Hydroxyl, Fe$^{2+}$, and *Acidithiobacillus ferrooxidans* Jointly Determined the Crystal Growth and Morphology of Schwertmannite in a Sulfate-Rich Acidic Environment

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**ABSTRACT:** Schwertmannite, ubiquitously found in iron and sulfate-rich acid mine drainage, is generated via biological oxidation of ferrous ions by *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*). However, little information on the mechanisms of biogenic schwertmannite formation and crystal growth is available. This study deliberately investigated the relationships among mineral morphology, solution chemistry, and phase transformation of schwertmannite in *A. ferrooxidans*-containing ferrous sulfate solutions. The formation of schwertmannite could be divided into three stages. In the first nucleation stage, crystallites are presented as nonaggregative or aggregative forms via a successive polymerization process. In the second stage, ellipsoidal aggregates, which are identified as ferrihydrite and/or schwertmannite, are formed. In the third stage, needles appear on the surface of ellipsoidal aggregates, which is caused by the phase transformation of ferrihydrite or schwertmannite to lepidocrocite and goethite through a Fe$^{2+}$ (aq) catalysis-driven pathway. After three stages, a typical characteristic "hedgehog" morphology finally appears. In addition, *A. ferrooxidans* could significantly speed up the mineral transformation. Solution pH affects the morphology of schwertmannite by acid leaching. The experimental results also reveal that the formation of schwertmannite depend on the content of hydroxyl complexes or the transformation of the monomers to polymers, which are greatly affected by the solution pH.

1. **INTRODUCTION**

Schwertmannite, a poorly ordered nanocrystalline ferric oxyhydroxysulfate mineral with a variable chemical formula $\text{Fe}_8\text{O}_8(\text{OH})_8\text{X}_2\text{SO}_4\text{X}_x$ ($1 \leq x \leq 1.75$), is widely distributed in sulfate-rich acid mine drainage (AMD) environments.\textsuperscript{1−3} Because of its high specific surface area, schwertmannite can serve as a potent adsorbent and repository for a variety of nutrients and toxic metals including phosphates, metalloids, and metal ions.\textsuperscript{2,4−7} In addition, schwertmannite is a bioavailable ferric oxyhydroxysulfate mineral prone to dissimilatory reduction by Fe(III)-reducing microbes. Accordingly, its formation and phase transformation involves natural cycling of iron, carbon, phosphorus, and many other metal elements.\textsuperscript{5,8−10} Thus, understanding the formation mechanisms of schwertmannite mediated by bacteria is beneficial for clarifying biogeochemical cycling of Fe and developing novel environmental remediation materials.

In AMD environments, the abiotic oxidation of Fe$^{2+}$ is greatly limited under low pH (<4.0) conditions.\textsuperscript{11} However, acidophilic Fe(II)-oxidizing bacteria such as *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*) accelerate the oxidation of Fe$^{2+}$ by 10$^{6}$−10$^{7}$ time in an acidic environment.\textsuperscript{12} *A. ferrooxidans*, an acidophilic chemolithoautotrophic bacterium capable of oxidizing ferrous ions and elemental sulfur, is widely distributed in the AMD environment.\textsuperscript{13−15} Fe$^{2+}$ is firstly oxidized to Fe$^{3+}$, subsequently the formed Fe$^{3+}$ further coordinates with SO$_4$$^{2−}$ ions and OH$^{−}$ to generate schwertmannite in the AMD system.\textsuperscript{16−19} This process can be expressed by the following reactions

\[
4\text{Fe}^{2+} + \text{O}_2 + 4\text{H}^{+} \rightarrow 4\text{Fe}^{3+} + \text{H}_2\text{O} \quad (1)
\]

\[
8\text{Fe}^{3+} + x\text{SO}_4^{2−} + (16 - 2x)\text{H}_2\text{O} \\
\rightarrow \text{Fe}_8\text{O}_8(\text{OH})_{8-2x}\text{SO}_4\text{X}_x + (24 - 2x)\text{H}^{+} \quad (2)
\]

