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Invited Review

Research progress in enhanced bioleaching of copper sulfides under the intervention of microbial communities

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Abstract: Compared with the traditional pyrometallurgical process, copper bioleaching has distinctive advantages of high efficiency and lower cost, enabling efficiently extracts of valuable metal resources from copper sulfides. Moreover, during long-term industrial applications of bioleaching, many regulatory enhancements and technological methods are used to accelerate the interfacial reactions. With advances in microbial genetic and sequencing technologies, bacterial communities and their mechanisms in bioleaching systems have been revealed gradually. The bacterial proliferation and dissolution of sulfide ores by a bacterial community depends on the pH, temperature, oxygen, reaction product regulation, additives, and passivation substances, among other factors. The internal relationship among the influencing factors and the succession of microorganism diversity are discussed and reviewed in this paper. This paper is intended to provide a good reference for studies related to enhanced bioleaching.

Keywords: bioleaching; copper sulfide; heap leaching; microbial community succession; enhanced mechanism

1. Introduction

Copper, which is one of the most important metals, is widely used in the aerospace, construction, military, electronic communications, and other fields. However, complex and difficult-to-treat primary copper sulfide ore accounts for a large proportion of the total copper ore reserves in China [1–2]. This situation has led to higher costs and technological requirements when traditional mineral processing and pyrometallurgical methods are applied to this ore [3–4]. Specifically, the copper sulfide is mainly divided into primary copper sulfide ore and secondary copper sulfide ore, which includes other associated minerals. The copper sulfide contains minerals such as chalcopyrite, chalcocite, and enargite; thus, the leaching reactions are extremely complicated. Since the 1950s, bioleaching technology has been proposed with the development of acidic bacteria like *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans* [5]. As Eqs. (1) and (2) show, the bacteria oxidize ferrous and sulfur species in the copper sulfide bioleaching process:

$$4FeSO_4 + 2O_2 + 2H_2SO_4 \xrightarrow{A.f\ bacteria} 2Fe_2(SO_4)_3 + 2H_2O \tag{1}$$

$$2S + 3O_2 + 2H_2O \xrightarrow{T.t\ bacteria} 2H_2SO_4 \tag{2}$$

The metal minerals are dissolved, and valuable ions are released into the solution.

Because of the advantages of copper bioleaching (e.g., environmentally friendliness and low cost), it is commonly used to extract valuable metals from waste or low-grade ores [6]. Currently, copper bioleaching accounts for more than 30% of the worldwide production of cathode copper. Unlike the copper oxide and ammonia leaching process, which is based on chemical acid/ammonia leaching [7–8], bioleaching with bacteria is simultaneously influenced by coupled and more complicated conditions. The basic scheme of a bioleaching process such as heap bioleaching is drawn in Fig. 1. The bioleaching process of copper sulfide heap is conducted under atmospheric exposure and

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involves multiple industrial procedures, multi-phase media, and multiphysics, which provides an opportunity for researchers to intervene in and intensity the bioleaching process.

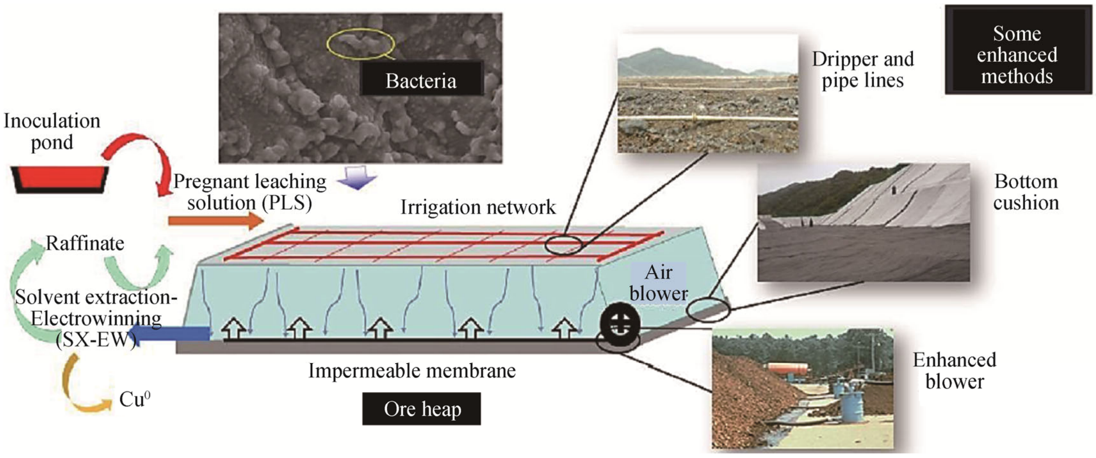

Fig. 1. Schematic of copper bioleaching and extraction via heap leaching.

Although copper bioleaching has been successfully applied in China, the United States, Chile, and Canada, such as at the Zijinshan Copper Mine, the Lince-Michilla mine, and the Bingham mine [9–11], numerous technological obstacles and variables remain existed, such as pH, selection of microorganism, temperature, and particle size distribution, among other factors [12]. The choice of microbial community is regarded as one of the essential factors that influences the copper extraction efficiency [13–14]. For instance, bacterial activity will decrease and the bacteria will even die under low-pH conditions [15]; in addition, the originally connected pore structure inside the ore heap will tend to recombine and close with the formation of passivation substances and the reactant ratio between ferrous and sulfide ions will influence the reaction kinetics [16]. Microorganisms are commonly separated from *in situ* acid mine drainage (AMD) and need to proliferate in the leachate solution. As a result, the aforementioned environmental factors are closely related to the biological activity. Accompanying the development of genetics testing and targeted DNA identification methods, some enhanced bioleaching methods related to the bacterial community have been presented gradually.

