

## Bioleaching of Pb–Zn–Sn chalcopyrite concentrate in tank bioreactor and microbial community succession analysis

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**Abstract:** The variation of microbial community structure was investigated for the tank bioleaching process of Pb–Zn–Sn chalcopyrite concentrate in the presence of mixed moderately thermophilic bacteria. The parameters, such as pH value, solution potential and concentrations of metal ions, were determined by the method of polymerase chain reaction-restriction fragment length polymorphism (PCR–RFLP) to analyze the succession of microbial community. The results showed that a final copper extraction rate of 85.6% could be obtained after tank bioleaching for 30 d. The *Acidithiobacillus caldus* was the dominant population with abundance of about 73.80% in the initial stage, then *Sulfobacillus thermosulfidooxidans* dominated from the 18th day to the end of bioleaching, while the abundance of *Leptospirillum ferriphilum* changed slightly. A higher solution potential within a certain range and appropriate concentration of ferric ions were essential for this tank bioleaching of chalcopyrite.

**Key words:** chalcopyrite; tank bioleaching; microbial community; PCR–RFLP technique

### 1 Introduction

Chalcopyrite ( $\text{CuFeS}_2$ ) accounts for about 70% of copper reserves in the world [1–3], but due to its special crystal structure, the extraction rate of copper using traditional hydrometallurgy process is too low. Compared with the conventional processes, bioleaching technology possesses many advantages, such as mild reaction conditions, environmental benefits, low energy consumption, low cost and short flow process [4,5]. Therefore, bioleaching technology capable of extracting copper from low-grade chalcopyrite is becoming increasingly important.

Tank leaching is one of the commonly used processes in the industrial bioleaching, due to its advantages of mass transfer effect (stirring, ventilatory, etc). This process can be effectively controlled, the parameters of leaching in the process can be regulated, and then accurate experimental data can be obtained [6]. Large amounts of elemental sulfur, polysulfides, jarosite and other substances would form on the surface of

chalcopyrite, which could cause passivation phenomenon in the leaching process [7,8], especially by mesophilic microorganisms [9,10]. Using moderately thermophilic bacteria for leaching chalcopyrite, not only reaction rate can be accelerated, but also excessive chalcopyrite passivation can be avoided to some extent [11–13]. More and more researchers are interested in applying moderately thermophilic bacteria to the bioleaching of chalcopyrite due to their resistance to high pulp density and high metal concentration [14]. And many researchers found that mixed culture could accelerate the rate of bioleaching process and increase the copper extraction rate of chalcopyrite [15–17]. However, the variation of microbial community structure and the corresponding influence in tank bioleaching of chalcopyrite are not explicit enough as well as the relationship between microbial community structure and bioleaching process.

In this work, a mixed culture of three kinds of moderately thermophilic bacteria (*Acidithiobacillus caldus*, *Leptospirillum ferriphilum*, *Sulfobacillus thermosulfidooxidans*) was used for the bioleaching of chalcopyrite, PCR–RFLP technique was used for the

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research of community succession, and the parameters, such as pH, solution potential ( $\varphi_h$ ) and concentrations of metal ions were determined to analyze the bioleaching process.

## 2 Experimental

### 2.1 Minerals

The minerals used in the test were collected from Meizhou of Guangdong province in China. The samples were crushed, ground and screened to be less than 75  $\mu\text{m}$ . X-ray diffraction (XRD) and chemical elements analyses showed that the mineral samples consisted of 61.7% chalcopyrite, 29.7%  $\text{Zn}_{0.825}\text{Fe}_{0.175}\text{S}$ , 3.9% blende, 3.7% lead sulfate, and 1.1% seligmannite.

