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ORIGINAL PAPER

Comparison of chalcopyrite bioleaching after different microbial enrichment in shake flasks

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Abstract The bioleachings of chalcopyrite ore were compared after inoculating different cultures enriched from the original acid mine drainage sample. The results showed that the higher bioleaching performance was achieved for inoculation with the enrichment D (0.5 % S, 2 % iron and 1 % chalcopyrite) compared to other enrichment systems. The generated ferric precipitation during bioleaching had a key influence on the final copper extraction. After enrichment, higher ratio of iron-oxidizer and higher ratio of sulfur-oxidizer existed in enrichment B and C, respectively. These caused the different bioleaching behaviours from other systems. Maintaining a suitable equilibrium between iron- and sulfur-oxidizers is significant to decrease ferric precipitation or postpone its formation, finally prolong efficient bioleaching period and improve copper extraction.

Keywords Bioleaching · Chalcopyrite · Enrichment · Microbial community · Ferric precipitation

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Introduction

In recent decades, many progresses on bioleaching copper sulfide ores have been made on laboratorial/commercial-scale in China,Australia and Chile (Brierley and Brierley 2001; Demergasso et al 2010; Ruan et al 2011). Until now, industrial application for bioleaching, however, is dedicated mainly to copper extraction from secondary copper sulfide ore. Heap bioleaching of chalcopyrite is a developing technology and there are many difficulties to overcome. Many efforts have been developed to improve its bioleaching performance by optimizing the bioleaching temperature, the inocula and redox potential (Córdoba et al 2008; Gautier et al 2008; Third et al 2002). These endeavors have partially enhanced copper extraction and have been very useful in the understanding of bioleaching mechanism. Some reports showed that mixed cultures have more strong ability to bioleaching sulfide mineral than pure cultures (Akcil et al 2007; Qiu et al 2005), this is because various species with different physiological and ecological functions co-exist in the same bioleaching system and play complementary roles to leach metal. Actually, it is impossible only a single species in commercial operation heap under usually open environment. Therefore, the microbial ecology aspect should be regarded as the key for understanding the mechanism and improving the bioleaching efficiency (Johnson 2001). Molecular ecology is an emerging technique for correlating heap leach parameters—such as pH, redox potential, temperature, and leach rates—with microbial activities and changes in microbial communities in heap leaching operations. It is conceivable that the correlation of microbial community dynamics and leaching parameters may be used to improve bioleaching performance in heap leaching operations in the future.

Therefore, inocula containing suitable microbial consortia are very important as well as an optimized bioleaching process. These inocula in heap come mainly from acid mine drainage (AMD), sulfur spring, or other natural acidic water. It, however, is not very ideal to directly inoculate these wild species due to the low microorganisms' concentration, weak tolerance to poor mineral environments and the susceptibility to high heavy-metal toxicity (Zhen et al 2008). Enrichment and adaptation are usually applied before being inoculated for bioleaching. Due to the different selective pressures and inhabitant environments between original and enriched environments, it is unavoidable to cause the difference of their microbial community composition and richness when preparing inocula by enrichment approach. The challenge aroused is to ensure that the less abundant species are not washed out and maintain/improve their activities during enrichment. Although it was been highlighted on the importance of assembling microbial consortia to process different mineral ores and concentrates (Rawlings and Johnson 2007), the significance was neglect to improve the bioleaching performance from the viewpoint of how to prepare inoculum with suitable consortia.

In this report, several different enrichment ways on the same original AMD sample were used to generate various secondary inoculum consortia and subsequent chalcopyrite bioleaching tests. Their bioleaching performances were compared and analyzed.

Materials and methods

Mineral and solid residues characteristics

Chalcopyrite ore in this study was determined by using inductively coupled plasma-atomic emission spectroscopy for its chemical composition analysis and following by X-ray diffraction for its phase composition analysis (Table 1). Leached residues were also quantified by X-ray diffraction based on their peak intensity ratios to external standards sample. Over 90 % of the ore had a particle size <45 μm.