In this paper, some enhanced bioleaching methods with interventions of microbial communities are reviewed. Some technological breakthroughs related to community identification, temperature, pH, heap structure, hydrology, flow behavior, forced aeration, passivation, reactant intervention, and additives are presented and reviewed systematically. Future challenges and prospects based on current status are also discussed. We regard this paper as a basic reference intended to stimulate research on copper bioleaching enhancement methods, especially research related to microbial community regulation.

2. Research promotion and breakthroughs of enhanced bioleaching

2.1. Identification and mutagenesis of dominant bacterial community

Although the utilization of AMD to leach copper sulfide ore has thousands of years of history, the real separation, acclimation, and recognition of leaching bacteria communities from AMD was first presented by Colmer *et al.* in the 1950s [17]. Some acid bioleaching microorganisms and their diversity characteristics [18] are shown in Fig. 2. Commercial heap practices were successfully developed; studies of bacterial successions gradually started after this period [19].

Through 16S rRNA, de novo, atomic force microscopy, and other testing scanning methods, the complicated internal microbial composition of AMD has been gradually revealed. Types of leaching bacteria, the internal microorganism community, and its mechanism were not known until these *in situ* bacterial detection and sequencing technologies were developed [20]. These technological innovations enabled genetic-level studies of microbial communities and their diversity. In addition to the indigenous bacteria of ore heaps, some artificial exogenous microorganisms have been added to leaching solutions [21]. Even some nonmetallic leaching bacteria [22] have been utilized, and some of them have presented good synergistic effects [23] that led to enhanced copper extraction.

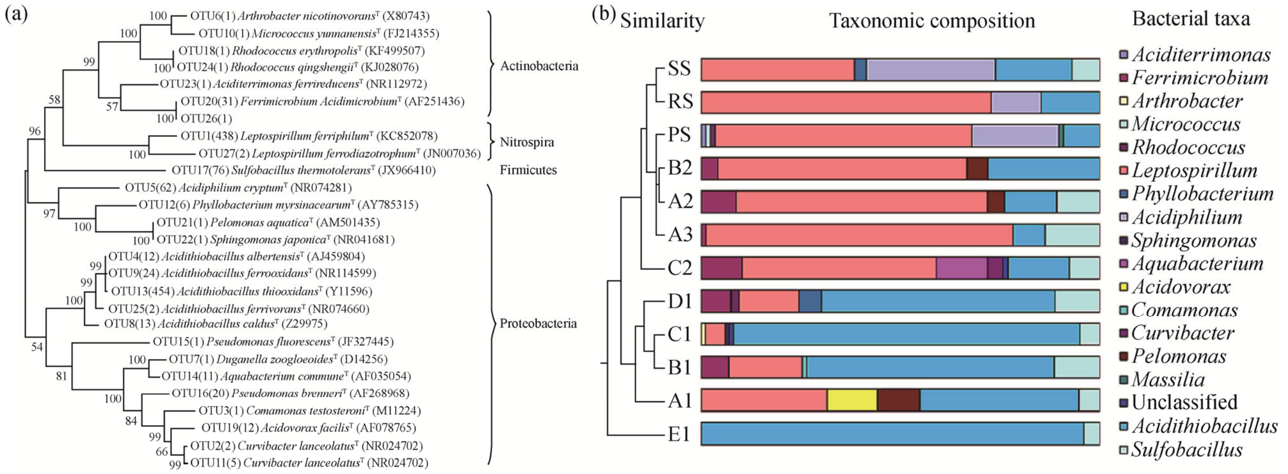

Fig. 2. Biodiversity and conservation of the microbial community structure in Zijinshan Copper Mine: (a) Phylogenetic affiliation of 16S rRNA gene sequences; (b) Samples similarity tree and samples community composition bar graph. Reprinted from *Minerals Engineering*, 94, X.Y. Liu, B.W. Chen, J.H. Chen, M.J. Zhang, J.K. Wen, D.Z. Wang, and R.M. Ruan, Spatial variation of microbial community structure in the Zijinshan commercial copper heap bioleaching plant, 76-82, Copyright 2016, with permission from Elsevier.

Accompanying the synergistic bioleaching of multiple microbes, the bacterial consortium inside AMD has strong potential to extract the valuable metals [24–25], and this community includes varieties of bacteria species such as *Acidithiobacillus thiooxidans Licanantay*, *Acidiphilium multivorum Yenapatur*, and *Leptospirillum ferriphilum Pañiwe*. Thus, the mixed bacterial population includes both dominant bacteria and vulnerable bacteria. This difference in population and the continuous succession of dominant bacteria are important causes of the difference in the final extraction rates. Thus, species interaction and diversity shifts have been discussed recently [26–28]. For instance, sulfur oxidizers can achieve higher copper extraction rates than ferrous oxidizers under different initial proportions of mixed microorganisms [29]. In addition, some new microorganism strains have been detected in neutral and slightly alkaline leaching conditions ($6 \leq pH \leq 8$) of low-grade copper sulfides [30–31]. On the basis of the energy source, these processes can be divided into obligate chemolithoautotrophs, facultative chemolithoautotrophs, chemolithoheterotrophs (mixotrophs), and phototrophs. Among them, *Thiobacillus*, *Halothiobacillus*, *Thermithiobacillus*, *Thiomonasand*, and *Starkeya* are regarded as dominant sulfur-oxidizing strains under alkaline leaching conditions [32–35]. However, subject to the effects of the external environment, the extraction efficiency of mixed microorganisms tends to be lower, especially in industrial heaps [36], and the dominant microbial community under the mixed culture shifts frequently.

