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ORIGINAL ARTICLE

Effects of pH value on the expression of key iron/sulfur oxidation genes during bioleaching of chalcopyrite on thermophilic condition

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Abstract

Physicochemical factors such pH value would affect the microbial metabolism during chalcopyrite bioleaching. To this end, the effects of pH on the expression of critical functional genes during bioleaching were evaluated. A mixed culture of moderate thermophiles was used for chalcopyrite bioleaching at initial pH values of 1.0, 2.0, and 3.0, and bioleaching processes were monitored via measuring the physicochemical parameters. Quantitative real-time PCR assay was used to monitor the dynamics of microbial community structures and the expression of critical iron/sulfur oxidation genes (4Fe-4S ferredoxin and sulfate adenylyltransferase genes, respectively). Redundancy analysis and calculation of correlation coefficients were used to reveal linkages between gene expression and various physicochemical factors. The leaching processes at initial pH of 1.0 and 3.0 were prolonged compared with that at initial pH of 2.0. It was shown that *Sulfobacillus thermosulfidooxidans* and *Acidithiobacillus caldus* were the dominant species during the early stage in free and attached cells, respectively, while *Ferroplasma thermophilum* became predominant in the later phase. The gene expression in *Sulfobacillus thermosulfidooxidans* and *Ferroplasma thermophilum* was greatly affected by pH values. On the other hand, the relationship between pH and gene expression in *Acidithiobacillus caldus* was not significant. The study unraveled the importance of pH value on chalcopyrite bioleaching, and pH selectively influenced the expression of key functional genes of some specific species.

Keywords Bioleaching chalcopyrite · pH · Functional genes · Moderate thermophiles

Introduction

Bioleaching of refractory copper sulfide, e.g., chalcopyrite, as an alternative for traditional pyrometallurgical methods, is a copper extraction process with low operation costs and environmental benefits (Feng et al. 2015; Wang et al. 2017). Nevertheless, the development of the basic theory in chalcopyrite bioleaching has been on the road. Many scientific problems such as the interactions between microorganisms and the bioleaching parameters remain to be unresolved, which hindered the progress of biohydrometallurgy. Environmental factors such as temperature, pH, and redox potential would affect bioleaching (Halinen et al. 2009a, b; Wang et al. 2016). Among them, pH is of great significance, which would affect the growth activities of microorganisms and structure of microbial communities, thereby influencing the leaching rate (Liu et al. 2010). On the one hand, bioleaching microorganisms are extremely acidophiles; high pH environments would be harmful to the oxidation ability of microorganisms. Besides, during heap leaching, elevated pH would reduce permeability of bioheap due to ferric ion precipitation (Ojumu and Petersen 2011). On the other hand, low pH values (e.g., lower than 0.8) are also detrimental to bioleaching, inhibiting microbial growth and oxidative activity (Watling et al. 2013).

In recent years, design and engineering issues have been considerably developed in chalcopyrite bioleaching; nonetheless, aspects concerning microbial metabolism remain elusive. Analyzing the expression of the critical functional genes helps to reveal the roles and metabolic mechanisms of microorganisms and microbial communities involved in leaching processes. To date, only several studies have examined the expression of functional genes during bioleaching using mesophilic bacteria (Zhu et al. 2012). In addition, few have been carried out to investigate the expression of functional genes on different

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environmental conditions, for example, on moderate thermophilic conditions and/or at different pH values (Marín et al. 2017). Previously, the expression of genes involved in sulfur (sulfate adenylyltransferase gene, *sat*) and iron oxidation (4Fe-4S ferredoxin gene, *fed*) during bioleaching of chalcopyrite concentrate at an initial pH of 2.0 was discussed (Zhou et al. 2015). Nevertheless, except chalcopyrite, the ore sample contains many other components such as sphalerite, steatite, and galena, which may lead to a bias for evaluation of the gene expression during chalcopyrite bioleaching. Furthermore, detailed information is limited concerning the effects of varied pH on the expression of critical iron and sulfur-oxidation genes.

Ferrous- and sulfur-oxidizing acidophiles are the predominant species involved in bioleaching. They obtain energy via the oxidation of ferrous iron and sulfur; the former provides ferric iron as the oxidant for chalcopyrite dissolution and the latter produces protons to maintain the low pH value in bioleaching solution, respectively (Vera et al. 2013). Ferredoxins are small, acidic, electron transfer proteins that are ubiquitous in biological redox systems (Zeng et al. 2007). One of the ferredoxins, which has one [4Fe-4S] cluster and coded by the *fed* gene, is a member of electron transport chain and functions in oxidation of ferrous iron in bioleaching system (Lin et al. 2013). The gene *sat* codes for the enzyme catalyzing adenosine-5′-phosphosulfate (APS) and pyrophosphate to generate ATP and sulfate, which is the final stage of the sulfite oxidation (Bick et al. 2000; Christel et al. 2016). In order to unravel the relationships between pH and the two genes during chalcopyrite bioleaching, an enrichment culture of moderate thermophiles was employed. The copper leaching, dynamics of microbial community structure, and expression of *sat* and *fed* in free and attached cells were monitored at different initial pH values. It represents the first to discuss the effects of pH on the expression of critical iron- and sulfur-oxidation genes during chalcopyrite bioleaching processes by moderate thermophiles in detail.