### 2.2 Microorganisms

Three strains of bacteria used in this work were obtained from the Key Laboratory of Biometallurgy of Ministry of Education, Central South University, China. The mixed culture was composed of *Acidithiobacillus caldus*, *Leptospirillum ferriphilum* and *Sulfobacillus thermosulfidooxidans*. The medium for the domestication of mixed microorganisms was composed of  $(\text{NH}_4)_2\text{SO}_4$  (3.0 g/L),  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$  (0.5 g/L),  $\text{K}_2\text{HPO}_4$  (0.5 g/L), KCl (0.1 g/L),  $\text{Ca}(\text{NO}_3)_2$  (0.01 g/L) and 5% chalcopyrite concentrate. The domestication process was conducted in a stirred tank at 45  $^\circ\text{C}$ , pH value was adjusted to 1.5–1.7, and stirring rate was adjusted to 300 r/min.

### 2.3 Bioleaching

The leaching experiment was conducted in a stirred tank with a volume of 10 L, which was connected with an inflatable equipment, and water of 45  $^\circ\text{C}$  was used for heat preservation (Fig. 1). Strains were inoculated into the stirred tank with 10% pulp density, and the concentration of inoculated strains was  $2.5 \times 10^8 \text{ mL}^{-1}$ , which was domesticated before. The whole bioleaching time was 30 d. Leaching conditions were as follows: the temperature 45  $^\circ\text{C}$ , pH value 1.5–1.7 and stirring speed

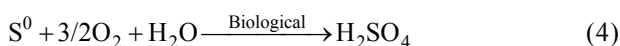
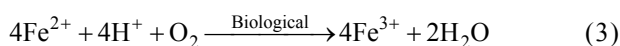
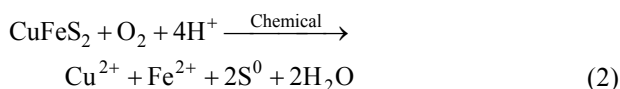
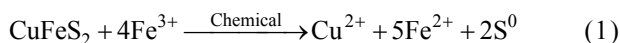
300 r/min. During the bioleaching process, the variation of pH values and potentials was recorded regularly, as well as the concentration of copper, iron ions and the bacteria concentration in the bioleaching solution. All the potential values were expressed against the Ag/AgCl electrode (3 mol/L KCl).

### 2.4 Restriction fragment length polymorphism analysis (RFLP)

Total DNA of the bacteria was extracted from 2 mL leaching solution every 6–8 d, and the primers 27f and 1492r were used in 16S-rDNA amplification system: ddH<sub>2</sub>O 36  $\mu\text{L}$ , 10 $\times$ PCR buffer 5  $\mu\text{L}$ , dNTPs 4  $\mu\text{L}$ , primers 27f 1  $\mu\text{L}$  and 1492r 1  $\mu\text{L}$ , TaqDNA polymerase 1  $\mu\text{L}$ , DNA template 2  $\mu\text{L}$ , total 50  $\mu\text{L}$ . The amplification program was as follows: 94  $^\circ\text{C}$  (5 min); 94  $^\circ\text{C}$  (5 s), 55  $^\circ\text{C}$  (45 s), 72  $^\circ\text{C}$  (90 s, 32 cycles); 72  $^\circ\text{C}$  (10 min). Plastic cutting recovery kit was used on 16S-rDNA. The pGEM-T vector connection was in a water bath at 16  $^\circ\text{C}$  overnight, and then transformed into *E. coli* Top10 competent cells. Coated tablet by a blue–white screening method was used to pick out the positive clones, amplify gene fragment after transferring tablet, then the restriction enzymes *Msp* I and *Rsa* I digestion of the amplified fragment was digested at 37  $^\circ\text{C}$  for 18 h. Finally, 3% agarose gel for restriction analysis was used to obtain the macrorestriction map, sequencing to show the different types of clones (OTU), and the identification of bacterial species.

