Article

The Important Role of Dissolved Oxygen Supply Regulated by the Hydraulic Shear Force during the Biosynthesis of Iron Hydroxysulfate Minerals

Jun Yang, Rui Wang, Heru Wang and Yongwei Song *

Department of Environmental Engineering, School of Information and Safety Engineering, Zhongnan University of Economics and Law, Wuhan 430073, China; z0004221@zuel.edu.cn (J.Y.); wr980317@163.com (R.W.); z0004382@zuel.edu.cn (H.W.)

* Correspondence: songyongwei@zuel.edu.cn; Tel.: +86-027-8838-5169

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Abstract: The severity of environmental pollution from acid mine drainage (AMD) is increasingly garnering attention. In this study, the effects of hydraulic shear forces (achieved by regulating the shaking table's rotation speed) on Fe^{2+} bio-oxidation and Fe^{3+} hydrolytic mineralization in an acidic 9K medium- FeSO_4 -*Acidithiobacillus ferrooxidans* system (simulated AMD) are investigated. Results reveal that a higher shaking speed favors a higher oxidation rate of Fe^{2+} , whereas a very low or high shaking speed restricts the removal of Fe^{3+} . Shaking table rotation speeds of 120–180 rpm were preferred for biomineralization treatment in the simulated AMD. As the initial concentration of Fe^{2+} in the system decreased from 9.67 to 0 g/L in 40 h, the dissolved O_2 (DO) in the solution dropped to its lowest concentration after 20 h and then increased to its initial level between 40 and 120 h. However, the corresponding total Fe (TFe) precipitation efficiency increased with the increasing mineralization time after 40 h. The effect of O_2 supply time on biomineralization revealed that DO was mainly used in Fe^{2+} bio-oxidation. After Fe^{2+} was completely oxidized by *A. ferrooxidans*, the precipitation efficiency of TFe was independent of the O_2 supply.

Keywords: acid mine drainage; *Acidithiobacillus ferrooxidans*; hydraulic shear force; dissolved oxygen; biomineralization; iron hydroxysulfate minerals

1. Introduction

When exposed to air and water, sulfidic wastes undergo atmospheric and aqueous oxidation and tend to generate acid mine drainage (AMD), which often contains elevated concentrations of soluble Fe (Fe^{2+} and Fe^{3+}), SO_4^{2-} and heavy metals [1,2]. The oxidation of pyrite to sulfate in AMD systems is described by the following two end-member reactions that utilize either O_2 or Fe^{3+} as oxidants [3].

AMD corrodes the underground pipes, drainage pumps and other related equipment and cause acid and heavy metal pollution of vast water resources and soils, considerably affecting the daily productivity and life. Therefore, AMD is a global environmental problem even though several predictive and preventive techniques have been developed.

Currently, neutralization is the most mature and widely used method for handling AMD [4–6]; however, this method has many limitations. For example, some soluble Fe in AMD remains as Fe^{2+} and the pH (>8.5) required for the complete precipitation of Fe^{2+} in the hydroxide form is significantly

higher than that required for the complete precipitation of Fe^{3+} ($\text{pH} = 3.5$), considerably decreasing the demand for the neutralization reagent. When AMD passes through a limestone ditch, Fe^{2+} , Fe^{3+} and SO_4^{2-} easily produce high amounts of flocculent sediments comprising $\text{Fe}(\text{OH})_2$, $\text{Fe}(\text{OH})_3$ and CaSO_4 . These compounds adhere to the limestone particle surfaces, acting as barriers and preventing further reaction between the reagent and water. Thus, the filter medium is blocked in the dewatering stage, inducing poor dehydration performance and reduced treatment efficiency [7–9]. Obviously, quickly transforming Fe^{2+} to Fe^{3+} and controlling the formation of $\text{Fe}(\text{OH})_3$ and CaSO_4 are critical issues that must be resolved to optimize the lime neutralization technology in case of AMD.

