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ORIGINAL PAPER

Bioleaching of chalcopyrite concentrate using *Leptospirillum ferriphilum*, *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* in a continuous bubble column reactor

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Abstract To estimate the bioleaching performance of chalcopyrite for various hydraulic residence times (HRTs), laboratory-scale bioleaching of chalcopyrite concentrate was carried out in a continuous bubble column reactor with three different HRTs of 120, 80 and 40 h, respectively. An extraction rate and ratio of 0.578 g Cu l^{-1} h^{-1} and 39.7%, respectively, were achieved for an HRT of 80 h at a solids concentration of 10% (w/v). Lower bioleaching performances than this were obtained for a longer HRT of 120 h and a shorter HRT of 40 h. In addition, there was obvious competition between *Leptospirillum ferriphilum* and *Acidithiobacillus ferrooxidans* to oxidize ferrous iron, causing large compositional differences between the microbial communitys obtained for the different HRTs. *Leptospirillum ferriphilum* and *Acidithiobacillus thiooxidans* were found to be the dominant microbes for the longer HRT (120 h). *Acidithiobacillus ferrooxidans* became the dominant species when the HRT was decreased. The proportion of *Acidithiobacillus thiooxidans* was comparatively constant in the microbial community throughout the three process stages.

Keywords Bioleaching · Chalcopyrite · Bubble column reactor · Microbial community · Hydraulic residence times (HRTs)

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Introduction

Chalcopyrite is often associated with pyrite, pyrrhotite, sphalerite, galena, quartz, calcite, and dolomite, so it is difficult and expensive to extract copper from these complex ores using traditional technology. In addition, chalcopyrite is very resistant to chemical attack by the reagents used in conventional hydrometallurgy. Significant attention has focused on the development of biohydrometallurgy in recent years [1–3], due to its relative simplicity, eco-friendly operation and low capital requirement.

It is generally accepted that ferric ions and acid contribute significantly to the bioleaching of chalcopyrite. Also, elemental sulfur, an intermediate in the leaching process, can lie on the surface of chalcopyrite, inhibiting mineral dissolution [4, 5]. Sulfur-oxidizing microorganisms can remove elemental sulfur that has accumulated on the mineral's surface and decrease the pH value due to their ability to oxidize the elemental sulfur to sulfuric acid. In addition, iron-oxidizing microorganisms can oxidize Fe^{2+} and regenerate the Fe^{3+} that is depleted during chalcopyrite bioleaching. Thus, sulfur- and iron-oxidizing microorganisms are often mixed and then inoculated into leaching systems because of their cooperative bioleaching of sulfide minerals [6]. Further, it is reasonable to speculate that the microbe population itself and changes in the microbial community consisting of

sulfur- and iron-oxidizing microorganisms can significantly affect the bioleaching performance of chalcopyrite. In turn, different chalcopyrite compositions can also affect the composition of the microbial community and changes in it due to the different Fe/S ratios of the different chalcopyrite compositions. Attaining an understanding of the interaction between sulfur- and iron-oxidizing microorganisms and chalcopyrite is the key to improving the bioleaching performance of chalcopyrite from a microbial ecology point of view. Molecular phylogenetic techniques such as FISH (fluorescent in situ hybridization) [7], SSCP (single-strand conformation polymorphism) [8] and PCR–restriction fragment length polymorphism (PCR–RFLP), have been successfully and widely applied to ecological analyses in a mixed culture or natural microbial consortia. Therefore, these techniques can potentially be used to acquire the above understanding. However, little attention has been paid in the previous literature to changes in the bacterial diversity of sulfur- and iron-oxidizing bacteria present in continuous-flow bioreactors for different HRTs.

Several reports have recently shown an interest in using thermophilic microorganisms (operating at 50–85°C) to extract copper due to an improvement in the kinetics of mineral dissolution. However, operations at high temperature decrease the solubility of O_2 and CO_2 in the bioleaching medium, resulting in limited growth of the thermophilic microorganisms. Further, these microorganisms (operating at 70–85°C) have been reported to be highly sensitive to solid concentrations and shear conditions, which severely limits their bioleaching performance at a higher concentrations than 10% w/v solids [9–12]. At present, most research and commercial operations remain focused on bioleaching using mesophiles.