The resulting schwertmannite possesses a characteristic "hedgehog" morphology, which is composed of spherical nuclei of hundreds of nanometers in diameter and radialized needles.\textsuperscript{1,20}
Microorganisms and their extracellular polymeric substances (EPS) can adhere to nanoparticles and may influence the particle growth process, aggregation, and transformation. Fortin et al. found that the cells of *A. ferrooxidans* were encapsulated by jarosite-like minerals during the bacterium-mediated mineral formation process. Similar results were also obtained by Ferris, who found that iron minerals were directly related to EPS, the cell wall, the periplasm, and the cytoplasmic membrane. However, the encrustation phenomenon on the cell surface was not observed during the *Ferrovum myxofaciens*-mediated mineralization process. Liao et al. demonstrated that schwertmannite could be formed by *A. ferrooxidans* in FeSO₄ solution at pH 1.6 with EPS stripping. Song et al. found that the morphology of schwertmannite would change if regulated by *A. ferrooxidans* with EPS stripping. In addition, for the H₂O₂-mediated schwertmannite synthesis, the morphology exhibited nanoparticle aggregated particle or characteristic hedgehog structure, depending on H₂O₂ supply rate. Even though the mineral morphology of biogenic schwertmannite mediated by *A. ferrooxidans* has been well characterized, the specific formation and crystal growth pathway still remain unknown.

Therefore, the objectives of this study were (i) to investigate the evolution process of morphology and corresponding solution chemistry during the biological synthesis process of schwertmannite; (ii) to identify the mineral phases in different growth stages; and (iii) to explore the key factors affecting schwertmannite formation. The findings will shed light on the development of more comprehensive models for nanoparticle formation, crystal growth, and phase transformation of schwertmannite. More significantly, it will clearly elucidate the role of microorganisms in the formation of biogenic schwertmannite.

### 2. RESULTS AND DISCUSSION

#### 2.1. Identification of Crystal Growth, Particle Morphology, and Solution Chemistry in the Nucleation Stage

The formation of schwertmannite driven by *A. ferrooxidans* in simulated AMD during the initial 3 h was observed by atomic force microscopy (AFM) (Figures 1a, S2). The particles were smaller than 4 nm in height in the first 40 min, and were similar to those in FeSO₄ solution dropped on quartz slides as control (Figure S2). The formed precipitates at 55 min were nonaggregative or aggregative crystallites with sizes below 26 nm (Figure 1a). Similarly, this phenomenon was also observed by cryo-transmission electron microscopy (TEM) during the nucleation stage of iron(III) oxide. Subsequently, the particle size progressively increased as the reaction time increased (Figures 1b, S2).

Ferron assays were performed to identify the forms of existence of ferric ions, and quantify the concentration variation of Fe(a), Fe(b), and Fe(c) over the reaction time. Fe(a) referred to Fe monomers, such as Fe(OH)₃(H₂O)₁⁺, Fe(OH)₂⁺, and FeSO₄⁺, and Fe(b) referred to Fe polymers, like [Fe₂₋(OH)₅(OH₂)]₃⁺. Fe(c) was considered as the Fe species that could not react with the ferron reagent, namely stable precipitation. As shown in Figure 1d, the concentration of Fe(a) and Fe(c) gradually increased within 3 h, whereas Fe(b) exhibited an opposite tendency. The previous report demonstrated that Fe oxide was formed via a successive polymerization process. Hydrolyzed Fe monomers possessed strong hydrolysis capacity and were hydrolyzed to form oligomers, such as dimers and trimers. Then these oligomers polymerized to form nuclei, which then organized into particles. For example, Zhu et al. explored the formation pathways of ferrhydrite in acidic solution, and found that Fe existed mainly as Fe(H₂O)₆³⁺ (Fe(a)), μ-oxo aquo dimers (Fe(b)) and ferrhydrite (Fe(c)) forms. The formed sulfate-complexed ferrhydrite-like molecular clusters were stable when the simulated AMD was neutralized with NaHCO₃ ([HCO₃⁻]/[Fe³⁺] = 0.5 and 0.6). When the [HCO₃⁻]/[Fe³⁺] ratio was increased to 1.0, a ferrhydrite-like molecular cluster was formed initially but subsequently converted to schwertmannite. For the biological synthesis of schwertmannite, the conversion from Fe₅⁶⁺ monomers to oligomers was the first key step, and ferrhydrite might be the main crystallite formed in 55 min.
Solution turbidity used to monitor the crystallization process displayed a steady state during the first 150 min, and then sharply increased to the maximum value of 2250 NTU in 48 h (Figure 1c). This phenomenon was consistent with the morphology change presented in AFM images, which indicated that the crystallite continuously grew into a number of particles. Correspondingly, the solution pH increased from 2.5 to 2.8 within 3 h (Figure 2a), which is ascribed to the consumption of protons caused by oxidation of ferrous ion to ferric ion (Reaction 1). Previous works conducted by Liao et al. showed that a rising trend of pH of the solution was observed during the early stage of the reaction when the initial pH of the solution was as low as 2.6 or below.\(^\text{20}\) The Fe\(^{2+}\) concentration drastically decreased from 98.4 to 76.7% in this stage, while resultant Fe\(^{3+}\) exhibited an opposite trend increasing from 1.65 to 13% (Figure 2b). This indicated that the generation efficiency of Fe\(^{3+}\) from bio-oxidation of Fe\(^{2+}\) was much higher than that by the hydrolysis of ferric ions in this stage.

