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Insights into the enhancement mechanism coupling with adapted adsorption behavior from mineralogical aspect in bioleaching of copper-bearing sulfide ore by *Acidithiobacillus* sp.

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Abstract

The enhancement mechanism of adapted adsorption behavior in bioleaching of copper-bearing sulfide ore by *Acidithiobacillus* sp. was systematically investigated from a mineralogical aspect and compared to adsorption-deficient (DF) and adsorption-unadapted (UA) systems. With the assistance of adapted adsorption behavior, both iron and sulfur metabolism was enhanced, which was proved by a series analysis of key chemical parameters, including scanning electron microscopy (SEM) and X-ray diffraction (XRD). SEM analysis revealed smaller jarosite and S^0 granules along with more potential adsorption sites on the ore surface, thus indicating a stronger "contact" mechanism. XRD analysis showed that more chemical derivatives were generated owing to active iron/sulfur metabolism. Additionally, attached and free biomasses of *A. ferrooxidans* and *A. thiooxidans* were increased by 33.3%-58.9% and 25.0%-33.9%, respectively. Moreover, the final concentration of extracted copper ion was improved by 22.8% (*A. ferrooxidans*) and 28.9% (*A. thiooxidans*), respectively. All results proved that the adsorption behavior coupled to attached cells was greatly stimulated and accelerated by the adapted evolution and further contributed to higher bioleaching efficiency. The adapted method and its mechanism will be useful to further guide similar bioleaching processes in the near future.

Keywords: Bioleaching of copper-bearing sulfides ore · *Acidithiobacillus* · Adapted adsorption behavior · Mineralogical enhancement mechanism

1. Introduction

A vast majority of low-grade ores cannot be economically utilized by the traditional smelting method and are deposited at mines.[1-3] The substantial discard of ores are accumulating, resulting in resource waste and potential environmental problems. The copper-bearing sulfide ore such as chalcopyrite ($CuFeS_2$), (>70% of world copper reserves), is general common low-grade and refractory that also faces the aforementioned problems.[4,5] In the last decades, bioleaching was recognized as a green and economical technology for recovering these waste ores.[6-8] However, owing to the complicated and refractory structure, the commercial application of bioleaching of copper-bearing sulfide ore is still not satisfactory.[9,10] Recently, bioleaching of copper-bearing sulfide ore has attracted increasing attentions, especially because of the growing copper consumption and environmental stress worldwide.

To improve bioleaching process, it is essential to deep understand the bioleaching mechanism in detail. Various mechanisms have been proposed for illustrate the bioleaching process of sulfide ore (pyrite, sphalerite, chalcopyrite etc.), such as surface attaching, oxidation reactions, elemental transformation, interfacial evolution, bio-molecules changes, and surface erosions.[11] Two indirect mechanisms via thiosulfate or via polysulfides were found in pyrite bioleaching with *A. thiooxidans*.[12] The sphalerite bioleaching process was divided into two steps.[13] The rapid surface attaching of microorganisms was the key to enhance leaching efficacy, resulting in the oxidation of the pyrite and concomitant bio-generation of ferric ions and protons. Then, the continued regeneration of ferric ions by planktonic bacteria and the oxidation of the elemental sulfur reaction product further contributed the higher leaching efficacy. Currently, "indirect contact" and "direct contact" mechanisms were proposed to better understand the bioleaching process.[14,15] The two mechanisms derived from bio-oxidation reactions from different spaces. In the former mechanism, bacteria oxidize soluble ferrous ion to ferric ion and sulfur to sulfate ion in the micro-liquid environment. Ferric ions oxidize the sulfide ore in an acidic environment. In the latter mechanism, bacterial attachment is important physiologically, but ferric ions oxidize the sulfide minerals in the solid-liquid interface environment. The specifics of bacterial (electro) chemical interactions with mineral surfaces and/or their direct contact (enzymatic) contribution to sulfide dissolution are unknown. In these mechanisms, the adsorption behavior of attached cell was prerequisite for the subsequent iron/sulfur metabolism.[16] Previous researches have also intensively studied the performance of adsorption behavior, such as the effect of single factor such as extracellular polymeric substance (EPS), mineral or bacterial attachment selection in various bioleaching processes.[16,17] It was reported that a multilayered biofilm around with EPS of *A. ferrooxidans*, *A. thiooxdians* and *Leptospirillum ferrooxidans* was pivotal in "contact" mechanisms.[18,19] The attached behaviors of different strains even with closely related genetic relationship were diverse, while the mineral-selection in the attached process of the same strain was also different.[14,20] However, most researchers focused on the adsorption kinetics, association factor and adsorption characteristics between different species. To date, to the best of our knowledge, the detailed mechanism of adsorption behavior in bioleaching of copper-bearing sulfide ore, especially the efficient strategy for enhancing the performance of adsorption behavior remains poorly understood.

In our previous study, an acidophilic strain *A. thiooxidans* ZJJN-3 was isolated from industrial bio-heap

leachate.[21] *A. thiooxidans* ZJJN-3 and *A. ferrooxidans* CUMT-1 was applied in chalcopyrite bioleaching to form an efficient catalytic system.[22] An integrated fermentation strategy was also proposed for enhancing chalcopyrite bioleaching in a 7-L bioreactor.[23] In this study, the typical bioleaching strains (*A. ferrooxidans* and *A. thiooxidans*) were employed for exploring the enhancement mechanism of adapted adsorption behavior in bioleaching of copper-bearing sulfide ore. First, the effects of adapted adsorption behavior on sulfur and iron metabolism were analyzed and compared to adsorption-deficient (DF) and adsorption-unadapted (UA) systems. Second, the effects of adaptive adsorption behavior on ore such as morphological, componential and functional group differences were also investigated by scanning electron microscopy (SEM), and X-ray diffraction (XRD), respectively. Finally, the efficiency of adaptive adsorption behavior on improving cell growth and bioleaching efficiency was further verified.

2. Materials and methods

2.1. Strain and growth condition

(Position for Table 1)

A. ferrooxidans CUMT-1 was kindly donated by Professor Leng from the China University of Mining and Technology, Xuzhou, Jiangsu, China. *A. thiooxidans* ZJJN-3 was isolated from leachate of industrial bio-heap (low-grade secondary sulfide, 20 million m^3) in Zijinshan Copper Mine, Longyan, Fujian, China. The strain was previously identified by analysis of physiological and molecular characteristics.[21] It was deposited in the China Center for Type Culture Collection with the number M2012104. The detailed strain characteristics are listed in Table 1. *A. ferrooxidans* was cultured in 9K media and *A. thiooxidans* was cultured in Starkey media. The basal salts of 9K media were listed as follows (g/L): $(NH_4)_2SO_4$ 3.0, K_2HPO_4 0.5, $MgSO_4{\cdot}7H_2O$ 0.5, KCl 0.1, $Ca(NO_3)_2$ 0.01. Energy substrate: 44.7 g/L $FeSO_4{\cdot}7H_2O$. The basal salts of Starkey media were listed as follows (g/L): $(NH_4)_2SO_4$ 3.0, KH_2PO_4 3.5, $MgSO_4$ 0.5, $CaCl_2{\cdot}2H_2O$ 0.25. Energy substrate: 10 g/L S^0. Trace elements were listed as follows (mg/L): Na_2SO_4 50.0, $FeCl_3{\cdot}6H_2O$ 11.0, H_3BO_3 2.0, $MnSO_4{\cdot}H_2O$ 2.0, $ZnSO_4{\cdot}7H_2O$ 0.9, $Na_2MoO_4{\cdot}2H_2O$ 0.8, $CoCl_2{\cdot}6H_2O$ 0.6, $CuSO_4$ 0.5, Na_2SeO_4 0.1. For both bacetrail systems, the initial pH of the media was adjusted to 2.2 and the strains were adapted by 3.0% (w/v) of copper-bearing sulfide ore sample at 30 °C and 170 rpm. Strains were incubated into fresh media once a month.