## 3 Results and discussion

The mechanism of chalcopyrite bioleaching is mainly to oxidize insoluble chalcopyrite and form metal ions of  $\text{Cu}^{2+}$  and  $\text{Fe}^{2+}$  dissolved into the leaching solution [18]. The oxidation process begins with the oxidation of chalcopyrite by  $\text{Fe}^{3+}$  and  $\text{Fe}^{3+}$  accepts electrons released by  $\text{S}^{2-}$  as rupture of chemical bonds. In this process, the role of bacteria in the bioleaching process is to oxidize  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$  (Eq. (3)), and  $\text{S}^0$  to  $\text{SO}_4^{2-}$  (Eq. (4)). The dissolution of chalcopyrite during bioleaching can be represented as the following reactions [19]:



### 3.1 Bioleaching

The growth curve of bacteria is shown in Fig. 2. The bacteria concentration grew steadily from the 1st to

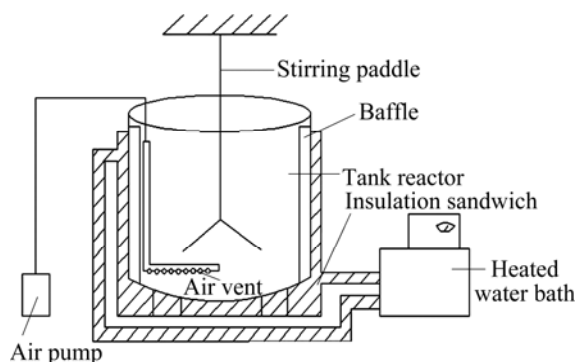


Fig. 1 Tank bioreactor used for bioleaching of chalcopyrite concentrate

the 13th day, and reached a stable state of  $10^9 \text{ mL}^{-1}$  in the subsequent leaching time. The bacteria grew rapidly to the logarithmic growth phase, and no obvious lag phase could be found. Bacteria with high activity were obtained after a long period of domestication before bioleaching. The bacteria concentration then steadily increased to  $10^9 \text{ mL}^{-1}$  after 13 d. In the subsequent bioleaching process, a large number of metabolites and metal ions were obtained because of consumption of energy substances, which led the bacteria to be stable.

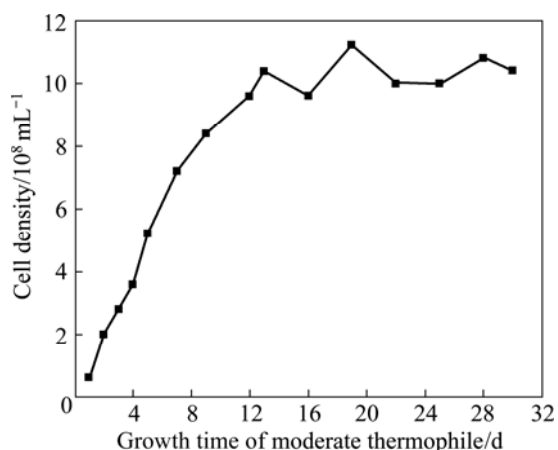


Fig. 2 Growth curve of moderate thermophile in tank bioleaching

The variation of potentials is shown in Fig. 3. The potential increased sharply from the 1st to 3rd day, and reached a peak of 540 mV, and then sharply decreased to 390 mV in the following 4 d. Finally, a steady trend of growth was obtained in the subsequent leaching process. In the initial stage of bioleaching, ferrous ions were rapidly oxidized, so the ferric ions concentration in the leaching solution increased (Eq. (3)), resulting in a sharp rise of potentials. Then the ferric ions were reduced to ferrous ions (Eq. (1)), and a large number of ferrous ions were released from eroded chalcopyrite surface

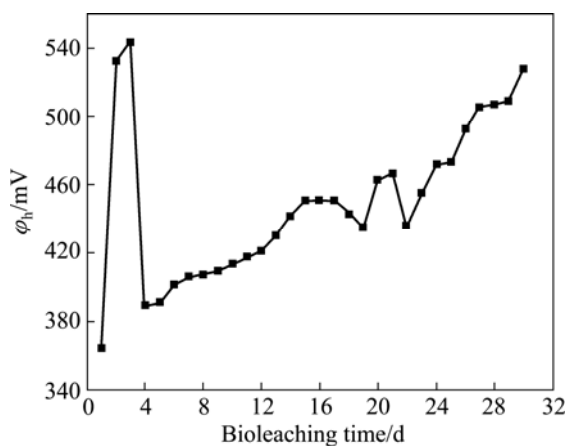
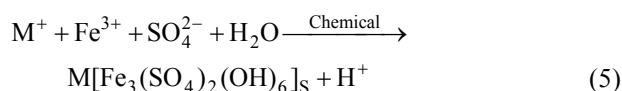