Materials and methods

Minerals

The chalcopyrite was obtained from Guangzhou in Guangdong Province, China. Prior to bioleaching experiments, the mineral sample was ground and passed through a sieve with a pore size of 0.075 mm. The ore is of high purity, which contains chalcopyrite (97.83%) and quartz (2.17%). The elemental compositions are Cu (33.1%), Fe (29.8%), S (35.4%), and Si (1.7%).

Microorganisms

The moderately thermophilic culture has been described in detail previously (Zhou et al. 2015). The culture has been acclimated by increasing pulp density of chalcopyrite concentrate for more than 5 years and used as a seed culture in our lab. After a long time acclimation, the microorganisms in the mixed culture can tolerate high concentrations of copper and iron.

Bioleaching tests

Bioleaching was carried out in a 3-L glass cylindrical reactor with a mechanic stirrer operating at 300 r/min. Chalcopyrite (250 g) and modified 9 K medium (Zhou et al. 2009) were added into the reactor to obtain a final volume of 2500 mL. Cells in the moderately thermophilic culture were harvested by filtration to remove ore residues and then centrifugation at 10,000×*g* for 10 min. The harvested cells were washed twice by sterilized acidified water (pH 2.0) to remove organic residues and metal ions. Then, the cells were inoculated into the reactor at an initial cell density of 2.0×10^7 cell/mL. Thermostatic bath was used to keep the temperature constant at 45 °C. Air was blown into the base of the reactor at an approximate rate of 360 mL/min by an ACO-005 air pump (Sensen Group Co., Ltd., Zhoushan). Water loss by evaporation was compensated by addition of sterilized deionized water. Bioleaching of chalcopyrite was conducted in three stirred tank reactors A, B, and C, which were carried out at initial pH values of 1.0, 2.0, and 3.0, respectively. In addition, abiotic control at each initial pH value was conducted (only data for abiotic control at initial pH value of 2.0 was shown and designated as reactor D). All the experiments were conducted in triplicate.

Redox potential (Ag/AgCl), pH value, and the concentrations of Cu^{2+}, Fe^{2+}, and total iron in solution were analyzed as described previously (Zeng et al. 2010) at regular intervals. For DNA extraction and cell counting, attached microorganisms were detached from the minerals according to previous study (Zeng et al. 2011). Free and attached cells were counted with a blood cell counting chamber under an optical microscope.

Microbial community succession analysis

The bioleaching solution was collected at regular intervals for extracting DNA. Total DNA was extracted using the TIANmap Bacteria DNA kit (TIANGEN Biotech Co., Ltd., Beijing) as per the manufacturer's instructions and checked by ethidium bromide-UV detection on an agarose gel and quantified using a Nanodrop ND-1000 spectrophotometer (NanoDrop Technologies, Wilmington, USA). The extracted DNA was stored at − 80 °C until use.

The amount of each microorganism was detected by quantifying the copy number of 16S rRNA gene (Wang et al. 2012). A total of 5 μL purified DNA was used as template for quantitative real-time PCR. Specific primers of 16S rDNA of the four species, PCR procedure, and the absolute

quantification of 16S rDNA were described in previous study (Zhang et al. 2009). All the tests were carried out in triplicate.

Quantitative real-time PCR determination of expression levels of *sat* and *fed*

Primers for genes *sat* and *fed* have been described previously (Zhou et al. 2015). Conventional PCR was performed by using these primers with DNA extracted from the moderately thermophilic culture as templates, and the PCR products were checked by 2% agarose gel electrophoresis. PCR products were purified for sequencing to verify the accuracy of the primers.

The bioleaching solution was withdrawn every 5 days for extracting RNA. To collect free cells, bioleaching solution was rapidly cooled and centrifuged at 2000×*g* at 4 °C for 3 min to remove the ore residue. After that, the liquid was centrifuged at 8000×*g* at 4 °C for 10 min to collect the free cells.