Dump bioleaching is often adopted to process the low-grade chalcopyrite and waste tailings due to its simple operational requirements and low cost. Nevertheless, it is not a perfect technology to process the high-grade chalcopyrite and concentrate due to its long operational times and low leaching rates compared to tank bioleaching. Instead, stirred tank reactors show promise as a technology for extracting metal from concentrate ore because of their high capability/volume ratio, high microorganism growth activities, and comparatively high leaching rates. However, the intensity of shear or turbulence produced to achieve the desired level of agitation may affect the microorganism performance [13]. The use of slurry bubble columns, in which the air current is used as a stirring system, can provide an alternative that overcomes this limitation [14]. In fact, in such simply-constructed reactors, shear and turbulence are usually smaller than in agitated tank reactors. Thus, the hydrodynamic environment of such a reactor is more suitable for cells that are susceptible to physical damage caused by mechanical agitation or fluid turbulence. Other advantages such as high gas dispersion efficiency, good heat and mass transfer characteristics, and rapid mixing are also applicable to the continuous bubble column reactor [15]. In addition, such a reactor can be better utilized because of reduced delays when filling and discharging slurry compared with batch process reactors in industrial operations.

In this study, laboratory-scale bioleaching experiments were carried out in a continuous-flow bubble column reactor for extracting copper from a chalcopyrite concentrate. The iron-oxidizing species *Leptospirillum ferriphilum* (*L. ferriphilum*), the sulfur- and iron-oxidizing species *Acidithiobacillus ferrooxidans* (*A. ferrooxidans*), and the sulfur-oxidizing species *Acidithiobacillus thiooxidans* (*A. thiooxidans*) were mixed and inoculated into the reactor. To elucidate the relationships among the biological and chemical parameters, the microbial community (especially the ecological composition of iron-oxidizers and sulfur-oxidizers), the bioleaching rate and ratio, the pH, the redox potential and the total Fe were determined and analyzed in a bubble column reactor for different HRTs.

Materials and methods

Ore characteristics

Chemical analysis of the sample used in the experiments revealed that it contained 29.1% Cu, 30.25% Fe, 35.34% S, 4.9% Pb and 0.41% Mo, according to inductively coupled plasma–atomic emission spectroscopy (ICP-AES). X-ray diffraction (XRD) analysis of the ore showed that chalcopyrite ($CuFeS_2$) (83.4%) was the major component and pyrite (FeS_2) (10.3%) was a minor component, together with small amounts of galena (PbS) (5.7%) and molybdenite (MoS_2) (0.6%). Over 90% of the ore had a particle size of 45 μm.

Bioleaching experiments

Experiments for bioleaching chalcopyrite were carried out in a 5 l tapered glass column reactor shown schematically in Fig. 1. The top of the column had an inner diameter of 0.23 m and a total height of 0.35 m, and the carrier-occupied volume was 4 l. The iron-free 9K medium [16] with a pulp density of 10% (w/v) employed in the experiments was sterilized and pumped through a peristaltic pump. The pH of the feed slurry and process temperature were 2.0 and 33°C, respectively. *L. ferriphilum*, *A. ferrooxidans* and *A. thiooxidans* had been separately

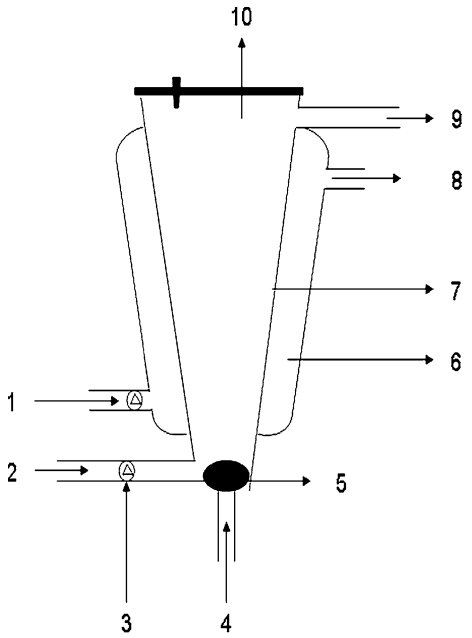

Fig. 1 Experimental apparatus. *1*, Inlet for water circulation; *2*, inlet for fresh feed; *3*, peristaltic pump; *4*, inlet for air; *5*, air distributor; *6*, circulation of water in the jacket; *7*, column; *8*, outlet for water circulation; *9*, effluent solution; *10*, outlet for air

