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The Effect of Heterogeneous Seed Crystals on Arsenite Removal as Biogenic Scorodite

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With the aim to effectively oxidize and remove highly toxic As(III) from acidic metal-refinery wastewaters, the seeding effect of different heterogeneous minerals was investigated on the formation of biogenic scorodite ($FeAsO_4{\cdot}2H_2O$), using the Fe^{2+}/As(III)-oxidizing thermo-acidophilic archaeon *Acidianus brierleyi*. Heterogeneous hematite-seeds exhibited even greater As-removal efficiency relative to homogeneous scorodite-seeds. While the effect of magnetite-seeds was mostly comparable to scorodite-seeds, feeding goethite or ferrihydrite negatively affected the speed of As precipitation, forming jarosite or jarosite/scorodite mixture, respectively, instead of scorodite. Similarly to those formed on scorodite-seeds (TCLP As leachability; 0.49 mg/L), the final scorodite products formed on hematite-seeds or magnetite-seeds were also highly stable (0.51 mg/L or 0.39 mg/L, respectively), well below the US standard of 5 mg/L. The effectiveness of hematite seeding was also demonstrated in the lower-temperature scorodite crystallization reaction (45°C), where Fe^{2+}-oxidizing moderately-thermophilic acidophilic bacterium *Acidimicrobium ferrooxidans* was employed, after the complete As(III) oxidation by *Thiomonas cuprina*. The overall results suggested that the effectiveness of hematite was, at least partly, attributed to its highly-positive surface charge. This effect was retained even when cells attached onto the hematite surface. This made the mineral an effective absorbent for anionic As(V) and SO_4^{2-}, consequently speeding up the reaction by shortening the steady-state induction period between the two As-removal stages, during the biogenic scorodite crystallization process. [doi:10.2320/matertrans.M-M2019858]

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1. Introduction

Water contamination with highly toxic arsenic (As) is a growing problem to be solved in mining industries, in order to ensure the future copper supply from primary copper sulfide deposits which contain As-bearing minerals such as enargite (Cu_3AsS_4) and tennantite ($Cu_{12}As_4S_{13}$). Mineralization of soluble As into highly stable scorodite ($Fe^{III}As^{V}O_4{\cdot}2H_2O$) is considered one of the most advantageous approaches of As immobilization prior to disposal.[1)]

The concentrated soluble As(V) (~hundreds of millimolar) can be chemically mineralized into scorodite via hydrothermal[2,3)] or atmospheric reactions[4–10)] at temperatures mostly 95–160°C.[11)] Nonetheless, residual As ions after such chemical reactions (in the form of As(V), as well as As(III) which had persisted the pre-oxidation step) yet remain to be treated. Some metallurgical operations also produce As(III) solutions at more dilute concentrations (~25 mM), at which chemical reactions become less effective.[11)]

In order to realize scorodite crystallization at such thermodynamically less feasible As concentrations under even milder conditions (e.g., lower temperatures, the lower doses of chemical reagents), microbiological approaches were proposed.[11–18)] Our previous studies demonstrated the simultaneous microbial Fe^{2+} and As(III) oxidations by the thermo-acidophilic archaeon *Acidianus brierleyi* (70°C), leading to one-step biogenic scorodite crystallization without adding chemical oxidants.[15,16)] Factors such as the initial Fe^{2+}/As(III) ratio, initial pH (pH_{ini}) and seeding were shown to influence the reaction kinetics of biogenic scorodite formation.[18)] Unlike in abiotic studies targeting high As(V) concentrations where the pH_{ini} generally set at around $\leq$1.0,[5–10)] the necessity was indicated of starting the biogenic reaction at a relatively high pH_{ini} of 1.5, in order to precipitate As from dilute solutions.[11)] This is due to the biogenic scorodite crystallization proceeding via the two-stage As precipitation (at pH_{ini} 1.5), driven by the SO_4^{2-}-mediated phase transformation (Appendix A1):[18)] The first-stage As-removal is triggered by microbial Fe^{2+} and As(III) oxidations, precipitating amorphous precursors consisting of basic ferric sulfate ($MFe_x(SO_4)_y(OH)_z$) and ferric arsenate ($FeAsO_4{\cdot}(2+n)H_2O$). This first stage is followed by an induction period (dissolution-recrystallization of amorphous precursors proceeds), wherein re-dissolved metal ions locally concentrated on the precursors surface give the driving force for the second-stage As-removal as crystalline scorodite ($Fe(AsO_4)_{0.94}(SO_4)_{0.08}{\cdot}1.69H_2O$). Lowering pH_{ini} from 1.5 to 1.2 resulted in the seemingly different, single-stage As-removal mechanism, where the immediate dissolution of amorphous precursors under more acidic pH likely led to the apparent diminishment of the inducing period.[11)] However, the final As-removal at pH_{ini} 1.2 was relatively incomplete compared to that at pH_{ini} 1.5. Therefore, the two-stage As-removal was indeed found to play an important role in effective biogenic scorodite crystallization.[18)]

In abiotic scorodite crystallization studies by other researchers, the effect of seed-feeding (homogeneous scorodite or other heterogeneous minerals) was shown to be one of the influential factors:[3,5,10,19)] Feeding scorodite-seeds allowed lowering the reaction temperature for scorodite crystallization (from 95 to 80°C).[3)] Use of heterogeneous-seeds such as hematite and gypsum were shown to be equally effective as homogeneous scorodite-seeds, where the authors suggested that hematite can outperform scorodite due to its "fine" properties.[5)] It was also suggested that hematite-seeds paly a role as the Fe^{3+} source for scorodite formation.[10)]

In our biogenic studies, the positive effect of scorodite-seeds was also noted.[11,17)] Since biomineralization reactions

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involve the interactions between the mineral, microbial cells (physicochemical as well as enzymatic effects) and reactant ions, the function of seed minerals within the biomineralization process can be complex. In one study, the scorodite-seeds directly influenced to push the microbial (enzymatic) activity; inhibition of microbial Fe^{2+}- and As(III)-oxidizing abilities by co-existing Cu^{2+} ions was readily alleviated by feeding scorodite-seeds. There, it was suggested that the surface of scorodite-seeds played a role in providing immediate support for microbial colonization and enabled more robust microbial reactions.[17] In another study targeting a low initial As(III) concentration of 4.7 mM, the advantages in feeding biogenic-seeds, rather than chemically-synthesized-seeds, was found.[11] The morphological and structural differences between biogenic and chemically-synthesized scorodite-seeds significantly affected the formation process of biogenic scorodite; feeding the former was found more effective not only in accelerating the reaction but also in forming more recalcitrant products.[11] By feeding homogeneous scorodite-seeds, such complex interactions consequently accelerated the reaction kinetics by shortening the induction period during the biogenic scorodite crystallization process (Appendix A1).[18]

As mentioned above, although a few studies are available testing the heterogeneous mineral seeding in chemical scorodite synthesis, its function is largely unclear. Furthermore, its effect in biogenic scorodite crystallization process is yet unknown. Therefore, this study aimed to; (i) compare the effect of different seed minerals (magnetite, hematite, goethite, ferrihydrite) on the formation and stability of biogenic scorodite produced at 70°C (by liquid, XRD, SEM and TCLP analyses), (ii) assess the seeding mechanism of selected heterogeneous minerals (by zeta-potential and XANES analyses), and (iii) apply the most effective heterogeneous mineral in the lower temperature biogenic reaction at 45°C.