Fig. 3 Variation of solution potential (vs Ag/AgCl) during tank bioleaching

(Eq. (2)), resulting in a sharply decline of potentials in a short time. In the subsequent bioleaching process, with the increase of the bacteria concentration, the oxidation rate of ferrous ions gradually accelerated, and potential gradually increased, accompanied by a decline of pH values with the hydrogen ions produced in the process of bioleaching (Eq. (4)). Potential was mainly determined by  $c(\text{Fe}^{3+})/c(\text{Fe}^{2+})$ , and  $c(\text{H}^+)/c(\text{OH}^-)$ .

Figure 4 shows the variation of ferrous ion, ferric ion and total iron concentrations in the chalcopyrite bioleaching. In the bioleaching process, the iron ion concentration of the bioleaching solution is an important leaching parameter, and ferrous iron concentration and ferric iron concentration can directly affect the bioleaching of chalcopyrite. Figure 4 shows that the total iron concentration decreased significantly from the 6th to 14th day, which may mainly attribute to the formation of precipitates in this period [20]:



where M is a monovalent cation, such as  $\text{H}_3\text{O}^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{NH}_4^+$ . The passivation phenomenon is a major affecting factor in the chalcopyrite bioleaching. Passivation layer coated on the surface of chalcopyrite can inhibit the leaching reactions, and most of the precipitate appears in the form of jarosite [21]. PINCHES et al [22] considered that jarosite layer formed in the leaching process had a compact structure, slowing down the chalcopyrite bioleaching rate. KINNUNEN et al [23] discovered that jarosite mainly formed on the surface of sulfur-rich layer.

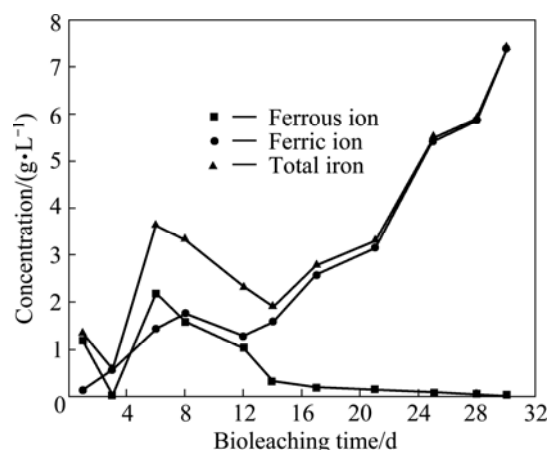


Fig. 4 Variation of ferrous iron, ferric iron and total iron concentration during tank bioleaching

Figure 5 shows the variation of copper ions concentration in 30 d. It increased to 9.20 g/L in the first 8 d, and then remained at a stable state of about 9.00 g/L from the 8th to 16th day. The concentration of copper

started to increase again along with the increase of ferric ions concentration and solution potential, and reached 17.36 g/L finally, with a final copper extraction rate of 85.6%. The results indicated that a relatively higher potential within a certain potential range was beneficial for chalcopyrite bioleaching, and appropriate concentration of ferric ions was also essential for the bioleaching of chalcopyrite in this work.