### 2.2. Evolution of Particle Morphology and Phase Identification of Ellipsoidal Aggregates

Schwertmannite further grew into compact ellipsoidal aggregates of about 217.4–600.6 nm in size with a relatively smooth structure during the reaction from 3 to 12 h (Figure 1a,b). The morphology of these ellipsoidal aggregates was consistent with that of chemosynthetic schwertmannite formed by adding \(\text{H}_2\text{O}_2\) at one time.\(^\text{21}\) The solution turbidity correspondingly increased from 96.1 to 879 NTU, while solution pH decreased from 2.8 to 2.4. It showed that a pH increase caused by bio-oxidation of Fe\(^{2+}\) would be quickly counteracted by the proton release from the subsequent hydrolysis of Fe\(^{3+}\), which finally led to the decrease of solution pH.

In addition, the mineral phase was identified by X-ray diffraction (XRD) analyses in Figure 3a. In the light of the patterns of ferrihydrite (PDF 29-0712) and schwertmannite (PDF 47-1775), the diffraction peaks and relative intensities of precipitate collected at 3 h agreed well with the standard ferrihydrite and schwertmannite, respectively. To further investigate the mineral species, the precipitate was analyzed via Mössbauer spectra at 77 K in Figure 3b, which was identified as the mixture phase of 2-line ferrihydrite (67.6%) and schwertmannite (32.4%).

As shown in Table S1, the proportion of ferrihydrite decreased from 67.6 to 7.2% as the mineral grew from 3 to 120 h, while the schwertmannite portion was increased from 32.4 to 38.5%. This indicated that partial ferrihydrite was transformed into schwertmannite. A previous study also demonstrated that ferrihydrite particles formed during AMD neutralization by \(\text{NaHCO}_3\) could rapidly convert into schwertmannite.\(^\text{21}\) They also proposed that schwertmannite could be directly formed by the dissolved Fe species, indicating that ferrihydrite was not an indispensable precursor for its formation. Since the Gibbs free energy for the formation of ferrihydrite \((\Delta G = -469.9 \pm 1.4 \text{ kJ mol}^{-1})\) was much lower than that of schwertmannite \((\Delta G = -34.12 \text{ kJ mol}^{-1})\), ferrihydrite was preferably formed in the initial stage because of the lower nucleation energy barrier.

#### 2.3. Formation of the Hedgehog Morphology via Mineral Phase Transformation

Along with the prolonged reaction time of 12 h, some readily observed bulges appeared on the surface of ellipsoidal aggregates (Figures 1a, S3). Furthermore, TEM images of mineral collected at 36 h and 120 h indicated that the bulges evolved into needles radiating from the surface of ellipsoidal aggregates (Figure S4), while the average diameter of ellipsoid increased gradually from 929.9 to 2024 nm within 84 h (Figure 1b). These images clearly proved that needles formed and grew on the surface of ellipsoidal aggregates instead of growing from a small central nucleus. Notably, the typical hedgehog morphology of schwertmannite was well developed after 48 h with needles surrounding the aggregates (Figure 1a).