Table 1 Composition analysis of chalcopyrite by ICP-AES and XRD

Chemical composition	Wt%	Phase composition	Wt%
Cu	21.9	Chalcopyrite ($CuFeS_2$)	61.7
Fe	18.8	Sphalerite (ZnS)	29.6
S	32.7	Galena (PbS)	3.9
Pb	6.8	$PbSO_4$	3.7
Zn	19.8	Seligmannite ($PbCuAsS_3$)	1.1

Enrichment and adaption

The original microbial sample was obtained from an AMD in a copper mine, China. One part of this sample was subjected to the microbial community composition analysis, and another part was used for the enrichment and adaption for the subsequent bioleaching tests. For enrichment and adaption, the original microbial sample was inoculated into the flasks containing sterile 1 % chalcopyrite; 2 % ferrous sulfate and 1 % chalcopyrite; 0.5 % sulfur and 1 % chalcopyrite; 0.5 % sulfur, 2 % ferrous sulfate and 1 % chalcopyrite to generate enrichment A, B, C, D, respectively. All enrichment cultures in the research were grown in 9 K basic medium with the initial pH of 2.0 as well as the above corresponding energy resource (Silverman and Lundgren 1959). Each enriched culture grown in the corresponding medium was re-inoculated into next fresh medium for their continued enrichment and adaptation when the total cell concentration of 1×10^8 cells/ml was obtained.

Bioleaching experiments

Chalcopyrite used in this study was sterilized separately by autoclaving for 40 min at 121 °C and other medium for 20 min at 121 °C. After three re-inoculations, 3 ml of final enriched cultures were inoculated into 200 ml 9 K sterile medium with pulp density of 4 % sterile chalcopyrite for bioleaching tests. As a control, equal microbial concentration from original microbial sample without enrichment and adaptation was also directly inoculated into medium with chalcopyrite. The initial total microbial concentration in all bioleaching tests were 1.5×10^6 cells/ml or so. All cultures were incubated at 30 °C and agitated at 180 rev/min. Aliquots of bioleaching solution were sampled to analyze the concentration of Cu, redox potential and pH at intervals of 3 days. Each test was replicated in three parallel flasks.

Analysis methods

Cells adsorbed on mineral were harvested according to our previous description (Xia et al 2012). Total cell numbers were counted using a Thoma chamber and microscopy. The copper concentration in solution was determined by an atomic absorption spectrophotometer. The pH was measured by using a pHS-3C acid meter. The redox potentials, which indicated the Fe(III)/Fe(II) ratio, were measured with Pt electrode, and a saturated calomel electrode as the reference electrode.

Microbial community analysis

Extraction of total DNA containing free/adsorbed cells and subsequent PCR-RFLP (polymerase chain reaction-restriction

fragment length polymorphism) assays were carried out according to our previous report (Xia et al 2012).Hundred clones were randomly selected from each clone library corresponding to each sample and subjected to OUT analysis (operational taxonomic units). According to rarefaction analysis, the number of selected clones could make sure that each library would be sequenced enough to extrapolate the total sequence diversity. The RFLP patterns were grouped, and the clone containing representative patterns was selected for its 16S rDNA sequencing. The clones in each OUT were enumerated so as to analyze the community composition.

Results and discussion

Bioleaching experiments

Although these various enriched cultures were originated from the same original sample, they exhibited the different bioleaching performances on chalcopyrite after enrichment and adaptation (Fig. 1). During the first 18 days, the highest leaching rate and copper extraction were achieved in the flask inoculated with enrichment B, however, after the 18th day, the bioleaching rate decreased obviously and the final percentage of copper extraction was only 49.8 %. On the contrary, high bioleaching rate was maintained in those tests inoculated with enrichment A, C, D and their final copper extraction percentages were 67.0, 71.2, 75.1 %, respectively. Compared with enriched cultures, the original sample as expected showed lower copper bioleaching activity (28.5 %). These indicated that it is significant to how to enrich and adapt the original environmental sample in order to improve the copper extraction in bioleaching process.

