



Adsorption and leaching behaviors of chalcopyrite by two extreme thermophilic archaea

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Abstract: The adsorption and leaching of chalcopyrite by two extreme thermophilic archaea (*A. brierleyi* and *S. metallicus*) and their mixture were studied. The results revealed that the chalcopyrite leaching rate of *S. metallicus* was slightly higher than that of *A. brierleyi*; the mixed system showed the highest rate. Community structure analysis during the leaching process showed that *S. metallicus* was maintained in a predominant state. However, the proportion of *A. brierleyi* in the community increased during leaching. Copper concentrations, which increased faster in the mixed system than in the single-organism systems during later stages, was related to the change of *A. brierleyi* in the community. Langmuir parameter analysis revealed no competitive adsorption between these two thermophilic archaea. Furthermore, qPCR (quantitative polymerase chain reaction) confirmed that adsorption was promoted between *A. brierleyi* and *S. metallicus* during mixed leaching. These findings can improve our understanding of the adsorption behaviors of mixed extreme microbial populations on mineral surfaces.

Key words: thermophilic archaea; *A. brierleyi*; *S. metallicus*; chalcopyrite; adsorption; leaching

1 Introduction

Rich, global ore resources are becoming increasingly scarce because of their continuous development and utilization. Researchers in China have efficiently and rationally developed and utilized metal mineral resources classified as low-grade and difficult to process [1]. Recently, bioleaching has emerged as an effective method for processing low-grade minerals because of its low cost, energy consumption, and environmental impact [2–4]. According to their optimum growth temperatures, common metallurgical microorganisms have been classified into mesophilic, moderately thermophilic, and extremely thermophilic ones. Mesophilic and moderately thermophilic organisms are currently used in industrial applications [5]. However, the application of extreme thermophilic microorganisms is rare. Extreme thermophilic archaea show high temperature resistance, high extraction speed, and high leaching rates compared to mesophilic bacteria; thus, such organisms have great potential in industrial applications [6] and have become a focal point of

research.

The most important factors influencing bioleaching are microorganisms which play very important roles in mineral leaching. The roles of various microorganisms in the leachate are highly complex and may be both positive and negative. The microorganisms are considered to coexist in synergistic, competitive, or mutually beneficial symbiotic relationships [7]. These different relationships are based on different physiological and biochemical characteristics of the various microorganisms, such as carbon fixation [8], sulfur oxidation and reduction [9], nitrification fixation, oxidation of organic matter [10,11], and ferrous iron oxidation and reduction capability [12]. Competitive relationships in bio-metallurgy mainly refer to substrate competition between organic and inorganic electron donors [13–15]. Environmental factors, such as temperature, pH, metal ion concentrations, and substrate concentrations, also strongly influence the competitive relationships in bio-metallurgy [16]. In contrast, in the physiological complementation, microorganisms synergistically oxidize and decompose minerals in mixed culture and display significant promoting effects. For example, chalcopyrite leaching efficiency in mixed

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leaching systems of *Leptospirillum ferrooxidans* (*L. ferrooxidans*) and *Acidithiobacillus thiooxidans* (*A. thiooxidans*) was significantly higher than that in any single-organism system [17–19]. This was primarily because despite their inability to directly oxidize and decompose minerals, sulfur-oxidizing bacteria produce acid, remove sulfur passivation film, and prevent the formation of jarosite. Additionally, related studies showed that minerals can only be oxidized and decomposed when *Ferroplasma* and *A. thiooxidans* coexist [17]. Regardless of the relationship between microorganisms, during the leaching process, the surface of the mineral is adsorbed first [20,21]. This step can also determine the relationship between populations during the leaching process to some extent. Microbial adsorption on the mineral surface is a prerequisite for effective interfacial interactions and plays an important role in the mineral dissolution process. It has been reported that the surface properties of minerals and different energy metabolism strategies of microorganisms affect the adsorption behaviors, thereby affecting microbial–mineral interactions.