2. Experimental

2.1 Microorganism

Thermophilic microorganism used for biogenic scorodite crystallization test at 70°C: The acidophilic Fe^{2+}- and As(III)-oxidizing archaeon *Acidianus* (*Ac.*) *brierleyi* DSM 1651^T was maintained and pre-grown in 200 mL heterotrophic basal salts (HBS) medium (450 mg/L $(NH_4)_2SO_4$; 50 mg/L KCl; 50 mg/L KH_2PO_4; 500 mg/L $MgSO_4 \cdot 7H_2O$; 14 mg/L $Ca(NO_3)_2 \cdot 4H_2O$; 142 mg/L Na_2SO_4; pH 1.5 with H_2SO_4 in 500 mL Erlenmeyer flasks) containing 18 mM Fe^{2+} (1000 mg/L; as $FeSO_4 \cdot 7H_2O$), 13 mM As(III) (1000 mg/L; as $NaAsO_2$), and 0.02% (w/v) yeast extract. The flasks were incubated at 70°C, shaken at 100 rpm.

Moderately-thermophilic microorganisms used for biogenic scorodite crystallization test at 45°C: The acid-tolerant As(III)-oxidizing bacterium *Thiomonas* (*Th.*) *cuprina* Hö5 (DSM 5495) was maintained and pre-grown in 200 mL HBS medium (pH 3.5 with H_2SO_4 in 500 mL Erlenmeyer flasks) containing 0.1% (w/v) elemental sulfur (35°C, shaken at 100 rpm). The acidophilic Fe^{2+}-oxidizing bacterium *Acidimicrobium* (*Am.*) *ferrooxidans* ICP (DSM 10331) was maintained and pre-grown in HBS medium (pH 1.5) containing 18 mM Fe^{2+} and 0.02% yeast extract. The flasks were incubated at 45°C, shaken at 100 rpm.

2.2 Biogenic scorodite crystallization test using different heterogeneous seed minerals (*Ac. brierleyi*; 70°C)

Pre-grown *Ac. brierleyi* cells were washed and inoculated at 1.0×10^7 cells/ml in 500 mL flasks containing 200 mL HBS medium (pH 1.5) with 18 mM Fe^{2+}, 13 mM As(III) and 0.02% yeast extract. Different types of seed minerals (biogenic scorodite as homogeneous control, and four heterogeneous minerals; Table 1) were fed at 0.5% (w/v). Flasks were incubated for 14 days at 70°C, shaken at 150 rpm. Liquid samples were regularly taken to monitor pH, Eh (vs. SHE) and cell density, and filtered to determine the concentrations of total Fe and As by ICP-OES (Optima8300, PerkinElmer), As(III) by the molybdenum blue method, and Fe^{2+} by the o-phenanthroline method. Precipitates were collected on day 14 and freeze-dried overnight for X-ray diffraction analysis (Ultima IV, Rigaku; Cu Kα 40 mA, 40 kV) and SEM observation (VE-9800, KEYENCE).

Biogenic scorodite-, goethite- or ferrihydrite-seeds were produced according to the procedures described in Refs. 11), 20) or 21), respectively. Hematite- and magnetite-seeds of two different particle sizes (fine or coarse) were used. The purchased coarse magnetite was ground and sieved to obtain fine magnetite-seeds (Table 1).

2.3 Toxicity characteristic leaching procedure (TCLP) test

Stability of the final secondary mineral products was evaluated by following the TCLP according to the EPA method 1311.[22] Secondary minerals (from 2.2; collected on day 14) were transferred into 25 mL vials containing 10 mL acetate buffer (pH 4.9) at a pulp density of 5% (w/v) and

Table 1 Different types of seed minerals fed for biogenic scorodite formation.

Seed Minerals		Chemical Formula	Particle Size (μm)	Note
Homogeneous seed	Scorodite (biogenic)	$Fe^{III}As^{V}O_4 \cdot 2H_2O$	10-40	11)
Heterogeneous seeds	Hematite	$Fe^{III}{}_2O_3$	2-40 2000-5000	Wako Pure Chemicals; 096-04825 Kojundo Chemicals; FEO19GB
	Magnetite	$Fe^{II, III}{}_3O_4$	-50 2000-5000	Kojundo Chemicals; FEO18GB
	Goethite	$Fe^{III}O(OH)$	5-50	20)
	Ferrihydrite	$Fe^{III}{}_2O_3 \cdot 0.5H_2O$	20-100	21)

incubated at 25°C, rotated at 30 rpm for 18 hours. Liquid samples were filtered (0.45 μm glass fiber) to measure total soluble Fe and As concentrations. Tests were conducted in duplicates.

2.4 Zeta-potential measurement

The interactions between solid particles (seed minerals or *Ac. brierleyi* cells) and anionic reactant species (As(III), As(V) or SO_4^{2-}) were studied: Suspensions of individual ground mineral (biogenic scorodite, hematite or magnetite; 0.2% (w/v)) or *Ac. brierleyi* cells (5×10^7 cells/mL) were prepared in 10 mM NaCl solutions (each at pH 2.3, 3.0 or 4.0; HCl or NaOH). The NaCl solutions were pre-mixed with 0.2 mM of As(III) (as $NaAs^{III}O_2$), As(V) (as $KH_2As^{V}O_4$) or SO_4^{2-} (as K_2SO_4), prior to thoroughly mixing with solid particles for 1 hour (70°C, shaken at 150 rpm). Interactions between seed minerals and *Ac. brierleyi* cells were also studied by mixing each one of the minerals with cells (pH 2.3). The suspensions were then transferred into the capillary cell for the zeta-potential measurement (Malvern ZETASIZER Nano series). The measurements were conducted in triplicate.

