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Characterization of apparent sulfur oxidation activity of thermophilic archaea in bioleaching of chalcopyrite

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Abstract: The apparent sulfur oxidation activities of four pure thermophilic archaea, *Acidianus brierleyi* (JCM 8954), *Metallosphaera sedula* (YN 23), *Acidianus manzaensis* (YN 25) and *Sulfolobus metallicus* (YN 24) and their mixture in bioleaching chalcopyrite were compared, which were characterized indirectly by the evolution of the cells concentration, pH value and sulfate ions concentration in solution. The results show that the mixed culture contributed significantly to the raising of leaching rate, which suggests that the mixed culture had a higher sulfur oxidation activity than the pure culture. Meanwhile, the results also indicate that the changes of parameters characterizing the sulfur oxidation activity of thermophilic archaea are often influenced by many factors, so it is hard to reflect accurately the specific sulfur oxidation activities among the different sulfur-oxidizing microbes when bioleaching chalcopyrite at different conditions. Accordingly, an efficient method to characterize microbial sulfur oxidation activity appears to be desirable.

Key words: thermophilic archaea; sulfur oxidation activity; characterization; bioleaching

1 Introduction

Microorganisms are important in the bioleaching of the sulfide ores in heap or tank reactor [1]. Currently, the mesophilic microorganisms are widely used in the hydrometallurgical field such as leaching of secondary metal sulfides minerals and pretreatment of metal sulfide-containing gold ores [2]. However, the copper extraction yields in bioleaching of chalcopyrite, the most widespread and refractory primary metal sulfide mineral, with mesophilic microorganisms are always unsatisfiable [3]. One of the reasons is the high temperature produced from mineral oxidation in the reactor and some zones in leaching heap, which influences the growth of mesophilic bacteria and even causes their death [4]. In contrast, thermophilic microorganisms, because of the inherent advantages of tolerating high temperature, can significantly improve the leaching kinetics of sulfide minerals thus accelerate the reaction rate and shorten the leaching cycle, and can prevent excessive passivation of primary sulfide minerals which could obstruct the conduction of bioleaching [5]. And the leaching rate by thermophilic microorganisms is often several times higher than that by mesophilic bacteria, in the bioleaching of recalcitrant chalcopyrite [6]. Therefore, it is important to select the thermophilic microorganisms with strong sulfur oxidation activity to achieve an effective dissolution of sulfide minerals.

Obviously, the sulfur oxidation activity of thermophilic archaea is an important factor to intrigue great interest of researchers [7,8]. However, literatures issued focused on the aspects of the bioleaching kinetics and leaching mechanisms while few information discussed about the bioleaching behaviors and sulfur oxidation activities of thermophilic archaea, especially when they were in mixed cultures [9−11]. Therefore, further study on such subjects related to the pure and mixed thermophilic archaea is important to understand the mechanism of thermophilic bioleaching. In this study, four representatives of thermophilic archea *Acidianus brierleyi* (*A. brierleyi*, JCM 8954), *Metallosphaera sedula* (*M. sedula*, YN 23), *Acidianus manzaensis* (*A. manzaensis*, YN 25) and *Sulfolobus metallicus*

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(*S. metallicus*, YN 24), with widespread occurrence and leaching capacity, were selected to perform the experiments.

Hence, two attempts of the present study were: 1) to compare the sulfur-oxidizing activities of four pure and mixed thermophilic archaea consisting of *A. brierleyi, M. sedula, A. manzaensis* and *S. metallicus*, and 2) to investigate the relationship between the apparent sulfur oxidation activity and the bioleaching capability of thermophilic archaea to promote the understanding of bioleaching capability as well as bioleaching mechanism by various thermophilic archaea, and further to find a desirable method for characterizing efficiently the sulfur oxidation activity of various thermophilic archaea.

2 Experimental

2.1 Microorganisms and culture media

Four thermophilic archea used in the present work were *A. brierleyi* (JCM 8954), *M. sedula* (YN 23), *A. manzaensis* (YN 25) and *S. metallicus* (YN 24), which were conserved by the Key Laboratory of Biometallurgy of Ministry of Education of China.

The basic media contained 3.0 g/L $(NH_4)_2SO_4$, 0.5 g/L K_2HPO_4, 0.1 g/L KCl, 0.5 g/L $MgSO_4\cdot 7H_2O$, 0.01 g/L $Ca(NO_3)_2$ and 10 g/L S, with 0.2 g/L yeast extracts.