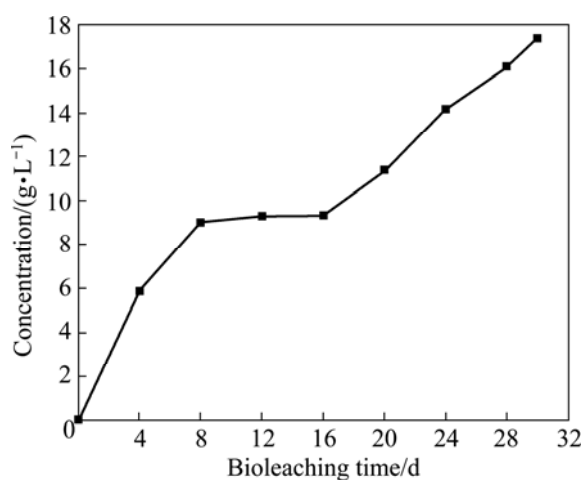


Fig. 5 Copper extraction during tank bioleaching

### 3.2 Microbial community succession

RFLP technique [24] was used to analyze the proportion of various bacteria in the tank bioleaching. The results showed that the *Acidithiobacillus caldus* (*At. caldus*) was the dominant population with an abundance of 73.80% in the initial stage, the *Sulfobacillus thermosulfidooxidans* became the dominant population with an abundance of 54.2% from the 18th day, and gradually increasing to the abundance of 66.70% on the 28th day, while the abundance of *Leptospirillum ferriphilum* just changed slightly, which always kept at a low value (Fig. 6). Equations (1) and (2) show that  $S^0$  can form on chalcopyrite surface in the initial stage of bioleaching [25], which might prevent the contact between chalcopyrite and leaching solution, and then inhibit the leaching process. *Acidithiobacillus caldus* could oxidize elemental sulfur to form sulfuric acid (Eq. (4)), so it dominated in the initial stage of bioleaching. When sulfur layer was dissolved, the ferric ions could erode chalcopyrite, and were reduced to be ferrous ions, which could provide energy substances for *Sulfobacillus thermosulfidooxidans* and *Leptospirillum ferriphilum*. Additionally, with the low resistance of *Acidithiobacillus caldus* to the metal ions like ferric ions and zinc ions, the proportion of *Acidithiobacillus caldus* gradually decreased in the subsequent bioleaching process, while *Sulfobacillus thermosulfidooxidans* dominated in the final stage of bioleaching.

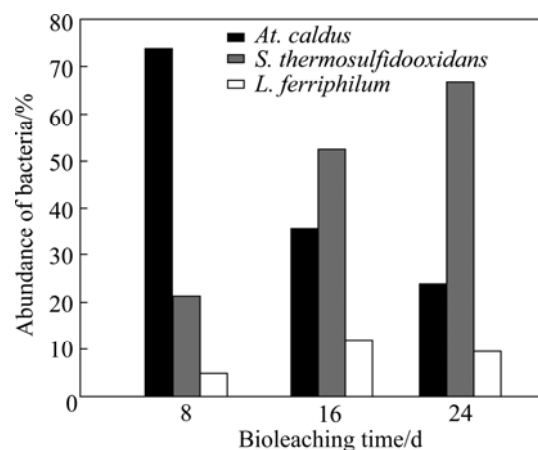


Fig. 6 Microbial community succession during tank bioleaching

## 4 Conclusions

1) A high leaching efficiency with copper extraction rate of 85.6% could be obtained in tank bioleaching of Pb–Zn–Sn chalcopyrite concentrate in the presence of mixed moderately thermophiles.

2) In the tank bioleaching process, the *Acidithiobacillus caldus* was the dominant population in the initial stage. The *Sulfobacillus thermosulfidooxidans* dominated from the 18th day, while the abundance of *Leptospirillum ferriphilum* just changed slightly.

3) The total iron concentration decreased significantly from the 6th to 14th day, which may be mainly attributed to the formation of iron precipitates in this period.

4) A relative high potential within a certain potential range was beneficial for chalcopyrite bioleaching, and appropriate concentration of ferric ions was essential for chalcopyrite bioleaching.