For harvesting of sessile cells, bioleaching solution was rapidly cooled and centrifuged at 2000×*g* at 4 °C for 3 min. The supernatant was removed and the obtained pellets were resuspended with 5 mL RNAprotect Bacteria Reagent (QIAGEN, Germany) in a 50-mL centrifuge tube. Then, 1 g of glass beads with a diameter of 0.2 mm was added into the tube and the tube was vortexed on a vortexer for 5 min at the maximum speed. Subsequently, the mixture was centrifuged at 2000×*g* at 4 °C for 3 min to separate the ore residue and the solution. The solution was decanted and the ore residue was resuspended with another 5 mL RNAprotect Bacteria Reagent. The resuspension, vortexing, and centrifugation steps were repeated twice. Finally, about 15 mL solution was obtained and centrifuged at 8000×*g* for 10 min at 4 °C to collect the sessile microorganisms.

Total RNA was extracted using Bacterial Total RNA Extraction Kit (Zoman Biotechnology Co., Ltd., Beijing). The quality of total extracted RNA was confirmed by 1% agarose gel electrophoresis and quantified using the Nanodrop ND-1000 Spectrophotometer. Genomic contamination was removed by gDNA Wiper kit (Zoman). To confirm that trace DNA has been digested, partial sequences of 16S rDNA were amplified using the specific primers for each microorganism with genomic DNA or the product of gDNA Wiper kit as templates. Positive and negative results were obtained when using the former and the later as templates, respectively. After digestion of genomic DNA, the product was retro-transcribed into cDNA with Reverse Transcriptase (Zoman) and random primers following the manufacturer's instructions. The cDNA was diluted by tenfold and stored at − 80 °C until use.

Each quantitative real-time PCR mixture (final volume 50 μL) contained 25 μL of SYBR Green Real-time PCR Master Mix (Zoman), 1 μL of each sense/anti-sense primer (10 μM), 5 μL of cDNA template, and 18 μL of DNase/RNase-free water. Real-time PCR procedure was carried out as described above while only the annealing temperatures for each primer pair were adjusted. The 16S rRNA gene for each species was used for normalization (Liljeqvist et al. 2013). The copy number was translated into amount of equal volume of leachate (1 mL) or equal weight of mineral (1 g). The logarithm of cDNA copy number (relative to 16S rRNA copies × 10^{-5} of each species, respectively) was shown. All the tests were carried out in triplicate.

Statistical analyses

Redundancy analysis (RDA) was used to evaluate the linkages between gene expression and Cu (II), Fe (II), and Fe (III) concentrations and pH with 499 permutations. Further, correlation coefficients between various parameters and gene expression were calculated using the Pearson method. The analyses were performed in R (version 3.3.1).

Results and discussion

Bioleaching of chalcopyrite at different initial pH values

Bioleaching of chalcopyrite by a mixed culture of moderate thermophiles at initial pH values of 1.0, 2.0, and 3.0 was carried out in glass cylindrical reactors A, B, and C, respectively. Abiotic control at initial pH value of 2.0 (reactor D) was shown. As shown in Fig. 1 a, the copper concentrations increased after a short period of lag phase in reactors A and B, while in reactor C the lag phase was much longer. Besides, the copper concentration was higher in reactor A than that in reactor B before day 8. It was speculated that protons plays a major role in the first 8 days because chalcopyrite is acid-soluble (Vera et al. 2013). After that, the copper extraction in reactor B exceeded that in reactor A. On day 34, the copper extraction in reactor B reached the highest of 56.8% (18.79 g/L) while those in reactors A and C were 47.3% (15.65 g/L) and 30.9% (10.22 g/L), respectively. The highest copper extractions in reactors A and C were 54.7% (18.12 g/L) on day 42 and 51.1% (16.90 g/L) on day 50, respectively. The overall trends showed that compared with bioleaching at initial pH of 2.0, changing the initial pH values to 1.0 or 3.0 resulted in extended leaching periods.

Fe (II) is the energy source for iron-oxidizing bacterium, and Fe (III) is the main oxidant in bioleaching; therefore, concentrations of Fe (II) and Fe (III) would directly affect bioleaching. As can be seen from Fig. 1 b, the concentration of ferric iron in bioleaching systems at initial pH values of 1.0 and 2.0 was much higher than that at initial pH 3.0. In reactors A and B, concentration of ferrous iron increased but that of ferric iron kept at a low level during the first 12 days. After