2.2. Ore sample composition and pretreatment

(Position for Table 2)

The copper-bearing sulfide ore sample was collected from Dongguashan copper mine, Tongling, Anhui, China. The main mineralogical compositions of the primary ore were chalcopyrite, pyrite, pyrrhotite, and magnetite. The detailed elements and contents of the ore sample were assayed by atomic absorption spectrometry (Spectr AA-220, Varian, USA) as Table 2. The ore sample was sieved through a 300-mesh grid, with controlling particle diameter <48 μm. The ore sample was sequentially washed with 2 M HCl, distilled water, and pure ethanol. Then, the ore sample was dried at room temperature and reserved in a vacuum desiccator.

2.3. Experimental procedure

2.3.1. Procedure for DF system

The DF system was designed as described here. The leaching solution was allowed to rest without agitation for 1 h to allow the ore particles to settle. Then, supernatant was transferred into another flask. The leached ore sample was collected by centrifugation (3K15, Sigma, Germany) at 380 × *g* for 2 min. The ore sample was suspended in 30 mL of fresh basal salts of 9K or Starkey media. Then 1.0 g of 0.2 mm glass beads was added and shaken with a vortex (lab-dancer, IKA, Germany) for 5 min. The ore sample was centrifuged and shaken again. The supernatant was then transferred into another tube. Almost no dissociative DNA was tested in the supernatant by this method according to the report of Gehrke et al. (1998), indicating that all attached cells were removed without breaking.[24] Moreover, no additional cells were separated with an additional shaken-operation process, proving that all attached cells were separated from the ore surface with the above operation. The ore sample was added into its original bioleaching supernatant. The above procedure was completed every five days.

2.3.2. Procedure of adsorption-adapted (AD) evolution

The AD evolution was processed as described as here. *A. ferrooxidans* and *A. thiooxidans* were independently cultured with 3.0% (w/v) of copper-bearing sulfide ore for 15 days. The leaching solution was stilled for 1 h and the supernatant was transferred into another flask. The ore sample was collected by centrifugation (3K15, Sigma, Germany) at 380 × *g* for 2 min, and suspended in 30 mL of fresh basal salts

of 9K or Starkey media. Then, 1.0 g of 0.2-mm glass beads was added and shaken with a vortex (lab-dancer, IKA, Germany) for 5 min. Most of the attached cells with poor adsorption performance were washed off while stronger ones were preserved. The ore sample was centrifuged, as before, and added into fresh media. This above process was repeated once every two weeks. After being repeated for 6 months, the attached cells were shed and collected, as in 2.3.1. The attached cells were used as the adapted strain for the bioleaching experiment.

2.3.3. Bioleaching experiment

Six bioleaching experiments with different performances of adsorption behavior were designed as follows: *A. ferrooxidans* CUMT-1 (DF); *A. ferrooxidans* CUMT-1 (unadapted; UA); *A. ferrooxidans* CUMT-1 (AD); *A. thiooxidans* ZJJN-3 (DF); *A. thiooxidans* ZJJN-3 (UA); *A. thiooxidans* ZJJN-3 (AD). The bioleaching experiments were carried out in 500-mL shaker flasks; 100 mL media was added into the *A. ferrooxidans* (9K basal salts media) and *A. thiooxidans* (Starkey basal salts media) systems. Then, 3.0 g of copper-bearing sulfide ore sample was added into each flask. The cell density in each system was controlled at 5.0×10^7 cells/mL after inoculation. The bioleaching experiments were carried out at 30 °C and 170 rpm. To balance the system from evaporation loss, 2.0 mL of sterile water was supplemented into each system once a day. The whole bioleaching cycle lasted 40 days.

2.4. Analytical methods

2.4.1. pH and Eh measurement

The pH value was measured by a pH meter (PHB-3TC, Sartorius, Germany). The Eh value was monitored by a Pt electrode (E-431Q, ASI, USA) with a calomel electrode (Hg/Hg_2Cl_2) as reference.

2.4.2. Sulfate ion assay

The concentration of sulfate ions was detected according to the chromic acid-barium colorimetric assay using a spectrophotometer (IV-1100D, Meipuda, China). Concentration of sulfate ion (mg/L) = $201.6 \times OD_{420\ nm} - 26.029$ (r^2=0.998).

2.4.3. Ferrous and ferric ion assay

The concentrations of ferrous and ferric ions were measured according to the *o*-phenanthroline spectrophotometry assay using a spectrophotometer. Concentration of ferrous ion (mg/L) = $5.077 \times OD_{508}$

$_{nm}$ – 0.0765 (r^2=0.999). Concentration of ferric ion (mg/L) = 5.102 × $OD_{508\ nm}$ – 0.143 (r^2=0.998).

2.4.4. SEM analysis

The ore sample was previously dried at room temperature and preserved in a vacuum desiccator. The morphology and surface of the ore were observed with an SEM (Quanta-200, FEI, Netherlands). The suspension solution of ore sample was added at specimen holder. After the natural volatilization, the sample was firmly immobilized and a 30 nm thick conductive coating of gold was applied to the surface. The scanned condition was set at 25 kV.

2.4.5. XRD analysis

The ore sample was previously washed with deionized water and dried at room temperature under the vacuum condition. Then the ore was covered on the center depression of the detection plate. The ore sample was scanned according to 2-Theta range 3-90 (°) by an X-ray diffractometer (D8, AXS, Germany). The detailed data were analyzed by the MDI Jade 5.0 (Materials Data Ltd., USA) integrating PDF card library.

2.4.6. Detection of free, attached and total cell density

One milliliter of bioleaching sample was centrifuged at 380 × *g* for 2 min to separate the supernatant from the ore precipitation. The free cell density was counted by a single span microscope. Meanwhile, the bottom ore sample was re-suspended in 5.0 mL of fresh basal media. Then, 0.2 g of 0.2-mm glass beads were added and shaken with a vortex for 5 min. This sample was centrifuged and the mixing process was repeated once. The attached cell density was counted in the supernatant as before. The total cell density was the sum of the free and attached cell densities.