2.2 Mineral samples

The mineral sample used in the experiments contained chalcopyrite ($CuFeS_2$) as the main mineral phase, and galena (PbS), calcite ($CaCO_3$) and calcium fluoride (CaF_2) as the minor phases, according to the X-ray diffraction characterization (Fig. 1). Chemical analysis showed that the mineral sample contained 34.63% Cu, 25.35% Fe and 30.45% S. The mineral was ground to fine powder and then passed through a sieve with a pore size of 0.075 mm.

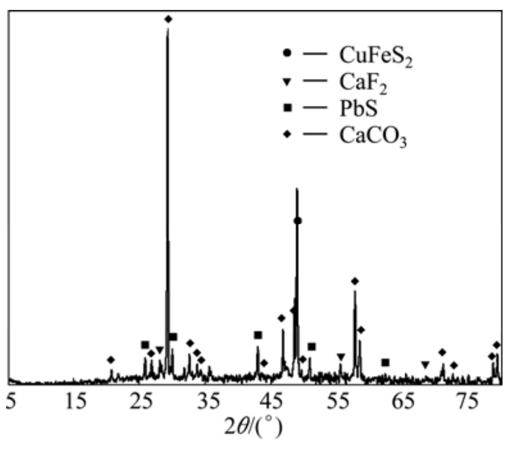

Fig. 1 XRD pattern of chalcopyrite concentrate used in this study

2.3 Bioleaching experiments

The bacterial leaching experiments were carried out in 250 mL Erlenmeyer flasks containing 100 mL basic medium supplemented with 20 g/L chalcopyrite as the sole energy source on a rotary shaker at 170 r/min. The initial pH of the culture was adjusted to 1.5 with diluted sulfuric acid. The experiments were carried out in duplicate. Evaporated water was compensated by additional distilled water.

Cells used in all leaching tests were previously cultivated to their logarithm phase, harvested by centrifugation and washed twice with sterilized distilled water, and then resuspended in fresh leaching solution. In pure culture, the cell was inoculated at 65 °C with the initial biomass of about 1×10^7 cell/mL. The mixed culture was inoculated at 60, 65, 70 and 75 °C. Each mixed culture was inoculated with approximately equal numbers of cells from each of the four pure strains and reached the initial biomass of 1×10^8 cell/mL. The abiotic controls were also designed.

2.4 Analyses

In bioleaching experiments, sample solutions were taken out at the same intervals to measure the cells concentration, pH value, sulfate ion, copper and iron ions. Copper and total iron concentrations in the solution were determined by atomic adsorption spectrophotometry. Free cells in solution were observed and counted under an optical microscope with a Neubauer chamber counter. The pH values in the solution were measured with a pH-meter (SJ-4A). Sulfate ions were quantified by a colorimetric method.

3 Results and discussion

3.1 Apparent sulfur oxidation activities of four pure thermophilic archaea

The apparent sulfur oxidation activities of four pure thermophilic archaea mentioned above were characterized based on the evolutions of cells concentration, pH value and sulfate ion concentration during bioleaching progress, and the results are presented in Figs. 2, 3 and 4, respectively.

As can be seen from Fig. 2, almost all thermophilic archaea showed an obvious lag phase. The cells entered logarithmic growth phase from the 2nd day and reached the maximum of $(3.6-4.79)\times10^7$ cell/mL after 8 days, then decreased with the increase of cells death. Among the pure thermophilic archaea, *A. brierleyi* grew better than *M. sedula* and *A. manzaensis*, while *S. metallicus* did not grow well such that its cell concentrations remained low and could not be detected at last. The reason may be that, as one of thermophiles, the performance of the strain *S. metallicus* is unstable.

