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ORIGINAL PAPER

Comparison of bioleaching behaviors of different compositional sphalerite using *Leptospirillum ferriphilum*, *Acidithiobacillus ferrooxidans* and *Acidithiobacillus caldus*

Lexian Xia · Songlin Dai · Chu Yin · Yuehua Hu · Jianshe Liu · Guanzhou Qiu

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Abstract Two sphalerite samples with different iron/sulphur (Fe/S) ratios, Shuikousan ore (Fe/S 0.2) and Dachang ore (Fe/S 0.52), were processed using three microbial species, *Leptospirillum ferriphilum*, *Acidithiobacillus ferrooxidans* and *Acidithiobacillus caldus.* Following 20 days of bioleaching in shake flask cultures, a higher zinc (Zn) extraction (96%) was achieved with Shuikousan ore than with Dachange ore (72%). The extraction efficiency increased when elemental S was added to Dachang ore to attain the same Fe/S ratio as that for Shuikousan ore. Following the addition of S, the redox potential, pH and total dissolved Fe for Dachang ore demonstrated similar behaviors to those of Shuikousan ore. *Acidithiobacillus caldus* and *L. ferriphilum* became the dominant species during the bioleaching of sphalerite with a high Fe/S ratio. In contrast, the dominant species were *A. ferrooxidans* and *A. caldus* during the bioleaching of sphalerite with a low Fe/S ratio. These results show that the Fe/S ratio has a significant influence on the bioleaching behavior of sphalerite and the composition of the microbial community.

Keywords Bioleaching · Community composition · Sphalerite

L. Xia · Y. Hu · J. Liu (✉)
Key Laboratory of Biohydrometallurgy of Ministry of Education, School of Resources Processing and Bioengineering, Central South University, 410083 Changsha, People's Republic of China
e-mail: ljscsut@126.com

C. Yin · G. Qiu (✉)
Department of Bioengineering, School of Resources Processing and Bioengineering, Central South University, 410083 Changsha, People's Republic of China
e-mail: qgz@126.com

S. Dai
Dongguan institute of Environmental sciences, 523011 Dongguan, People's Republic of China

Introduction

The general technological approach used for extracting metallic zinc (Zn) is still the Roast–Leach–Electrowinning (RLE) process, which is currently responsible for more than 85% of the world's total Zn production [1]. However, the high SO_2 emissions and sulphuric acid consumption associated with the RLE process are increasingly challenging the future acceptability of this technology [2].

In terms of the processing of low-grade, overburden and waste sulfide minerals from current mining operations, bacterial leaching technology has attracted considerable interest because it is low-cost and relatively more environmentally friendly [3, 4]. Many microbes, such as *Leptospirillum ferriphilum*, *Acidithiobacillus ferrooxidans* and *Acidithiobacillus caldus*, have been shown enhance bioleaching in commercial applications for the extraction of cobalt, nickel, cadmium, antimony, Zn, lead, gallium, indium, manganese, copper, and tin from sulfur (S)-based ores [5–7].

Several important factors affect the growth of bacteria and, consequently, the dissolution process, including temperature, pH, availability of nutrients, sulfide minerals, O_2 and CO_2, pulp density, metal toxicity, among others [5, 8]. Most of the microorganisms involved in bioleaching can grow by oxidizing S and ferrous ions derived from minerals, with the S and ferrous ion ultimately being oxidized to sulfuric acid and ferric ions, respectively. It has also been reported that both protons and ferric ions are significant leaching reagents in the extraction of Zn from sphalerite minerals [9, 10]. Thus, it is reasonable to speculate that the different sphalerite minerals with various ratios of iron (Fe) and S have an important influence on the growth and community composition of these S- and ferrous-oxidizing bacteria. Conversely, the composition of the different microbial communities containing S- and ferrous-oxidizing bacteria can also affect bioleaching performance. Moreover, in most natural sphalerites, Zn can be substituted by Fe at various levels in the crystal lattice, and the mineralogical property leads to various ratios of Fe, Zn and S for natural sphalerite. Therefore, a knowledge of the bioleaching behaviors of sphalerite of different mineralogical composition and the associated changes in the composition of the microbial community would be valuable.