2.5 Low-temperature biogenic scorodite crystallization test using hematite-seeds (*Th. cuprina* at 35°C and *Am. ferrooxidans* at 45°C)

Pre-grown *Th. cuprina* cells were washed and inoculated (at 1.0×10^7 cells/ml) in 500 ml flasks containing 200 ml of HBS medium (pH 3.0) with 13 mM As(III). Flasks were incubated at 35°C, shaken at 100 rpm. After completion of microbial As(III) oxidation (within 7 days), the culture pH was re-adjusted to 1.5 or 1.2, followed by inoculation of pre-grown *Am. ferrooxidans* cells (at 1.0×10^7 cells/mL) and addition of 13 mM Fe^{2+} ($[Fe^{2+}]_{ini}/[As(V)]_{ini}$ molar ratio = 1.0). Flasks were then incubated for 35 days at 45°C, shaken at 100 rpm. Liquid samples were regularly taken to monitor pH, Eh (vs. SHE) and cell density, and filtered to determine the concentrations of total Fe As, Fe^{2+} and As(III), as described in 2.2.

2.6 X-ray near edge structure (XANES) analysis

Pre-grown *Am. ferrooxidans* cells were washed and inoculated at 1.0×10^7 cells/mL in 500 ml flasks containing 200 ml of HBS medium (pH 1.5) containing 13 mM As(V) and 13 mM Fe^{2+} ($[Fe^{2+}]_{ini}/[As(V)]_{ini}$ molar ratio = 1.0). Flasks were incubated at 45°C, shaken at 100 rpm. Biogenic scorodite precursors were recovered at 3 h and on day 1. Matured biogenic scorodite was recovered on day 50 as control. To avoid Fe^{2+} oxidation during sample preparation, storage and transportation, precipitates were immediately collected and freeze-dried, followed by mixing with boron nitride to form tablets. The tablets were immediately vacuum-sealed for measurements. The Fe K-edge XANES spectra were collected (transmission mode; 6800–8600 eV) at SAGA-LS (1.4 GeV, 75.6 m), using standard chemicals of $FeSO_4 \cdot 7H_2O$ (Wako chemicals; No. 7782-63-0) and $Fe_2(SO_4)_3 \cdot nH_2O$ (Wako chemicals; No. 15244-10-7). An energy axis was calibrated by shifting the 1st inflection point of a Fe foil absorption spectrum (the 1st peak of the derivative spectrum) to 7112 eV.

3. Results and Discussion

3.1 Effect of different heterogeneous seed minerals on biogenic scorodite crystallization by *Ac. brierleyi* (70°C)

3.1.1 As(III) and Fe^{2+} oxidation and precipitation

The trend of microbial As(III) oxidation was similar in all cases (completed by day 4), except with ferrihydrite-seeds where As(III) oxidation became somewhat slower (75% As(III) oxidized by day 4; Fig. 1(a)). Also, compared to the case of homogeneous biogenic scorodite-seeds, the effect of feeding different heterogeneous minerals on microbial Fe^{2+} oxidation was negligible, where Fe^{2+} oxidation mostly completed by day 2 in all cases (Fig. 1(b)).

Goethite- and ferrihydrite-seeds were partially dissolved at the initial stage by protonation upon contact with the acidic solution (as seen by an increase in total Fe concentration; Fig. 2(b)). This was accompanied by a pH-jump (Appendix A2(a)). Eventually, precipitation of As (Fig. 2(a)) and Fe (Fig. 2(b)) were slower and less complete when goethite- or ferrihydrite-seeds were fed, leaving 1.7 mM or 2.4 mM of soluble As, respectively on day 12. The slowest As(III) oxidation and precipitation observed with ferrihydrite-seeds (Fig. 2(b)) coincided with an apparent suppression of the planktonic cell density (Appendix A2(b)), possibly partially due to encrustation of cells in primary or secondary Fe^{3+} minerals.

Feeding magnetite-seeds was shown equally effective to the scorodite-seeds in reducing the length of induction

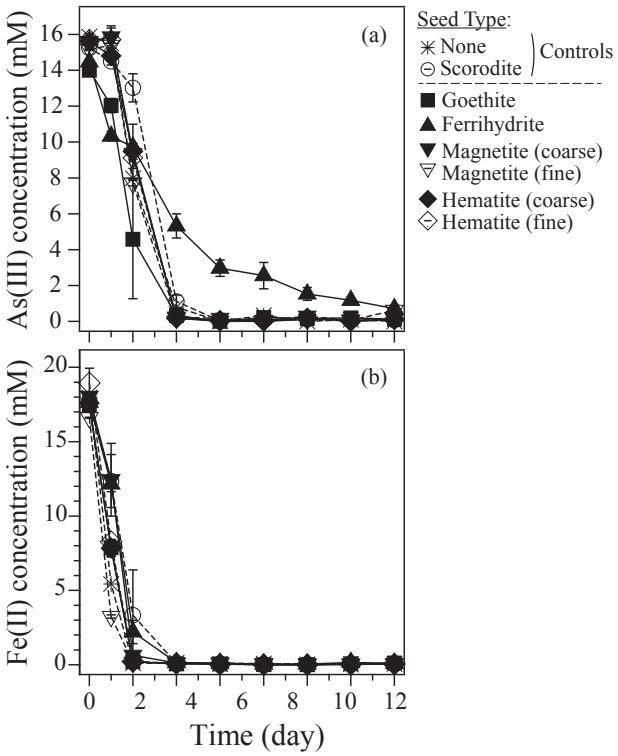

Fig. 1 Microbial As(III) (a) and Fe^{2+} (b) oxidation by *Ac. brierleyi* at 70°C. Heterogeneous-seeds, such as goethite-seeds (■), ferrihydrite-seeds (▲), magnetite-seeds (coarse ▼; fine ▽) and hematite-seeds (coarse ◆; fine ◇), were fed to cultures, in comparison with seed-free (∗) and homogeneous biogenic scorodite-seeds (○). Data points are mean values from duplicate flasks; error bars depicting averages are sometimes invisible as these were smaller than the data point symbols.