The pH values were determined to promote the understanding of various bioleaching behaviours of inoculums after different enrichment and adaptation. In all the bioleaching tests, two clear phases can be distinguished, as shown in Fig. 2. During the first phase, there is an increase of the pH followed by a decrease. However, there was an obvious difference in the period that took from the rise phase of pH to the decrease phase in the different bioleaching tests. The final pH value were also various from 2.40 (original sample), 2.10 (enrichment A), 1.50 (enrichment B), 1.05 (enrichment C) to 1.47 (enrichment D). The final pH value during the process of bioleaching chalcopyrite depends on four different reactions: acid leaching, iron oxidation, ferric precipitation and sulfur oxidation which take place in bioleaching tests. Iron oxidation and acid leaching will lead to the increase of pH value, on the contrary, sulfur oxidation and ferric precipitation decrease the pH value (Eqs 1−4).

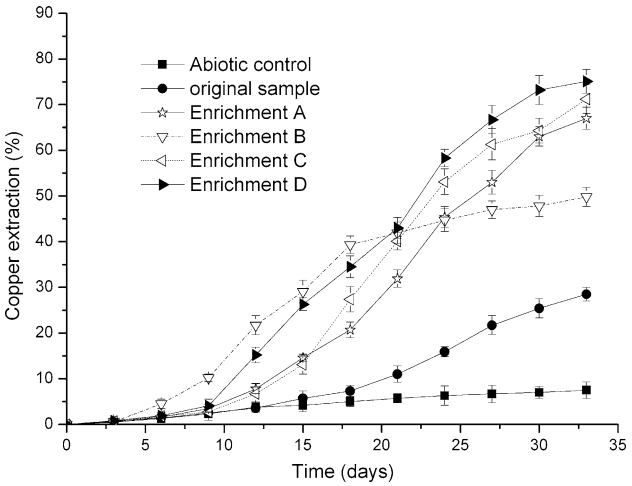

Fig. 1 Percentage of copper recovery obtained by using inoculation with different enriched cultures

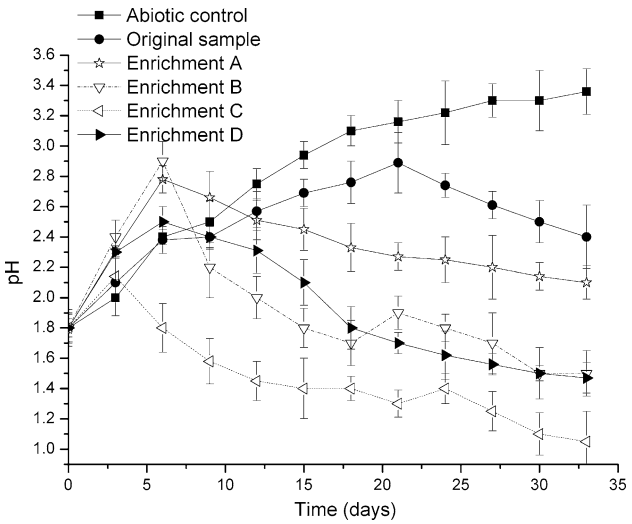

Fig. 2 Change of pH in various tests inoculated with different enriched cultures

$$4Fe^{2+} + O_2 + 4H^+ \xrightarrow{\text{Iron oxidizing metabolism}} 4Fe^{3+} + 2H_2O \quad (1)$$

$$CuFeS_2 + 4H^+ \rightarrow Fe^{2+} + Cu^{2+} + 2H_2S \quad (2)$$

$$2S^o + 2H_2O + 3O_2 \xrightarrow{\text{Sulfur oxidizing metabolism}} 2SO_4^{2-} + 4H^+ \quad (3)$$

$$3Fe^{3+} + K^+ + 2HSO_4^- + 6H_2O \rightarrow KFe_3(SO_4)_2(OH)_6 + 8H^+ \quad (4)$$