However, for a solution having $\text{pH} < 3.5$, the air oxidation rate constant of Fe^{2+} is only $10^{-3.5}/\text{d}$ [10]. Bosecker [11] indicates that *A. ferrooxidans* increases the oxidation rate of Fe^{2+} by approximately 10^5 – 10^6 in an acid sulfate environment. Furthermore, this oxidation is frequently accompanied by the hydrolysis and mineralization of the produced Fe^{3+} to form iron hydroxysulfate minerals such as schwertmannite and jarosite [12,13]. Equations (3)–(5) represent the schwertmannite and jarosite formation processes [14].

M^+ represents K^+ , NH_4^+ , Na^+ and H_3O^+ . In addition, the iron hydroxysulfate minerals can be directly synthesized via chemical methods. For example, under acidic conditions, schwertmannite can be obtained via iron salt hydrolysis or ferrous salt oxidation ($\text{FeSO}_4\text{-H}_2\text{O}_2$) [15–17]. Iron hydroxysulfate minerals are ideal materials for the adsorption and coprecipitation of heavy metals in AMD [18–20]. Therefore, the *A. ferrooxidans*-mediated biomineralization is an efficient AMD treatment method, exhibiting significant economic and environmental benefits.

The primary factors controlling the formation of iron hydroxysulfate minerals are the reaction time, temperature, acidity, Fe^{2+} concentration and the monovalent cation type and concentration (K^+ , Na^+ , NH_4^+ , etc.) [21–24]. In an extremely acidic environment, the *A. ferrooxidans*-mediated biomineralization involves the biologic oxidation of Fe^{2+} and thus facilitate Fe hydrolysis. Previous studies showed that schwertmannite formation usually occurs in Fe and sulfate-rich solutions in a pH range of 2.5–4.5, with lower pH values promoting the precipitation of jarosite. For instance, Bigham et al. [25] demonstrated that schwertmannite, formed through the bio-oxidation of Fe^{2+} by *A. ferrooxidans*, was the only mineral phase at $\text{pH} = 3.0$. Liao et al. [26] observed that the formed ferric hydroxysulfate minerals were a mixture of Na-jarosite and schwertmannite at an initial pH of 3.5; however, a pH of 1.6–3.4 produced only pure schwertmannite. As an aerobic autotrophic bacterium, *A. ferrooxidans* utilizes Fe^{2+} as the electron donor and O_2 as the electron acceptor for oxidation to Fe^{3+} . It also uses CO_2 as the carbon source and N and P as the nutrient elements. Further, it employs the energy obtained via the oxidation of Fe^{2+} to support its growth and reproduction [27,28]. Many studies have investigated the effects of O_2 supply on Fe^{2+} oxidation, growth and metabolism of *A. ferrooxidans*. For example, Ohmura et al. [29] indicated that the growth of *A. ferrooxidans* is inhibited at a dissolved O_2 (DO) concentration of less than 0.29 mg/L and terminated at 0.2 mg/L. Goodman et al. [30] reported that bacteria can survive under anaerobic conditions when the oxidation potential of *A. ferrooxidans* for pyrite (FeS_2) decreases rapidly at DO levels of lower than 3 $\mu\text{mol/L}$. Magdalene et al. [31] reported positive correlations between the concentration of DO and the oxidation rate of FeS_2 as well as the cell synthesis of *A. ferrooxidans*. O_2 is vital with respect to the oxidation ability, growth and reproduction of *A. ferrooxidans*. In acidic sulfate environments, such as AMD, the Fe^{2+} bio-oxidation and Fe^{3+} hydrolysis reactions are coupling reactions of the *A. ferrooxidans*-mediated biomineralization. However, the effect of the DO supply level on the Fe^{3+} hydrolysis reactions in the system has been rarely investigated. Liao et al. [26] observed that a significant amount of yellowish hydroxysulfate precipitates was generated by statically incubating the FeSO_4 solution containing *A. ferrooxidans* for

40 h, implying that DO may not be indispensable throughout the experiment for the biosynthesis of the iron hydroxysulfate minerals. However, this assumption is not supported by direct evidence.

The objective of this study was to investigate the influence of DO on the formation of iron hydroxysulfate minerals by *A. ferrooxidans*. Proper hydraulic shear force can maintain the uniformity of a three-phase system (gas–liquid–solid), ensuring a high concentration of DO in the solution and maintaining good heat transfer in the system [32,33]. The effects of the hydraulic shear force on the Fe^{2+} oxidation, total Fe (TFe) precipitation, DO concentration, iron hydroxysulfate mineral yield or phase were investigated by adjusting the rotating speed of the shaking table. These investigations provide the necessary theoretical basis for promoting the regulation and biologic transformation of Fe in AMD to iron hydroxysulfate minerals.