Currently, petals or Venn figures are used for the differential identification of microbial population structures to determine the shared genetic groups after leaching [37]. To closely distinguish the roles of different bacteria species during the bioleaching of copper sulfides, free microorganisms and attached microorganisms [38] have been studied. According to current research, even though the proportion of free bacteria is large, the attached bacteria could be the key factor in the interfacial reaction [39–40], which directly affects copper leaching. Moreover, the comparative metagenomics [41] and some novel sequencing technologies such as high-throughput/resolution chromosome conformation capture (Hi-C) have been discussed as efficacious approaches to determining the structural mechanisms of chromosomes during the bioleaching process [42] and to identifying the genetics through structural differentiations. Gene compilation will be a prominent research field for bacterial cultivation and effective leaching, such as implantation of highly environmentally adaptive genes via targeted compilation.

2.2. pH stress and its enhanced mechanisms

In the current operation of industrial bioleaching heaps, whether a copper oxide ore heap or secondary sulfide heap, acid leaching is the main method and the pH of the solution is typically below 3.0 during the leaching process. The pH is constantly and spontaneously changing with the bioleaching reactions, and the external modification of pH affects the bacterial succession, sulfide bioleaching kinetics, amounts of ferrous and copper ions, ratio of ferric and total iron, the oxidation–reduction potential (ORP) value, and other key parameters [43]. The pH is adjusted to enhance the bioleaching progress of moderate thermophiles. The effects of different pH [44] on the leaching parameters are shown in Fig. 3. The effect of pH on the leaching process is substantial when pH is between 1.25 and 2.5. Specifically, a lower pH value (1.25) tends to promote the ferrous oxide oxidation process and increase the amount of copper ions in the solution. The oxidation–reduction potential gradually increases with increasing pH value.

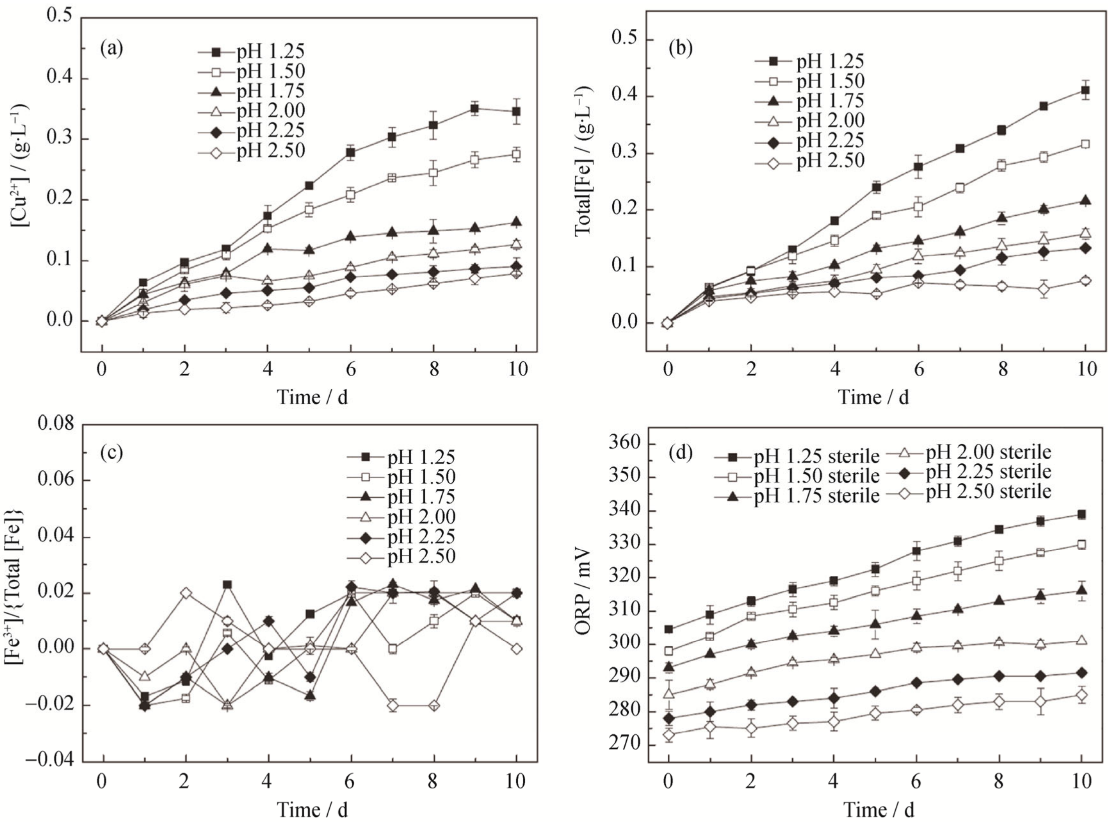

Fig. 3. Bioleaching parameters of chalcopyrite in the sterile control at a constant pH of 1.25, 1.50, 1.75, 2.00, 2.25, and 2.50: (a) [Cu^{2+}]; (b) Total [Fe]; (c) [Fe^{3+}]/{Total [Fe]}; (d) ORP value. Reprinted from *Minerals Engineering*, 98, H.C. Liu, J.L. Xia, Z.Y. Nie, C.Y. Ma, L. Zheng, C.H. Hong, Y.D. Zhao, and W. Wen, Bioleaching of chalcopyrite by *Acidianus manzaensis* under different constant pH, 80-89, Copyright 2016, with permission from Elsevier.