This trend was well coincident with the turbidity evolution plotted against the reaction time. The turbidity continuously decreased after 48 h, which might be attributed to the phase transformation.\(^\text{1}\) To illustrate this point, HRTEM was performed to observe the high-resolution images of bulges on the surface of ellipsoidal aggregates collected at 36 h. Lattice fringes with an average spacing of 3.31 and 2.57 Å were...
Figure 4. SEM images showing the morphology of 9 h mineral (pristine mineral) (a), when they were immersed in 30 mM FeSO₄ solution (pH = 2.6) (b); inactive *A. ferrooxidans* suspension (pH = 2.6) (c); 30 mM FeSO₄ solution with inactive *A. ferrooxidans* suspension (pH = 2.6) (d); aqueous solution with pH = 2.6 (e) and aqueous solution with pH = 2.0 (f), respectively.

clearly observed, which were closely matched the spacing of the [003] and [021] planes in ferrihydrite and goethite, respectively (Figure S5). Hockridge et al. also reported that the aggregates formed in the early stages of chemosynthetic schwertmannite composed of nanometer-scaled ferrihydrite crystallites, whereas mature needles on the surface of ellipsoidal aggregates were made up of goethite nanoparticles. Moreover, Mössbauer analysis of the 120 h sample showed (Figure 3b) that the mineral components were identified as ferrihydrite (7.2%), schwertmannite (38.5%), lepidocrocite (45%), and goethite (9.3%). Compared with the ellipsoidal aggregates with a relatively smooth surface, the needles on the surface of ellipsoidal aggregates were lepidocrocite and goethite.

Meanwhile, as shown in Figure 2b, the fraction of Fe²⁺ decreased and reached nearly zero after 48 h. However, the fraction of Fe³⁺ exhibited an opposite trend, in which the percentage of Fe³⁺ gradually increased in this stage and stabilized at approximately 72.7% after 48 h. In addition, SO₄²⁻ gradually decreased to 90% after 48 h and remained constant afterward. Obviously, these results indicated that the Fe³⁺ bio-oxidation had been terminated after 48 h, and the resultant Fe³⁺ was not completely transformed into schwertmannite. Correspondingly, only 28% of iron and 10% of sulfate were incorporated into the precipitate after 120 h of the reaction. This result was in agreement with those reported by previous studies, in which total iron precipitation efficiency was stable at about 30% during schwertmannite formation by *A. ferrooxidans* oxidation. However, solution pH still gradually decreased after 48 h (Figure 2a) due to the hydrolysis of resultant Fe³⁺, which would be discussed further later.

2.4. Effects of Solution Chemistry on Mineral Morphology. The mineral obtained at 9 h was used to explore the formation process of needles on the surface of ellipsoidal aggregates in different solution chemistry. Compared with the pristine mineral (Figure 4a), the already-formed mineral sample immersed in 30 mM FeSO₄ solution for 108 h (pH = 2.6) exhibited a small number of needles growing on the surface of ellipsoidal aggregates (Figure 4b). On the contrary, no significant changes were observed for the samples immersed in aqueous solution at pH = 2.6 in the presence (Figure 4c) or absence (Figure 4e) of inactive *A. ferrooxidans* suspension. Moreover, the typical morphology of abundant needles radiating from the surface of ellipsoidal aggregates was observed when the mineral was immersed in 30 mM FeSO₄ aqueous solution containing inactive *A. ferrooxidans* suspension (pH = 2.6). The findings demonstrated that Fe²⁺ (aq) contributed to the formation of needles on the surface of ellipsoidal aggregates, and *A. ferrooxidans* accelerated this process. Many researchers also reported that ferrihydrite or schwertmannite could transform into lepidocrocite and goethite through a Fe²⁺ (aq) catalysis-driven pathway. It was also well documented that there was a long-range electron transfer pathway between the microbial–mineral interface. It was just like hematite that could act as a conductor and accelerate electron directly from Fe²⁺ to cells. However, Fe²⁺ (aq) catalysis-driven formation of needles on the surface of schwertmannite in the presence of *A. ferrooxidans* cell still was not reported. Here, we speculated that some active substances generated from *A. ferrooxidans* cell could accelerate the electron transfer between Fe²⁺ and minerals. Further studies are needed to clarify the mechanism involved in mineral phase transformation driven by Fe²⁺ (aq) in the presence of *A. ferrooxidans*.

As shown in Figure 4f, the mineral immersed in aqueous solutions with pH = 2.0 generated some hypoplastic ellipsoidal aggregates, indicating that the mineral was partially dissolved. The discrepancies of mineral morphology presented in Figures 4e,f was due to the decline of solution pH, which was caused by Fe²⁺ (aq) catalysis-driven formation of needles on the surface of schwertmannite. Indeed, the solubility of goethite, schwertmannite, and 2-line ferrihydrite was found to increase in acidic solution, which might accelerate the dissolution of ellipsoidal aggregates, and further led to needle extension caused by a decline of solution pH. These results explicitly indicated that the morphology of schwertmannite was jointly dependent on the solution pH, Fe²⁺, and *A. ferrooxidans*. 