2. Materials and Methods

2.1. Preparation of the Modified 9K Liquid Medium

The modified 9K liquid medium was prepared as follows: 3.5 g of $(\text{NH}_4)_2\text{SO}_4$, 0.119 g of KCl, 0.058 g of K_2HPO_4 , 0.0168 g of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ and 0.583 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ in 1 L of distilled water, adjusted to pH = 2.50 with 1:1 (v/v) H_2SO_4 , followed by sterilization using a sterilizer for 30 min at 121 °C [34].

2.2. Preparation of the *A. ferrooxidans* Resting Cells

A. ferrooxidans (CGMCC0727) was obtained from the China General Microbial Culture Collection Center (Beijing, China). Sterilized $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was added to an Erlenmeyer flask containing 225 mL of the modified 9K liquid medium, which was inoculated with 25 mL of *A. ferrooxidans* to obtain an Fe^{2+} concentration of 9.67 g/L. After evenly mixing the solution, the flask was shaken and cultured in a shaker at 28 °C and 180 rpm, and the culture was terminated later in the exponential growth stage (approximately 3 d). The culture medium was filtered using 1004-055 Whatman filter paper (Grade 4: 20–25 μm) to collect the iron hydroxysulfate minerals, whereas the filtrate was centrifuged at 7720 rpm to collect the concentrated bacteria and then washed twice using dilute H_2SO_4 (pH = 1.50) to eliminate the various doped ions. Finally, the centrifuged cells were resuspended in dilute H_2SO_4 at an optimum pH of 2.50, producing the *A. ferrooxidans* resting cells [26]. The density of *A. ferrooxidans* was approximately 5×10^8 cells/mL.

2.3. Effect of Hydraulic Shear Force on Fe^{2+} Bio-Oxidation and Fe^{3+} Hydrolysis by *A. ferrooxidans*

In a series of 500-mL Erlenmeyer flasks, 225 mL of the modified 9K liquid medium and 25 mL of the *A. ferrooxidans* resting cells were added to produce an inoculation with 10% (v/v) *A. ferrooxidans*. The effective volume of the prepared 9K- FeSO_4 -*A. ferrooxidans* system was 250 mL, where the *A. ferrooxidans* density was approximately 5×10^7 cells/mL. Then, based on the Fe^{2+} concentration of 9.67 g/L, $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ was accurately added and shaken for complete dissolution. The pH values of the solutions were adjusted to 2.50 by adding 1:1 (v/v) H_2SO_4 . The Erlenmeyer flasks were sealed with eight layers of gauze and placed in the shaker at 0, 60, 120, 180 and 240 rpm at a constant temperature of 28 °C for a 120-h biomineralization reaction. During the reaction period, the Fe^{2+} concentration, TFe precipitation efficiency and DO concentration were measured and calculated via regular sampling (after waiting for mineral precipitation, extract 1 mL of the supernatant passed through a 0.22- μm filter membrane). At the end of the culture, the iron hydroxysulfate minerals were collected using 1004-055 Whatman filter paper (Grade 4: 20–25 μm), which was rinsed twice with deionized water to eliminate the ions adsorbed on the minerals and the minerals were weighed after drying at 60 °C for 24 h, with each treatment conducted in triplicate.

2.4. Effect of the O₂ Supply Time on the *A. ferrooxidans*-Mediated Biomineralization

The DO concentration in the solution returned to the initial level and remained stable after 9.67 g/L of Fe²⁺ in the system was completely oxidized by *A. ferrooxidans* at shaking speeds of 120 and 180 rpm (40 h). However, the TFe precipitation efficiency continuously increased between 40 and 120 h. This response provides motivation to investigate the role of DO in the *A. ferrooxidans*-mediated biomineralization.