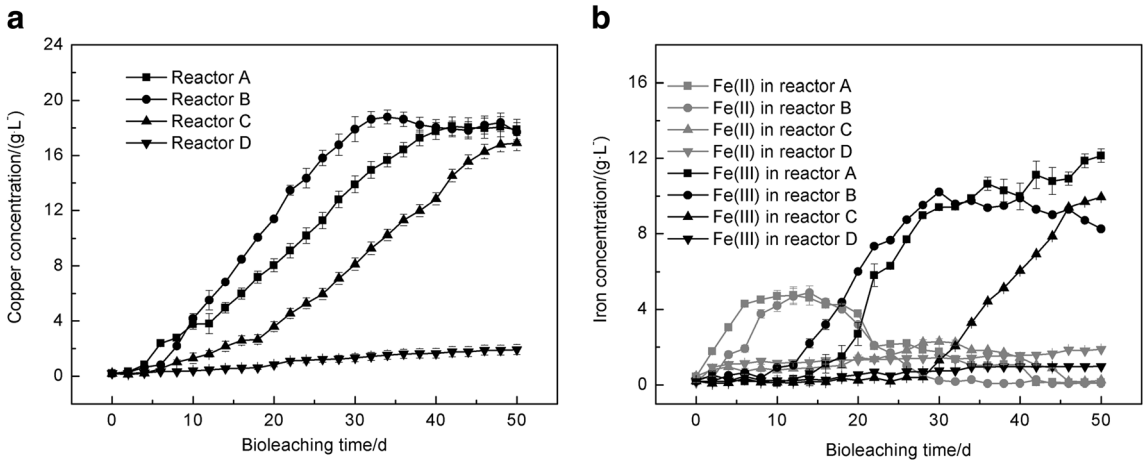

Fig. 1 The variations of **a** copper concentration and **b** ferrous and ferric iron concentrations in reactors A, B, C, and D. Initial pH values for reactors A, B, and C were 1.0, 2.0, and 3.0, respectively. Reactor D was an abiotic control with an initial pH value of 2.0

that, microorganisms grew rapidly which led to a decreased concentration of ferrous iron. However, in reactor C, the ferrous and ferric iron stayed low until day 28; then, the ferric iron increased and ferrous iron decreased. High pH greatly promoted the formation of iron precipitates and therefore reduced the available ferric ion in solution (Ojumu and Petersen 2011). In addition, more iron precipitated at higher pH indicates that more copper would be adsorbed to or entrained in the iron precipitates (Hille et al. 2010). These resulted in a lower copper extraction in reactor C.

As can be seen from Fig. 2 a, on the second day, the pH values in reactors B and C reached the highest of 2.25 and 3.40, respectively, while in reactor A, the pH increased until day 6, and the highest pH value was 1.65. The increase of pH during the early time was due to the dissolution of chalcopyrite by proton attack. As bioleaching progressed, because the acid-producing reactions catalyzed by sulfur-oxidizing microbes were speeding up, the pH values in all the three reactors decreased continually. Meanwhile, the activities of iron-oxidizing microorganisms were also increased; therefore, the ratio of Fe (III) to Fe (II) increased, which led to a rise in redox potential of the bioleaching system (Fig. 2b).

Figure 3 showed the variations of free and attached cell densities in the three reactors. The variation of the cell number was fairly in accordance with the trend of copper extraction (Fig. 1a). Cells in reactors A and B grew better than those in reactor C before day 36 (free cells) and day 44 (attached cells). The free cells in reactors A and B reached the maximum amounts of 1.15×10^9 and 1.44×10^9 cell/mL, and the maximum sessile cell amounts were 5.61×10^9 and 8.17×10^9 cell/g ore, respectively. This was due to that the extremely acidophilic bioleaching microorganisms grew optimally at pH < 3, and that relatively lower pH can offer more protons to leach chalcopyrite, releasing more sulfur species and ferrous iron as energy sources for microbial growth. After day 36, free cells in reactors A and B entered the decline phase while the cell number in reactor C kept increasing because the conditions (e.g., pH) in reactor C were relatively favorable for the growth of microorganisms.

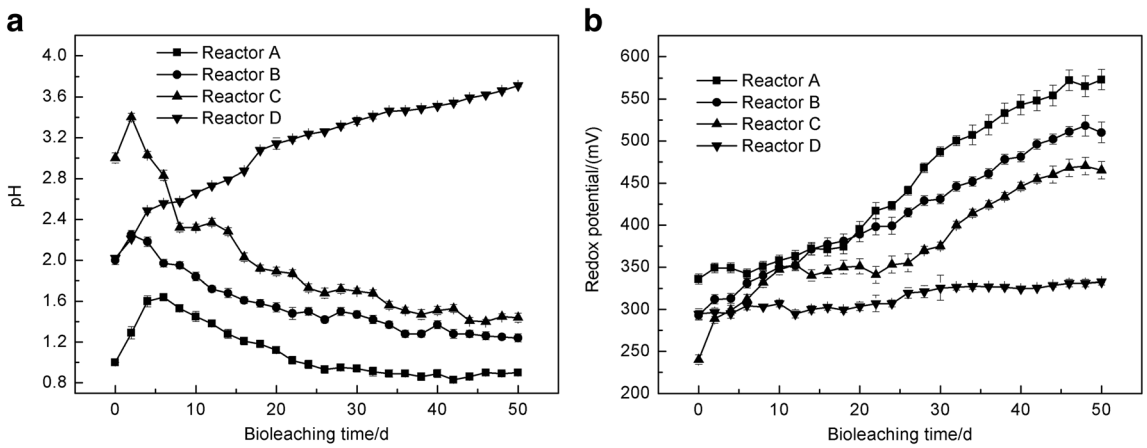

Fig. 2 The variations of **a** pH and **b** redox potential in reactors A, B, C, and D

