease of goethite. The Sb(V) sorption on $\text{GR1(CO}_3^{2-})$ seems to drive the mineral's stability. In contaminated subsurface environments, $\text{GR1(CO}_3^{2-})$ displays the ability to remove arsenic (As) species and to be a host phase for As (Perez et al., 2021). It is, therefore, necessary to study the fate of Sb(V) sorbed on $\text{GR1(CO}_3^{2-})$ particles to decipher the role played by these metastable Fe(II)/Fe(III) minerals in natural settings in regards to the (im)mobilization of metalloids. Our study's significance is that the parameters governing the biogeochemical flux of Fe(II) should be understood before considering iron oxides and secondary minerals as a reliable Sb(V) sink for long-term storage in Sb-contaminated environments.

5. CONCLUSIONS

This study highlights how Sb affects the rate and the extent of Fh bio-reduction as well as the nature of biogenic minerals. By using complementary characterization methods, we were able to capture the precipitation of a relative abundance of various biogenic minerals with a relative abundance that were governed by multiple biomineralization pathways. The presence of Sb reduced by almost 50% the rate of bio-reduction and enhanced the extent of reduction of Fh with a Sb/(Sb + Fe) molar ratio of 0.1. Note should also be taken of the total Fh transformation irrespective of the Sb(V) concentration. Thus, magnetite precipitated in the absence of Sb(V), while $\text{GR1}(\text{CO}_3^{2-})$, goethite and an unidentified Fe(II)-Sb-bearing solid formed in its presence. Importantly, this study shows that the efficiency of Sb in stabilizing the metastable $\text{GR1}(\text{CO}_3^{2-})$ by mitigating its mineralogical transformation toward a thermodynamically more stable mineral such as goethite. The question raised by this study is, how does Sb(V) interact with $\text{GR1}(\text{CO}_3^{2-})$ crystals? A natural next step would be to carry out research to reveal the interaction of Sb(V) with $\text{GR1}(\text{CO}_3^{2-})$ at the crystallographic level.

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FIGURES CAPTIONS

Figure 1: X-ray diffraction pattern of Fh (black line) and Sb substituted Fh (Fh_Sb(0.04) blue line, Fh_Sb(0.06) red line and Fh_Sb(0.1) green line). The two maxima (0.258 nm and 0.149 nm) are consistent with 2-line Fh.

Figure 2: Bio-reduction of Fh (black symbols) and Sb substituted Fh, (Fh_Sb(0.04) Fh_Sb(0.06) and Fh_Sb(0.1) blue, red and green symbols respectively) measured as $\text{Fe(II)}_{\text{tot}}/\text{Fe}_{\text{tot}}$. The initial rate of reduction was computed from the first derivation of a nonlinear curve fit represented by the dotted lines. Errors bars represent standard deviation of three triplicates batches incubations.

Figure 3: X-ray diffractograms of microbially reduced Fh (black line) and Sb substituted Fh. (Fh_Sb(0.04), Fh_Sb(0.06) Fh_Sb(0.1) blue, red and green lines respectively. “GR1”: green rust 1, “M”: magnetite, “S”: siderite and “*” unidentified phase.

Figure 4: TMS spectra of solid phases obtained after the bio-reduction of Fh at different temperature. Black dots: experimental data; red dashed line: global fitted curve; solid line: components.

Figure 5: Raman spectra of biogenic and abiotic minerals **A:** Raman spectra for two locations (red and black spectra) on bio-reduced Fh_Sb(0.04) sample and two locations (green and blue spectra) on bio-reduced Fh_Sb(0.1) sample. **B:** Raman spectra of (a) abiotic GR1 precipitate, (b) abiotic GR1 precipitate with phosphate, (c) abiotic GR1 precipitate and addition of Sb(V) ~ 10 minutes after the precipitation, (d), (e) two locations for abiotic GR1 precipitated with

1155 Sb(V). The analyses were done after a week of aging. M: Magnetite, S: siderite, GR: Green
1156 Rust, ?: unassigned phase.

1157

1158 **Figure 6:** TMS spectra of solid phases obtained after the bio-reduction of Sb substituted Fh at
1159 16 K. Black dots: experimental data; red dashed line: global fitted curve; solid line:
1160 components.

1161

1162 **Figure 7:** XPS high resolution spectra of (A) Fe2p_{3/2} core level before (upper panel) and after
1163 bio-reduction (lower level), (B) Sb 3d_{5/2} core level before (upper panel) and after bio-
1164 reduction (lower level), (C) C1s core level before (upper panel) and after bio-reduction (lower
1165 level).

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1167 **Figure 8:** Relative abundance distribution of biogenic minerals as a function of Sb/(Sb + Fe)
1168 based on Mössbauer data.

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1171 TABLES CAPTIONS:

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1173 **Table 1:** Rate and extent of Fh and substituted Fh bio-reduction. Aqueous Sb were measured
1174 at the end of the bio-reduction period (*i.e.* 25 days). Numbers in parenthesis are the standard
1175 deviation of triplicates analyses.

1176

1177 **Table 2:** Mössbauer hyperfine parameters at 15 K and relative abundance of components in
1178 bio-reduced Fh and bio-reduced Fh_Sb series. CS = center shift relative to α -iron at room

1179 temperature, $\Delta(\epsilon)$ = Quadrupole splitting (shift), H = magnetic hyperfine field, RA = Relative
1180 abundance.
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