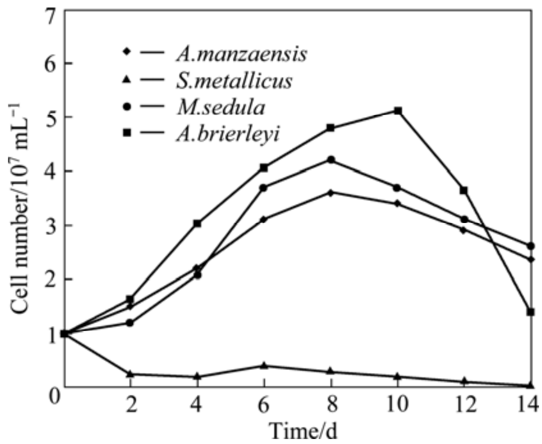

Fig. 2 Growth of four pure thermophilic archea, *A. brierleyi*, *M. sedula, A. manzaensis* and *S. metallicus* in solution in bioleaching chalcopyrite at initial pH=1.5 and 65 °C (Pulp density 2%; initial biomass 1×10^7 cell/mL)

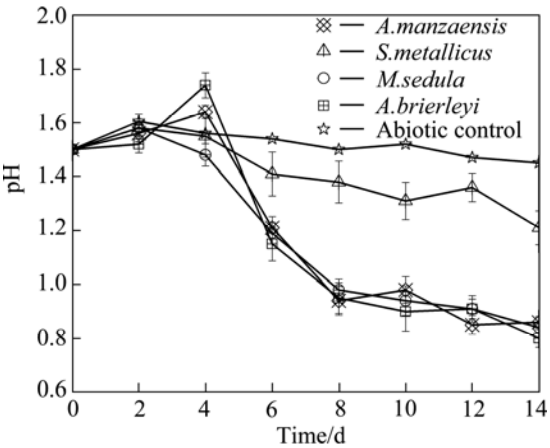

Fig. 3 Comparison of evolution of pH value of *A. brierleyi, M. sedula, A. manzaensis* and *S. metallicus* in bioleaching chalcopyrite at initial pH=1.5 and 65 °C (Pulp density 2%; initial biomass 1×10^7 cell/mL)

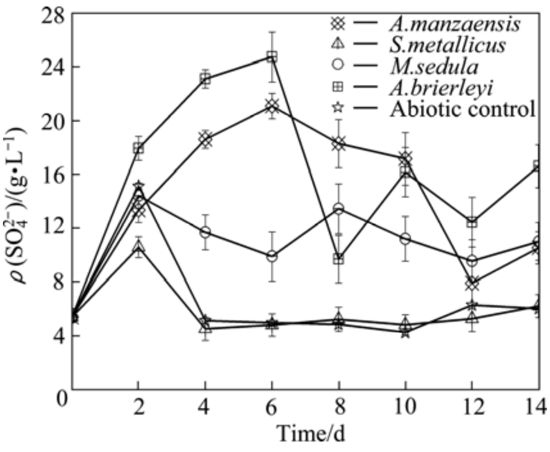

Fig. 4 Comparison of evolution of sulfate ion concentration of *A. brierleyi, M. sedula, A. manzaensis* and *S. metallicus* in bioleaching chalcopyrite at initial pH=1.5 and 65 °C (Pulp density 2%; initial biomass 1×10^7 cell/mL)

Figure 3 shows that the pH fluctuated through the whole process. At the initial days, all pH values maintained a rise trend, which may be mainly related to the attack of H^+ by the mineral, regardless of an increase trend in the cells concentration. After that, the pH values dropped dramatically except in the case of *S. metallicus* and the control test, in which both of them kept the pH values at the level of 1.2−1.5. In this phase, the elemental sulfur in the mineral sample was gradually oxidized to sulfuric acid, reflected by a decrease of the pH and an increase of the cells concentration (Fig. 2), which indicated that the strains had the strong sulfur oxidation activities[1,12,13]. However, the evolutions of sulfate ions concentration appeared to be inconsistent with the result, because there was a great fluctuation of sulfate ions concentration, which was likely to suggest that there were intermediate compounds formed under the action of thermophilic archaea in bioleaching of chalcopyrite (Fig. 4). It was reported that the nature of the intermediate compounds would impede the effectiveness of the conduction of biological oxidation [14−16]. Consequently, these preliminary results suggested that the parameters mentioned above were hardly capable of accurately reflecting the specific sulfur oxidation activities of various thermophiles.