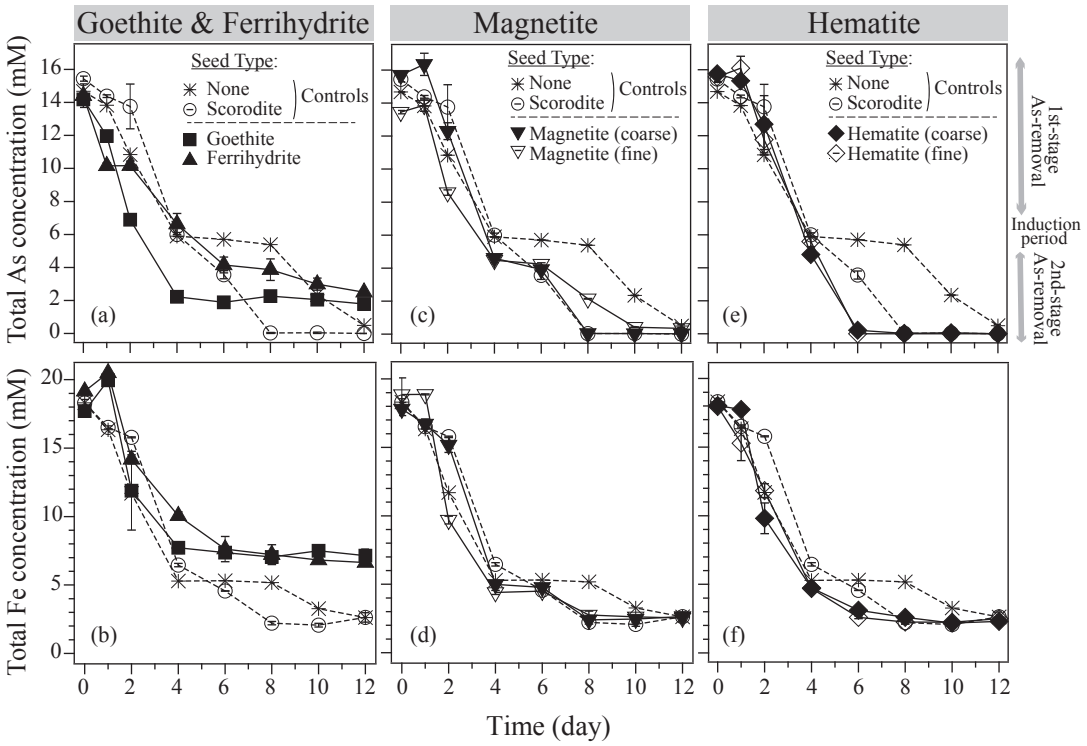

Fig. 2 Precipitation behavior of As (a), (c), (e) and Fe (b), (d), (f) on different heterogeneous-seeds, in comparison with seed-free (∗) and homogeneous biogenic scorodite-seeds (○), during biogenic scorodite formation in *Ac. brierleyi* cultures at 70°C. (a), (b) Ferrihydrite-seeds (▲) or goethite-seeds (■). (c), (d) Magnetite-seeds (coarse ▼; fine ▽). (e), (f) Hematite-seeds (coarse ◆; fine ◇). Data points are mean values from duplicate flasks; error bars depicting averages are sometimes invisible as these were smaller than the data point symbols.

period, and thus speeding up the overall reaction (Fig. 2(c)). While the final As removal was greater with scorodite-seeds ($As_{soluble}$ < detection limit of 50 μg/L; on day 12) than with magnetite-seeds (0.02 mM $As_{soluble}$ with coarse seeds, 0.3 mM $As_{soluble}$ with fine seeds; on day 12) (Fig. 2(c)). When hematite-seeds were fed, the speed of As removal increased further, due to the apparent diminishment of the induction period (Fig. 2(e)): Hematite also allowed complete As removal by day 12 ($As_{soluble}$ < detection limit). In the case of hematite- and magnetite-seeds, the trend of pH was similar to that of scorodite-seeds control (Appendix A2(a)), and no negative effect was noted on the planktonic cell density (Appendix A2(b)). Singhania *et al.* suggested that the seeding effect of hematite in chemical scorodite synthesis lies in its "fine" property.[5] However, the results here showed that the reaction efficiency did not correlate with the particle size (specific surface area) of hematite- and magnetite-seeds (Fig. 2(c), (e)). Therefore, there should be a different underlying mechanism involved in this phenomenon.

3.1.2 Type and stability of the resultant secondary mineral products

The resultant secondary minerals were recovered on day 14 and analyzed by XRD (Fig. 3). It was previously shown that while biogenic scorodite is formed without feeding any seed minerals (as was also shown in Fig. 3(a)),[16] the addition of homogeneous biogenic scorodite-seeds can facilitate its reaction speed.[11,17] The results here indicated that heterogeneous-seeds such as magnetite- and hematite-seeds could also act to facilitate As and Fe precipitation (Fig. 2(c)–(f)) to form biogenic scorodite (Fig. 3(e), (f)). On the other hand, use of ferrihydrite- or goethite-seeds, which negatively affected the speed of As and Fe precipitation (Fig. 2(a), (b)), led to the formation of jarosite (Fig. 3(c)) or jarosite/scorodite mixture (Fig. 3(d)), respectively. This can be at least partially explained by the initial imbalance of the Fe/As ratio,[16] as well as the presence of hydroxyl groups in ferrihydrite- and goethite-seeds. The SEM images of the surface of hematite-seeds (Fig. 4(a), (b)) and magnetite-seeds (Fig. 4(c), (d)) before and after the deposition of scorodite particles are shown. Compared to those formed on scorodite-seeds (TCLP As leachability; 0.49 mg/L) or without seeds (0.33 mg/L),[11] the final scorodite products formed on hematite-seeds or magnetite-seeds were also highly stable (0.51 mg/L or 0.39 mg/L, respectively), well satisfying the US standard of 5 mg/L (Fig. 5). Jarosite-containing As precipitates formed on ferrihydrite- and goethite-seeds were shown to be much less stable (Fig. 5).