The zeta potential and isoelectric point are closely related to pH and, importantly, depend on the mineral electrical surface and the oxidation sequence of the reaction. As pH increases, the zeta potential of the bacteria gradually decreases. When pH is lower than the isoelectric point, the surface of the bacteria is positively charged. When pH is greater than the isoelectric point, the surface of the bacteria is negatively charged. After bacteria contact the surface of the copper sulfide ore, the zeta potential of the ore is substantially reduced [45]. In addition, pH strongly affects bacteria adsorption, the leaching reaction process, and the progression of sulfur oxidation [46]. The most typical of these processes is chalcopyrite bioleaching using *Acidithiobacillus ferrooxidans*. pH controls the secretions of the polysaccharide and extracellular protein substances [47]. Because of the alkaline passivation substances and a decrease in the amount of ferrous iron [48], a higher pH adversely affects bacteria proliferation and subsequent copper leaching. In addition, according to the results of real-time polymerase chain reaction (qRT-PCR) testing, excessive pH stimulation reduces the leaching activity of bacteria, especially free bacteria in the pregnant leaching solution (PLS), and decreases the copper extraction rate from 87.5% (pH 2.0) to 64.0% (pH 2.0) [49]. The initial pH affects the diversity of the microbial community [50], and relevant research [51–52] has revealed that a lower pH value and higher mineral content can accelerate the complexity of the bacterial community during chalcocite bioleaching. Denaturing gradient gel electrophoresis has been used to analyze the differences in the initial pH value. Under different pH values, the number of copper ions adsorbed by leaching bacteria were observed to first decrease and subsequently increase. Specifically, the number of adsorbed copper ions is small at pH 1.5 and 2.0; conversely, the amount of adsorbed copper ions is higher at pH 1.0 during chalcopyrite bioleaching [53].

2.3. Heap structure and its quantitative characterization

The heap structure strongly influenced the microbial community succession and the leaching efficiency, and it directly affects the solution flow behavior, which dominates the distribution and successions of bacterial communities inside the heaps [54]. Because of differences in particle size and pretreatment methods of ores, the porosity and permeability of the initial heaps differ. Consequently, bacterial community succession and leaching efficiency are difficult to predict. In this regard, Jia *et al.* [55] revealed the relationship between the heap structure and the bacterial community.

Currently, the sampling and detection of PLS separated from different areas inside ore heaps is widely used to reveal the quantitative characteristics of the microbial community at different ore locations and pore structures. For instance, as shown in Fig. 4, Hao *et al.* [56] used three dumping methods (layered dumping, agglomerated dumping, and pelletized sintering dumping) to form three types of pore structures, which revealed variations in the composition of the bacterial community under different pore structures during 83 days of leaching. The composition of bacterial communities was similar under different pore sizes; however, the specific proportion of bacterial species differed substantially, as shown in Fig. 4(b). These differences in the microbial community proportion are one of the main causes of different biochemical reaction processes under different pore structures and locations inside heaps [57].

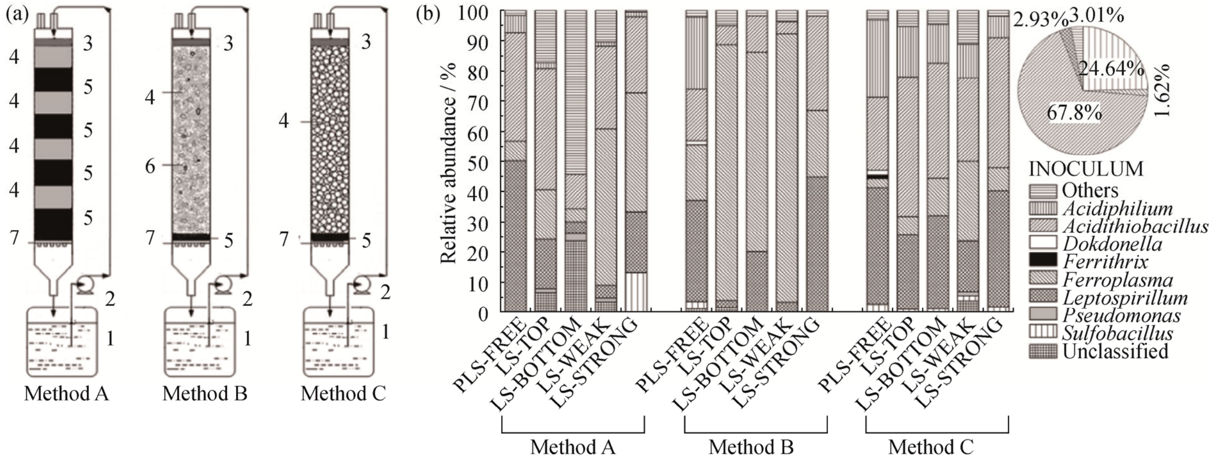

Fig. 4. Effect of heap structure on the microbial community structures: (a) different heap construction and structure; (b) successions of microbial communities under different dumping methods. 1—glass container; 2—peristaltic pump; 3—sterile sponge; 4—flotation tailings; 5—quartz sand; 6—Yulong mineral; 7—support plate. Reprinted from *Minerals Engineering*, 98, X.D. Hao, Y.L. Liang, H.Q. Yin, L.Y. Ma, Y.H. Xiao, Y.Z. Liu, G.Z. Qiu, and X.D. Liu, The effect of potential heap construction methods on column bioleaching of copper flotation tailings containing high levels of fines by mixed cultures, 279-285, Copyright 2016, with permission from Elsevier.

Moreover, a heap structure can be characterized through quantitative or qualitative characterization of the pores between adjacent ores and of the fractures on the ore surface. Micro-computed tomography (μCT), X-ray computed tomography (CT), Ultraviolet (UV) fluorescence, and other noninvasive techniques [58–60] have been used to obtained images of the initial pore structures. For instance, Lin *et al.* [61] used the watershed algorithm and the lattice Boltzmann algorithm in MATLAB to accurately extract the pore structure and obtain a physical model of a heap. By comparing the pore structure characteristics with the microbial community succession, they characterized the effects of the heap structure.