2.4.7. Copper ion assay

The concentration of copper ion was monitored by flame atomic absorption spectrometry (Spectr AA-220, Varian, USA). Concentration of copper ion (mg/L) = 6.852 × $\lambda_{325\ nm}$ – 0.0301 (r^2=0.999).

2.5. Statistical analysis

All experiments were performed in triplicate. The statistical analysis of experimental data was performed by one-way analysis of variance and expressed as mean values ± SD. The software SPSS 17.0 (SPSS Inc., Chicago, USA) was used for the statistical analysis.

3. Results and discussion

3.1. Effects of adapted adsorption behavior on iron and sulfur metabolisms

3.1.1. Iron metabolism

Changes in ferrous and ferric ions, the main parameters of iron metabolism, in different systems are shown in Fig. 1. The main biochemical reactions in copper-bearing sulfide ore sample (mainly chalcopyrite as example) are summarized as Eqs. (1) - (7). In the *A. ferrooxidans* system, the highest concentrations of ferrous ions in each system were (in mg/L) 200.5 (DF), 327.4 (UA) and 356.3 (AD). In the *A. thiooxidans* system, the values were (in mg/L) 65.4 (DF), 75.3 (UA) and 94.5 (AD). It was reported that the adsorption behavior of attached cells at the early stage is beneficial to further concentrate ferric ions and attack chalcopyrite, as shown Eq. (1).[9,21] The greatest increase in ferrous ions was 63.3%, which was tested in the *A. ferrooxidans*-DM system. It was owing to that the ferrous ion metabolism was more closely related with *A. ferrooxidans* than the pure sulfur oxidizer-*A. thiooxidans*. Furthermore, compared to reductive sulfur, ferrous ion was more easily utilized by *A. ferrooxidans* in a multiple-energies system.[25,26] The similar trend as ferrous ions was tested in ferric ions. In the *A. ferrooxidans* system, the highest concentrations of ferric ions in each system were (in mg/L) 435.2 (DF), 637.0 (UA) and 673.2 (AD). In *A. thiooxidans* system, the values were (in mg/L) 82.4 (DF), 123.2 (UA) and 137.8 (AD). These results indicated that the adapted evolution of adsorption behavior was favorable for enhancing iron metabolism.

$$CuFeS_2 + 4Fe^{3+} = Cu^{2+} + 2S^0 + 5Fe^{2+} \quad (1)$$

$$CuFeS_2 + 4H^+ + O_2 = Cu^{2+} + 2S^0 + Fe^{2+} + 2H_2O \quad (2)$$

$$4Fe^{2+} + 4H^+ + O_2 = 4Fe^{3+} + 2H_2O \quad (3)$$

3.1.2. Sulfur metabolism

(Position for Fig. 1 and Table 3)

Similarly, the changes in pH and sulfate ion concentrations in different systems were also investigated (Fig. 1). After the adaptive phase, the pH gradually decreases along with the dissolution of the ore. In both the *A. ferrooxidans* and *A. thiooxidans* systems, the pH of the DM system was always the lowest, while the highest value was tested in DF systems. In the *A. ferrooxidans* system, the final pHs were 1.92 (DF), 1.82 (UA), and 1.74 (AD). In the *A. thiooxidans* system, the final pHs were 1.82 (DF), 1.63 (UA), and 1.51

(AD). In the DF system, the granular sulfur on the ore surface could not be used by attached cells and thus formed the S^0 passivation layer. The subsequent oxidization process was greatly inhibited. Conversely, in the AD system, the adsorption behavior of attached cells was enhanced by the directionally domesticated evolution. Most of the sulfur could be more efficiently utilized, as shown Eqs. (4) and (5), and produced sulfuric acid. Moreover, the pH decline range of *A. thiooxidans* (1.82 to 1.51) was higher than that of *A. ferrooxidans* (1.92 to 1.74), with the assistance of enhanced adsorption behavior.

Comparatively, the concentration of the sulfate ions steadily increased and achieved stability on day 20. The trend of the sulfate ions in both *A. ferrooxidans* and *A. thiooxidans* systems was similar to the pH data. In the *A. ferrooxidans* system, the highest concentrations of sulfate ions in each system were (in g/L) 2.93 (DF), 3.56 (UA) and 3.88 (AD). In the *A. thiooxidans* system, the highest concentrations of sulfate ions in each system were (in g/L) 1.24 (DF), 2.83 (UA) and 3.86 g/L (AD). Compared to *A. ferrooxidans*-AD, the increased range of sulfate ion concentration in the *A. thiooxidans*-AD system, via enhancing adsorption behavior, was more significant. The dependence on the adsorption behavior with pure-sulfur oxidizer such as *A. thiooxidans* was generally stronger, especially in eliminating S^0 membrane compared to multiple-energies oxidizer.[21] It was also verified that the attached biomass of the *A. thiooxidans* system on the sulfur surface was almost twice times of the *A. ferrooxidans* system.[22] The above result was also closely consistent with the data on the changes in pH. These results all indicated the higher efficacy of adaptive adsorption behavior on sulfur metabolism, especially for *A. thiooxidans*. The detailed comparison of key chemical parameters in different systems is also listed in Table 3.

$$2S^0 + 3O_2 + 2H_2O = 2SO_4^{2-} + 4H^+ \quad (4)$$

$$S_xO_y^n + O_2 + H_2O \rightarrow SO_4^{2-} + H^+ \quad (S_xO_y^n \text{ represents reduced sulfur, such as } SO_3^{2-}) \quad (5)$$

$$3Fe^{3+} + 2SO_4^{2-} + 6H_2O + K^+ = KFe_3(SO_4)_2(OH)_6 + 6H^+ \quad (6)$$

$$2FeS_2 + 2Fe^{3+} + 3H_2O = 4SO_3^{2-} + 4Fe^{2+} + 6H^+ \quad (7)$$

3.2. Effects of adaptive adsorption behavior on mineralogy

3.2.1. Ore morphology

(Position for Fig. 2)

To better understand the mineralogical effects of adapted adsorption behavior, the morphologies of ore

samples in different bioleaching systems were observed by SEM (Fig. 2). The morphological differences of between DF, UA, and AD systems were significant, from either *A. ferrooxidans* or *A. thiooxidans*, In the *A. ferrooxidans*-DF system, the ore surface was smooth and jarosite precipitation was tiny. Owing to the absence of attached cells, the "contact" mechanism was greatly inhibited and there was not enough energy substrate (ferrous ion) released from the ore for cell growth. The concentration of ferric ion sequentially decreased and further reduced jarosite production. In the *A. ferrooxidans*-UA system, more compact jarosite appeared on ore surface. With the assistance of adsorptive behavior, the iron metabolism was accelerated and produced more jarosite. In the *A. ferrooxidans*-AD system, the jarosite precipitation became significantly smaller and more potential adsorption sites were observed on the ore surface; jarosite formation was moderately inhibited by lower pH, as shown in Eq. (6), although with more active metabolism. The potential adsorption site indicated the stronger adsorption behavior via the adapted evolution.