3.2 Surface interaction between seed crystals, cells and anionic reactant species

The zeta-potential-pH diagram in Fig. 6(a)–(c) showed that the hematite surface was most positively charged compared to magnetite and scorodite. Among the three minerals tested, the surface charge of hematite was most significantly lowered by the addition of anions (especially As(V) and SO_4^{2-}), suggesting that As(V) (mostly in the form of $H_2AsO_4^-$ at pH 2–4[23]) and SO_4^{2-} ions readily adsorbed onto the positively-charged hematite surface (Fig. 6(a)). While As(III) is mostly uncharged (as H_3AsO_3) at highly acidic pHs,[23] the minerals surface charge was generally less affected (Fig. 6(a)–(c)). Relative to that of the minerals, the surface charge of microbial cells was nearly neutral and

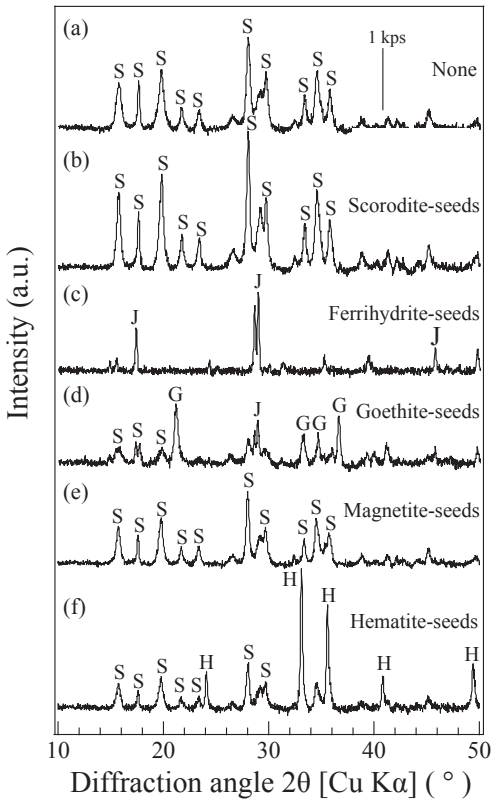

Fig. 3 XRD patterns of different seed minerals after the As precipitation reaction (collected on day 14) in *Ac. brierleyi* cultures at 70°C: (a) No seeds, (b) biogenic scorodite-seeds, (c) ferrihydrite-seeds, (d) goethite-seeds, (e) magnetite-seeds, (f) hematite-seeds. Symbols: S (scorodite; PDF No. 00-026-0778), J (jarosite; PDF No. 01-075-3295), G (goethite; PDF No. 01-077-9313), H (hematite; PDF No. 01-071-5088).

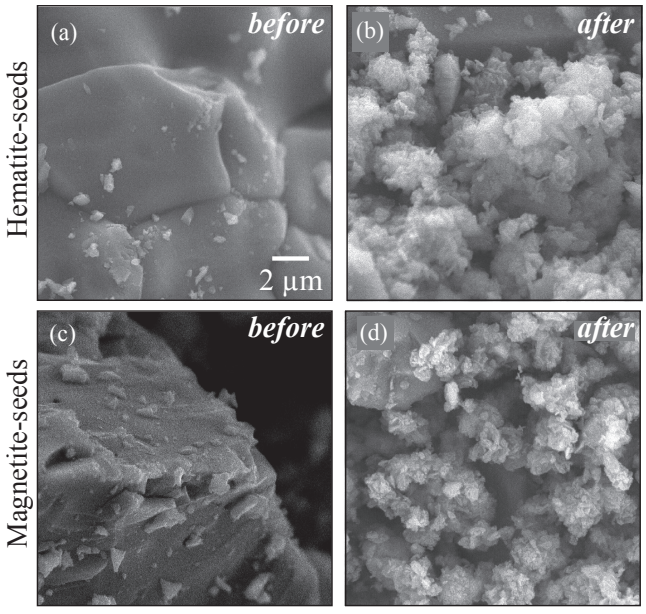

Fig. 4 SEM images of hematite-seeds (a), (b) and magnetite-seeds (c), (d), before (a), (c) or after (b), (d) deposition of biogenic scorodite in *Ac. brierleyi* cultures at 70°C. The scale bar indicates 2 μm for all images.

mostly unaffected by the presence of anions (Fig. 6(d)). Together with the zeta-potential distribution data measured at pH 2.3 (Fig. 6(e)–(g)), the observations can be summarized as follows: (i) Hematite surface is most positively charged (~50 mV) and readily attracts negatively charged As(V) and

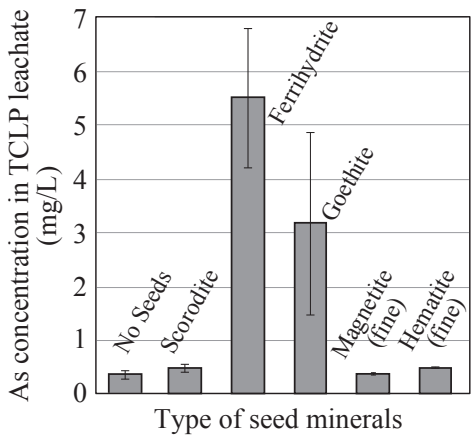

Fig. 5 TCLP test results for As-precipitates formed in *Ac. brierleyi* cultures (70°C), fed with different seed-minerals. Data are mean values from duplicate tests.

SO_4^{2-} anions (peak shifted to ~10 mV). Hematite particles mostly retained the original positive charge even when mixed with *Ac. brierleyi* cells (~45 mV). This likely facilitated adsorption of anionic species onto the hematite/cell mixture (peak shifted to around +15 mV) (Fig. 6(e)). (ii) Magnetite surface (~43 mV) was also shown to attract adsorption of As(V) anions (peak shifted to ~3 mV). When *Ac. brierleyi* cells are mixed with magnetite, two peaks (original cell peak at ~3 mV, plus a new small cell/magnetite peak at ~15 mV) appeared indicating the charge shift of magnetite towards negative direction due to the cell attachment. Still, some As(V) adsorption onto the cell/magnetite surface was observed as a new peak emerged at ~7 mV (Fig. 6(f)). (iii) Scorodite is also highly positively charged (~45 mV) and As(V) likely adsorbed onto its surface (peak shifted to ~35 mV). Nonetheless, the extent of As(V) sorption seemed less significant with scorodite than with other two minerals (Fig. 6(c), (g)). *Ac. brierleyi* cells were also previously shown to attach onto the positively-charged crystalline scorodite surface.[17] Cells attachment lowered the scorodite surface charge to ~25 mV, and therefore, no new peak emerged which suggests As(V) adsorption onto the cell/scorodite surface (Fig. 6(g)).