Another series of 500-mL Erlenmeyer flasks with 250 mL of the 9K-FeSO₄-*A. ferrooxidans* solution (pH of 2.50, Fe²⁺ concentration of 9.67 g/L and *A. ferrooxidans* density of approximately 5×10^7 cells/mL) was prepared. The prepared system was divided according to the O₂ supply times as follows. In treatment (1) (“unlimited O₂ supply”), the Erlenmeyer flasks were sealed with eight layers of gauze and placed in a shaker at 28 °C and 180 rpm for biologic oxidation and hydrolytic mineralization. During the culture, the DO concentration, Fe²⁺ oxidation and TFe precipitation efficiency were measured and calculated at 0 and every 12 h up to 120 h (after waiting for mineral precipitation, extract 1 mL of the supernatant passed through a 0.22-μm filter membrane). In treatment (2) (“limited O₂ supply at 0 h”), the eight layers of gauze in treatment (1) were replaced with rubber plugs equipped with samplers, suction ducts and DO monitors (Figure 1). The suction ducts were connected to vacuum pumps, and pumping was initiated, creating a relative pressure of −0.098-MPa in Erlenmeyer flasks. Subsequently, the catheter valves were closed. Then, the Erlenmeyer flasks were placed in a shaker at 28 °C and 180 rpm for the biomineralization reaction. Sampling was performed and parameters were calculated according to treatment (1). Treatment (3) (“limited O₂ supply at 36 h”) involved biomineralization reactions according to treatment (1) for assessing the oxidation efficiency of the Fe²⁺ in the 9K-FeSO₄-*A. ferrooxidans* system. After the Fe²⁺ oxidation efficiency reached 100% (monitoring determined that the time was 36 h), the eight layers of gauze were replaced by rubber plugs as in treatment (2), and the vacuum pumps were initiated to ensure that the relative pressure in the flask was −0.098 MPa. Further, the catheter valves were closed. The Erlenmeyer flasks were placed in the shaker at 28 °C and 180 rpm for the reaction to proceed, and sampling was performed for determining and calculating various parameters; each treatment was performed in triplicate.

Figure 1. Erlenmeyer flask with a (a) sampler, (b) suction tube and (c) dissolved O₂ (DO) monitor installed on the rubber stopper.

After 120 h of the biomineralization reaction, the iron hydroxysulfate minerals were collected using Whatman No. 4 filter paper, washed twice with deionized water to eliminate the adsorbed ions, and weighed after drying at 60 °C for 24 h. The minerals obtained from the three treatments were identified via X-ray diffraction (XRD) analyses, and their characteristics were observed via scanning electron microscopy (SEM).

2.5. Determination Methods

The pH values of the solutions were determined using a pH S-3C precision pH meter, whereas the DO concentrations were measured using a portable dissolved oxygen analyzer (JPB-607A). The Fe^{2+} and TFe concentrations were determined via the 1,10-phenanthroline method using a spectrophotometer [35]. Then, the Fe^{2+} oxidation efficiency and the TFe precipitation efficiency were calculated according to the following formulas.

Fe^{2+} oxidation efficiency (%) = $[(C_{\text{Fe(II)}} - C'_{\text{Fe(II)}})/C_{\text{Fe(II)}}] \times 100\%$, where $C_{\text{Fe(II)}}$ is the initial Fe^{2+} concentration (mg/L) and $C'_{\text{Fe(II)}}$ is the Fe^{2+} concentration (mg/L) at different times.

TFe precipitation efficiency (%) = $[(C_{\text{TFe}} - C'_{\text{TFe}})/C_{\text{TFe}}] \times 100\%$, where C_{TFe} is the initial TFe concentration (mg/L) and C'_{TFe} is the TFe concentration (mg/L) at different times.