The same phenomenon was more obvious in the *A. thiooxidans* system, to some extent. In the *A. thiooxidans*-DF system, the ore surface was smoother and the sulfur granule was extremely exiguous. In the *A. thiooxidans*-UA system, some rill and micro-pore appeared on the ore surface. In the *A. thiooxidans*-AD system, there were more sulfur granules and the ore surface was significantly rougher. Meanwhile, more potential adsorption sites indicated a stronger "contact" mechanism with adaptive attached cells. It was reported that the dependence on adsorption behavior with *A. thiooxidans* was stronger because most of the main energy source (S^0) was generated on the ore surface.[26] Additionally, due to the active chemical ion status, more unknown derivatives were also generated and coupled with the ore.

3.2.2. Ore components

(Position for Fig. 3)

The XRD analysis was performed to investigate the composition of ore samples in different bioleaching systems (Fig. 3). The main components were $CuFeS_2$, $KFe_3(SO_4)_2(OH)_6$, S, Fe_7S_8, Fe_3O_4, FeS_2 and $CaSO_4{\cdot}2H_2O$. Compared to the *A. ferrooxidans*-DF system, there were fairly larger amounts of precipitate peaks with ore such as jarosite and granular sulfur in the UA system and especially the AD system. The higher activity of iron/sulfur metabolism was achieved by stronger adsorption behavior and further produced more crystal forms. The result was also closely consistent with the morphological differences

(Fig. 2). Compared to the *A. thiooxidans*-DF system, the sulfur peaks were more in the UA system, especially in the AD system, indicating that more crystal forms of elemental sulfur were generated with more active sulfur metabolism. It was reported that, to some extent, sulfur was generally coupled with amorphous iron or other oxy-hydroxides.[18] Meanwhile, the $CaSO_4 \cdot 2H_2O$ peak was significant in the UA and AD systems, indicating higher concentration of sulfate ions. Additionally, minor accumulation of other unknown peak in these precipitates was due to previous washes with deionized water prior to XRD detection. It was also reported that the abundant inorganic ions and microbial organic compounds in bioleaching systems contribute to more complicated derivatives.[20]

3.3. Efficacy of adapted adsorption behavior for enhancing biomass and copper recovery

3.3.1. Biomass

(Position for Fig. 4)

With the assistance of adapted evolution, the attached biomass was significantly improved (Fig. 4A). In the *A. ferrooxidans* system, the highest attached biomass of each system was 0.62 (DF), 1.29 (UA) and 1.72 × 10^7 cells/mL (AD). The attached biomass was improved by 33.3% via the adapted evolution. Free biomass was also increased sequentially from 21.6 into 27.0 × 10^7 cells/mL (25.0%); more energy and nutrients were released owing to a stronger "direct contact" mechanism. The phenomenon was more obvious in *A. thiooxidans* system. The highest attached biomass of each system was 0.81 (DF), 1.65 (UA) and 2.62 × 10^7 cells/mL (AD). Attached biomass was improved by 58.9% via adapted evolution. Moreover, the free biomass increased from 11.8 to 15.8 × 10^7 cells/mL (33.9%) with stronger adsorption behavior because *A. thiooxidans* is pure-energy oxidizer (sulfur), which is different from the multiple energy oxidizer-*A. ferrooxidans*. *A. thiooxidans* relied more on its attached cells because the main energy substrate (S^0) largely existed on the ore surface. Sulfur granules were primarily oxidized into some intermediate status such as $S_4O_6^{2-}$ or $S_4O_5^{2-}$, with the assistance of adsorption behavior. Then, the reduced and soluble sulfur was thoroughly utilized via a "non-contact" mechanism. These data are consistent with the TEM images of flagella and capsule, which also indicated a stronger requirement of attached cells by *A. thiooxidans* ZJJN-3.[21]

3.3.2. Copper recovery

The copper recovery efficiency was significantly enhanced by the adaptive evolution of attached cells (Fig. 4B and Table 3). In the *A. ferrooxidans* system-AD, the efficiency was improved by 93.5% and 22.8%, compared to the DF and UA systems, respectively. The improvement in the *A. thiooxidans*-AD system was more significant. The efficiency was improved by 154.2% and 28.9%, compared to the DF and UA systems, respectively. In other words, more than 48.3%-60.7% of the bioleaching efficiency was contributed by directly domesticating attached cells. Compared to *A. ferrooxidans*, the efficacy of adaptive evolution was more prominent with *A. thiooxidans*. The result was also closely consistent with the chemical, mineralogical, and biological parameters, thereby proving the efficacy of adapted evolution.

3.4. Overall assessment effects of adsorption behavior in bioleaching copper-bearing sulfide ore

(Position for Fig. 5 and Table 4)

The microenvironments of bioleaching of copper-bearing sulfide ore were divided into solid-liquid and liquid microenvironments based on the biochemical reaction site (Fig. 5, chalcopyrite as example). Apparently, attached cells adsorbed onto ore surface in the solid-liquid microenvironment. The biochemical reaction in the liquid microenvironment was subsequently influenced by the surface adsorption process. The "direct contact" and "indirect contact" bioleaching mechanisms were derived from these two different microenvironments.[25,27] The role of attached biomass in bioleaching of copper-bearing sulfide ore was characterized by the aspects of iron and sulfur metabolisms.

In the "direct contact" mechanism's iron metabolism (*A. ferrooxidans* CUMT-1), attached cells adsorbed onto the ore surface and oxidized ferrous ion into ferric ion, as shown in Eq. (3). The ore surface was sequentially attacked by generated ferric ion as Eq. (1) and dissolved copper ion. The resulting ferrous ion entered into the ion cycle again. The dissolution process of ore took place at the interface between the cells and the ore surface. Extracellular polymeric substance (EPS), consisting of some polysaccharides, proteins, and nucleic acids, generally served as the reaction space.[28] The increasing concentrations of ferrous and ferric ions in the liquid microenvironment gradually initiated and enhanced the "indirect contact" mechanism.[29,30] Also, the accumulated ferric ions partly participated during the formation of jarosite, as shown in Eq. (6).

In the "direct contact" mechanism's sulfur metabolism (*A. thioodidans* ZJJN-3/*A. ferrooxidans*

CUMT-1): in the "direct contact" mechanism, sulfur colloids were subsequently oxidized into intermediate compounds such as $S_4O_6^{2-}$ or $S_4O_5^{2-}$. Also, redundant sulfur gathered as micro-particles (S_8) and formed a passivation layer. This reduced sulfur dissolved into the liquid microenvironment and was oxidized, as shown in Eq. (5). Then, the hydrogen ions entered into the solid-liquid microenvironment and attacked ore surface, as shown in Eq. (2). The copper ion was finally released. Reduced sulfur and hydrogen ions in the liquid microenvironment gradually initiated and enhanced the "indirect contact" mechanism. With the assistance of adsorption behavior, more hydrogen ions, ferrous ions, ferric ions, sulfur compounds, and free biomass were created in the bioleaching system. The whole bioleaching system was directly or indirectly affected by these oxidizing and reductive agents. Therefore, in both iron and sulfur metabolism, adsorption behavior acted as an initiator and accelerator. Our research was the first time to reveal the enhancement mechanism coupling with adapted adsorption behavior from mineralogical aspect in bioleaching of copper-bearing sulfide ore.