2.4. Hydrology and microbial colonization

The leaching solution is an important medium for the biochemical reaction, mass transfer, heat transfer, soluble oxygen concentration, free bacterial migration, and ion exchange. Poor solution flow behavior adversely affects the ore leaching process and metal-ion extraction, and these negative effects such as preferential flow [62–64] have been observed in bioleaching dumps in Dexing Copper Mine. Therefore, the percolation process plays an important role in improving the leaching efficiency. Especially for the heap bioleaching systems of copper sulfides, the stagnant liquid and the preferential flow coexist and form complex unsaturated flow behavior through the particles in the heap [65–66]. As a result, the hydrology characteristics play an important role in microbial colonization and in the extraction efficiency. Current research reveals that preferential flow commonly occurs when solution flows through the interface between ore particles of different size [67–68]. When the liquid preferentially flows to the interchange regions of the coarse–fine ore particles, the solution is easily split and laterally moved and a large number of flow channels are generated in the fine ore layer. At the interface of fine–coarse ore particles, many small tributaries converge, re-form the solution preferential flow, and pass down.

If the microorganisms evenly reach the reaction interface of the ore in the heap, the sulfide ores can be leached more efficiently. Improved heap hydrodynamics techniques, such as lower irrigation rates and multiple-lift and thin-lift irrigation, have been proposed [69–70]. Heap liquid addition affects the liquid holdup value [71–72] and the bacterial concentration inside heaps; it is also regarded as one of the important parameters regulating the bioleaching process. The liquid holdup in different areas of heaps is substantially uneven. Chiume *et al.* [73] revealed that an excessive irrigation rate leads to a high liquid holdup and high free bacterial concentration but less adsorbed bacteria inside the leaching solution; they also found that a low irrigation rate favors adsorption of more bacteria. To reduce the stagnant liquid regions where inefficient metal leaching occurs, the acid solution containing bacterial community can be injected into the inner part of the heap via the side of the heap. With artificial intervention of the solution flow paths, the preferential flow paths are effectively broken and the bioleaching of stagnant regions can be reduced [74–75]. Fig. 5 shows that the distribution of the solution and microorganisms inside the heap are uneven and unstable [76]. Moreover, Fig. 5(b) shows that the amount of solution is positively correlated with the bacterial concentration. In Fig. 5(b), the liquid holdup of the bacteria solution in the central portion of the heap increases from 8.27% to 19.19%. As shown in Fig. 5(c) shows, a comparison of ports reveals a preferential flow channel for the bacteria community inside heaps.

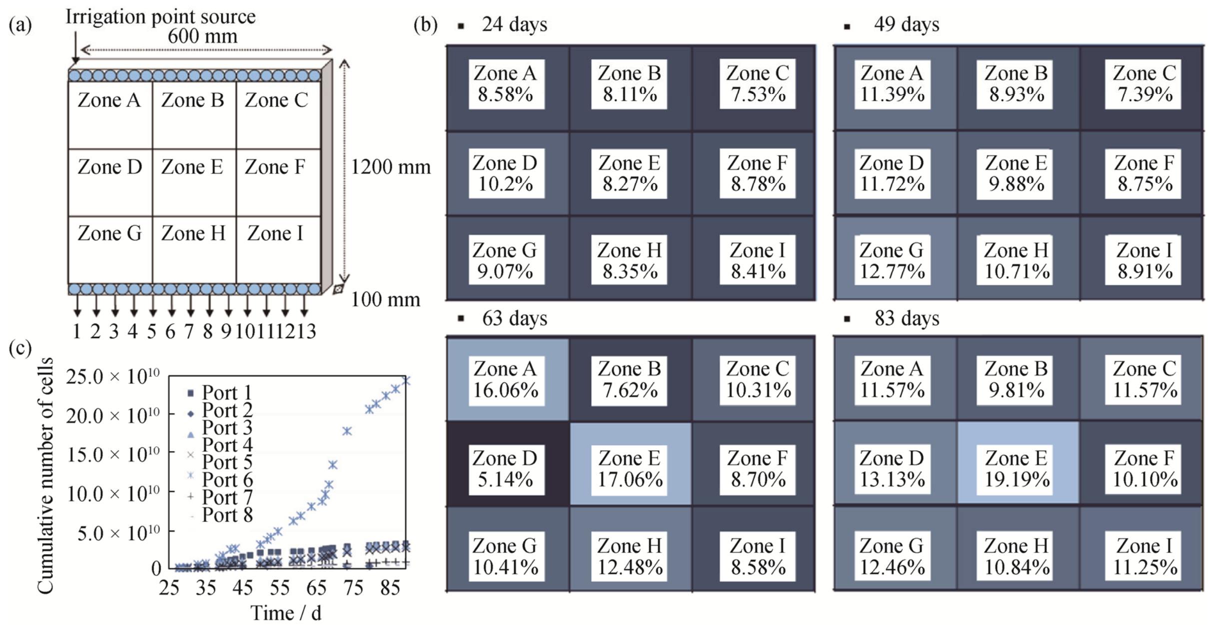

Fig. 5. Effects of hydrology on microorganism propagation: (a) schematic of the "ore-slice" box reactor; (b) moisture content (expressed as a weight percentage) in the different zones of the box reactor; (c) cumulative number of cells exported in the leachate passing through the outlet ports. Reprinted from *Hydrometallurgy*, 150, M.A. Fagan, I.E. Ngoma, R.A. Chiume, S. Minnaar, A.J. Sederman, M.L. Johns, and S.T.L. Harrison, MRI and gravimetric studies of hydrology in drip irrigated heaps and its effect on the propagation of bioleaching micro-organisms, 210-221, Copyright 2014, with permission from Elsevier.