After bioleaching for 3 days, the pH in test with the enrichment C began to decrease and the change of pH from increase to decrease occurred earlier than other tests. This indicated that sulfur oxidation and/or ferric precipitation were stronger than other tests in the beginning of

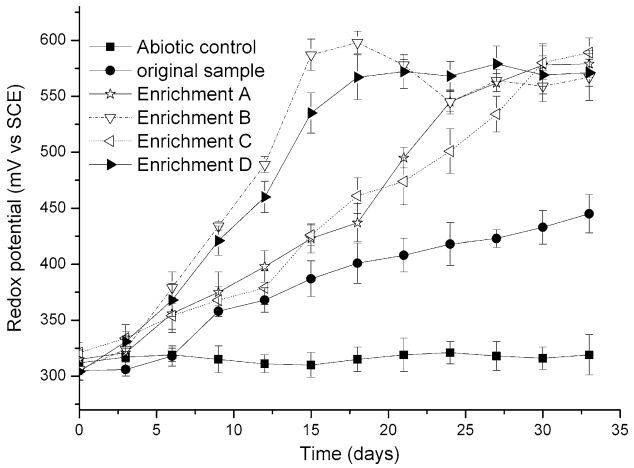

Fig. 3 Change of redox potential as a function of time

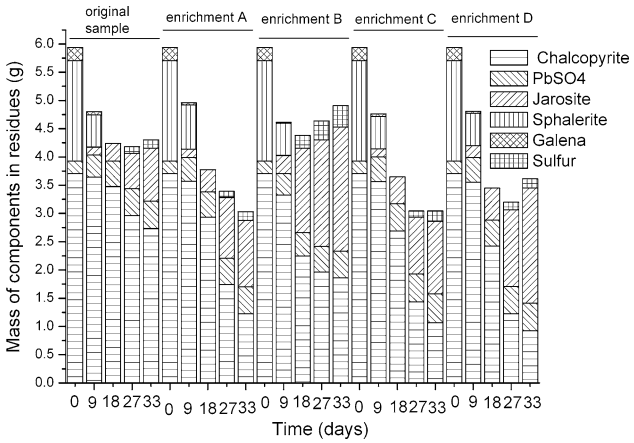

Fig. 4 Variation of the residues component in bioleaching tests inoculated with different enriched cultures as a function of leaching time based on XRD analysis

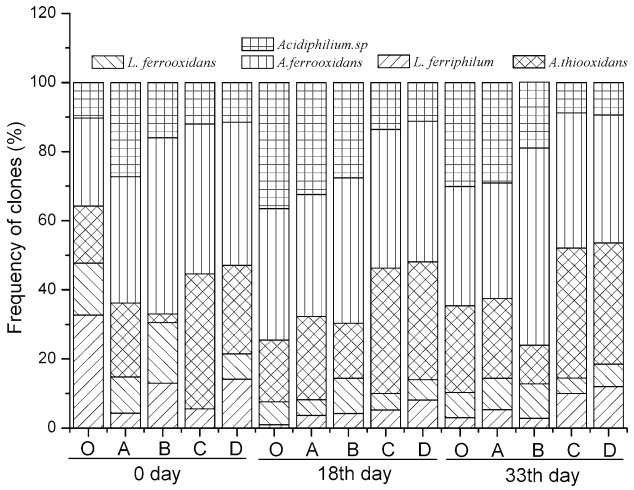

Fig. 5 Change of microbial community composition after the different enrichment treatments. *O* original sample, *A* enrichment A, *B* enrichment B, *C* enrichment C, *D* enrichment D