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2.5. Roles of Solution OH\(^{-}\) on the Formation of Schwertmannite. The distribution of Fe\(^{3+}\) species was calculated using Visual MINTEQ, 3.1 software. In the pH range of 2–3, Fe was mainly existed in the forms of FeSO\(_4\)^{2–} and Fe(SO\(_4\))\(_2\)^{3–}, meanwhile, a minor portion of Fe\(^{3+}\) existed as FeOH\(^{2+}\) and Fe(OH)\(_2\)^{+} complexes (Figure S6). However, the content of hydroxyl complexes was scarcely observed when the pH was below 2.0. As stated previously, the mineral formed at 3 h contained 67.6% of ferrihydrite, a precursor of schwertmannite (Table S1), which corresponded to the solution environment with pH 2.8 (Figure 2a). Therefore, it was speculated that the concentration of Fe(b) gradually decreased and nearly disappeared after 12 h (Figure 1d). The conversion from Fe(a) to Fe(b) involved two main bridge transformation mechanisms, namely olation and oxolation.\(^4\) Olation referred to the replacement of aquo-ligands by hydroxo-ligands to form bridging OH\(^{−}\) to Fe\(^{3+}\) according to Reaction 3.

\[
\text{Fe}^{III} - \text{OH} + \text{Fe}^{III} - \text{OH}_2 \rightarrow \text{Fe}^{III} - \text{OH} - \text{Fe}^{III} + \text{H}_2\text{O} \tag{3}
\]

Oxolation entailed OH\(^{−}\) nucleophilic substitution with adjacent Fe\(^{3+}\) to form bridging O\(^{2−}\) according to Reaction 4.

\[
\text{Fe}^{III} - \text{OH} + \text{Fe}^{III} - \text{OH} \rightarrow \text{Fe}^{III} - \text{O} - \text{Fe}^{III} + \text{H}_2\text{O} \tag{4}
\]

For the hydrolytic polymerization reactions, the OH\(^{−}\) group was essential to promote bridge formation.\(^10\) In addition, it tended to consume OH\(^{−}\) to form bridge groups and simultaneously release water molecules as more closely interconnected bonding networks progressively formed.\(^4\)\(0\)\(4\)\(9\)\(5\)

To better understand the effects of solution pH on the formation process of schwertmannite, four treatments were performed, in which solution pH was set as initial pH 2.2 and 2.5, and constant pH 2.2 and 2.5 during the reaction process. As shown in Figure 5a, the pH in the treatment with initial pH 2.2 and initial pH 2.5 were decreased to approximately 2.0 after 72 h, while it remained stable in constant pH 2.2 and constant pH 2.5 groups. Figure 5b showed that the oxidation efficiency of Fe\(^{2+}\) sharply increased during first 36 h and reached nearly 100% after 48 h in all treatments, implying that the solution pH had little influence on the rate of bio-oxidation of Fe\(^{2+}\). A similar phenomenon was also observed by Liao et al. during the biological preparation of schwertmannite under different pH conditions.\(^2\)\(0\) The Fe precipitation efficiency in the treatments with initial pH 2.2, initial pH 2.5, and constant pH 2.2 gradually increased to 21.9, 31.5, and 66.2% after 72 h, respectively (Figure 5c). However, the Fe precipitation efficiency in the treatment with constant pH 2.5 was rapidly increased to 91.3% within 36 h, then slightly reached 96% after 72 h. These results clearly illustrated that the addition of OH\(^{−}\) during the formation of biogenic schwertmannite greatly enhanced the yield of the mineral. Hence, approximately 30% of Fe precipitation efficiency in the treatments without the addition of any OH\(^{−}\) during schwertmannite formation should be attributed to the insufficient hydroxyl complexes or the restriction of transformation from Fe(a) to Fe(b) caused by the successive decline of solution pH. Further studies were needed to identify the Fe species present in the nucleation stages of schwertmannite at the molecular scale to better understand its formation mechanisms.