Few studies have examined the interactions between extreme thermophiles in bioleaching. However, the use of thermophilic archaea in leaching processes has broad application potential. Therefore, *Acidianus brierleyi* and *Sulfolobus metallicus* were selected as representative thermophilic leaching microorganisms, to study mutual relationships in the adsorption of chalcopyrite surfaces and their influence on leaching processes. Both archaea are thermophilic autotrophic paleontological archaea that can grow in the presence of ferrous, sulfur, and reducing inorganic sulfur compounds. According to previous studies, *A. brierleyi* has strong adaptability and sulfur oxidation capacity and *S. metallicus* has a very strong ferrous oxidation ability, but it is sensitive to environmental conditions [22–24]. Thus, both strains are representative of extremely thermophilic archaea.

2 Experimental

2.1 Organisms and minerals

Chalcopyrite was supplied by the Key Lab of Biohydrometallurgy of the Ministry of Education, Central South University, Changsha, China. Pure chalcopyrite minerals were manually selected and ground to 0.045–0.074 mm in diameter. For adsorption and zeta potential measurements, chalcopyrite was ground with a diameter <5 μm using agate. The mass fractions of elemental copper, iron, and sulfur were 31.45%, 26.74%, and 31.87%, respectively.

A. brierleyi and *S. metallicus* were obtained from the Key Lab of Biohydrometallurgy of the Ministry of Education, Central South University, Changsha, China.

They were inoculated in basal salt medium and supplemented with 1% elemental sulfur and 0.02% yeast extract [25]. The pH of the medium was adjusted to be 2.0 with 2.5 mol/L H_2SO_4 . The basal salt medium consisted of 3.09 g/L $(\text{NH}_4)_2\text{SO}_4$, 0.1 g/L KCl, 0.5 g/L K_2HPO_4 , 0.5 g/L $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, and 0.01 g/L $\text{Ca}(\text{NO}_3)_2$.

2.2 Bioleaching experiment

Wide-mouth conical flasks (250 mL) and basal salt medium with pH 2.0 were utilized in the leaching experiment. The pH required no adjustment. The leaching test was performed in a high-temperature water bath oscillator at 68 °C and a rotation speed of 170 r/min. Chalcopyrite powders were placed into the flask to a mineral concentration of 0.2% (w/v). In the single-archaea leaching test, the leaching microorganisms were present at a concentration of 1×10^7 cell/mL. The mixed sample consisted of equal amounts of the two archaea in a total concentration of 1×10^7 cell/mL. A blank control group without archaea was also used. For the initial mixed-system experiment, 18 parallel samples with the same initial leaching conditions were used, with three parallel samples in each group. Thus, six flasks were used at each stage and were divided into two groups. One group was used to extract DNA and slag from the solutions, while the other group was used to construct adsorption curves. The microorganisms were collected and centrifuged at 3000 r/min for 2 min. The supernatant was separated, and then the slag was mixed with sterile PBS buffer. After magnetic stirring at high speed for 10 min, the washing solution was collected under aseptic conditions for DNA extraction. The process was repeated until no further organisms were microscopically identified in the slag. The supernatant was collected, and its slag washing solution was centrifuged to collect microorganisms for later use.

2.3 Experimental sampling and analysis of leaching process

The pH levels and redox potential were measured every two days. Additionally, 2 mL solution was extracted and used to measure the Cu^{2+} , Fe^{2+} , and Fe^{3+} contents. Collected samples were replaced with the same volumes of medium. Evaporation losses from the solution during testing were replaced with the same amount of sterile water. pH values were measured using a pH meter (PHS-3C), and the oxidation–reduction potential (φ) was measured by an inductively coupled plasma atomic emission spectrometer (ICP–AES) combined with Ag/AgCl platinum (3 mol/L KCl) (bpb-922). The concentrations of Cu^{2+} and total iron in the solution were determined by atomic absorption spectrophotometry. Additionally, Fe^{2+} levels were

determined by potassium dichromate titration, and the Fe^{3+} concentration was calculated by subtracting the Fe^{2+} concentrations from the total iron concentrations.