The density of *A. ferrooxidans* was measured using the double-layer plate method [36]. Briefly, 0.1 mL of the heterotrophic yeast strain *Rhodotorula* sp. R30 solution was spread onto the bottom layer made of 2% agar in plate. Then, the mineral-salt medium containing 0.4% agarose with respect to *A. ferrooxidans* were poured on it as the upper layer; after the plates were cold, 0.1 mL of the bacterial suspension containing *A. ferrooxidans* was evenly spread on the superstratum and cultured for 15 d at 30 °C. Further, the bacterial colony on the plate was assessed. The precipitated mineral phases were analyzed via XRD (Bruker D8A25, Bruker Corporation, Karlsruhe, Germany) using $\text{CuK}\alpha$ radiation (40 kV, 40 mA); the samples were scanned from $2\theta = 10^\circ$ to 80° with an increment step of 0.01° and a scanning speed of $6^\circ/\text{min}$. The morphology of the precipitates was examined via field-emission scanning electron microscopy (FE-SEM, SU8010) [37], whereas the specific surface areas of the secondary iron minerals were determined using the Brunauer–Emmett–Teller (BET) adsorption method (Micromeritic ASAP 2020) [38].

3. Results and Discussion

3.1. Effect of Hydraulic Shear Force on the Fe^{2+} Oxidation and TFe Precipitation Efficiency

Generally, the faster the rotating speed of the shaker, the more will be the consumption of Fe^{2+} by *A. ferrooxidans* (Figure 2). During biologic oxidation, *A. ferrooxidans* oxidizes the Fe^{2+} in the system to Fe^{3+} . Further, Fe^{3+} precipitates through hydrolysis with other metal ions or SO_4^{2-} in the solution as iron hydroxysulfate minerals, eliminating soluble Fe [25,39]. Bai et al. [40] indicated that the Fe^{3+} supply rate was an important factor affecting mineral formation in the biomineralization system containing *A. ferrooxidans*, with a higher rate promoting mineral formation. Increasing the hydraulic shear force accelerates the supply rate of the oxidation product Fe^{3+} during biomineralization, with TFe precipitation efficiencies of 5.28%, 26.33% and 39.65% at 0, 60 and 120 rpm in the system at the end of the biomineralization reaction, respectively.

However, under approximately identical Fe^{3+} supply rates, very high shaker speeds (240 rpm) adversely affected the removal of TFe from the 9K- FeSO_4 -*A. ferrooxidans* system. Thomas [41] and Doran [42] indicated that bacterial mineralization is weakened because of their inability to adhere to the solid surface in a strong stirring environment, significantly reducing the TFe removal efficiency of the solution. The changes in TFe precipitation efficiency directly reflect the formation potential of the iron hydroxysulfate minerals in the system [40]. Based on Figure 3, at the end of biomineralization, the TFe precipitation efficiencies of the system at 0, 60, 120, 180 and 240 rpm were 5.28%, 26.33%, 39.65%, 41.28% and 33.38%, respectively, with corresponding iron hydroxysulfate mineral masses of 0.24, 0.87, 1.25, 1.47 and 1.02 g.

A. ferrooxidans is an exclusive aerobic microbe. With O_2 as the only electron acceptor for Fe^{2+} oxidation, *A. ferrooxidans* utilizes the energy released by this oxidation for its growth and reproduction. Therefore, sufficient supply of O_2 is a necessary condition for the growth of *A. ferrooxidans* [27,28]. The DO variations in the 9K- FeSO_4 -*A. ferrooxidans* system under different hydraulic shear forces are displayed in Figure 4.

Figure 2. Effect of hydraulic shear force on the (a) Fe²⁺ concentration and (b) TFe precipitation efficiency in the 9K-FeSO₄-*A. ferrooxidans* system.

Figure 3. Comparison of the biosynthetic iron hydroxysulfate mineral mass with respect to different hydraulic shear forces.

When the reaction proceeds at 0 or 60 rpm, the DO concentration in the solution gradually decreases from approximately 9.50 mg/L at the beginning to 6.32 and 5.21 mg/L after 120 h, respectively. As the speed of the shaker was increased to 120 and 180 rpm, the DO concentration in the system (approximately 9.50 mg/L) initially decreased to 5.38 and 6.91 mg/L, respectively, after 20 h and then becomes 9.26 mg/L and 9.64 mg/L, respectively, after 40 h. If the nutrient supply is sufficient, the amount of *A. ferrooxidans* in the system can increase by a hundred times as the culture time increases when compared with that observed during the initial stage of the reaction [21]. In this case, the O₂ needed for the biologic oxidation of Fe²⁺ in the solution gradually increases, and the dissolution rate of the air O₂ is insufficient to support the O₂ consumption of *A. ferrooxidans* during the Fe²⁺ oxidation

process, gradually decreasing the solution's DO concentration. However, the concentration of Fe^{2+} will decrease gradually when the density of *A. ferrooxidans* is high. At this time, the Fe^{2+} concentration becomes the main limiting factor with respect to the biologic oxidation reaction [43]. Further, biologic oxidation wanes and the O_2 needed for oxidation reduces. Because of the O_2 obtained from the air, the DO concentration in the solution slowly increases, attaining a stable state.