3.2 Effect of temperature on sulfur oxidation activities of mixed thermophilic archaea

Figure 5 shows sulfur oxidation activities of the mixed thermophilic archaea during bioleaching chalcopyrite at different temperatures. For the growth of each mixed culture, the strains grew particularly well due to the preference of mixed culture for mixotrophic or heterotrophic growth [17,18]. There was almost no lag phase and the logarithmic growth phase and stationary

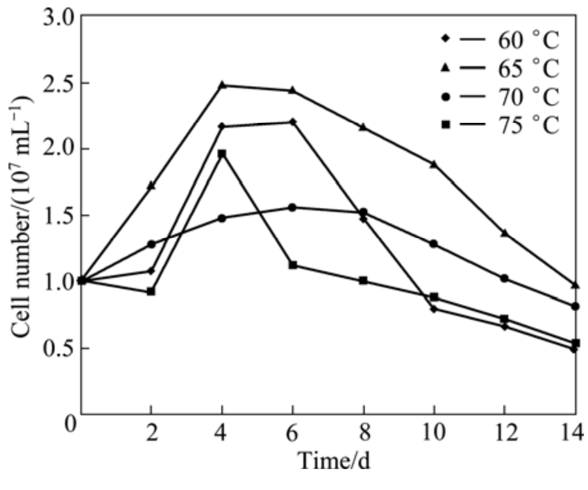

Fig. 5 Effect of temperature on growth of mixed thermophilic archaea in bioleaching chalcopyrite at various temperatures and an initial pH of 1.5 (Pulp density 2%; initial biomass 1×10^8 cell/mL)

phase were particularly longer at these conditions, in which the maximum cell concentration was (2.0−2.5)×10^8 cell/mL.

Meanwhile, the data showed that the optimum temperature for the growth of the mixed culture, that is, the temperature at which the highest cells concentration of these thermophilic archaea were measured, was 65 °C, but the highest sulfate ions concentration was not necessarily obtained at that temperature. For example, the mixed thermophilic archaea grew slowly at 70 °C, at which the maximum sulfate ion concentration was obtained (Fig. 6). Moreover, it was particularly evident that pH value was decreased with the increase in temperature regardless of the thermophilic archaea available in leaching [19]. This means that it is insignificant to compare pH values between 60 °C and 75 °C (Fig. 7). Likewise, these preliminary results suggested that the parameters hardly characterized the sulfur oxidation activity of thermophiles, which were strongly influenced by many factors.

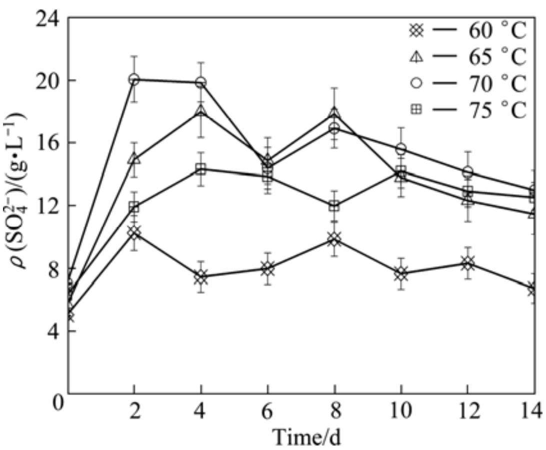

Fig. 6 Comparison of evolution of sulfate ion concentration of mixed thermophilic archaea in bioleaching chalcopyrite at various temperatures and an initial pH of 1.5 (Pulp density 2%; initial biomass 1×10^8 cell/mL)

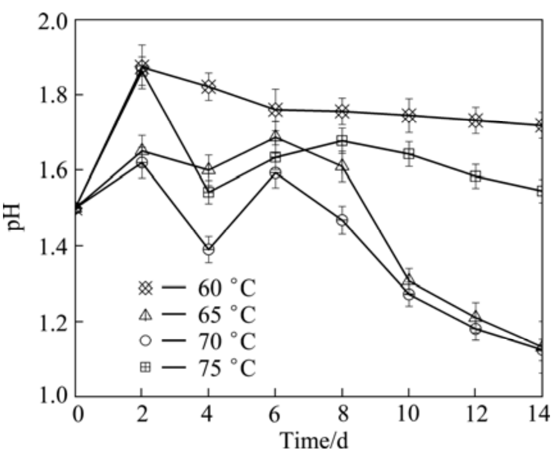

Fig. 7 Comparison of evolution of pH value of mixed thermophilic archaea in bioleaching chalcopyrite at various temperatures and an initial pH of 1.5 (Pulp density 2%; initial biomass 1×10^8 cell/mL)

3.3 Relationship between sulfur oxidation activity and bioleaching capability of thermophilic archaea

The experimental results indicated that the optimum temperature for the growth of the thermophilic archaea was 65 °C. In order to investigate the bioleaching capability of thermophilic archaea with different sulfur oxidation activities, chalcopyrite bioleaching experiments were performed. The tests were carried out at the same conditions, with initial pH 1.5, pulp density 20 g/L and initial biomass 1×10^7 cell/mL. After being leached for 14 d, the concentrations of leached copper and total iron in culture liquid were analyzed and are shown in Table 1.