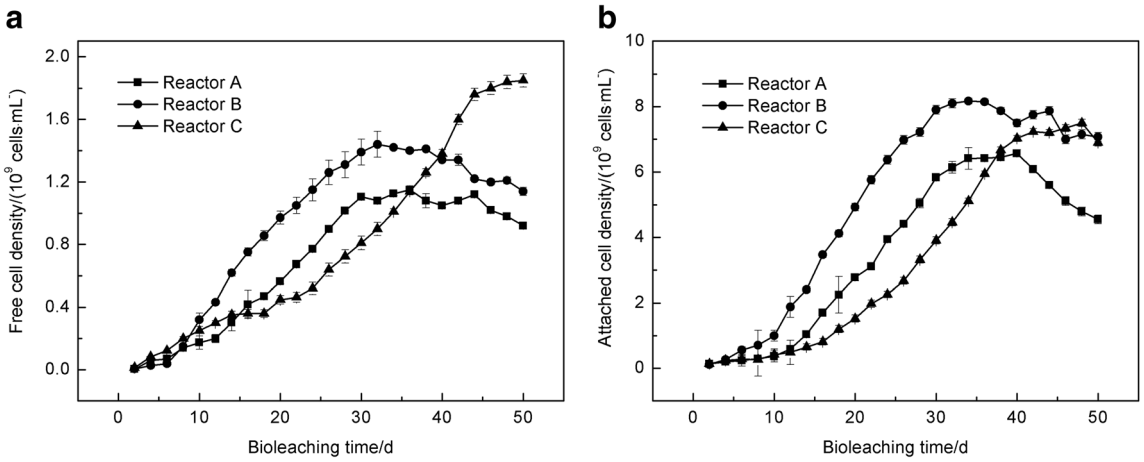

Fig. 3 The variations of **a** free cell density and **b** attached cell density in reactors A, B, and C

The dynamics of microbial community structures

The dynamics of microbial community structure at different initial pH values were analyzed by quantitative real-time PCR based on quantification of 16S rRNA gene. Figure 4 a showed the variation of free cells in the proportions of *Sulfobacillus thermosulfidooxidans*, *Acidithiobacillus caldus*, *Leptospirillum ferriphilum*, and *Ferroplasma thermophilum*. As it can be seen, in the first 15 days of bioleaching, *S. thermosulfidooxidans* took up the largest proportion in the leachate. In reactors A, B, and C, percentages of *S. thermosulfidooxidans* were 79.3%, 62.4%, and 67.0%, respectively. *Sulfobacillus thermosulfidooxidans* can oxidize both ferrous iron and inorganic sulfur compounds (ISC), and the optimal growth pH is 1.5–1.7 and 2.4–2.5 when utilizing Fe^{2+} and ISC as energy sources, respectively (Guo et al. 2014; Yu et al. 2014). This may explain why *S. thermosulfidooxidans* can grow well in reactors of which the initial pH values are 1.0 and 3.0. Thereafter, the content of *F. thermophilum* increased rapidly and became a predominant microorganism, which accounting for more than 90% of the population in the later stage (after day 30). *Ferroplasma thermophilum* grows optimally at extreme low pH and the growth is promoted by organic matters such as those produced by microbial metabolism (Zhou et al. 2008). That is why *F. thermophilum* was rarely detected in the early stage, but became dominant as bioleaching continued. Few cells for *L. ferriphilum* were detected in all three reactors. This could be due to that the optimum growth temperature of *L. ferriphilum* is around 40 °C, and it may be at a disadvantage compared to the other three species (Coram and Rawlings 2002).

As can be seen from Fig. 4 b, the variations of sessile microorganisms were similar to that of the free cells. Notably, it was *A. caldus* rather than *S. thermosulfidooxidans* that dominated the communities in the initial stage (the first 10 days). On day 5, the proportions of *A. caldus* were 52.6%, 71.0%, and 60.5% in reactors A, B, and C, respectively. The dominance of attached *A. caldus* cells may be owing to the formation of elemental sulfur on the surface of chalcopyrite and that *A. caldus* can use elemental sulfur as an energy source more effectively than *S. thermosulfidooxidans*. Moreover, the oxidation of elemental sulfur can offer more energy for growth of the sulfur-oxidizing microorganisms than the oxidation of ferrous iron for growth of the iron-oxidizing microorganisms (Zhu et al. 2012). Additionally, there was a smaller difference among reactors A, B, and C in attached microbial community