subcultured in chalcopyrite medium, with several transfers, so they were well adapted to the chalcopyrite medium before bioleaching. They were then mixed and inoculated (initial cell number of each species was 10^8 cells ml^{-1}) into the bubble column reactor. The feed rates were 0.1, 0.05 and 0.033 l h^{-1}, and thus the HRTs for the fluidized bed volume were 40, 80 and 120 h, respectively. After start-up (20 days), aliquots of effluent solution were taken from the reactor to analyze the concentration of Cu, the total Fe, the pH and the redox potential (Eh) at regular intervals. The column was designed to taper from the top downwards in order to fully stir and suspend the chalcopyrite slurry. An air current of 4 l h^{-1} was used as a stirring system to maintain adequate concentrations of CO_2 and O_2 for bacterial growth.

Analytical methods

The leached residues were analyzed by XRD. The concentrations of Cu and total Fe in solution were determined by atomic absorption spectrophotometry. The pH was measured using a pHS-3C acid meter. The Eh, which indicates the ratio Fe(III)/Fe(II), was measured with a Pt electrode, and a saturated calomel electrode was used as the reference electrode. The quantity of free bacteria in effluent solution was counted directly using a Thoma chamber with an optical microscope.

Bacterial population analysis

Preparation of total DNA and PCR amplification

When a steady-running state was obtained within the bubble column reactor after each change of HRT, the effluent was sampled and prepared for extraction of total DNA. In the steady-running state the effluent was filtered through a 0.22 μm pore-size membrane. The residue containing all biomass was used to extract the total DNA according to the procedure described by Zhou [17]. Community 16S rDNA genes were first amplified using the universal primer set 1492R (5′-CGGCTACCTTGTTACGACTT-3′) and 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) [18], and the PCR product was then separated by gel electrophoresis on a 1% agar gel in Tris/acetate buffer and analyzed by staining with ethidium bromide (EB) under UV light. The expected band was excised and purified with a commercial kit (gel extraction kit, Promega, Madison, WI, USA).

Cloning, analysis of the RFLP pattern and community composition

The purified 16S rDNA from the residue was cloned into the pGEM-T vector (Promega) and transformed into *Escherichia coli* TOP10 competent cells (Invitrogen, Carlsbad, CA, USA) for blue–white screening. About 60 white clones were randomly selected from each library. The inserted fragments were amplified with the vector-specific T7 and SP6 primers and digested by the restriction enzymes RsaI and MspI overnight at 37°C. The digested 16S rDNA was detected by 3.0% (w/v) agarose gel electrophoresis and EB staining. The RFLP patterns were identified and grouped, and the clone containing representative cloned fragments was selected for its 16S rDNA sequencing. Each operational taxonomic unit (OTU) (unique RFLP pattern) indicated one of the three bacterial species. The clones in each OTU were enumerated so as to analyze the bacterial population of each species (community composition).

Results and discussion

Effect of HRT variation on bioleaching performance

At first, the bubble column reactor was run for 4 days with a series of different HRTs (200, 180, 160, 140 and 120). The steady-running parameters, including pH, total Fe and Cu recovery, were obtained from the reactor for an HRT of 120 h (Figs. 2, 3 and Table 1). This indicated that the reactor reached a successful start-up after running for 20 days. The reactor was subsequently operated for 28, 30 and 30 days with HRTs of 120, 80 and 40 h, respectively.