## References

- WANG Yu-guang, SU Li-jun, ZHANG Li-juan, ZENG Wei-min, WU Jun-zi, WAN Li-li, QIU Guan-zhou, CHEN Xin-hua, ZHOU Hong-bo. Bioleaching of chalcopyrite by defined mixed moderately thermophilic consortium including a marine acidophilic halotolerant bacterium [J]. *Bioresource Technology*, 2012, 121: 348–354.
- WANG Shi-jie. Copper leaching from chalcopyrite concentrates [J]. *JOM Journal of the Minerals, Metals and Materials Society*, 2005, 57(7): 48–51.
- ZHANG Li-min, PENG Juan-juan, WEI Man-man, DING Jian-nan, ZHOU Hong-bo. Bioleaching of chalcopyrite with *Acidianus manzaensis* YN25 under contact and non-contact conditions [J]. *Transactions of Nonferrous Metals Society of China*, 2010, 20(10): 1981–1986.
- MARHUAL N P, PRADHAN N, KAR R N, SUKLA L B, MISHRA B K. Differential bioleaching of copper by mesophilic and moderately thermophilic acidophilic consortium enriched from same copper mine water sample [J]. *Bioresource Technology*, 2008, 99(17): 8331–8336.

- [5] XIA Jin-lan, YANG Yi, HE Huan, LIANG Chang-li, ZHAO Xiao-juan, ZHENG Lei, MA Chen-yan, ZHAO Yi-dong, NIE Zhen-yuan, QIU Guan-zhou. Investigation of the sulfur speciation during chalcopryrite leaching by moderate thermophile *Sulfobacillus thermosulfidooxidans* [J]. International Journal of Mineral Processing, 2010, 94(1–2): 52–57.
- [6] BRIERLEY C L. Bacterial succession in bioheap leaching [J]. Hydrometallurgy, 2001, 59(2): 249–255.
- [7] HE Huan, ZHANG Cheng-gui, XIA Jin-lan, PENG An-an, YANG Yi, JIANG Hong-chen, ZHENG Lei, MA Chen-yan, ZHAO Yi-dong, NIE Zhen-yuan, QIU Guan-zhou. Investigation of elemental sulfur speciation transformation mediated by *Acidithiobacillus ferrooxidans* [J]. Current Microbiology, 2009, 58(4): 300–307.
- [8] XIA Jin-lan, YANG Yi, HE Huan, LIANG Chang-li, ZHAO Xiao-juan, ZHENG Lei, MA Chen-yan, ZHAO Yi-dong, NIE Zhen-yuan, QIU Guan-zhou. Investigation of the sulfur speciation during chalcopryrite leaching by moderate thermophile *Sulfobacillus thermosulfidooxidans* [J]. International Journal of Mineral Processing, 2010, 94(1): 52–57.
- [9] DEVECI H, JORDAN M A, POWELL N, ALP I. Effect of salinity and acidity on bioleaching activity of mesophilic and extremely thermophilic bacteria [J]. Transactions of Nonferrous Metals Society of China, 2008, 18(3): 714–721.
- [10] ZENG Wei-min, WU Chang-bin, ZHANG Ru-bing, HU Pei-lei, QIU Guan-zhou, GU Guo-hua, ZHOU Hong-bo. Isolation and identification of moderately thermophilic acidophilic iron-oxidizing bacterium and its bioleaching characterization [J]. Transactions of Nonferrous Metals Society of China, 2009, 19(1): 222–227.
- [11] RODRIGUEZ Y, BALLESTER A, BLAZQUEZ M L, GONZALEZ F, MUNOZ J A. New information on the chalcopryrite bioleaching mechanism at low and high temperature [J]. Hydrometallurgy, 2003, 71(1): 47–56.
- [12] KONISHI Y, ASAI S, TOKUSHIGE M, SUZUKI T. Kinetics of the bioleaching of chalcopryrite concentrate by acidophilic thermophile *Acidianus brierleyi* [J]. Biotechnology Progress, 1999, 15(4): 681–688.