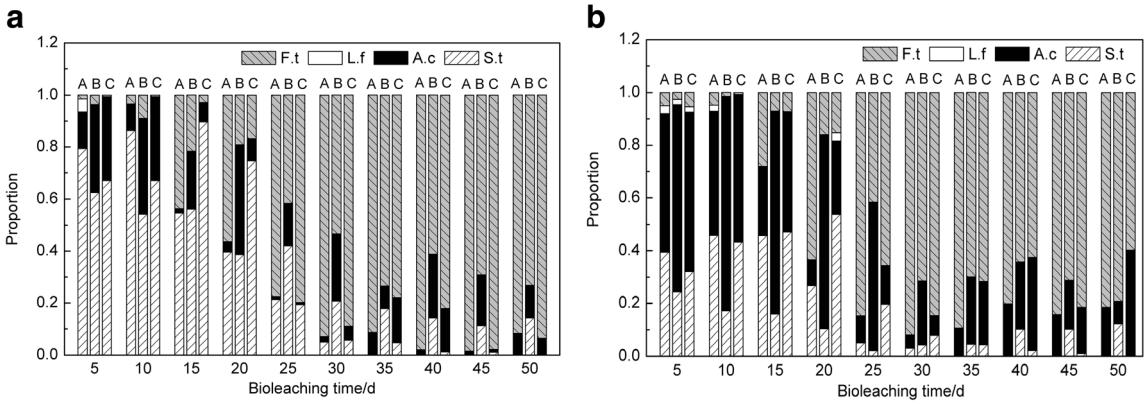

Fig. 4 The dynamics of **a** free cell community and **b** attached cell community in reactors A, B, and C. The labels "S.t,:" "A.c," "L.f," and "F.t" represented *S. thermosulfidooxidans*, *A. caldus*, *L. ferriphilum*, and *F. thermophilum*, respectively

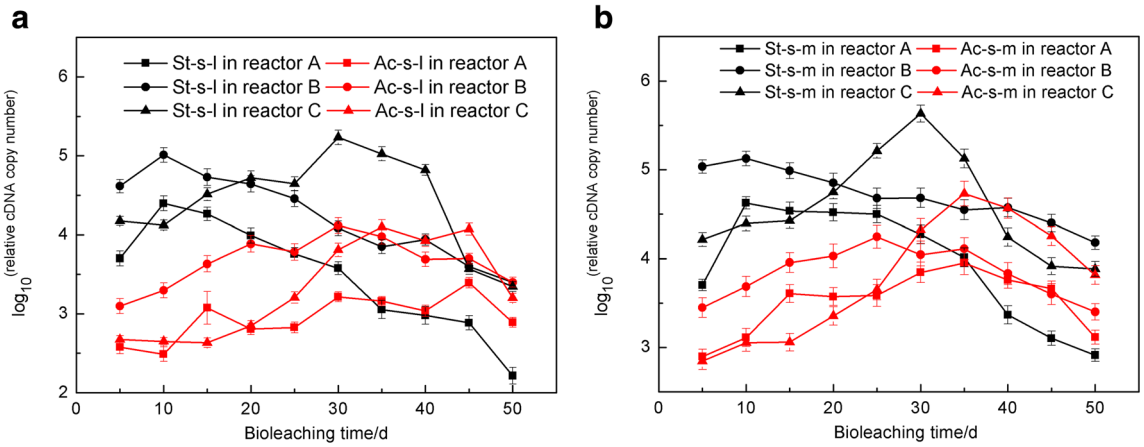

Fig. 5 Quantification of the *sat* expression in **a** free cells and **b** attached cells in reactors A, B, and C. St represented *S. thermosulfidooxidans* and Ac represented *A. caldus*. The label "s" meant *sat*, and "l" and "m" stood for free and attached cells, respectively. The labels also applied to Figs. 6 and 7. The logarithm of cDNA copy number (relative to 16S rRNA copies × 10^{-5} of each species, respectively) is shown

structure, suggesting that pH may impose less effects on attached cells.

Expression of selected functional genes

Figure 5 showed the expression of *sat* in reactors A, B, and C. As it can be seen, a similar trend was observed for both free and attached cells. Before day 25, the expression level of *sat* in *S. thermosulfidooxidans* in both free and attached cells were much higher than that in *A. caldus*, but the amount of *sat* expression in *S. thermosulfidooxidans* kept on declining after day 30. On the other hand, the expression level of *sat* in *A. caldus* increased before day 25. Finally, the expression levels were comparable in the two species.

When comparing the expression of *sat* among the three reactors, it was found that in both free and attached cells of *S. thermosulfidooxidans*, the gene was more expressed in reactor B than that in reactors A and C during the early stage. Nevertheless, as bioleaching continues, the gene expression began decreasing in reactors A and B on day 10 (pH 1.45 and 1.84, respectively), and in reactor C, it increased until day 30 (pH 1.70) and then decreased rapidly until day 40 as pH declined continuously. As to *sat* in *A. caldus*, the gene expression in both free and attached cells in reactor B was higher than that in the other two reactors in the initial stage. The highest levels of the gene expression for free and attached cells in reactor B occurred on days 30 (pH 1.47) and 25 (pH 1.50), respectively. The gene expression for free and attached cells in reactor C kept on increasing until day 35 (pH 1.56) it reached the highest level, on the contrary, the expression of *sat* in reactor A kept at a relatively low level.