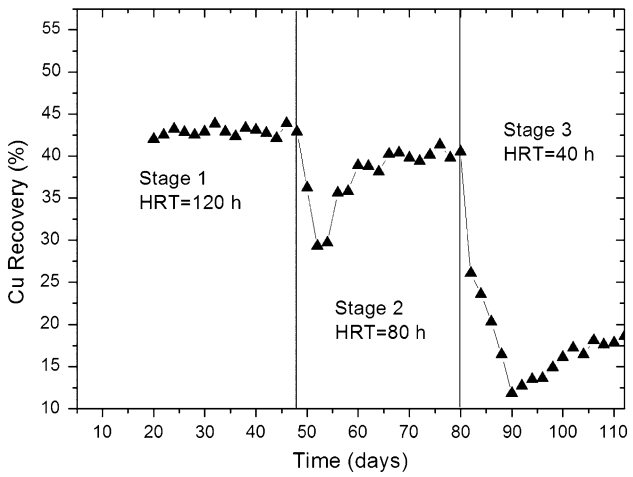

Fig. 2 Copper recovery vs. time

Figure 2 shows that the copper recovery ratio decreased as the HRT decreased from 120 to 40 h. With each change of HRT, average copper recovery ratios of 42.7, 39.7 and 18.1% were achieved for HRTs of 120, 80 and 40 h, respectively, when the running of the reactor had stabilized. Copper recovery ratios decreased by 21.6% with a decrease in HRT of 40 h on moving from stage 2 to stage 3. However, the copper recovery ratios only decreased by 3% with the same decrease in HRT of 40 h on moving from stage 1 to stage 2. In addition, the process efficiency significantly declined with every decrease in HRT, and the reactor slowly recovered to attain a new steady state: 18 days from stage 2 to stage 3, compared with 12 days from stage 1 to stage 2. This evidence indicates that a reactor inoculated with three species is not suited to running at HRTs that are too low due to its vulnerability to a high slurry feed and loading rate. For the investigated HRTs, the reactor is suited to running at an HRT of 80 h with an average copper extraction rate of 0.578 g Cu l^{-1} h^{-1} and a Cu recovery of 39.7%, compared with 0.414 g Cu l^{-1} h^{-1} and 42.7% for an HRT of 120 h, as well as 0.527 g Cu l^{-1} h^{-1} and 18.1% for an HRT of 40 h.

The evolution of pH in the effluent solution is shown in Fig. 3 as a function of time. The pH is comparatively constant with an initial value of 2 throughout the process stages, except for stage 1. At stage 1, the pH value of the effluent solution (about 1.8) is slightly lower than that of the feeding medium (about 2). The bioleaching performance of chalcopyrite is generally determined by two reaction products that form on the mineral surface and inhibit further dissolution; one of these is elemental sulfur (Eq. 1) and the other is ferric iron precipitation (Eqs. 4, 5) [19–21]. In the following five reaction equations, Eqs. 3 and 4 result in a decrease of pH value, while Eq. 2 results in an increase. Generally, reactions 1, 2 and 3 are necessary to dissolve the chalcopyrite, and reactions 4 and 5 only happen at high pH (above 1.7) and redox potential. Therefore, the pH values do not decrease if ferric iron precipitation does not form in the bioleaching system (Eqs. 4, 5). Otherwise, based on an analysis of reactions 1–5, the pH decreases. Thus, the decrease in pH values from 2 to 1.8 at stage 1 suggests the formation of ferric iron precipitation.

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

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{L.\ ferriphilum,\ A.\ ferrooxidans} 4Fe^{3+} + 2H_2O \quad (2)$$

$$2S^o + 2H_2O + 3O_2 \xrightarrow{A.\ ferrooxidans,\ A.\ thioxidans} 2SO_4^{2-} + 4H^+ \quad (3)$$

$$3Fe^{3+} + 2SO_4^{2-} + 6H_2O \rightarrow Fe_3(SO_4)_2(OH)_6 + 6H^+ \quad (4)$$

$$3Fe^{3+} + K^+ + 2HSO_4^- + 6H_2O \rightarrow KFe_3(SO_4)_2(OH)_6 + 8H^+ \quad (5)$$

An average Cu recovery of 42.7% was acquired at stage 1. Correspondingly, an average of 10.9 g l^{-1} iron should