Figure 4. Effect of the hydraulic shear force on the DO concentration of the 9K- FeSO_4 -*A. ferrooxidans* system.

Figure 2 shows that the TFe precipitation efficiency of the solution increases as the mineralization reaction time increases after the complete biologic oxidation of Fe^{2+} in the 9K- FeSO_4 -*A. ferrooxidans* system. Compared with Figure 4, for a biomineralization lasting for 40 h, the DO concentration in the system at 120 and 180 rpm returns to the initial level, indicating that DO was not involved in the hydrolytic mineralization of Fe^{3+} . Liao et al. [26] noted that after inoculating *A. ferrooxidans* into an $\text{Fe}_2(\text{SO}_4)_3$ solution and waiting for 40 h (equivalent to a rotation speed of 0 rpm), the Fe concentration in the solution gradually decreases, producing a small amount of minerals. However, the study lacked an in-depth discussion about the causes of this phenomenon.

3.2. Effect of the O_2 Supply Time on the Formation of Iron Hydroxysulfate Minerals

Three groups of control experiments were conducted to further determine the function of O_2 in Fe precipitate formation (Section 2.4). As shown in Figure 5, in the treatment “limited O_2 supply at 0 h,” the O_2 in air cannot dissolve in the 9K- FeSO_4 -*A. ferrooxidans* system, whereas the O_2 in the solution diffuses into the air owing to the difference in concentration. Accordingly, a marked inhibition of Fe^{2+} bio-oxidation and TFe precipitation efficiency could be observed, as indicated by the low Fe^{2+} oxidation efficiency (16.26%) and TFe precipitation efficiency (4.25%) at the end of the reaction (120 h). This suggests that relying only on the DO remaining in the initial reaction solution barely supports the O_2 requirement for the formation of iron hydroxysulfate minerals when *A. ferrooxidans* should utilize O_2 to oxidize Fe^{2+} and obtain energy for its growth [27,28].

The DO concentration in the “unlimited O_2 supply” treatment decreases to its lowest value (3.17 mg/L) at 36 h and then rapidly increases to the initial level (9.52 mg/L) and remains stable at 48 h. Furthermore, almost similar dynamic changes were observed with respect to the Fe^{2+} oxidation efficiency and TFe precipitation efficiency in the “unlimited O_2 supply” and “limited O_2 supply at 36 h” treatments. This suggests that the supply of O_2 had an important function in the oxidation of Fe^{2+} to Fe^{3+} by *A. ferrooxidans*; however, its function in the formation of iron hydroxysulfate minerals could be neglected possibly because the energy released from the oxidation of Fe^{2+} can be used for the subsequent hydrolysis reaction of Fe^{3+} into minerals [44,45]. This was advantageous to significantly

reduce the AMD biomineralization treatment cost because the O₂ charging operation of aeration can be stopped after complete Fe²⁺ oxidation by *A. ferrooxidans*.

Figure 5. Effects of O₂ supply time on the (a) DO concentration, (b) Fe²⁺ oxidation and (c) TFe precipitation efficiency during *A. ferrooxidans*-mediated biomineralization.