Figure 6 showed that *fed* was mainly expressed in *S. thermosulfidooxidans* in the early and middle terms. After that, the expression of *fed* decreased in *S. thermosulfidooxidans*. The amount of gene expression in *F. thermophilum* kept on increasing as pH dropped between days 10 and 30 and finally exceeded (free cells) or became comparable (attached cells) to those in *S. thermosulfidooxidans*. The results also showed that

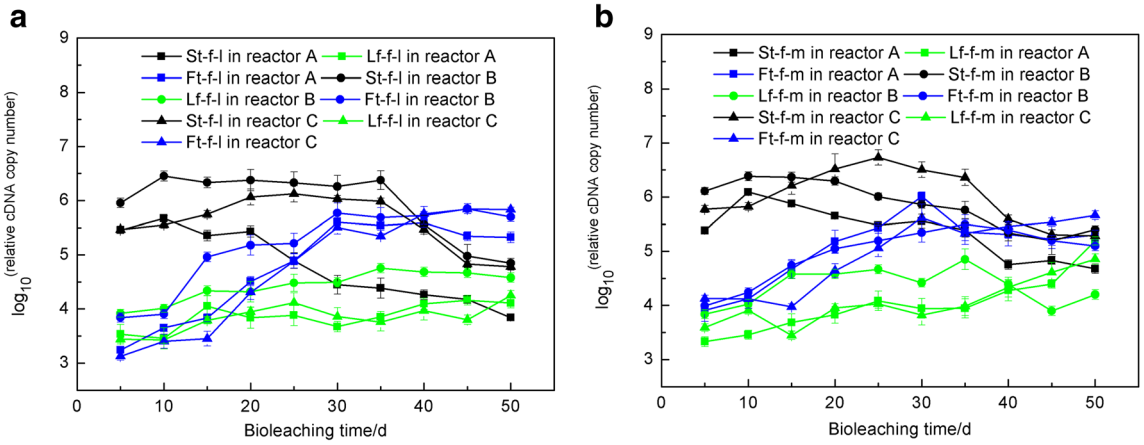

Fig. 6 Quantification of *fed* expressed in **a** free cells and **b** attached cells in reactors A, B, and C. Lf represented *L. ferriphilum* and Ft represented *F. thermophilum*. The label "f" meant *fed*. The labels also applied to Fig. 7. The logarithm of cDNA copy number (relative to 16S rRNA copies × 10^{-5} of each species, respectively) is shown

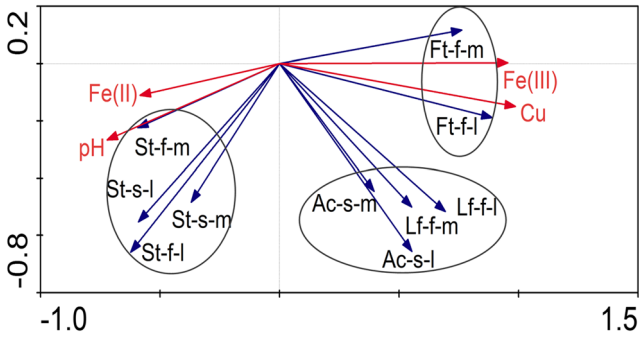

Fig. 7 Relationship between gene expression and pH, copper, Fe(II), and Fe (III) performed by RDA. Blue arrows represented the gene expression in the four species

the expression of *fed* in *S. thermosulfidooxidans* and *F. thermophilum* was highly related to the variation of pH.

In the leachate, the gene expression levels in *S. thermosulfidooxidans*, *L. ferriphilum*, and *F. thermophilum* were higher in reactor B than those in reactors A and C. The gene *fed* in *S. thermosulfidooxidans* was mostly expressed in the pH range 1.37–1.89. Previous study has shown that optimum pH for ferrous iron oxidation by *S. thermosulfidooxidans* is 1.5, indicating that expression of *fed* correlated well with iron oxidation of *S. thermosulfidooxidans* (Plumb et al. 2008). Similarly, the expression of *fed* was high in the later time, and this was consistent with that *F. thermophilum* oxidizes ferrous iron optimally at low pH values (Zhou et al. 2008). The copy number of *fed* expressed in *L. ferriphilum* was very low all the time though it kept a slightly rising trend on the whole. As for attached cells, the variation trend was similar to that of free cells.