Molecular phylogenetic techniques, such as PCR–denaturing gradient gel electrophoresis (PCR–DGGE), PCR–restriction fragment length polymorphism (PCR–RFLP) and micro-arrays, have been successfully and widely applied to ecological analyses of acid mine drainage [11–14]. It is therefore possible to investigate the relationship between sphalerites and the composition of the microbial community during the bioleaching sphalerite of different mineralogical composition by the above techniques. Clearly, the results of such experiments would accelerate our understanding of the interaction between S-oxidizing and ferrous-oxidizing bacteria and sphalerite as well as the bioleaching mechanism. To date, little ecological information is available on the bioleaching of sphalerites of different mineralogical composition.

In the study reported here, we compared the bioleaching of two sphalerite ores with different Fe/S ratios by inoculating the samples with three kinds of bacterial species, *L. ferriphilum*, *A. ferrooxidans* and *A. caldus*. The subsequent changes in the composition of the microbial community of the above three species were examined by PCR–RFLP during the bioleaching process.

Materials and methods

Ore characteristics

The two ore samples of sphalerite used in this work were obtained from Shuikousan (Hunan, China) and Dachang (Hubei, China), respectively. Their chemical composition is shown in Table 1. X-ray diffraction (XRD) and chemical analyses indicated that Zn is partly substituted by Fe in the crystal lattice of the two sphalerite samples. The ore samples were finely ground to a particle size (over 75%) of <0.074 mm. Prior to use, the sphalerite was washed with 2 *M* hydrochloric acid to remove any surface-oxidized deposits, rinsed repeatedly with distilled water, sterilized and dried at 100°C for 1 h.

Table 1 Chemical composition of sphalerite from Shuikousan and Dachang

Sample	Zn	Fe	S	Pb	Sn	Si	Mole ratio of Fe and S
Dachang	48.14	16.21	34.43	0.9	0	0.3	0.52
Shuikousan	50.54	11.27	32.12	3.1	0.33	4.45	0.2

Composition of each element is given as a mass fraction (5)

Zn, zinc; Fe, iron; S, sulphur; Pb, lead; Sn, tin; Si, silicon;

Bioleaching experiments

The bioleaching experiments were carried out in 500-ml flasks containing 200 ml sterilized, iron-free 9 K nutrient media into which equal amounts of *L. ferriphilum*, *A. ferrooxidans* and *A. caldus* were inoculated (initial cell number of each species 10^6 cells/ml). The bacteria were cultured on a rotary shaker (180 rpm) at 35°C. The pulp density and initial pH were 10% and 2.0, respectively. The initial pH of the medium in all flasks was around 2.0, but no pH control was exercised during the experiment. Aliquots of bioleaching liquid were sampled to analyze the concentration of Zn^{2+}, Fe^{2+}, total Fe, pH and redox potential (E_H) at regular intervals. Each bioleaching experiment was replicated in five parallel flasks.

Analysis methods

The components of the mineral sample and leached residues were analyzed by XRD. The concentrations of Zn and total Fe in solution were determined by atomic absorption spectrophotometer, and that of ferrous ions in solution was assayed by titration with potassium dichromate. The pH was measured using a pHS-3C acid meter. The E_H, which indicates the ratio of Fe(III)/Fe(II), was measured with a Pt electrode, and a saturated calomel electrode was used as the reference electrode.

Bacterial population analysis

Preparation of total DNA and PCR amplification

Culture flasks were removed from the shaker after bioleaching for 6, 12 and 18 days, respectively. The leaching solution from parallel flasks was filtered through a 0.22-μm pore-size membrane. The residue containing all biomass was used to extract total DNA according to the procedure described by Zhou et al. [15]. Community 16S rDNA genes were first amplified with the primer set 1492R

(5′-CGGCTACCTTGTTACGACTT-3′) and 27F (5′-AG AGTTTGATCCTGGCTCAG-3′) [16], 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).

Cloning, analysis of 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) for blue–white screening. About 100 white clones were randomly selected from each library. The inserted fragments were amplified with the vector-specific T7 and SP6 primers and digested by restriction enzymes *Hin*6I and *Msp*I 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 representative cloned fragments were selected for sequencing. Each operational taxonomic unit (OTU) (unique RFLP patterns) indicates one of three bacterial species. The clones in each OTU were accounted for in order to analyze the bacterial population of each species (community composition).