2.5. Reactants controlling of biochemical reactions

Microbial proliferation promotes the dissolution of minerals and also produces a large number of side-reaction products, which are usually colloidal and re-react with gangue minerals to form passivation layers such as jarosite, sulfur membrane, calcium sulfate, or polysulfide substances [77]. These porous passivation materials on the ore surface provide spaces where the reactant was attached at the beginning of the leaching reaction. However, the acid-resistant hard shell that formed in the later stage blocks the solution from reaching the ore reaction interface, which leads to a slower chemical reaction [78]. In this regard, the issue of how to control the chemical composition and formation time of the passivation layers during the bioleaching process has become an important research area to enhance the bioleaching process.

Thus, some current studies [79–81] have focused on the composition, proportion of reactant substances, and the dissolution process of sulfide minerals, especially for chalcopyrite and pyrite. The phase transformation process and speciation of copper, iron, sulfur, and polysulfide have been studied using X-ray photoelectron spectroscopy (XPS) and electrochemical analysis [82]. Specifically, for ion oxidizers and sulfur oxidizers enriched during the chalcopyrite bioleaching, Ma *et al.* [83] studied mechanisms of enhancing these two oxidizers to improve the bioleaching efficiency. Their results show that sulfur oxidizer *Acidithiobacillus thiooxidans* presented as the dominant bacteria inside the sulfur-enriched communities; thus, the intervention of sulfur-enriched biomes can effectively enhance the chalcopyrite leaching process and this enhancement is more significant than using the iron-biomes. Huang *et al.* [84] presented a model of copper sulfide bioleaching under external iron and sulfur intervention. Their results, which are shown in Fig. 6, obviously reveal that sulfur copper ore leaching is a complex biochemical reaction system in which multivalent species coexist. Additionally, studies of the bioleaching kinetics of copper sulfides have been widely carried out. Ahmadi *et al.* [85] presented a kinetic model under electrochemically controlled conditions, and Davis-Beimar *et al.* [86] used chloride-tolerant microorganisms to study the kinetics of copper sulfides with the presence of chloride.

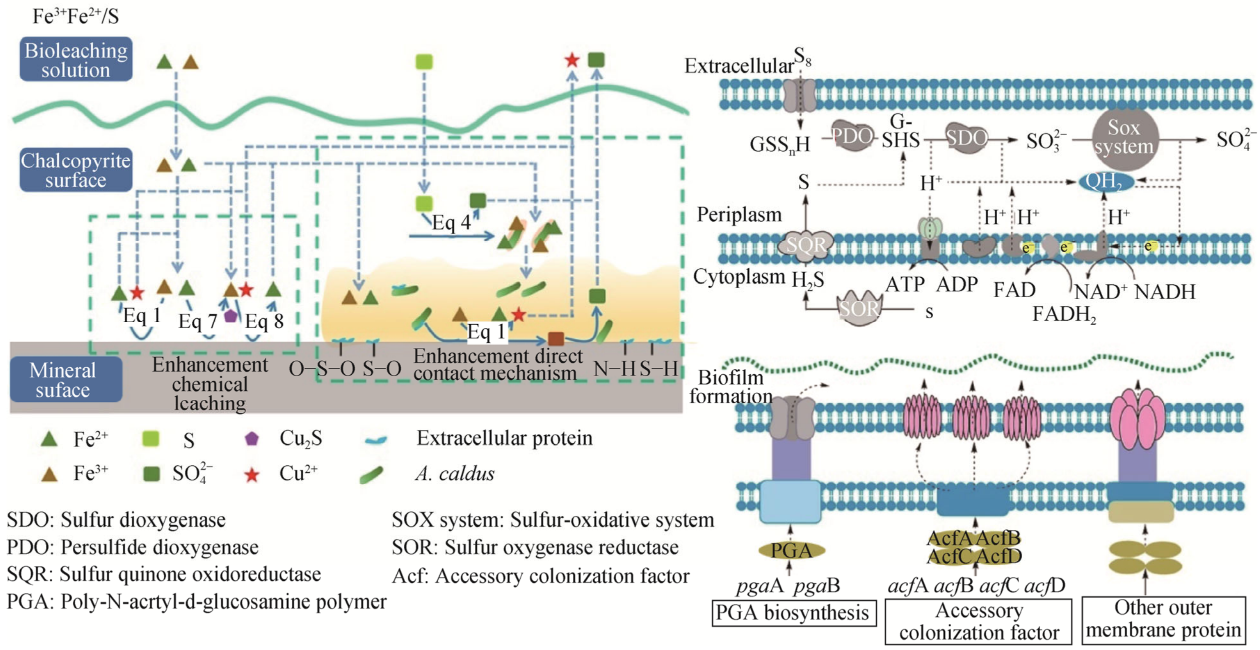

Fig. 6. Model of copper sulfide bioleaching under external iron and sulfur intervention. Reprinted from *Journal of Environmental Management*, 242, Z.Z. Huang, S.S. Feng, Y.J. Tong, and H.L. Yang, Enhanced “contact mechanism” for interaction of extracellular polymeric substances with low-grade copper-bearing sulfide ore in bioleaching by moderately thermophilic *Acidithiobacillus caldus*, 11-21, Copyright 2019, with permission from Elsevier.

The effect of energy sources on the adhesions of bacterial cells have also been investigated [87]. Additionally, some medium thermophiles and extreme thermophiles have been coupled to attain greater extraction efficiency, especially for complicated reactants [88]. Microbial derivatives are produced during the leaching process [89], and these proton-promoted derived metabolites or ligand-promoted derived metabolites enhanced the bioleaching process [90]. Complexed metals such as siderophores are inserted as a soluble organic-complex. In addition, this moderation process and control are also considered an effective method to avoid environmental pollution near industrial bioleaching areas [91], especially for abandoned acidic leaching solutions.