2.4 Analysis of microbiological communities

DNA extraction was conducted as described previously [26]. Free and attached microorganisms were harvested by filtration and centrifugation (12000 r/min, 20 min). The attached cells were collected from the ore in advance by repeated vortexing and resuspension. DNA was extracted immediately using the TIANamp® Bacterial DNA Kit (Tiangen, Beijing, China) according to the manufacturer's instructions. All tests were performed in triplicate. The real-time quantitative polymerase chain reaction (qPCR) was used to analyze the population dynamics during bioleaching. The quality of the amplified DNA fragment stained with ethidium bromide was examined with 1.5% agarose gel electrophoresis, followed by purification of the DNA in the bands using the QIAquick-spin PCR Purification Kit was used (Qiagen, Hilden, Germany). Next, DNA sequencing was performed by Shanghai Biotech, China, and BLAST analysis was performed in GenBank (<http://www.ncbi.nlm.nih.gov/BLAST/>). Each sample leaching system was evaluated as follows: pre-denaturation at 95 °C for 3 min, 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 30 s for a total of 40 cycles. qPCR data were analyzed using iCycler MyiQ software v1.0 (Bio-Rad, Hercules, CA, USA). The primers were arranged as described previously [27]. The six standard curves generated from known concentrations of PCR products were used to quantify the copy number of each conserved gene. The correlation coefficient of the standard curve of each gene was greater than 0.99, and the PCR amplification efficiency was between 90% and 110%. The number of cells for each strain was calculated from the total number of cells and percentage of each strain.

2.5 Strain adsorption experiments

The pH of NaCl solution with an ionic strength of 1×10^{-3} mol/L was adjusted to 2.0. The volume of the solution was 100 mL, and then 0.2 g chalcopyrite was added and shaken at 170 r/min and 68 °C for 1 h. In separate adsorption experiments, the concentration of archaea in the solution was adjusted to be 1.0×10^6 cell/mL. In the mixed archaea adsorption experiment, the same number of archaea was used to adjust the archaea concentration in the solution to be 1.0×10^6 cell/mL. The number of archaea in the separate adsorption experiments was directly counted using a microscope. In the mixed archaea adsorption experiment, the number of each archaea was determined by qPCR. To eliminate the measurement error, sample determination

and quantitative standard curve establishment were performed simultaneously in the same operation, and all experiments were performed three times. Both the initial archaeal amount (1.0×10^8 cells) and mineral mass (0.2 g) in the solution, and the number of archaea in the liquid at the time of adsorption equilibrium (C_L) were all used to calculate the number of adsorbed archaea (C_A) [28].

3 Results and discussion

3.1 Bioleaching experiment

Changes in planktonic conditions of the leaching-solution from the three experimental groups are shown in Fig. 1(a). During the early period, planktonic levels rose sharply, and then began to decrease during the middle and late periods. Compared with that of the single-archaea system, the decrease of the mixed system was slower. Figure 1(b) shows the changes in pH levels during the leaching process for the three experimental groups. While the pH of the blank control slowly decreased, the pH levels in the three experimental groups increased during the early stage and declined rapidly at the middle and later stages. As shown in Fig. 1(c), the φ of the blank control showed a steady and slow-rising trend. The experimental groups with the two single strains showed obviously upward trends, and only showed significant decrease near the end of the leaching process. The φ of the mixed archaea system changed similarly to those of the single-archaea systems at the early and middle stages of the leaching process. However, no significant decrease was observed at the end of the leaching process. This difference was similar to that between copper ion concentrations in the leachate of the mixed and single-archaea systems, as detailed in Fig. 1(d). The leaching rates of copper ions after leaching with *A. brierleyi* and *S. metallicus* were 60.01% and 59.61%, respectively, while the copper leaching rate of mixed archaea was 64.43%. Therefore, the leaching rate of the mixed thermophilic archaea system was significantly greater than that of the single-archaea systems, with the copper leaching rate increased by approximately 5%. As shown in Fig. 2, the concentration of ferrous ions in the archaeal system was low during the leaching process and showed minimal changes, because archaea oxidized ferrous ions released by the dissolution of chalcopyrite into ferric ions, and thus the iron in the leaching system is mainly in the form of ferric ion. The ferric ion concentration in the two groups of single archaea solution began changing in the later stage. The plateau concentration of the mixed archaea still shows a rapidly increasing trend. Compared to the copper ion concentration change of leaching in Fig. 1(d), according to the previous study, Fe^{3+} combines with SO_4^{2-} in solution to form jarosite. The formation of jarosite during