3.3 Effect of hematite-seeds on low-temperature biogenic scorodite crystallization by *Th. cuprina* and *Am. ferrooxidans* (35–45°C)

Since hematite-seed feeding was shown to be effective in biogenic scorodite crystallization using *Ac. brierleyi* (70°C), its effect was further tested in the lower-temperature system. Due to unavailability of moderately-thermophilic, extreme acidophiles which possess both Fe^{2+}- and As(III)-oxidizing abilities, the reaction here was set-up in two-steps: Firstly As(III) oxidation was conducted using the acid-tolerant As(III)-oxidizer, *Th. cuprina* (35°C, pH 3.0), followed by pH adjustment, Fe^{2+} addition and inoculation of Fe^{2+}-oxidizing *Am. ferrooxidans* (45°C, pH 1.5 or 1.2). Since As(III) oxidation was completed in the first step, all As species existed as As(V) at the time of Fe^{2+} addition. The trend in Fe^{2+} oxidation by *Am. ferrooxidans* was mostly identical in all conditions; cells were readily grown to ~8×10^7 cells/mL and Fe^{2+} oxidation completed by day 2

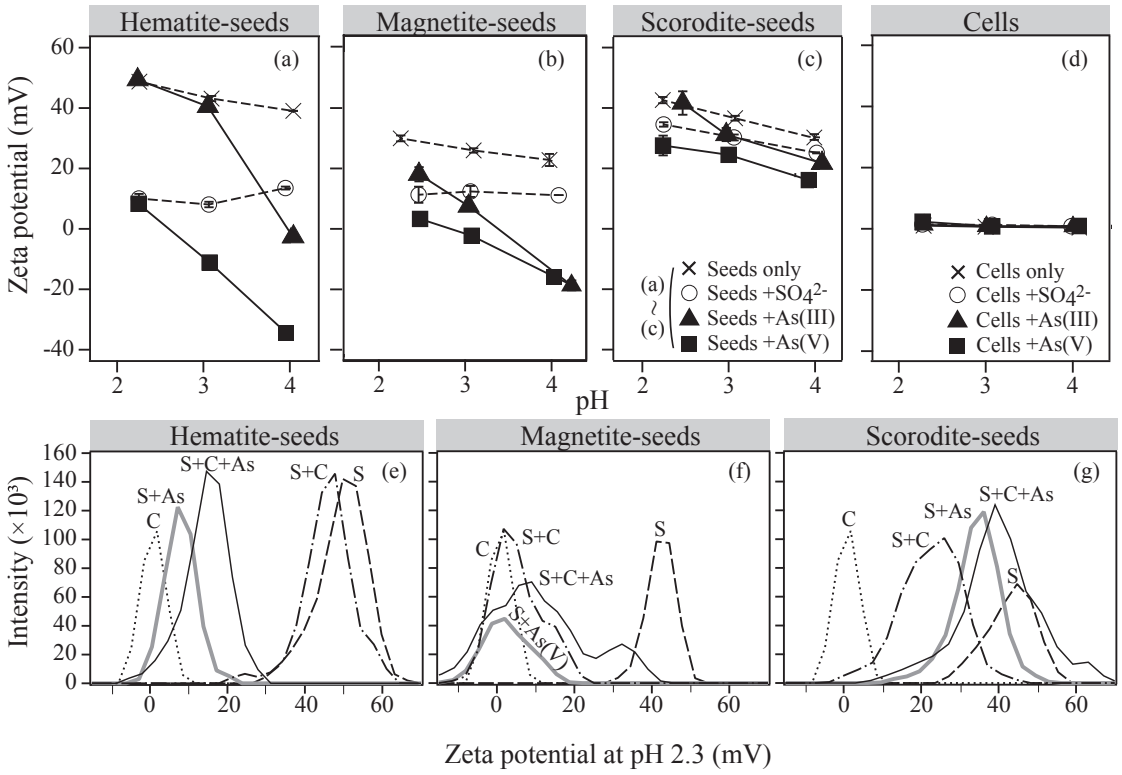

Fig. 6 Electrostatic interactions between different seed minerals, *Ac. brierleyi* cells and oxyanion reactants. (a)–(d) The zeta-potential-pH diagram for hematite-seeds (a), magnetite-seeds (b), biogenic scorodite-seeds (c) or *Ac. brierleyi* cells (d); seeds only (×), seeds+SO_4^{2-} (○), seeds+As(III) (▲), seeds+As(V) (■). (e)–(g) The zeta-potential distribution at pH 2.3 of hematite-seeds (e), magnetite-seeds (f) or biogenic scorodite-seeds (g); seeds only (dashed lines; S), cells only (dotted lines; C), seeds+cells (dash-dotted lines; S+C), seeds+As(V) (thick grey lines; S+As), seeds+cells+As(V) (solid lines; S+C+As). Data points in (a)–(d) are mean values from triplicate measurements; error bars are invisible as these were smaller than the data point symbols.

(data not shown). Figure 7 shows changes in the concentration of As (a) and Fe (b). At this low temperature (45°C) using pH_{ini} 1.5, the steady-state induction period between the two As-removal stages was prolonged (Fig. 7(a)), compared to the *Ac. brierleyi* system at 70°C (Fig. 2). This was likely due to a much slower reaction kinetics for dissolution and recrystallization occurring during this induction period (Appendix A1). However, the effect of hematite-seed feeding was obvious in shortening this induction period (Fig. 7(a), (b)). Our previous study at 70°C reported that lowering pH_{ini} from 1.5 to 1.2 leads to the single-stage As-removal mechanism, where the inducing period seemingly diminishes due to stronger acidity.[11] This test at 45°C showed that although the reaction does not even initiate at pH_{ini} 1.2 without seeds, feeding hematite-seeds can dramatically accelerate this single stage As-removal after some initial lag-phase (Fig. 7(a), (b)). In the 45°C system, the final products were identified to be scorodite (Fig. 7(c)). The final As-removal was less complete, leaving around 1 mM As(V) on day 34 (Fig. 7(a)) and the final scorodite products were less stable (TCLP As leachability ~5.3 mg/L, slightly above the US standard of 5.0 mg/L) than those formed at 70°C. However, feeding hematite-seeds made possible crystallization of biogenic scorodite even at 45°C, which, otherwise, hardly proceeds at this low temperature (Fig. 7).

In studies of chemical scorodite synthesis by other researchers (DMSP method), the importance of the initial formation of gel-like Fe^{2+}-As(V) precursors was reported. In the DMSP reaction using hematite-seeds, the overall Fe^{2+} concentration in the system was reported to be constant: The authors, therefore, suggested that Fe^{2+} ions once incorporated into the gel-like Fe^{2+}-As(V) precursors are then released upon their conversion to crystalline scorodite by reacting with Fe^{3+} deriving from hematite.[10]

In the process of our biogenic reaction using *Ac. brierleyi* (70°C), the oxidation of Fe^{2+} and As(III) mostly precede the precipitation of Fe and As.[11,16] Therefore, the formation of gel-like Fe^{2+}-As(V) precursors is unlikely the case.