Table 1 Results of bioleaching of chalcopyrite by thermophilic archaea in shake flask

Microorganism	$\rho(Cu^{2+})/(g\cdot L^{-1})$	$\rho(TFe)/(g\cdot L^{-1})$
Mixed thermophilic archaea	6.10	3.16
A. brierleyi	5.18	2.63
M. sedula	5.06	2.95
A. manzaensis	5.11	2.56
S. metallicus	2.31	1.05

In Table 1, the data show that copper ion concentration after 14 d reached 6.10 g/L in mixed culture, higher than 5.11 g/L in *A. manzaensis,* 5.06 g/L in *M. sedula*, and 5.18 g/L in *A. brierleyi*, which indicated that the chalcopyrite bioleaching capability of mixed culture was higher than that of pure culture. Table 1 also shows that the mixed culture with the highest concentration of copper has the highest total iron concentration 3.16 g/L, whereas the abiotic control experiment kept them at a low level of 0.08−0.59 g/L during leaching of chalcopyrite.

The analysis of data shows that the bioleaching of chalcopyrite and the recoveries of copper and iron varied in different tests, which were related to the sulfur oxidation activities of thermophilic archaea. At the first stage, the recoveries of copper and iron increased (data not shown) due to the acid consumption; meanwhile, the formed elemental sulfur was oxidized to sulfuric acid, causing an increase in sulfate concentration (Fig. 4). According to the chemiosmotic theory, a decrease in the metal iron oxidation activity of strains is expected when the pH increases [20]. Hence, it is vague to analyze the relationship among pH value and sulfur oxidation activity and bioleaching capability of strains. Moreover, the copper and iron dissolving rates in mixed cultures were more actively accelerated than those in pure cultures, which can be inferred that an enhanced capacity of mixed culture on bioleaching was caused by a higher sulfur oxidation activity, which provided adequate protons for attacking the mineral, that is, a higher ferrous oxidation activity, facilitated an oxidative attack [21, 22]. However, the results also faced a conflict with the other apparent indicators such as the evolutions of sulfate ions concentration. Accordingly, an efficient method to characterize bacterial sulfur oxidation activity in bioleaching chalcopyrite appears to be needed.

4 Conclusions

1) Comparison of the leaching rates of chalcopyrite of four pure thermophilic archea and their mixture shows that the copper concentrations in bioleaching from chalcopyrite are 6.10 g/L in mixed culture, 5.11 g/L in *A. manzaensis*, 5.06 g/L in *M. sedula*, and 5.18 g/L in *A. brierleyi*, and variation trends of the iron concentration are similar to the copper concentration. The results show that the mixed culture contributes significantly to the raising of leaching rate, which indicates that mixed culture has a higher sulfur oxidation activity than the pure culture.

2) Changes of apparent parameters characterizing the sulfur oxidation activities of thermophiles are difficult to explain accurately the sulfur oxidation activity of various strains, which are often influenced by many factors. For example, the sulfate ions concentration did not necessarily give the highest value in the case of mixed culture. Therefore, it is necessary to find a desirable method for characterizing efficiently the sulfate oxidation activity of various thermophiles.

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古菌生物浸出黄铜矿过程中的表观硫氧化活性表征

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摘　要：对 4 株纯的极端嗜热古菌及它们的混合菌在生物浸出黄铜矿过程中的硫氧化活性进行对比研究。结果表明，混合菌比纯菌拥有更高的硫氧化活性，它大幅度促进黄铜矿浸出率的提高。表征嗜热古菌硫氧化活性的参数值通常受很多因素的影响，以致在不同的硫氧化菌和不同的条件下生物浸出黄铜矿时，这些参数很难准确地反映出相应的硫氧化活性。因此，期待找到一种能有效表征浸矿菌硫氧化活性的方法。

关键词：嗜热古菌；硫氧化活性；表征；生物浸出

(Edited by Hua YANG)