Results and discussion

Bioleaching experiments

The main difference between the sphalerite samples in this study is the ratio of Fe and S contained in the mineral, as is shown in Table 1. The mole Fe/S ratio of of the Dachange sample is 0.52 and that of the Shuikousan sample, 0.2. Following 20 days of bioleaching in shake flask cultures, a higher zinc (Zn) extraction (96%) was achieved with Shuikousan ore than with Dachange ore (72%) (Fig. 1). However, less Zn extraction was extracted from Shuikousan ore during the initial 12 days of the experiment than from Dachang ore, whereas thereafter very little Zn was extracted from Dachang ore. Taking into consideration that we maintained the experimental factors identical in each bioleaching experiment, such as pH, temperature, pulp density and microbial species, we speculate that different Fe/S ratios led to the different extraction efficiencies from the two sphalerite samples.

The pH of the solution initially increased in all bioleaching systems. However, compared with the pH of the solution in the abiotic flasks, the pH of the inoculated solution decreased after several days. It has been reported that sphalerite can be leached by acid [4, 17] (Eq. 1). Therefore, the increase in the pH at the beginning of the experiment could be attributed to the consumption of protons in the chemical leaching of sphalerite (Eq. 1). The inoculated S-oxidizing bacteria (*A. ferrooxidans* and *A. caldus*) were able to grow through their oxidizing of the elemental S that is formed in the chemical leaching process (Eq. 2). Consequently, the bacterial oxidization of s could release protons to the solution and compensate for their consumption. The rate of released protons increased when the S-oxidizing bacteria shifted from the lag growth period to the exponential growth period after bioleaching for some days. That is, chemical leaching together with S oxidation determined the pH of the solution. Hence, the decrease in the pH in leaching systems inoculated with bacteria could be attributed to the rapid release of protons due to the rapid growth of S-oxidizing bacteria. The release of protons exceeds consumption during bacterial exponential growth. As a result, the pH decreases in systems with bacteria after some days, which is in contrast with the sterile systems in which the pH increases continuously.

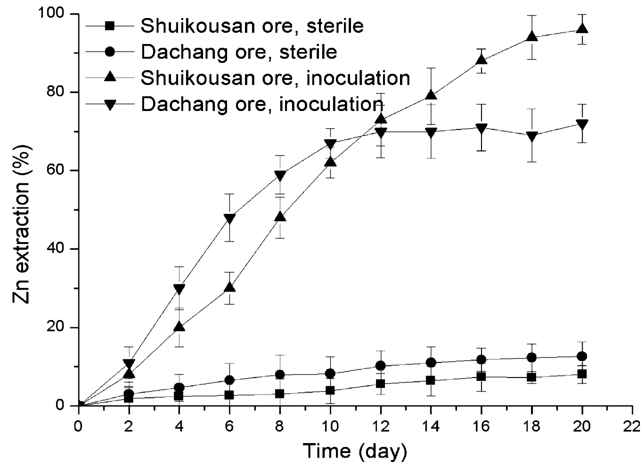

Fig. 1 Zinc (*Zn*) extraction from Shuikousan ore and Dachang ore in leaching experiment

$$ZnS + H^+ + \frac{5}{4}O_2 \rightarrow Zn^{2+} + \frac{1}{2}S^0 + \frac{1}{2}SO_4^{2-} + \frac{1}{2}H_2O \quad (1)$$

$$2S + 3O_2 + 2H_2O \xrightarrow{\text{bacteria}} 2H_2SO_4 \quad (2)$$

Iron plays an additional key role in the bioleaching of sphalerite. Ferric ions initially dissolve sphalerite to generate Zn ions and ferrous ions. The latter, as the energy source of bioleaching microorganisms, are ultimately oxidized to regenerate ferric ions by ferrous-oxidizing bacteria (*L. ferriphilum*, *A. ferrooxidans*) [4] (Eqs. 3, 4). Figures 2 and 3 show the change in total Fe and E_H in solution, respectively. At the beginning of bioleaching, both the E_H