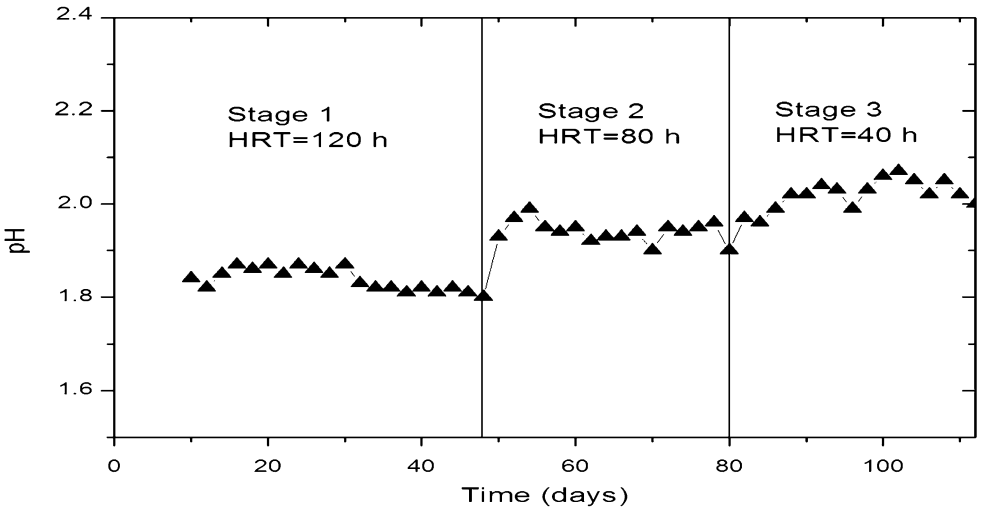

Fig. 3 pH values in effluent solution vs. time

dissolve from the chalcopyrite based on the stoichiometric ratio of Cu/Fe in chalcopyrite. Taking into account the dissolution of some pyrite (indicated by stage 3 in Table 1), a total iron concentration of more than 10.9 g l^{-1} should be detected in the effluent solution if ferric iron precipitation does not occur. However, a total iron concentration of 7.2 g l^{-1} was measured in the effluent solution at stage 1. Thus, during stage 1, ferric iron precipitate must have formed on the surface of the chalcopyrite, and this lead to only a slightly higher Cu recovery than during stage 2, although the former involved a much longer HRT. Further, the presence of ferric iron precipitation (jarosite) in effluent residues was also verified by XRD (Fig. 4).

Bacterial dynamics and the microbial community for various HRTs

In this study, we constructed a simple bioleaching ecological community consisting of three typical microbial species, *L. ferriphilum, A. ferrooxidans* and *A. thiooxidans*, which have been found to exist widely in much natural acidic mineral drainage and mesophilic bioleaching systems in other studies [22–24]. Figure 5 shows the evolution of free bacteria in effluent solution. A free cell concentration of 3.6×10^9 cells l^{-1} was obtained at stage 1 with an HRT of 120 h, 3.0×10^9 cells l^{-1} at stage 2, and 0.7×10^9 cells l^{-1} at stage 3. The cell concentration decreases with decreasing HRT. This suggests that the most serious consequence of low HRT is the washout phenomenon due to the entrainment of bacteria with the effluent [25]. In addition, the bioleaching time is short, and the mineral may pass through the reactor with a rather limited time exposure to bacterial activity at low HRT and high feed flow. Therefore, both short process times and low cell concentrations result in a low bioleaching performance at low HRT; for example, a Cu recovery of 39.7% was obtained for stage 2, and 18.1% for stage 3. However, given that the formation of ferric iron precipitation should be considered for high HRTs, a moderate HRT of 80 h is more beneficial for improving the bioleaching efficiency than low/high HRTs.