2.6. Temperature regulation during bioleaching process

The largest difference between bioleaching and chemical leaching is the involvement of microorganisms, and temperature directly affects the survival of bacteria in heaps. The most difficult factor to control in industrial bioleaching is the temperature of the heap [92]. The temperature distribution in an ore heap is affected by the natural air flow at the surface of the heap. The bioleaching reaction produces heat, which causes the initial temperature of the heap to exceed the surface. Bacterial growth is well known to require a suitable temperature; for example, the optimum temperature for proliferation of *Thiobacillus ferrooxidans* is 30 ± 2°C. The temperature range inside a heap varies dramatically [93], and the temperatures in the internal regions of a heap are too high because of difficulty in heat dissipation [94].

Researchers have found that, in general, the copper extraction of a chalcocite heap reaches approximately 80% (28 months, 25°C) [95]. This temperature differs from that inside ore heaps, which ranges from 30 to 65°C; thus, copper extraction and microbial diversity inside ore heaps will be different. Moderate thermophiles rather than extreme thermophiles dominate the communities at 65°C, which can obviously promote the second stage of copper sulfide bioleaching [96]. Indoor column experiments that the microbial diversity under the effect of different temperatures have been studied. Disentangling effects of temperature during bioleaching of low-grade copper sulfides have been discussed [97], and the relevant findings have inferred that the microbial community changes at different temperatures [98]. A higher temperature and greater microbial alpha of the initial bioleaching period lead to greater copper extraction. Thus, to adapt bioleaching to some extreme environmental temperature conditions, extremely thermophilic bacteria (70–80°C) and cold-tolerant bacteria (10°C or below) have been separated and gradually studied [99]. For medium thermophilic bacteria [100], increasing the temperature in the stirred reactor to approximately 42–50°C can enhance dissolution of chalcopyrite and reduce the redox potential of the PLS, enhancing the copper extraction rate from to 86% by 97%. In addition, in a moderate temperature environment, increasing the ambient temperature after the end of the bacterial growth period can enhance the leaching of the chalcopyrite and delay the formation of the passivation layer, which is beneficial to achieving a better leaching effect [101]. In summary, the heap temperature influences the bioleaching process in two different ways: it promotes the activity of bioleaching microorganisms and adjusts the composition ratio of bacterial species during bioleaching.

2.7. Forced aeration and its mechanisms

Forced aeration is mainly used to increase the oxygen concentration inside the leaching reaction system, and oxygen is an important solute involved in the microbial-assisted leaching reaction. In the 1920s, the earliest industrial application of forced aeration occurred at Bingham Canyon, Utah [102]. The commonly used leaching bacteria are aerobic microorganisms such as *Thiobacillus ferrooxidans*; thus, the concentration of dissolved oxygen in the PLS is an important indicator affecting the extraction rate [103–104]. Most importantly, the use of the forced aeration process enhances electrochemical reactions at the interacted surface of ores and solution [105] and accelerates the dissolution of ore minerals during bioleaching.

Because of the low oxygen concentration in the leaching solution, forced aeration from the bottom of the ore heap has been a successful practice for enhancing copper recovery (Fig. 1). For instance, an aerated heap achieved 80% copper recovery in 275 days, which is substantially greater than the 40% recovery from heaps without aeration in the same time period [106]. Forced aeration promotes microbial community succession and changes in the flow path inside the heap due to adjacent particle adhesions [107]. The original ore particles are redistributed, and the preferential flow channels are disrupted, making the solution distribution more uniform. This process indirectly changes the distribution of the microbial population and the diversity compared with those in the ventilation process [108]. In addition, artificial air flow removes heat from the heap, accelerates heat transfer during the forced aeration process [109], and is regarded as another common method to adjust the temperature distribution of an ore heap. The effect of forced aeration on the bioleaching efficiency using mixed bacteria has also been studied [110].

In summary, forced aeration plays several important roles: (1) the airflow breaks up blockages created by fine particles in the pore throat, thereby enhancing oxygen transfer; (2) the exogenous oxygen increases the dissolved oxygen in the PLS, thereby increasing the bacterial concentration and the bioleaching strength [111]; and (3) high-velocity airflow accelerates heat circulation, making the heat distribution more uniform and avoiding bacterial inactivation under high-temperature conditions and low bacteria activity under low-temperature conditions [112].

2.8. Additive agents and exogenous catalysts

The bioleaching process can be effectively regulated by adding exogenous substances and physical fields [113]. Additive agents or substances, including surfactants, silver ions, waste newspapers (WNs), activated carbon pipes, polyethylene glycol (PEG), graphene, and acid-processed rice straw [114–116], have been deliberately added to the reaction systems to enhance redox reactions. Such additive agents [117] will gain additional industrial applications in the future because of the simplicity and efficiency of this approach. The cost of additive agents is an important parameter in the industrial cases.

In particular, for primary copper sulfides [118], added activated carbon could decrease the bioleaching intensity of ferrous oxidation and iron dissolution. Tween-80 and Tween-20 are surfactants commonly used to enhance sulfur metabolism and the adsorption of extracellular polymeric substances by changing the contact angles [119]; for example, copper extraction of chalcopyrite improved by 16% in the presence of 10^{-2} g/L Tween-80. A few grams of silver (14.7 g Ag per 1 kg Cu) was found to enhance the leaching of chalcopyrite; however, the leaching efficiency of chalcopyrite decreased substantially when excessive silver was added (294.12 g Ag per 1 kg Cu) [120]. Because the WNs contain a large amount of cellulosic substances, they will be hydrolyzed and form polysaccharide substances, especially in an acidic environment. As shown in Fig. 7, the microbial bioleaching reaction leads to a substantial effect on the mineral surface [121−122]. The PEG has been added as an exogenous enhancer; it can promote the attachment of bacterial communities and eliminate sulfur during chalcopyrite bioleaching.