and dissolved total Fe are low, increasing during the bioleaching of the Shuikousan and Dachang ores. However, after 8 and 12 days of bioleaching of the Dachang ore, the E_H and the concentration of total Fe decrease, respectively. This evolution pattern of E_H and dissolved total Fe can be explained by bacterial growth. The initial biomass of the bioleaching bacteria in the solution is low; therefore, Zn extraction is mainly chemical at this time, leached by a few protons and ferric ions. When the growth rate of the bacteria increases, the bioleaching function improves, leading to an increase in the number of released protons and ferrous ions; in turn, the released ferrous ions are further oxidized to ferric ions and increase the E_H. The decrease in both the E_H and dissolved total Fe can be attributed to the formation of jarosite (Eq. 5). The study of Daoud [18] revealed that the main parameter affecting jarosite formation is pH, with low jarosite precipitation under conditions of pH 1.6–1.7 at an operating temperature of 35°C. Jarosite formation does not only decrease the number of ferric ions used as the oxidant but also inhibits the diffusion of reactants and products through the precipitation zone [19]. Hence, a low pH inhibits jarosite precipitation and leads to higher Zn extraction due to the decreased pH (<1.7) towards the end of the bioleaching process for the Shuikousan ore (Fig. 4). However, the system for Dachang ore maintains a high pH. Consequently, high jarosite precipitation occurred and possibly inhibited the bioleaching process after about 10 days. Finally, a much high ratio of Zn extraction (96%) was obtained for the Shuikousan ore than for the Dachang ore (72%). Thus, based on the results, we conclude that the difference in zinc extraction is the result of different behaviors of Fe(III) and pH due to different ratios of Fe/S in the two sphalerite samples.

To clarify the above hypothesis, we added an additional 2.85 g elemental S to the bioleaching system containing 20 g Dachang ore so as to reach the same Fe/S ratio as that of the Shuikousan ore. The evolution of the E_H and pH are shown in Figs. 5 and 6. Compared with the bioleaching system for Dachang ore without any additional elemental S, the E_H of the S-supplemented system did not decrease, and the pH of the latter system was lower at the end of the bioleaching process. At the end of the experiment, the S-supplemented system had achieved a Zn extraction efficiency of 90% (72% for the same system without the additional S). Therefore, the Zn extraction process and the behavior of the pH and E_H of the Dachang ore system with supplemented S resembled those of the Shuikousan ore system when the same Fe/S ratio was attained. Thus, the Fe/S ratio in sphalerite has a significant influence on bioleaching performance.

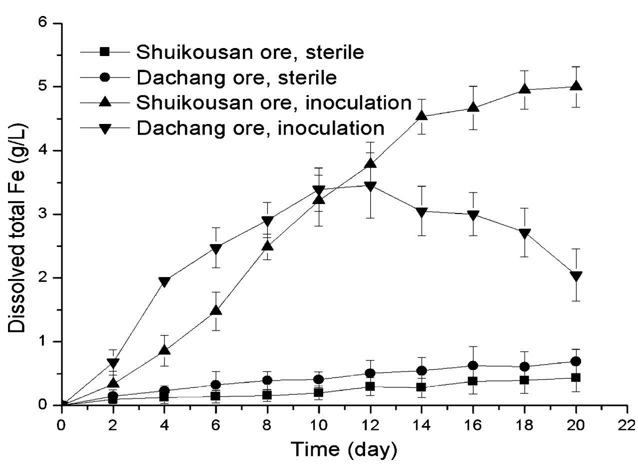

Fig. 2 Change of the total iron (*Fe*) concentrations during the leaching of Shuikousan and Dachang ore

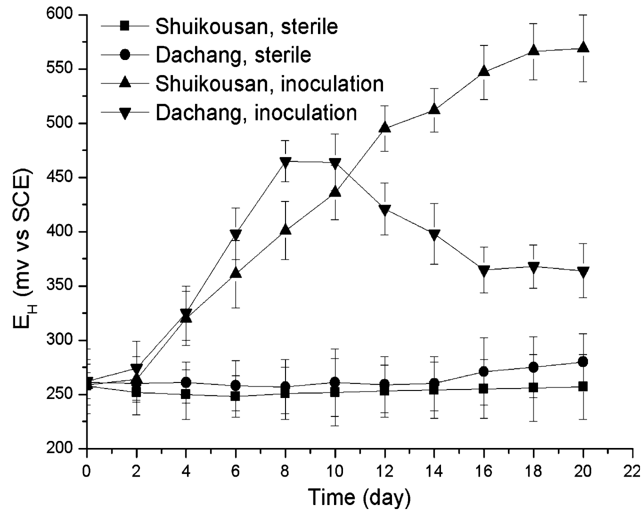

Fig. 3 Evolution of the redox potential (E_H) during the leaching of Shuikousan and Dachang ore