The proportions of the three species differ significantly at the three stages with various HRTs (Fig. 6). *L. ferriphilum* becomes the most dominant microbe with a compositional proportion of approximately 63.4% while *A. thiooxidans* is the second most dominant, with a compositional proportion of 29.9% at stage 1. Only 6.7% of the clones belong to *A. ferrooxidans* at stage 1. Therefore, *L. ferriphilum* and *A. thiooxidans* should be the main microbes to respond to the extraction of copper at high HRTs. An obvious difference that was observed between the three investigated stages is the compositional change in *A. ferrooxidans* from 6.7 to 34% and 46.3%, respectively, and in *L. ferriphilum* from 63.4% to 43% and 32.7% at stages 1, 2, and 3, respectively. It is not difficult to

Table 1 Concentration of theoretical/measured total iron in effluent solution (g l^{-1})

	Stage 1	Stage 2	Stage 3
Theoretical total iron in effluent	10.9	10.1	4.6
Measured total iron in effluent	7.2	9.2	4.8

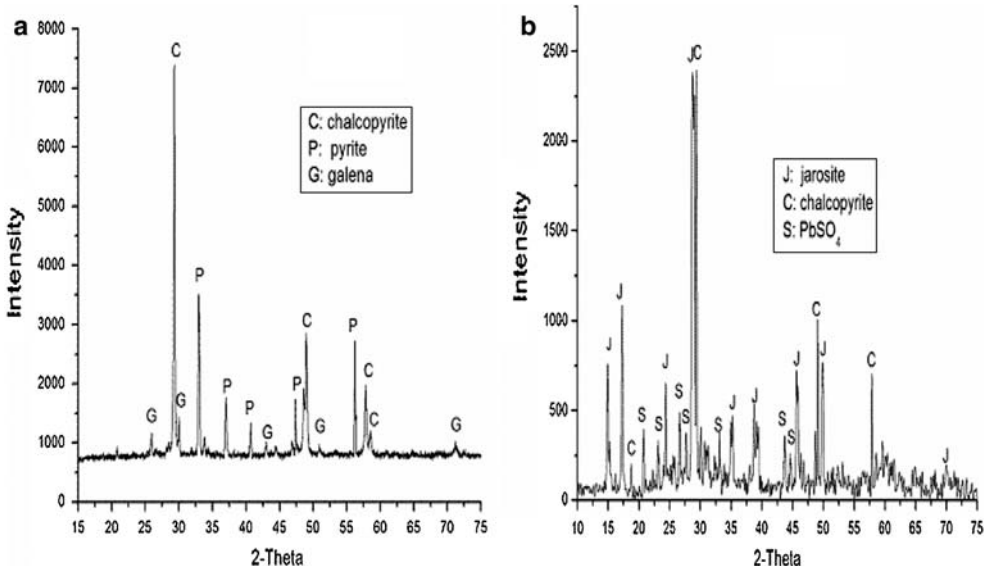

Fig. 4 X-ray diffraction patterns of the raw mineral (**a**) and its residues during stage 1 after bioleaching (**b**)