4. Conclusions

Bioleaching of copper-bearing sulfide ore was improved by directly adapting adsorption behavior, and its mineralogical enchantment mechanism was also successfully investigated and compared to DF and UA systems. With the assistance of adapted evolution, both iron and sulfur metabolism was greatly enhanced. Jarosite (*A. ferrooxidans*) and S^0 (*A. thiooxidans*) became significantly smaller along with more potential adsorption sites. More compound derivatives were generated because of active biochemical reactions. Attached biomass was increased and further contributed to higher free biomass. Moreover, the efficiency of copper recovery was improved by 22.8% (*A. ferrooxidans*) and 28.9% (*A. thiooxidans*), respectively. Taken together, these results indicated that this mechanism can be applicable to directly domesticate adsorption behavior for improving bioleaching of copper-bearing sulfide ore, especially with *A. thiooxidans*.

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Table 1

The main characteristics of strains used in the study.

Speices	Strain	Energy type	Optimal T/pH	Description and source
A.ferrooxidans	CUMT-1	Ferrous and sulfur oxidizer	30-35°C, pH 1.8-2.5	Waste acid mine drainge of coal ore, Jiangsu, China
A.thiooxidans	ZJJN-3	Sulfur oxidizer	28-30°C, pH 0-2.0	Leachate of Zijinshan Copper Mine, Fujian, China.

Table 2

The main characteristics of ore sample used in the study [a].

Parameter and unit	Value and description
Cu (%)	1.03 ± 0.05
S (%)	12.8 ± 0.21
Fe (%)	32.3 ± 0.53
Ca (%)	3.78 ± 0.32
Mg (%)	3.53 ± 0.25
Al (%)	1.66 ± 0.39
Zn (%)	0.051 ± 0.01
Mn (%)	0.044± 0.01
Ni (%)	0.029 ± 0.005
Pb (%)	0.028 ± 0.005
As (%)	0.0042 ± 0.001
Particle diameter (μm)	< 48[b]

[a] The ore sample was collected from the Dongguashan copper mine, Tongling, Anhui, China; the values of Ag, Au, Co, Cd and Hg were all below detection limitation (< 0.0002).
[b] The ore sample was ground and sieved through a 300-mesh grid, which controlled the particle diameter <48 μm.

Table 3

Comparison of key chemical and biological parameters between pre-leaching and after-leaching in DF, UA and AD systems.

	Parameter and unit	Pre-leaching	After-leaching					
			A. ferrooxidans			*A. thiooxidans*		
			DF	UA	AD	DF	UA	AD
Chem-indexes	pH	2.20	1.92	1.82	1.74	1.82	1.63	1.51
	ORP/mV	270	278	298	312	275	286	303
	Sulfate ion (g/L)	2.4/0.6	2.9	3.3	3.7	1.2	2.8	3.8
	Conversion ratio of sulfate ion %[a]	None	4.3	7.8	11.3	5.2	19.1	27.8
	Ferrous ion (mg/L)	None	162.5	312.5	356.3	65.4	75.3	94.5
	Conversion ratio of ferrous ion %[a]	None	1.7	3.2	3.7	0.7	0.8	1.0
	Ferric ion (mg/L)	None	432.2	564.5	675.3	82.0	123.2	137.8
	Conversion ratio of ferric ion %[a]	None	4.5	5.8	7.0	0.8	1.3	1.4
	Total iron (mg/L)	None	594.7	877.0	1031.6	147.4	198.5	232.3
	Conversion ratio of total iron %[a]	None	6.2	9.0	10.7	1.5	2.1	2.4
	Final copper ion (mg/L)	None	25.06	39.50	48.51	24.11	44.25	54.34
	Mineral color	Black	Tawny	Tawny	Tawny	Gray	Gray	Gray
	Mineral weight (g)	3.00	3.01	3.04	2.92	3.03	2.78	2.65
Bio-indexes	Free biomass (10^7 cells/mL)	5.0	17.9	21.6	27.0	8.3	11.8	15.8
	Attached biomass (10^7 cells/mL)	None	0.62	1.29	1.72	0.81	1.65	2.62
	Attached ratio (%)	None	3.34	5.97	6.37	8.90	12.27	14.22
	Total biomass (10^7 cells/mL)	5.0	18.51	22.89	28.72	9.11	13.45	18.42
	Daily productivity (10^7 cells/mL)	None	0.46	0.57	0.72	0.23	0.34	0.46

[a] It represents soluble ion (sulfate, ferrous, ferric, and total iron ion) in bioleaching system.

Figure captions

Fig. 1. Changes in key chemical parameters in different bioleaching systems. (A): *A. ferrooxidans*-DF; (B): *A. ferrooxidans*-UA; (C): *A. ferrooxidans*-AD; (D) *A. thiooxidans*-DF; (E) *A. thiooxidans*-UA; (F) *A. thiooxidans*-AD. (■) pH; (▲) Ferrous ion; (□) Ferric ion; (△) Sulfate ion.

Fig. 2. Morphological surface differences of the ore samples between different bioleaching systems. (A): *A. ferrooxidans*-DF; (B): *A. ferrooxidans*-UA; (C): *A. ferrooxidans*-AD; (D) *A. thiooxidans*-DF; (E) *A. thiooxidans*-UA; (F) *A. thiooxidans*-AD.