On the other hand, the lower-temperature biogenic reaction using *Am. ferrooxidans* (45°C) first completed As(III) oxidation, prior to Fe^{2+} addition. Therefore, the involvement of Fe^{2+}-As(V) precursors was thought possible. In order to clarify this, biogenic scorodite precursors formed at the early stage (at 3 h and on day 1) as well as matured biogenic scorodite (from day 50; as control) were collected to be analyzed for Fe speciation by XANES (Fig. 8). The results indicated that the majority of Fe in precipitates existed in the form of Fe^{3+}, rather than Fe^{2+}. Therefore, even when As(III) oxidation completed before the start of Fe^{2+} oxidation, the formation of Fe^{2+}-As(V) precursors was not evident in this biogenic process. Also, the soluble Fe^{2+} concentration readily decreased by oxidation to Fe^{3+} and the following precipitation. Dissolution of hematite-seeds were also not apparent in both cases (70°C and 45°C). This implies that As reacted with Fe originally present in the solution but not with Fe originating from the hematite mineral. Such differences between the chemical and biogenic processes may arise from different conditions used; i.e., initial Fe and As

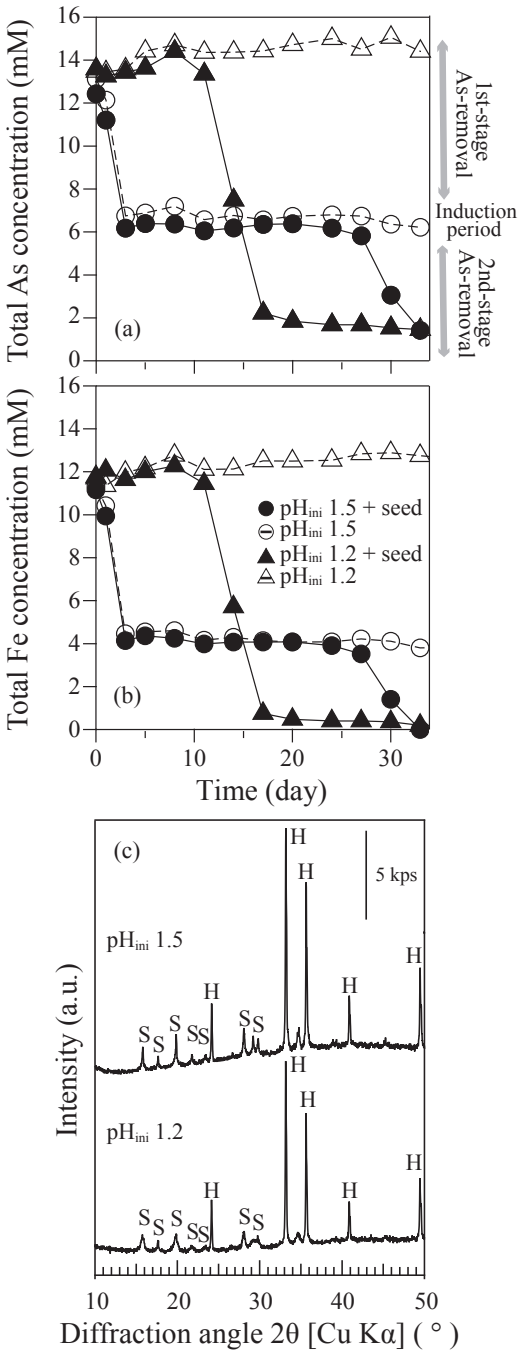

Fig. 7 Precipitation behavior of As (a) and Fe (b), and identification of the resultant products (c) in *Am, ferrooxidans* cultures at 45°C. (a), (b) The pH$_{ini}$ was set to either 1.5 (○, ●) or 1.2 (△, ▲) with hematite-seeds (solid symbols) or without seeds (open symbols). Data points are mean values from duplicate cultures; error bars depicting averages are sometimes invisible as these were smaller than the data point symbols. (c) XRD patterns of the resultant precipitates collected from hematite-seeded cultures on day 28. Symbols: S (scorodite; PDF No. 00-026-0778), H (hematite; PDF No. 01-071-5088).

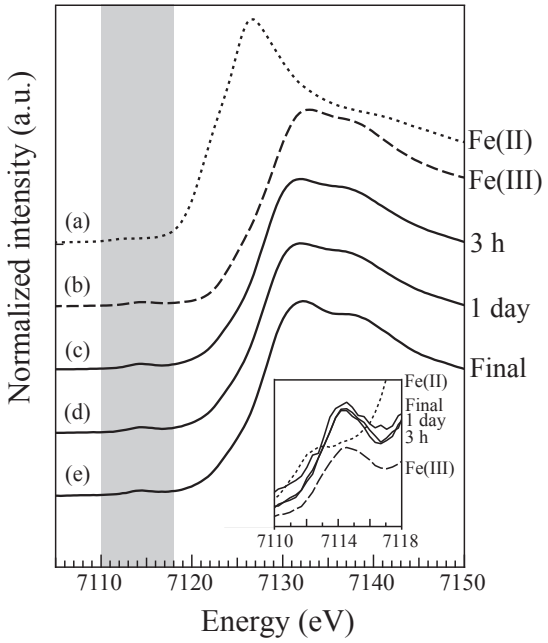

Fig. 8 Normalized Fe K-edge XANES spectra for Fe standards (a), (b) and biogenic scorodite samples produced in Fe^{2+}-oxidizing *Am. ferrooxidans* cultures at 45°C (c)–(e). The pre-edge region (shadowed) is enlarged in the inset.

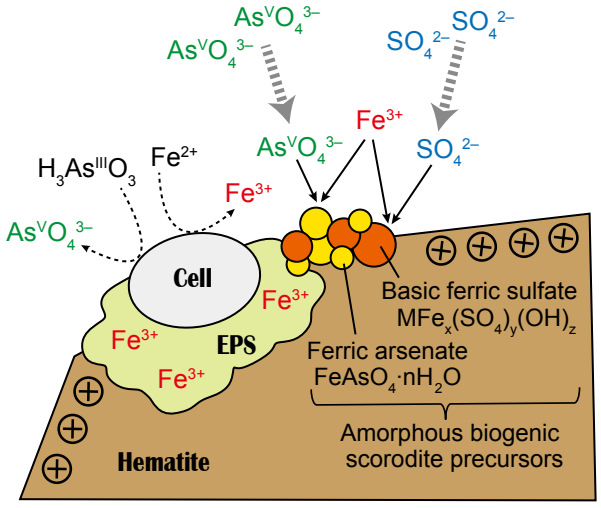

Fig. 9 Schematic illustration of the hematite-seeding effect during the biogenic scorodite crystallization process (based on the previously reported mechanism of biogenic scorodite crystallization process;[18] Appendix A1).

concentrations (several tens of times greater in the former), pH, temperature.