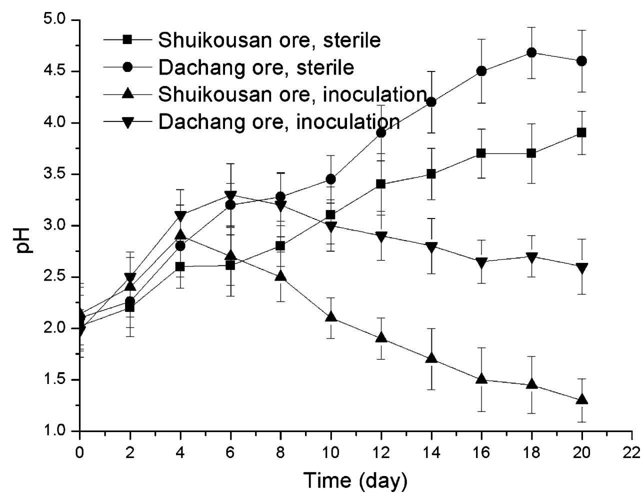

Fig. 4 The evolution of pH in the shake flask bioleaching systems as a function of time

$$ZnS + Fe_2(SO_4)_3 \rightarrow ZnSO_4 + S^0 + 2FeSO_4 \quad (3)$$

$$2FeSO_4 + H_2SO_4 + \frac{1}{2}O_2 \xrightarrow{\text{bacteria}} Fe_2(SO_4)_3 + H_2O \quad (4)$$

$$3Fe^{3+} + K^+ + 2HSO4^- + 6H_2O \rightarrow KFe_3(SO_4)_2(OH)_6 + 8H^+ \quad (5)$$

Bacterial population analyses

In this study, we used a simple bioleaching ecological community comprising three microbial species, *L. ferriphilum*, *A. ferrooxidans* and *A. caldus*. The proportions of these bacteria were continuously changing: at the initial stage of the experiment, the cell numbers of each species were the same, but by the end of the the bioleaching process of the Shuikousan and Dachang ore samples, the proportions were different (Figs. 7, 8). During the processing of Shuikousan ore, *L. ferriphilum* became increasingly the dominant microbe species, comprising approximately 33% of the microbial population at the start of the experiment and increasing to 61% after bioleaching for 18 days. In contrast, *A. ferrooxidans* decreased from an initial 33 to 9%, and *A. caldus* decreased from an initial 33 to 30% within this same time period. In comparison, in the Dachang ore bioleaching system, *L. ferriphilum* and *A. caldus* decreased from an initial 33 to 29.8 and 21.6%, respectively, after 18 days of bioleaching. However, *A. ferrooxidans* increased from an initial 33 to 48.6% within this same time period. 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 E_H, while *A. ferrooxidans* prefers to grow in the range pH 1.8–2.5 and low E_H [20–22]. In our bioleaching experiment for Dachang and Shuikousan ores, the bioleaching system of the Shuikousan ore samples had conditions of low pH (1.3) and high E_H (569 mV) at the end of the 18-day bioleaching experiment, which may explain why *L. ferriphilum* became the dominant microbe for the Shuikousan ore system and *A. ferrooxidans* became the dominant microbe for Dachang ore. In addition, the optimum growth temperature for *A. caldus* is not 35 but 45°C. Hence, *A. caldus* did not become the most abundant microbe in the bioleaching systems for Shuikousan and Dachang ores at 35°C, although it was present in the two systems at proportions of 21.6 (Dachange) and 30% (Shuikousan) at the end of the 18-day bioleaching experiment. The relatively higher abundance of *A. caldus* in the Shuikousan system (30%) may be a response to the lower pH in the bioleaching system for Shuikousan ore due to the ability of this species to oxidize reduced S to sulfuric acid.

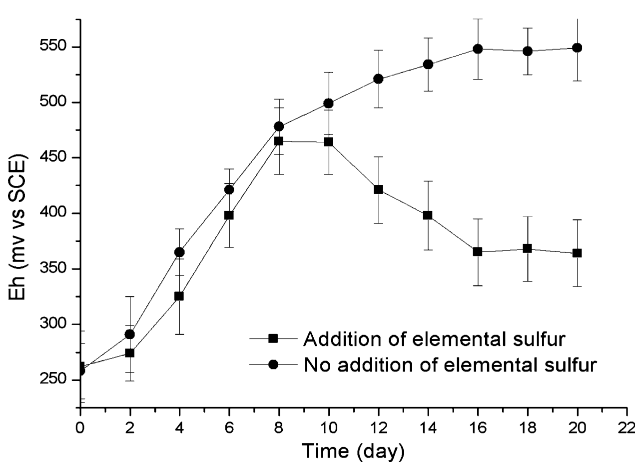

Fig. 5 Evolution of E_H in the Dachang ore system during the bioleaching process by three microbial species both with and without the addition of supplementary sulphur (S)