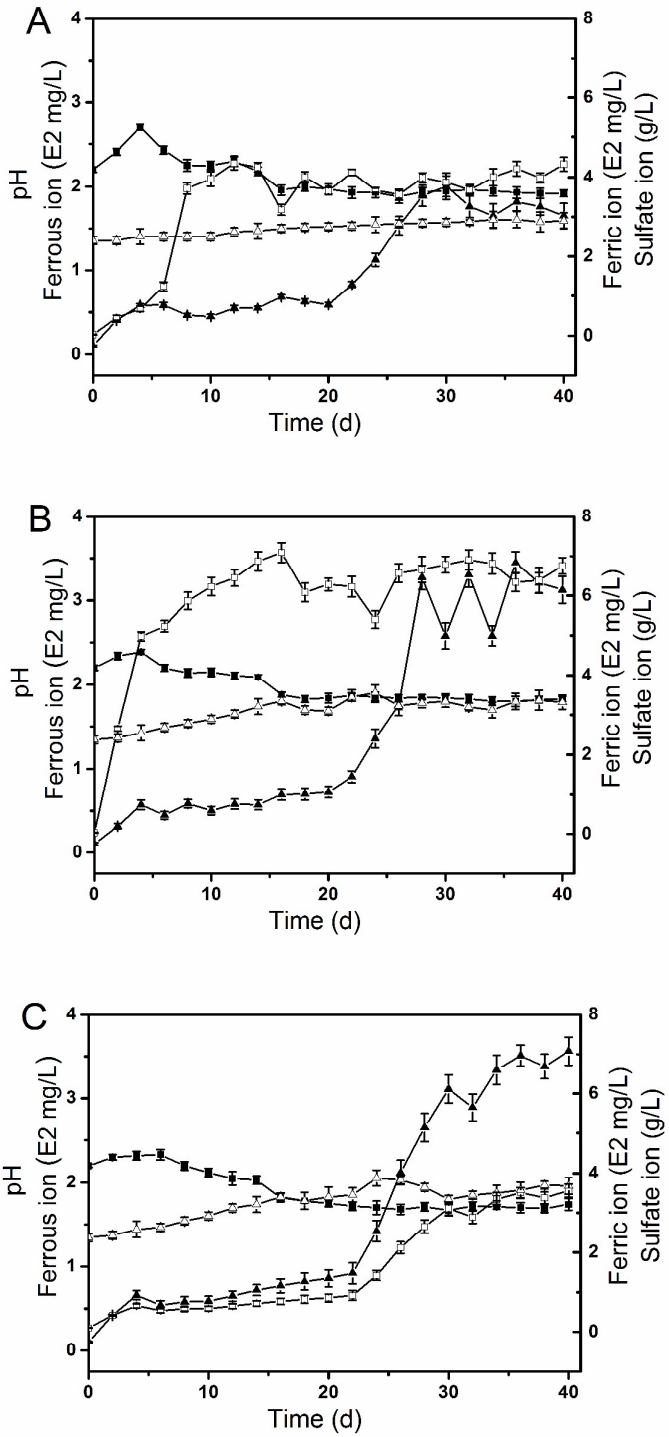
Fig. 3. XRD analysis of the ore samples in different bioleaching systems. (A): *A. ferrooxidans*-DF; (B): *A. ferrooxidans*-UA; (C): *A. ferrooxidans*-AD; (D) *A. thiooxidans*-DF; (E) *A. thiooxidans*-UA; (F) *A. thiooxidans*-AD. (■) Fe_3O_4; (▲) FeS_2; (●) $CuFeS_2$; (□) $CaSO_4 \cdot 2H_2O$; (△) $KFe_3(SO_4)_2(OH)_6$; (○) Fe_7S_8 (★) S.

Fig. 4. The highest biomass and final recovery efficiencies of copper ion in different systems. (A) The highest free and attached biomass; (B) The final recovery efficiency of copper ion. a-c and A-C represent the statistically significant differences (c > b > a; C > B > A). The recovery efficiency of *A. ferrooxidans*-DF or *A. thiooxidans*-DF system was selected as the standard and defined as 100%. The relative recovery efficiency of *A. ferrooxidans*-DF/AD system and *A. thiooxidans*-DF/AD system was calculated by dividing the recovery efficiency of *A. ferrooxidans*-DF or *A. thiooxidans*-DF system.

Fig. 5. Overall effects of the adsorption behavior in bioleaching of copper-bearing sulfide ore (chalcopyrite as example).

Figures

Fig. 1.



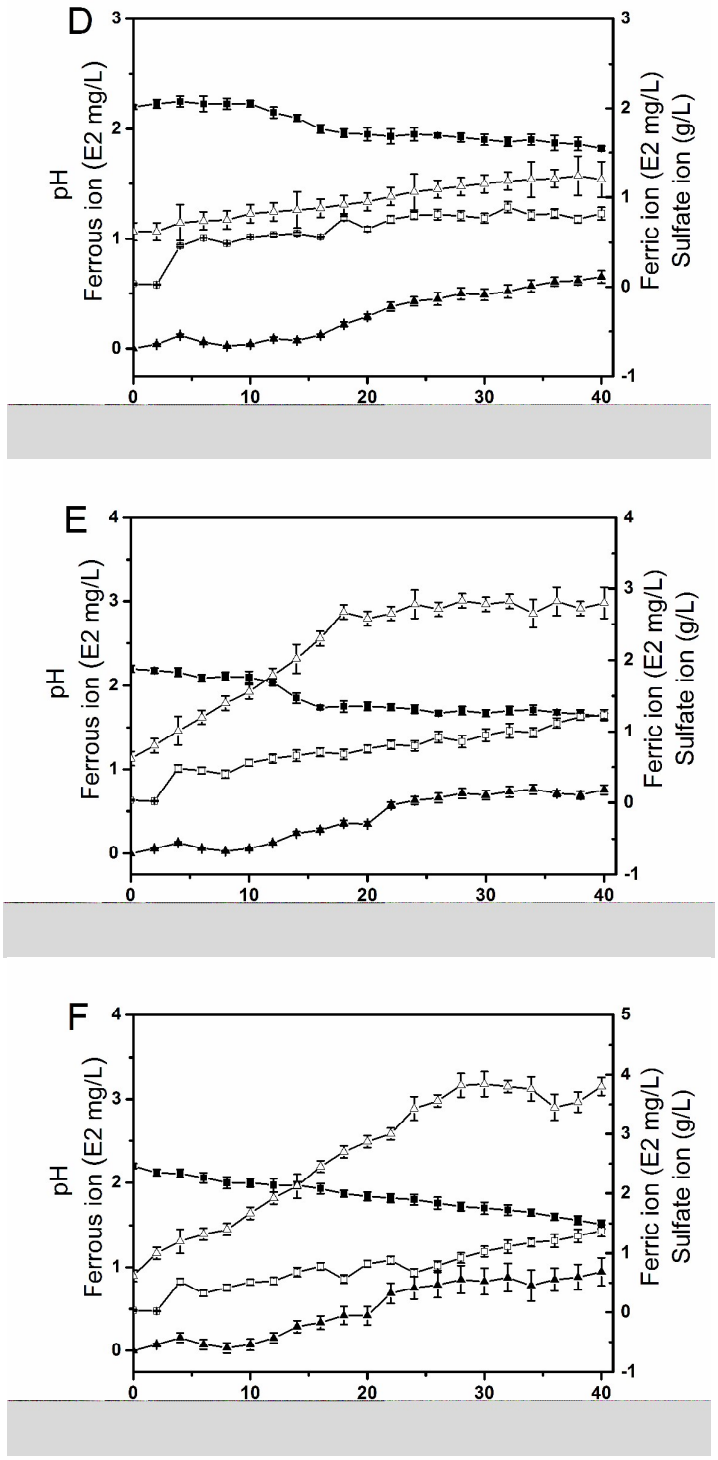


Fig. 2.