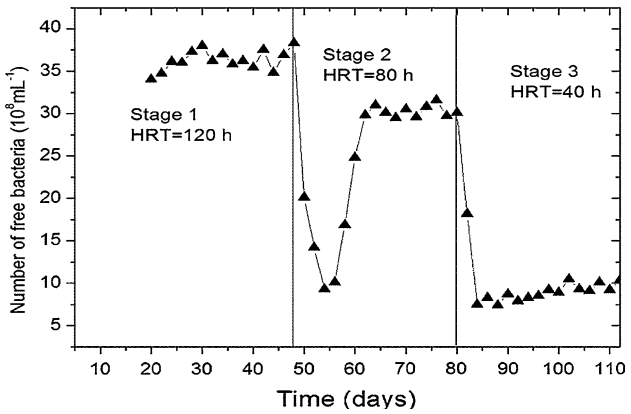

Fig. 5 Changes in the population of free bacteria in effluent solution

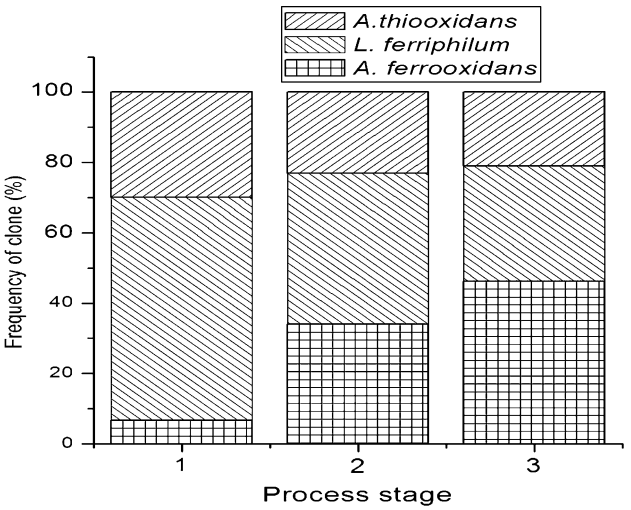

Fig. 6 Changes in the community composition of the three microbial species for the different HRTs in the steady-running state

understand these compositional changes in *L. ferriphilum* and *A. ferrooxidans*. When two kinds of iron-oxidizing species exist in the system together, it is inevitable that the bacteria will compete for ferrous iron as their energy substrate for growth. Thus, ferrous and ferric iron can significantly affect the microbial consortium. A number of researchers have reported that *L. ferriphilum* has a higher adaptability than *A. ferrooxidans* under conditions of low pH (<1.5) and high Eh, while *A. ferrooxidans* prefers to grow in the range pH 1.8–2.5 and at low Eh (<450 mV) [26, 27]. Figure 7 shows that a higher Eh than 450 mV was obtained during stage 1 (average 570 mV), while comparatively low Eh values occurred during stages 2 (average 495 mV) and 3 (average 400 mV). These results explain the compositional changes in *L. ferriphilum* and *A. ferrooxidans*. It should be noted that competition between *A. thiooxidans* and *A. ferrooxidans* was weak although the

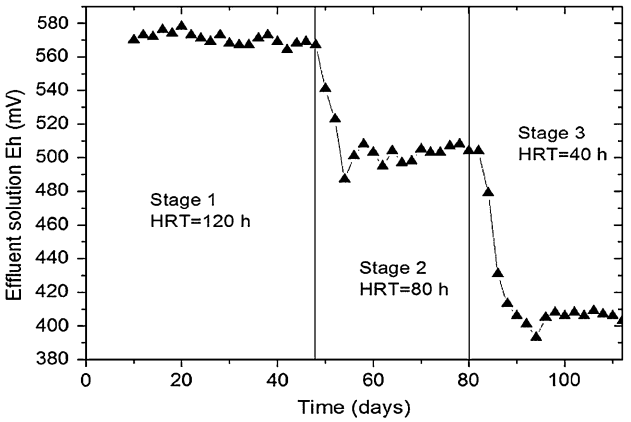

Fig. 7 Evolution of Eh in the effluent solution as a function of process time

latter oxidizes sulfur. Generally, *A. ferrooxidans* prefers ferrous irons to sulfur when both of these energy substrates exist [28, 29]. Thus, the proportion of *A. thiooxidans* was comparatively constant during the three stages.

Conclusions

A high recovery ratio and rate could be achieved in a continuous bubble column reactor for processing chalcopyrite concentrate through the inoculation of three mesophilic species: *L. ferriphilum*, *A. ferrooxidans* and *A. thiooxidans*. It is important to adopt suitable HRTs to optimize the recovery ratio and rate in a continuous-flow reactor. Among the three investigated HRTs of 120, 80 and 40 h, an HRT of 80 h was found to be the most applicable for achieving high copper recovery ratios and rates. When the reactor was in the steady-running state, the recovery ratio and extraction rate were 39.7% and 0.578 g Cu l^{-1} h^{-1}, respectively. At a higher HRT of 120 h, the formation of ferric iron precipitation inhibited the bioleaching process and reduced the Cu recovery, while a lower HRT of 40 h also decreases the Cu recovery due to a low concentration of biomass and the short process time for a high feed flow. In addition, analysis of the microbial community revealed that *L. ferriphilum* and *A. thiooxidans* became the dominant microbes and leached chalcopyrite for a high HRT of 120 h, *A. ferrooxidans* became dominant as the HRT was decreased. The proportion of *A. thiooxidans* was comparatively constant in the microbial community throughout the three process stages.

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