3.3. Characteristics of the Minerals Obtained from Different O₂ Supply Conditions

Generally, XRD is employed to analyze the mineral phases [46,47]. The XRD patterns of three biogenic iron hydroxysulfate minerals obtained using different O₂ supply times are presented in Figure 6. Based on standards [48], the product obtained from the “limited O₂ supply at 0 h” treatment after biomineralization for 120 h was schwertmannite, whereas the remaining two treatment systems produce a mixture of schwertmannite and jarosite. Based on Figure 5, the oxidation efficiency of Fe²⁺ can be determined using the O₂ supply time. At a low Fe²⁺ oxidation efficiency, the hydrolytic mineralization product of Fe³⁺ was mainly schwertmannite and a high Fe²⁺ oxidation efficiency promotes the formation of jarosite. Bai et al. [49] observed that either amorphous schwertmannite or crystalline jarosite existed at the critical point of jarosite formation in a FeSO₄-K₂SO₄-H₂O system with *A. ferrooxidans* and depended on the rate of Fe³⁺ supply. The low supply rate of Fe³⁺ obviously inhibited the incorporation of K⁺ into the iron hydroxysulfate mineral to form jarosite, improving the generation of amorphous schwertmannite in the system.

Figure 7 shows the SEM images of the iron hydroxysulfate minerals mediated by *A. ferrooxidans* under different O₂ supply times. Schwertmannite agglomeration was obvious during the “limited O₂ supply at 0 h” treatment. The mineral particles exhibit “chestnut shell” and “sea urchin” shapes, with their surfaces covered with needle-like burrs. Further, their average specific surface areas

(42.12 m²/g) were considerably higher than the range (4–14 m²/g) reported for schwertmannite by Regenspurg et al. [15]. Similar to the results of this study, Loan et al. [16,23,50] observed that the schwertmannite formed in an acidic mine environment dominantly displayed a spherical “sea urchin”-like structure, which had a diameter of only 300–500 nm and the surface of which was covered with 60–90-nm needle-like burrs. When compared with the structure of the minerals obtained via the “limited O₂ supply at 0 h” treatment, the apparent structure of the minerals obtained from the “unlimited O₂ supply” and “limited O₂ supply at 36 h” treatments clearly differ. The crystallinity of schwertmannite was poor with a distributed network, whereas the jarosite minerals display obvious crystallinity and were relatively dispersed on the schwertmannite surfaces. This is consistent with Sasaki and Konno’s results that biosynthetic jarosite comprises many micron-sized crystals with clear edges and corners and smooth surfaces [51]. The average specific surface areas of the mixtures obtained via the two treatments were 28.97 and 31.53 m²/g, respectively.

Figure 6. XRD patterns of the iron hydroxysulfate minerals mediated by *A. ferrooxidans* under different O₂ supply times, i.e., (a) limited O₂ supply at 0 h; (b) limited O₂ supply at 36 h and (c) unlimited O₂ supply.

Figure 7. Scanning electron microscopy (SEM) images of the iron hydroxysulfate minerals mediated by *A. ferrooxidans* under different O₂ supply times, i.e., (a) limited O₂ supply at 0 h; (b) limited O₂ supply at 36 h and (c) unlimited O₂ supply.

4. Conclusions

In this study, we demonstrated that in a 9K-FeSO₄-*A. ferrooxidans* system, the greater the hydraulic shear force (the higher the shaker speed), the higher will be the utilization of Fe²⁺ by *A. ferrooxidans*. Regardless, a very high shaker speed (e.g., 240 rpm) adversely impacted the hydrolytic mineralization and precipitation of Fe³⁺ in the system. Based on the Fe²⁺ biologic oxidation efficiency and TFe precipitation capacity, shaking table speeds of 120–180 rpm were optimum for efficiently synthesizing iron hydroxysulfate minerals. In addition, the effect of O₂ supply time on the *A. ferrooxidans* biomineralization process confirmed that the DO in the system was mainly consumed by the biologic oxidation of Fe²⁺. After Fe²⁺ was completely oxidized by *A. ferrooxidans*, the hydrolytic precipitation efficiency of the oxidation product Fe³⁺ was independent of the O₂ supply. The XRD and SEM analyses demonstrated that when Fe²⁺ is completely oxidized in the 9K-FeSO₄-*A. ferrooxidans* system, the O₂ supply did not alter the mineral precipitated via Fe³⁺ hydrolysis, mainly comprising partially amorphous schwertmannite and jarosite with good crystallinity.

Author Contributions: J.Y. conceived and designed this study. R.W. performed the experiments and conducted data collation and analysis. H.W. contributed to the revision of the manuscript. Y.S. collated the data, performed analysis and wrote the manuscript. All authors have read and agreed to the published version of the manuscript.

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