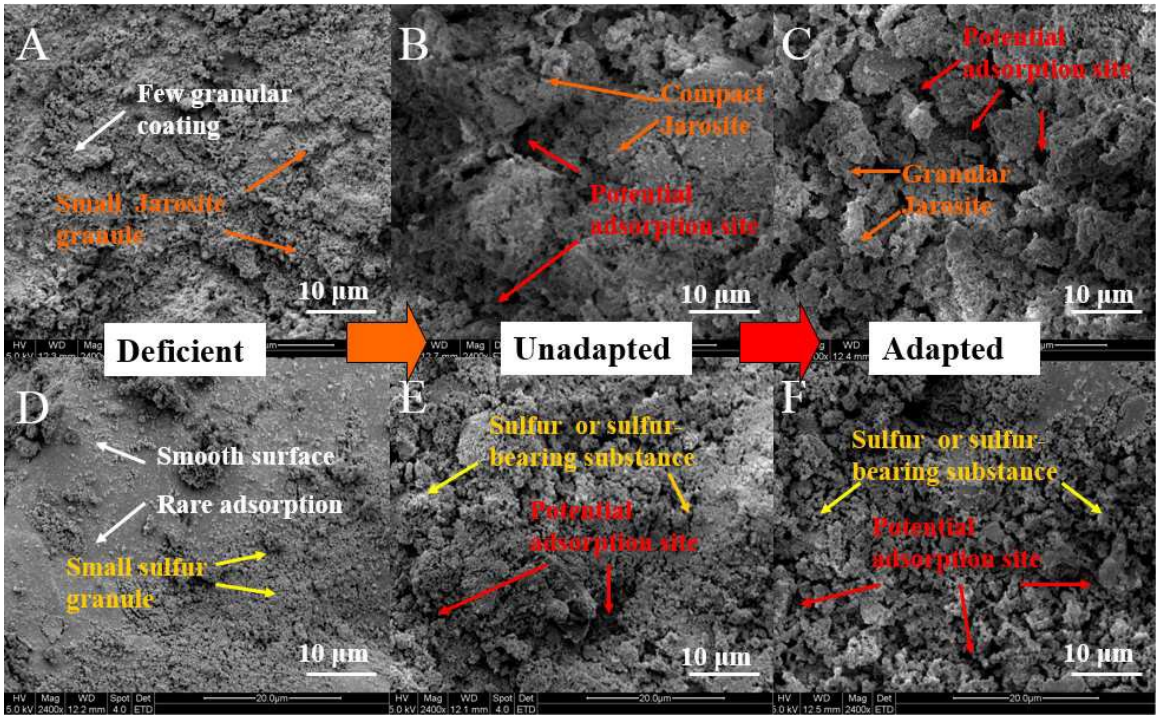
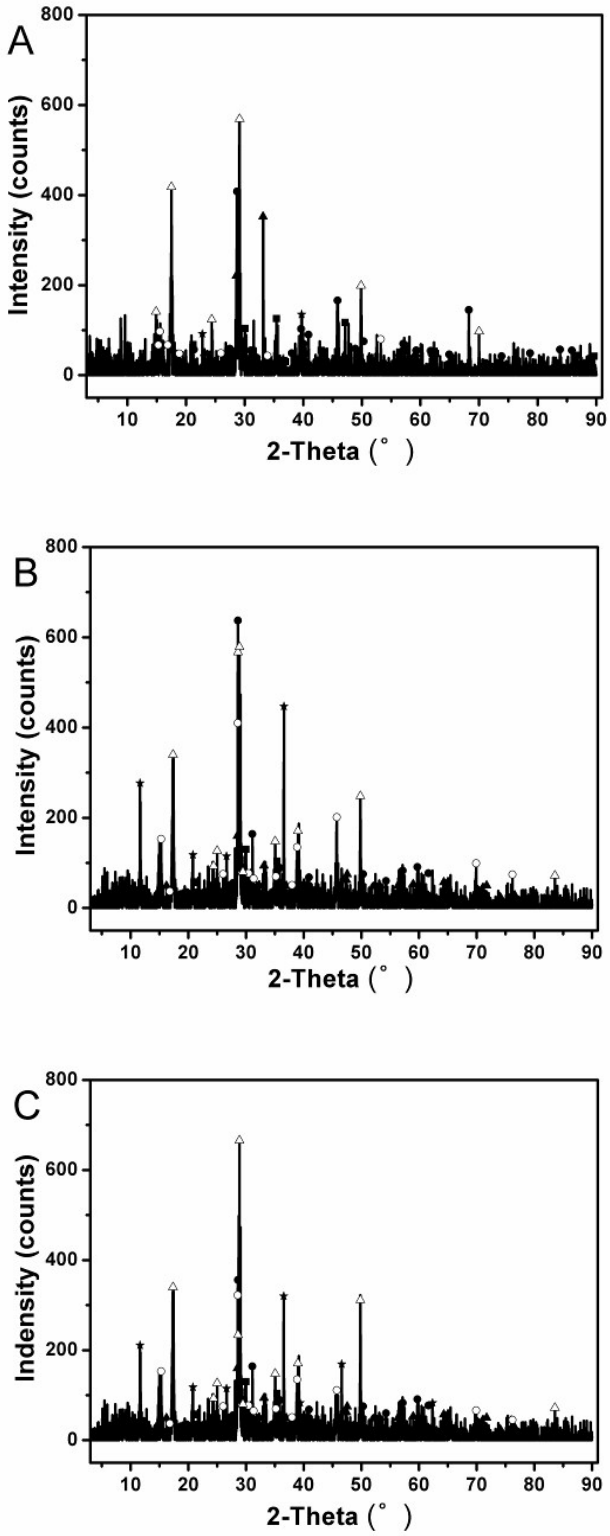
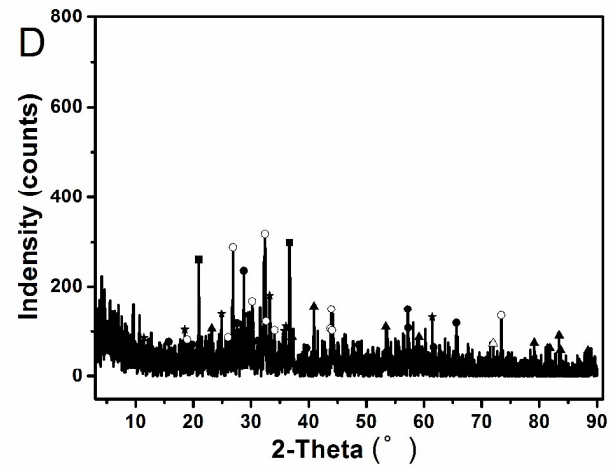
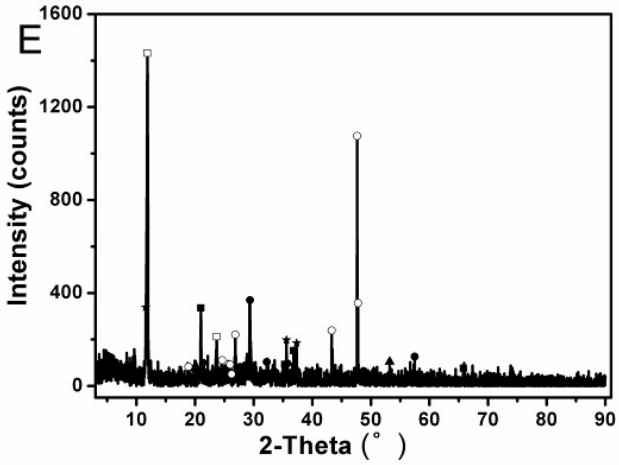


Fig. 3.