bioleaching process, however, it was reported that the formation of jarosite will be significantly inhibited under pH 1.8 (Daoud and Karamanev 2006; Pogliani and Donati 2000), moreover, the result of redox potential showed less than 450 mV indicating low Fe(III)/Fe(II) ratio in all tests during this term (Fig. 3). Thus, it can be neglected to the formation of ferric precipitation under such a low redox potential condition. Also, it should be noted that the inoculum in test (enrichment C) was enriched by sulfur and chalcopyrite. Therefore, it is possible that higher sulfur-oxidizing activity existed in the beginning of bioleaching process because the inoculum was enriched with sulfur medium. Therefore, the decrease of pH value after bioleaching for 3 days should not contributed to the ferric precipitation but to the increase of sulfur oxidation in the microbial consortia. Our previous work also showed that moderate external additional sulfur can increase the sulfur-oxidizing activity in microbial consortia (Xia et al 2012). However, sulfur oxidation may be low in other tests at the beginning of bioleaching process. Consequently, the change of pH from increase to decrease in other tests occurred later than the test with enrichment C. Taken together these results, it showed that the different enriched cultures coming from the same original sample have different influence on the change of pH and redox potential during the bioleaching process.

Assay of bioleaching residues

After bioleaching for 33 days, chalcopyrite remained to be main component in residues in tests with original sample and enrichment B (Fig. 4), this supported the results of low bioleaching performance in the original sample and enrichment B tests as well as the low bioleaching activities of inoculums from original sample and enrichment B (Fig. 1). Residues analysis revealed sphalerite and galena were easier to be leached than chalcopyrite in all tests (Fig. 4). However, galena was transformed into $PbSO_4$ to subsequent precipitate. Additionally, new components-sulfur and jarosite were generated at the middle or latter stages of bioleaching process. Previous studies have identified that these new components including sulfur, $PbSO_4$ and jarosite could cover the mineral surface and hinder the copper extraction by interrupting the contact between microorganisms and mineral (Córdoba et al 2009; Zhou et al 2009). However, it is impossible to generate quite amount of sulfur due to the existed sulfur oxidizer in all tests here (Fig. 5). This was also testified by the results from residues analysis. Therefore, jarosite is the key factor to inhibit the bioleaching compared to the new formed $PbSO_4$ and sulfur from viewpoint of amount. Further, it should be noted that higher amount of jarosite (1.49 g) has

been generated in enrichment B test after only bioleaching for 18 days compared to other tests (Fig. 4). This indicated that the earlier generation of jarosite caused low final copper extraction in enrichment B test. Additionally, at the final bioleaching stage, less biomass in original sample test (7.2×10^7 cells/ml) than other tests (more than 6.3×10^8 cells/ml) could be contributed to no adaptation for the original sample. Thus, this also explains why lower copper extraction percentage was achieved in original sample test than other tests. Consequently, adaptation and applicable enrichment are essential to ensure a high copper extraction before inoculating and leaching chalcopyrite. This could be contributed that the application of a suitable enriched culture can inhibit or delay the generation of passivation and prolong the effective leaching period, as well as achieving high cell concentration after adaptation.

Comparison of microbial communities

According to the PCR-RFLP assays, the original sample contains *Acidithiobacillus ferrooxidans* (*A. ferrooxidans,* 25.5 %), *Leptospirillum ferrooxidans* (*L. ferrooxidans*, 15 %), *Acidiphilium* sp. (10.3 %), *Acidithiobacillus thiooxidans* (*A. thiooxidans,* 16.5 %) and *Leptospirillum ferriphilum (L. ferriphilum,* 32.7 %) (Fig. 5). *L. ferrooxidans* were not detected. *A. ferrooxidans* (43.4 %) and *A. thiooxidans* (39 %) became more dominant in enrichment C with sulfur and chalcopyrite. This suggested that species with low competitive ability would be washed out of the consortia due to high selective stress during enrichment. *A. thiooxidans* decreased from 16.5 to 2.5 % and became the non-dominant species in enrichment B test. Obviously, *A. ferrooxidans* was the dominant species in all enrichment cultures. This may be related to that both ferrous iron and sulfur can be used as its growth source. However, *A. thiooxidans* only oxidize sulfur and *L. ferriphilum* only for iron, consequently, ferrous iron or sulfur in medium will have an important influence on microbial richness in community as a selective factor.