3. CONCLUSIONS

The formation process of biogenic schwertmannite was tracked by AFM and SEM analyses. Crystallites with simultaneous nonaggregative to aggregative behavior were firstly observed in the initial stage (Step I), which were formed via a successive polymerization reaction. Then the crystallite particles were further gathered together to form ellipsoidal aggregates (Step II) identified as ferrihydrite and schwertmannite. Finally, needles began to grow on the surface of ellipsoidal aggregates (Step III) owing to the phase transformation of ferrihydrite or schwertmannite to lepidocrocite and goethite driven by Fe\(^{2+}\) catalysis. In addition, A. ferrooxidans could also accelerate this process. Solution pH was a key factor determining the precipitation efficiency of Fe. Maintaining a constant pH by without further adjustment, labeled as initial pH 2.2 and initial pH 2.5, respectively. Correspondingly, contrast experiments maintained the solution pH of 2.2 and 2.5 throughout the trial, labeled as constant pH 2.2 and constant pH 2.5, respectively.

**Figure 5.** Variation of pH values (a), ferrous ion oxidation efficiency (b), and total iron removal efficiency (c) during the formation of schwertmannite driven by A. ferrooxidans in simulated AMD with or without pH regulation (80 mM FeSO\(_4\); A. ferrooxidans density: 6 × 10\(^{10}\) cell mL\(^{−1}\)). The experiments set the initial solution pH to 2.2 and 2.5 without further adjustment, labeled as initial pH 2.2 and initial pH 2.5, respectively. Correspondingly, contrast experiments maintained the solution pH of 2.2 and 2.5 throughout the trial, labeled as constant pH 2.2 and constant pH 2.5, respectively.
the biogenic mineral. The findings were conducive to seeking strategies to effectively treat AMD or obtain lots of functional environmental materials in light of the biomineralization approach.

4. MATERIALS AND METHODS

4.1. Preparation of *A. ferrooxidans* Resting Cell Suspension. *A. ferrooxidans* LX5 (CGMCC No. 0727) was obtained from China General Microbiological Collection Center (CGMCC) and cultured in sterile 9 K medium as described by Silverman and Lundgren.† The composition of the 9 K medium is as follows: 44.3 g of FeSO$_4$ 3.0 g of $(NH_4)_2$SO$_4$, 0.5 g of K$_2$HPO$_4$, 0.5 g of MgSO$_4$7H$_2$O, 0.1 g of KCl, and 0.01 g of Ca(NO$_3$)$_2$ in 1 L of deionized water, and the solution pH was adjusted to 2.5 with H$_2$SO$_4$ (50%, v/v). *A. ferrooxidans* was incubated in 500 mL Erlenmeyer flasks, each containing 250 mL of 9 K medium and 10% (v/v) inoculum at 28 °C and 180 rpm. *A. ferrooxidans* resting cells were prepared according to previous methods.™ Briefly, *A. ferrooxidans* at the end of the exponential growth phase (after cultivation for three days) was directly filtered through Whatman No. 4 filter paper to remove precipitates, and then the bacterial cells were collected after centrifuging the filtrate at 10 000 g for 10 min at 4 °C. The resultant cells were washed three times with H$_2$SO$_4$ solutions (pH = 1.5), and then resuspended in dilute H$_2$SO$_4$ solutions (pH = 2.5). After this, the bacterial density was subjected to measurement according to the double-layer plate method.™™

4.2. Biological Synthesis of Schwertmannite. The preparation of schwertmannite was conducted in 500 mL Erlenmeyer flasks. Briefly, 80 mM FeSO$_4$ solution (250 mL) was inoculated with *A. ferrooxidans* resting cell (6 × 10$^7$ cells mL$^{-1}$) and co-cultured for 5 days at an initial pH of 2.5 and 180 rpm. At a given time interval, 2 mL of suspension was withdrawn and determined for pH, Fe$_2^+$, total Fe, and SO$_4^{2−}$ after filtering through a 0.45 μm membrane. The resultant precipitate was collected by vacuum filtration and then washed three times with H$_2$SO$_4$ solution (pH = 2.0) and deionized water, respectively. Finally, the precipitate was freeze-dried for further determination.

In addition, the effect of initial solution pH on the mineral yield was also investigated by setting up four experimental groups. The first group was labeled as initial pH 2.2, in which solution pH was adjusted to 2.2 and no longer controlled during the reaction process. The second group was labeled as constant pH 2.2, in which solution pH was maintained at 2.2 by adding H$_2$SO$_4$ (10%, v/v) or NaOH (5 M) every 3 h. The other two groups were initial pH 2.5 and constant pH 2.5, respectively.