In previous studies, microbial gene expression during bioleaching operations has been analyzed (Zhu et al. 2012; Zhou et al. 2015; Marín et al. 2017). Nonetheless, the chalcopyrite concentrate samples used contained other components such as pyrite, sphalerite, galena, chalcocite, and they would influence the bioleaching behavior and metabolism of microorganisms. In order to elucidate the effects of pH on the microbial gene expression and minimize the disturbance of the impurities, a chalcopyrite sample of high purity was used in the present study. Comparatively, the microbial community structures and the gene expression were quite different from previous study by Zhou et al. (2015). For instance, in this study, the content of *F. thermophilum* was much higher than that in the previous study in the later stage. Furthermore, the expression levels of *fed* in *S. thermosulfidooxidans* were highest during the early term in this study, while in the previous study, *fed* in *S. thermosulfidooxidans* was mostly expressed in the mid phase. The results indicated that the component of minerals would greatly affect the growth and metabolism of microorganisms.

Statistical analyses

Redundancy analysis (RDA) was used to evaluate the relationship between pH, copper, Fe (II), and Fe (III) concentrations and gene expression. As can be seen from Fig. 7, the expression of *sat* and *fed* in *S. thermosulfidooxidans* was positively correlated with pH and Fe (II), but negatively related to Cu (II) and Fe (III), indicating that they were mostly expressed during the early time with high pH value and Fe (II) concentration and low Cu (II) and Fe (III) concentrations. On the contrary, the expression of *fed* in *F. thermophilum* was negatively related to pH and Fe (II), but positively related to Cu (II) and Fe (III), suggesting that it tended to have higher expression level on conditions with low pH and Fe (II) concentration and high Cu (II) and Fe (III) concentrations in the species. Comparatively, the expression of *sat* in *A. caldus* was largely unrelated to pH and Fe (II) concentration and slightly correlated to Cu (II) and Fe (III) concentrations, which is in accordance with that the gene *sat* in *A. caldus* was more likely to be expressed on moderate conditions (Fig. 5). Results of correlation coefficients using the Pearson method (Table 1) showed that expression of *sat* and *fed* in *S. thermosulfidooxidans* was

Table 1 Correlation coefficients (*r* values) between pH, copper, Fe(II) and Fe (III), and expression of *sat* and *fed*. The letters in italic stood for correlation between pH values and gene expression with significance ($p < 0.05$)

	pH		Fe(II)		Fe(II)		Cu(II)	
	r value	*p* value	*r* value	*p* value	*r* value	*p* value	*r* value	*p* value
Ac-s-l	−0.001	0.996	−0.287	0.391	0.563	0.071	*0.648*	*0.031*
Ac-s-m	−0.069	0.807	−0.065	0.848	0.394	0.230	0.465	0.149
Ft-f-l	*−0.583*	*0.022*	−0.494	0.122	*0.824*	*0.002*	*0.904*	*0.0001*
Ft-f-m	*−0.620*	*0.013*	−0.355	0.284	*0.717*	*0.013*	*0.739*	*0.009*
Lf-f-l	−0.278	0.316	−0.445	0.170	*0.650*	*0.030*	*0.756*	*0.007*
Lf-f-m	−0.338	0.218	−0.454	0.161	*0.644*	*0.032*	*0.625*	*0.039*
St-f-l	*0.711*	*0.003*	0.456	0.159	−0.584	0.059	−0.514	0.106
St-f-m	*0.653*	*0.008*	0.394	0.23	*−0.603*	*0.049*	−0.555	0.076
St-s-l	*0.685*	*0.005*	0.435	0.181	−0.592	0.055	−0.501	0.116
St-s-m	*0.595*	*0.019*	0.322	0.332	−0.411	0.209	−0.296	0.377

significantly related to pH, but largely not significantly related to Cu (II) and Fe (III) concentrations (except for St-f-m, which is significantly related to Fe (III)). The expression of *Fed* in *F. thermophilum* was significantly correlated to pH, Cu (II), and Fe (III) concentrations. The relationship between pH, Fe (II), and Fe (III) and gene expression in *A. caldus* was not significant.

Conclusion

Physicochemical parameters such as pH would, basically, affect microbial metabolism during chalcopyrite bioleaching, and we tested it from the viewpoint of the gene expression in the present study. Iron and sulfur oxidation are the foremost metabolic pathways in bioleaching; therefore, two iron- and sulfur-oxidizing-associated genes were selected. Our results revealed that pH influenced leaching time of duration and microbial structure dynamics. Meanwhile, pH significantly affected the expression of the selected genes in *S. thermosulfidooxidans* and *fed* in *F. thermophilum* but not in *A. caldus*, namely, it selectively affected the expression of critical functional genes of specific species.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Research involving human participants and/or animals This article does not contain any studies with human or animal subjects.

Informed consent Informed consent is not required in this study.

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