Based on the number of clones corresponding to the different OUTs, it was calculated on the ratio of iron-oxidizers to sulfur-oxidizers (in here, *A. ferrooxidans* is not statistically involved due to its both sulfur oxidation and iron oxidation). The higher ratio (0.9) of iron-oxidizers to sulfur-oxidizers was maintained in enrichment B test after inoculating and bioleaching for 18 days. This is related to its high initial ratio present in the inocula. The ratio was higher than that of other tests (0.42, 0.34, 0.27 and 0.41 for original sample, enrichment A, C and D, respectively) (Fig. 5). It revealed that higher iron-oxidizing activities and lower sulfur-oxidizing activities were present in enrichment B test than other tests. This also can be supported from the evidence of higher and more rapid increased redox potential in enrichment B test than other tests (Fig. 3). Consequently, more ferric ions were generated and leaded to the fast increase of redox potential, subsequently, an earlier formation of jarosite (Ahmadi et al 2011). At the same time, the lower sulfur-oxidizing activities may not completely oxidize sulfur covering the mineral surface. At this point, sulfur and jarosite on mineral surface will hinder the further copper release. This was also supported by the results in Fig. 4. Likewise, high abundance of *A. thiooxidans* in enrichment C and D tests (36.1 and 34.1 %, respectively) could be contributed to its high ratio in these initial inocula. This ensured that sulfur on mineral surface is most possibly oxidized to sulphuric acid that can inhibit the formation of jarosite and promote acid leaching. Thus, suitable equilibrium between iron- and sulfur-oxidizers is very beneficial to postpone the generation of passivation and prolong the efficient leaching period, and finally ensure high copper extraction. Due to the modification of microbial consortia in inoculums preparation by adding different ratio of energy source, it exhibits the different bioleaching efficiencies of the investigated chalcopyrite ore. Furthermore, this also reasoned that it is possible to further improve the bioleaching performance through optimizing the energy source composition and considering the influence of the ore composition during inoculums preparation, subsequently generating optimal microbial consortia for inoculating.

After 33 days, the ratio of *A. ferrooxidans* increased from 42.7 % in 18th day to 57 % in 33rd day in the enrichment B test. The compositions of consortia in other tests after 18th day are similar to those in 18th day and showed low compositional change at the final bioleaching stage (Fig. 5). This indicated that the influence of microbial composition in inocula became weaker as a function of bioleaching time. However, the starting microbial consortia composition and its composition change are very important influence on copper extraction. This is because starting microbial consortia would determine when to form passivation layer and what to passivation layer on mineral surface by affecting the redox, pH and ion concentration. Once the key factor-jarosite was formed early due to the influence of starting inocula with unsuitable microbial consortia, this will significantly limit the copper extraction in the middle and final of bioleaching process. After all, the formation of jarosite is a chemical non-reversible process even if microbes exist in bioleaching system and this is different from sulfur formation/dissolution process by sulfur-oxidizers.

Conclusions

Compared to original sample, higher copper extraction could be achieved by inoculating the enriched cultures with

sulfur and chalcopyrite or with iron and sulfur and chalcopyrite. Passivation layers composited by $PbSO_4$, sulfur and ferric precipitations (mainly by ferric precipitations) during bioleaching inhibited the bioleaching process and decreased the final copper extraction in all tests. Enriched cultures with suitable equilibrium between iron- and sulfur-oxidizers could achieve higher copper extraction. This could be attributed to the delayed formation of passivation and the prolonging of efficient leaching period.

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