4.3. Effect of Fe$^{2+}$, *A. ferrooxidans*, and pH on Schwertmannite Morphology. The mineral sample formed at 9 h were used to investigate the effect of Fe$^{2+}$, *A. ferrooxidans*, and pH on the morphology of schwertmannite during the biological synthesis process. The pristine mineral (0.14 g L$^{-1}$) was added to five different solutions: (1) FeSO$_4$ solution (30 mM, pH = 2.6), (2) inactive *A. ferrooxidans* suspension (pH = 2.6), (3) FeSO$_4$ solution (30 mM, pH = 2.6) containing inactive *A. ferrooxidans* suspension, (4) aqueous solution with pH 2.6, and (5) aqueous solution with pH 2.0. The resultant mixture solution with a total reaction volume of 250 mL was subjected to further reaction for 108 h. The parameters during the reaction process and procedures for mineral collection were consistent with those described above.

4.4. Analytical Methods. The morphology of the newly-formed mineral during the initial 3 h reaction in the biological synthesis system was observed by atomic force microscopy (AFM, MultiMode 8; Bruker, Billerica MA). The suspension (5 μL) was firstly dropped on a clean quartz slide, air dried for 12 h, and then was observed in air-contact mode. The data were analyzed using NanoScope Analysis software. The morphology of minerals collected at different times was observed by scanning electron microscopy (SEM, Hitachi, Japan) and transmission electron microscopy (TEM, JEOL-2011, Tokyo Japan). High-resolution TEM (HRTEM) images were obtained on a JEOL 3000 operated at 300 kV and analyzed using Digital Micrograph software. The mineral phase was determined by X-ray powder diffraction (XRD, MiniFlex II, Japan) using Cu Kα radiation (50 kV, 15 mA). Mössbauer spectra were recorded at 77 K using a conventional spectrometer (Wissel MS-500, Germany) in transmission mode. A Janis cryostat was used to cool the samples, and $^{57}$Co in an Rh matrix with activity of 25 m Ci was used as the source. The velocity calibration was done with an α-Fe absorber at room temperature, and spectra were interpreted using Recoil software and the Voigt-based fitting (VBF) method.

Solution pH was determined using a PHS-3C pH meter. Turbidity was monitored on a Hach TL2300 turbidity meter. Fe$^{2+}$ and total Fe concentrations were analyzed via the 1,10-phenanthroline colorimetric method with an UV-2202pcs ultraviolet visible (UV) spectrophotometer. The concentration of SO$_4^{2−}$ was determined by ion chromatography ( Dionex ICS-1100). The distribution of Fe$^{3+}$ species (monomer-Fe(a), polymer-Fe(b) and precipitate-Fe(c)) was investigated by the ferron (8-hydroxy-7-iodo-5-quinoline sulfonic acid) reaction. Fe(a) and Fe(b) were distinguished by different complex times, and Fe(c) was obtained by subtracting Fe(a) and Fe(b) from the total Fe concentration. In order to eliminate the influence of Fe$^{3+}$ reacting with the ferron reagent, the linear regression fitting between the Fe$^{2+}$ concentration and absorbance (600 nm) was estimated (Figure S1). Thus, the absorbance intensity of the corresponding Fe$^{3+}$-ferron complex was obtained after subtracting the absorbance values of the Fe$^{2+}$-ferron complex.

4.5. Calculation Approach for Fe$^{3+}$ Speciation. The distribution of Fe$^{3+}$ ions at different pH values was calculated using Visual MINTEQ 3.1 software. The concentrations of SO$_4^{2−}$ and Fe$^{3+}$ ions were set as 96.3/91.2 and 2.6/57.9 mM, respectively, which corresponded to maximum/minimum values of SO$_4^{2−}$/Fe$^{3+}$ during the formation of *A. ferrooxidans*-driven schwertmannite.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c05606.

Mössbauer spectral fitting parameters collected at 77 K for 3 and 120 h samples (Table S1); linear regression plots of absorbance intensity for the reaction of Fe$^{2+}$ with ferron (Figure S1); AFM, SEM, and TEM of schwertmannite formed by *A. ferrooxidans* in simulated acid mine drainage (Figures S2, S3, and S4); HRTEM images of schwertmannite bulges on the surface of...
ellipsoidal aggregates at 36 h (Figure S5); speciation of Fe$^{3+}$ as a function of solution pH (Figure S6); TEM of an ultrathin section of A. ferrooxidans showing the magnetosomes (Figure S7) (PDF)

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Notes

The authors declare no competing financial interest.

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