The overall results in this study suggest that the effect of hematite was not particle-size dependent, but at least partly attributed from its highly-positive surface charge. The hematite surface effectively acted to attract anionic reactants such as As(V) and SO_4^{2-}, as illustrated in Fig. 9. The As sorption behavior of hematite was also noted by other researchers.[24] Attached Fe^{2+}-oxidizing cells can also act to locally concentrate the reactant cations on the hematite surface, as they accumulate Fe^{3+} ions in their EPS (extracellular polymeric substances) region.[15] This consequently allowed the reduction of the induction period (dissolution and recrystallization phase) between the two As-removal stages during the process of biogenic scorodite crystallization (Appendix A1).

4. Conclusion

(1) Hematite-seeds feeding exhibited greater effectiveness in scorodite crystallization (in terms of the speed and completeness), compared to homogeneous scorodite-

seeds. The effect of magnetite-seeds was nearly comparable to scorodite-seeds.

(2) The final scorodite products formed on hematite-seeds or magnetite-seeds were also highly stable, compared to those formed on scorodite-seeds, well satisfying the US standard of 5 mg/L for As disposal.

(3) Feeding goethite or ferrihydrite negatively affected the biogenic scorodite crystallization reaction.

(4) The positive effect of hematite- or magnetite-seeding was independent of the mineral particle size.

(5) The effect of hematite likely attributed from its highly-positive surface charge, which locally concentrated anionic reactants, such as As(V) and SO_4^{2-}, on its surface. Together with Fe^{3+} ions accumulated on the magnetite surface via microbial attachment, this caused the reduction of the induction period (dissolution and recrystallization phase) between the two As-removal stages during the biogenic scorodite crystallization process.

(6) Based on the mechanism described above, feeding hematite-seeds made possible crystallization of biogenic scorodite even at 45°C, which, otherwise, hardly proceeds at this low temperature.

(7) Findings of this study contribute to developing more environmentally benign bioprocess for the removal of highly toxic As(III).

Acknowledgments

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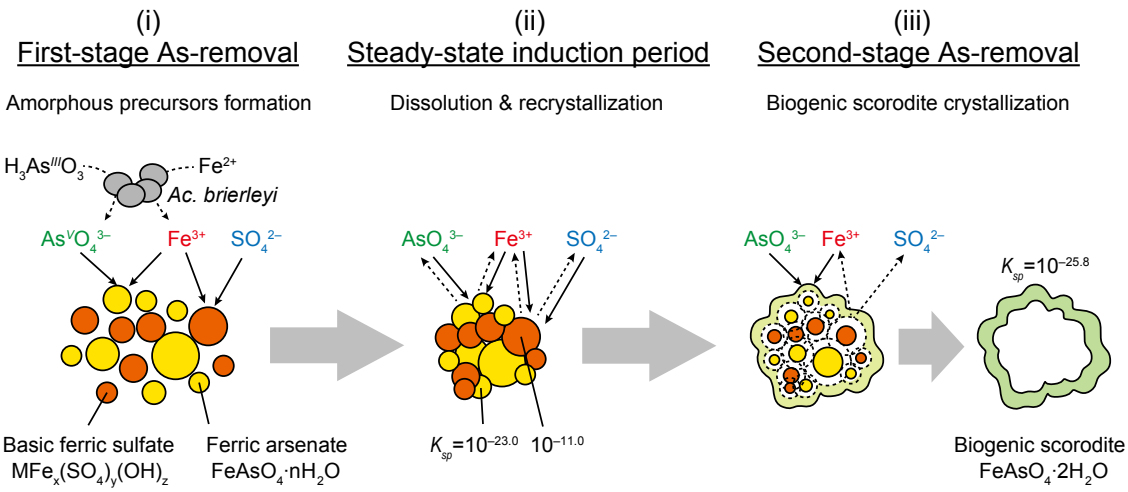
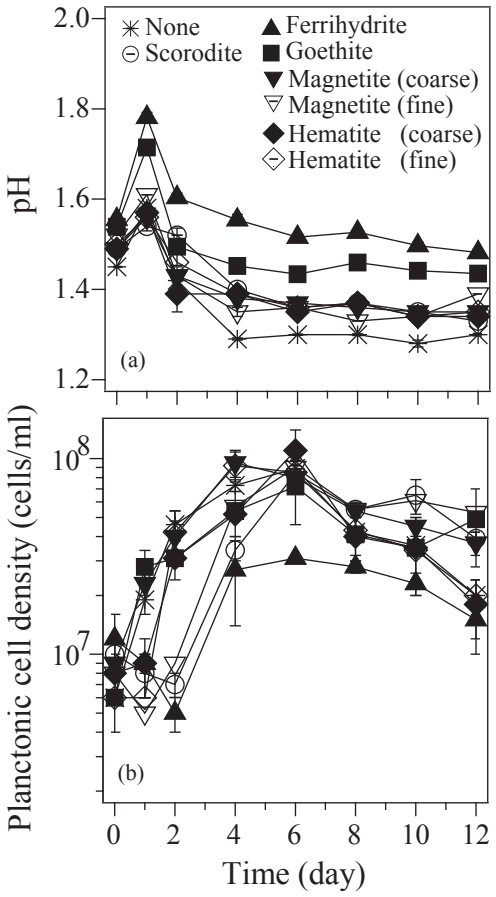


Appendix A1 (partly revised from the previous report[18]) The proposed mechanism of biogenic scorodite crystallization process via SO_4^{2-}-mediated phase transformation (using *Ac. brierleyi* at 70°C, pH 1.5; no initial seed feeding): (i) Microbial oxidation of Fe^{2+} and As(III) proceed readily, triggering the first-stage As-removal as amorphous precursors composed of basic ferric sulfate and ferric arsenate. (ii) During the induction period, the dissolution-recrystallization process takes place for phase transformation. (iii) By so doing, metal ions become locally concentrated on the precursors surface which gives the driving force for crystallization of biogenic scorodite, from even very dilute and seeded aqueous environment.



Appendix A2 Changes in the pH value (a) and planktonic cell density (b) during precipitation of As and Fe (as shown in Fig. 2): Ferrihydrite-seeds (▲), goethite-seeds (■), magnetite-seeds (coarse ▼; fine ▽), hematite-seeds (coarse ◆; fine ◇), biogenic scorodite-seeds (○), seed-free (∗). Data points are mean values from duplicate flasks; error bars depicting averages are sometimes invisible as these were smaller than the data point symbols.