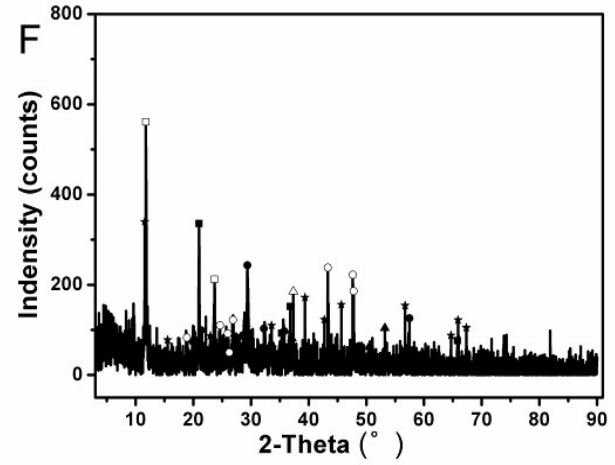


Fig. 4.

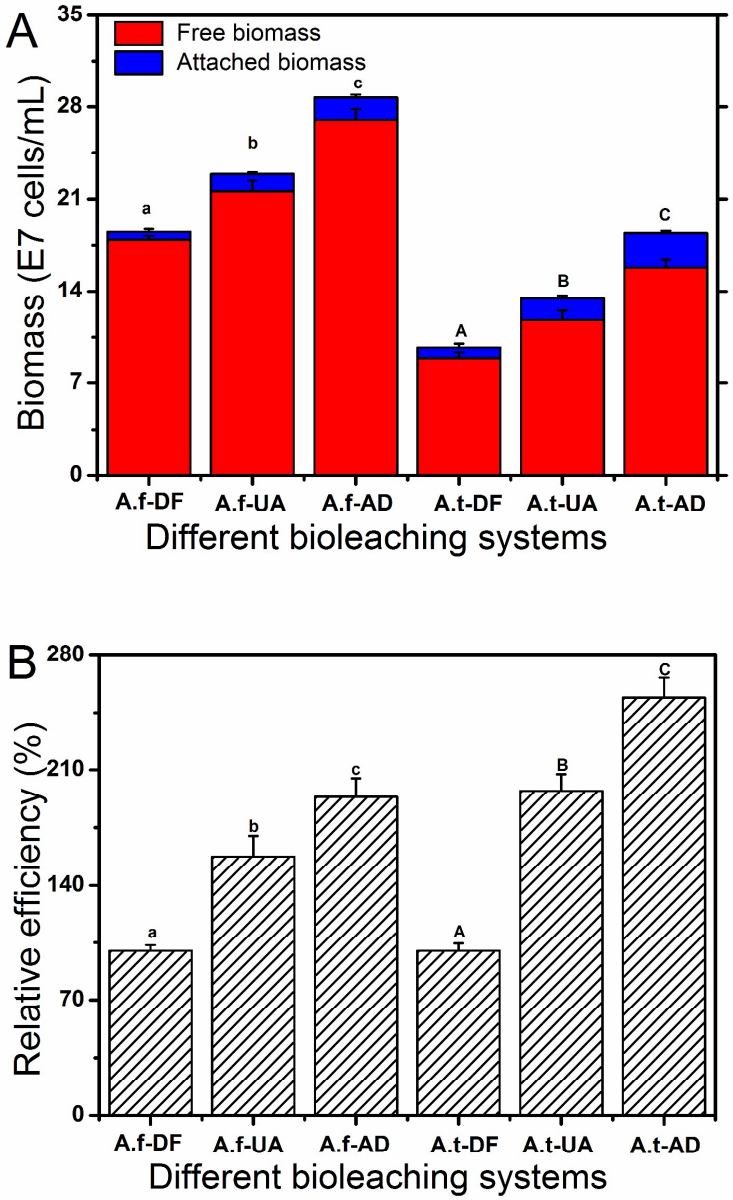
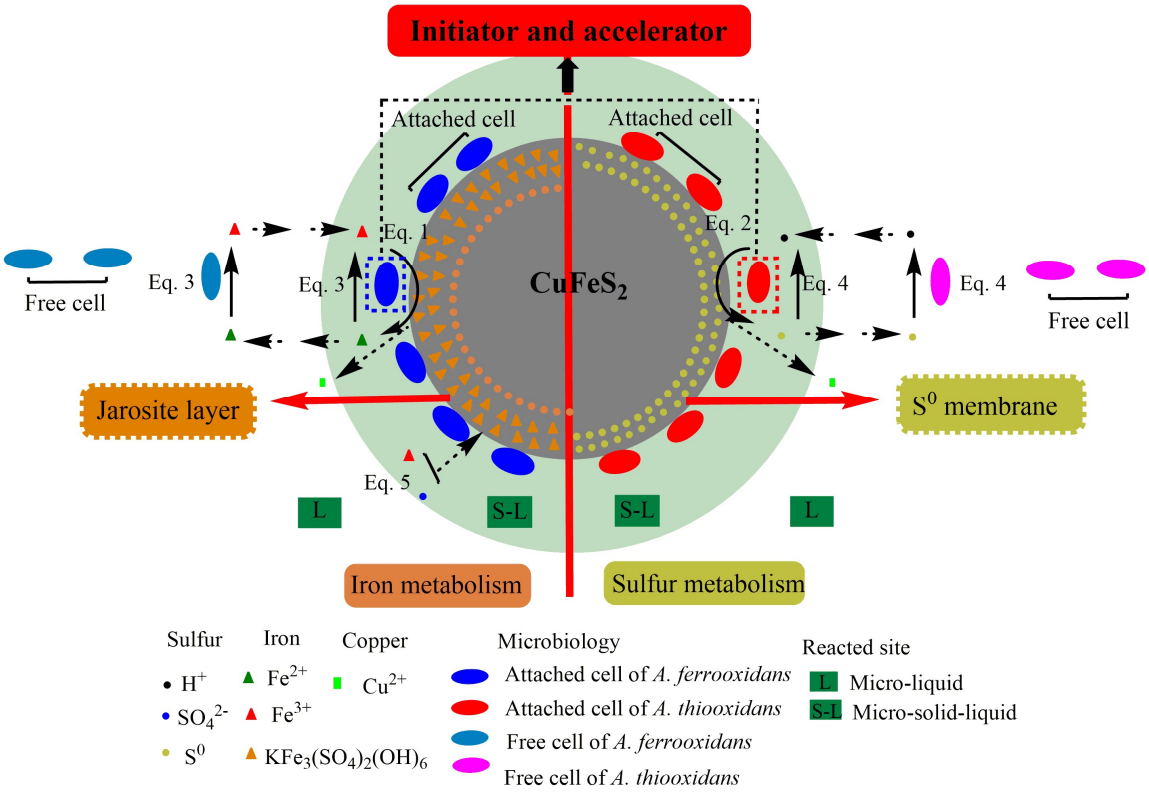


Fig. 5.



Graphical abstract