- [13] ZHOU Hong-bo, ZENG Wei-min, YANG Zhi-feng, XIE Ying-jian, QIU Guan-zhou. Bioleaching of chalcopryrite concentrate by a moderately thermophilic culture in a stirred tank reactor [J]. Bioresource Technology, 2009, 100(2): 515–520.
- [14] GERICKE M, GOVENDER Y, PINCHES A. Tank bioleaching of low-grade chalcopryrite concentrates using redox control [J]. Hydrometallurgy, 2010, 104(3–4): 414–419.
- [15] D'HUGUES P, FOUCHER S, GALLE-CAVALLONI P, MORIN D. Continuous bioleaching of chalcopryrite using a novel extremely thermophilic mixed culture [J]. International Journal of Mineral Processing, 2002, 66(1): 107–119.
- [16] QIU M Q, XIONG S Y, ZHANG W M, WANG G X. A comparison of bioleaching of chalcopryrite using pure culture or a mixed culture [J]. Minerals Engineering, 2005, 18(9): 987–990.
- [17] ZHANG Yan-sheng, QIN Wen-qing, WANG Jun, ZHEN Shi-jie, YANG Cong-ren, ZHANG Jian-wen, NAI Shao-shi, QIU Guan-zhou. Bioleaching of chalcopryrite by pure and mixed culture [J]. Transactions of Nonferrous Metals Society of China, 2008, 18(6): 1491–1496.
- [18] TRIBUTSCH H. Direct versus indirect bioleaching [J]. Hydrometallurgy, 2001, 59(2): 177–185.
- [19] SHU Rong-bo, RUAN Ren-man, WEN Jian-kang. Review on passivation of chalcopryrite during bioleaching process [J]. Chinese Journal of Rare Metals, 2006, 30(3): 395–400. (in Chinese)
- [20] ZHEN Shi-jie, QIN Wen-qing, YAN Zhong-qiang, ZHANG Yan-sheng, WANG Jun, REN Liu-yi. Bioleaching of low grade nickel sulfide mineral in column reactor [J]. Transactions of Nonferrous Metals Society of China, 2008, 18(6): 1480–1484.
- [21] MERUANE G, VARGAS T. Bacterial oxidation of ferrous iron by *Acidithiobacillus ferrooxidans* in the pH range 2.5–7.0 [J]. Hydrometallurgy, 2003, 71(1): 149–158.
- [22] PINCHES A, ALJAID F O, WILLIAMS D J A, ATKINSON B. Leaching of chalcopryrite concentrates with *thiobacillus ferrooxidans* in batch culture [J]. Hydrometallurgy, 1976, 2(2): 87–103.
- [23] KINNUNEN P M, HEIMALA S, RIEKKOLA-VANHANEN M L, PUHAKKA J. Chalcopryrite concentrate leaching with biologically produced ferric sulphate [J]. Bioresource Technology, 2006, 97(14): 1727–1734.
- [24] HE Zhi-guo, XIAO Sheng-mu, XIE Xue-hui, HU Yue-hua. Microbial diversity in acid mineral bioleaching systems of dongxiang copper mine and Yinshan lead–zinc mine [J]. Extremophiles, 2008, 12(2): 225–234.
- [25] ZENG Wei-min, QIU Guan-zhou, ZHOU Hong-bo, LIU Xue-duan, CHEN Miao, CHAO Wei-liang, ZHANG Cheng-gui, PENG Juan-hua. Characterization of extracellular polymeric substances extracted during the bioleaching of chalcopryrite concentrate [J]. Hydrometallurgy, 2010, 100(3–4): 177–180.

## 微生物浸出 Pb–Zn–Sn 黄铜矿及其生物群落的演替分析

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**摘 要:** 研究了 Pb–Zn–Sn 黄铜矿精矿在混合中度嗜热微生物槽浸过程中的细菌群落结构变化, 并监测浸出体系中金属离子浓度、溶液电位、溶液 pH 值变化, 通过聚合酶链式反应–限制性片段长度多态性(PCR–RFLP)技术分析微生物群落的结构变化。结果表明, 最终铜浸出率高达 85.6%, 在浸出前期, *Acidithiobacillus caldus* 为优势群落, 从第 18 天开始到浸出结束, *Sulfobacillus thermosulfidooxidans* 为优势群落, 但 *Leptospirillum ferriphilum* 丰度变化较小。试验结果表明, 适当较高的溶液电位和合适的铁离子浓度对黄铜矿精矿的生物浸出作用很关键。

**关键词:** 黄铜矿; 微生物槽浸; 微生物群落; 聚合酶链式反应–限制性片段长度多态性技术 PCR–RFLP

(Edited by Hua YANG)