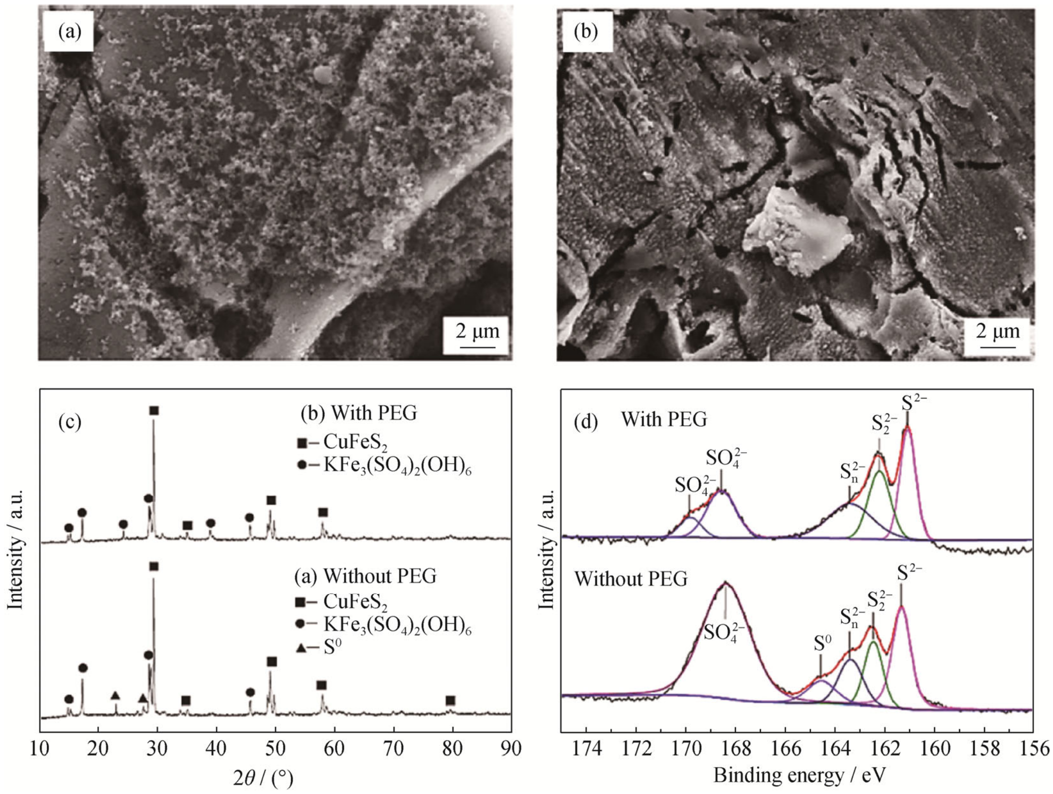

Fig. 7. Surface morphology and bioleaching parameters: (a) without PEG; (b) with PEG; (c) XRD patterns of bioleached chalcopyrite; (d) S 2p XPS spectrum of the surface of bioleached chalcopyrite. Reprinted from *Minerals Engineering*, 95, R.Y. Zhang, D.Z. Wei, Y.B. Shen, W.G. Liu, T. Lu, and C. Han, Catalytic effect of polyethylene glycol on sulfur oxidation in chalcopyrite bioleaching by *Acidithiobacillus ferrooxidans*, 74-78, Copyright 2016, with permission from Elsevier.

3. Challenges and prospects

Overall, as a highly efficient, low-cost, environmental friendly technique, bioleaching has several industrial applications in metal extraction from low-grade copper sulfides, electronic waste (or e-waste), slag, and mine tailings [123−125]. However, it still faces major challenges, including a change of the succession process of the leaching microbial community, poorly understood biochemical leaching mechanisms, disturbances of the microbial activity, and variations in leaching efficiency. On the basis of recent studies, we identified following future research areas:

(1) Passivation substances are an important indicator characterizing the degree of the leaching reaction. Under the condition of enhanced bioleaching, the coupling relationship between passivation and bacterial successions are not well understood. The order of the formation of various passivation substances and the coupling relationship between them are further related. In addition, the intrinsic relationship between the passivation and the dominant bacteria succession requires further research.

(2) The synergistic relationship between exogenous additives such as PEG, WNs, and native bioleaching bacteria is still not known. The intrinsic associations among the enhanced mechanism, microorganisms, and leaching time warrant further exploration. In the present literature survey,

some efficient enhanced methods have been mentioned; however, the key question concerning the inter-event time of these enhanced bioleaching methods remains inadequately investigated. The use of exogenous additives for enhanced leaching is a simple and easy approach. Operating costs and environmental pollution are also important considerations in enhanced leaching processes.

(3) Strategies to prevent leaching microorganisms from influencing the genetic diversity of local biomes while striving to achieve greater bacterial concentration and leaching rate must be developed given that the introduction of exogenous bacteria is certain to disturb the local microbial loop. Moreover, because of the acid spillover caused by poor control of the seepage range of the acidic solution, serious potential exists for soil pollution. The detection of the pollution in the groundwater system and in the surrounding land is not negligible and must be considered in the design of methods to strengthen the leaching.

(4) Irrespective of whether enhanced leaching can always play an active role in the whole leaching process, it will continue to be explored. To attain positive reinforcement, the optimal combination of coupled enhanced methods should be studied. In summary, enhanced bioleaching methods were presented from the perspectives of bacterial community structure, solution, pore-cracking structure, biochemical leaching reaction, use of exogenous additives, and multi-field multi-phase media control. These methods regulate bacterial community and leaching process of the leaching system, which is beneficial to extract copper sulfides. Follow-up studies will reveal strengthening mechanism of coupled enhanced methods, and the adverse effects of coupled leaching methods on heap surroundings will be comprehensively considered to improve extraction efficiency.

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