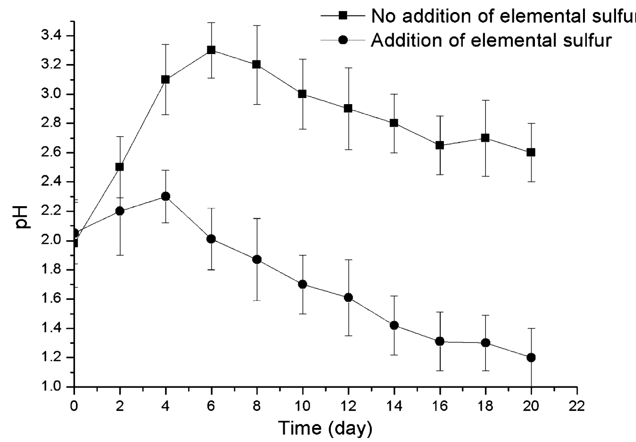

Fig. 6 Evolution of pH in the Dachang ore system during the bioleaching process by three microbial species both with and without the addition of supplementary S

Conclusions

Two sphalerite samples each with a different Fe/S ratio were processed by three microbial species, *L. ferriphilum*, *A. ferrooxidans* and *A. caldus*. The results show that the Fe/S ratio of the sphalerite had a significant influence on bioleaching. By the end of 18 days of bioleaching, a higher Zn extraction as well as a higher E_H, higher dissolved total Fe and lower pH were achieved for the Shuikousan ore than for Dachang ore bioleaching system. We also observed that different Fe/S ratios also led to the various changes of microbial community composition, with *L. ferriphilum* and *A. caldus* becoming the dominant microbes in the bioleaching for sphalerite with a high Fe/S

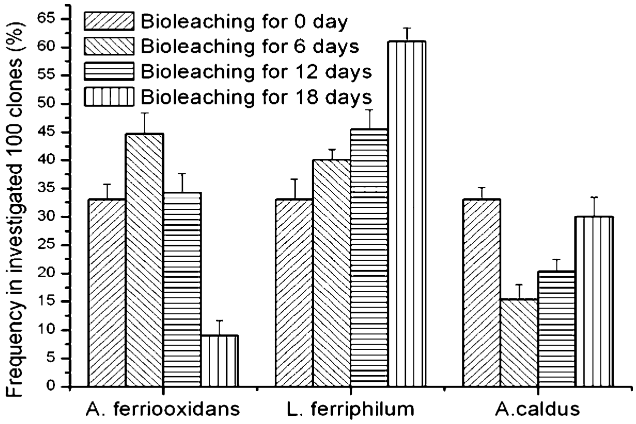

Fig. 7 Changes in the community composition of three microbial species as a function of bioleaching of Shuikousan ore

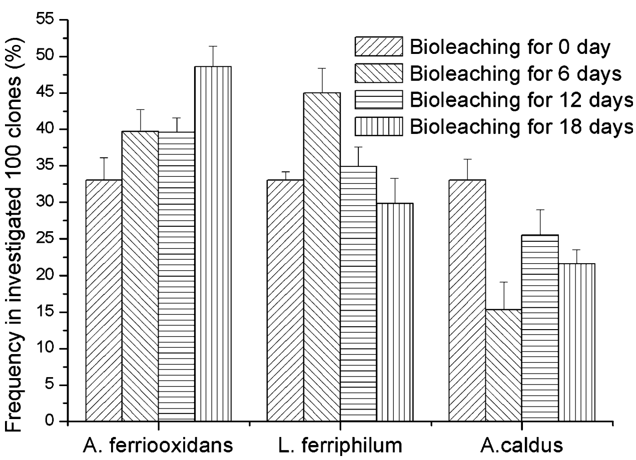

Fig. 8 Changes in the community composition of three microbial species as a function of the bioleaching of Dachang ore

ratio and, in contrast, *A. ferrooxidans* and *A. caldus* becoming the dominant microbes in the bioleaching of sphalerite with a low Fe/S ratio.

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