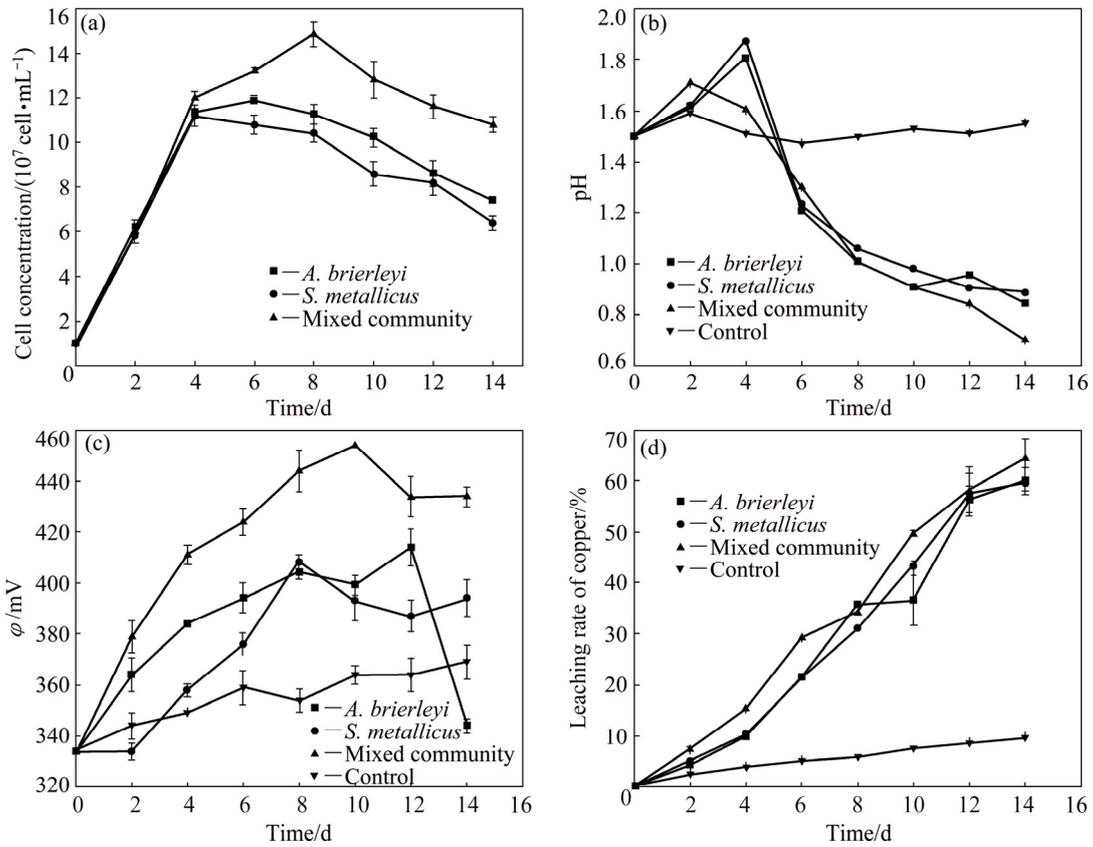


Fig. 1 Differences in chalcopyrite leaching of single- and mixed-archaea systems: (a) Cell concentration; (b) pH; (c) ϕ ; (d) Leaching rate of copper

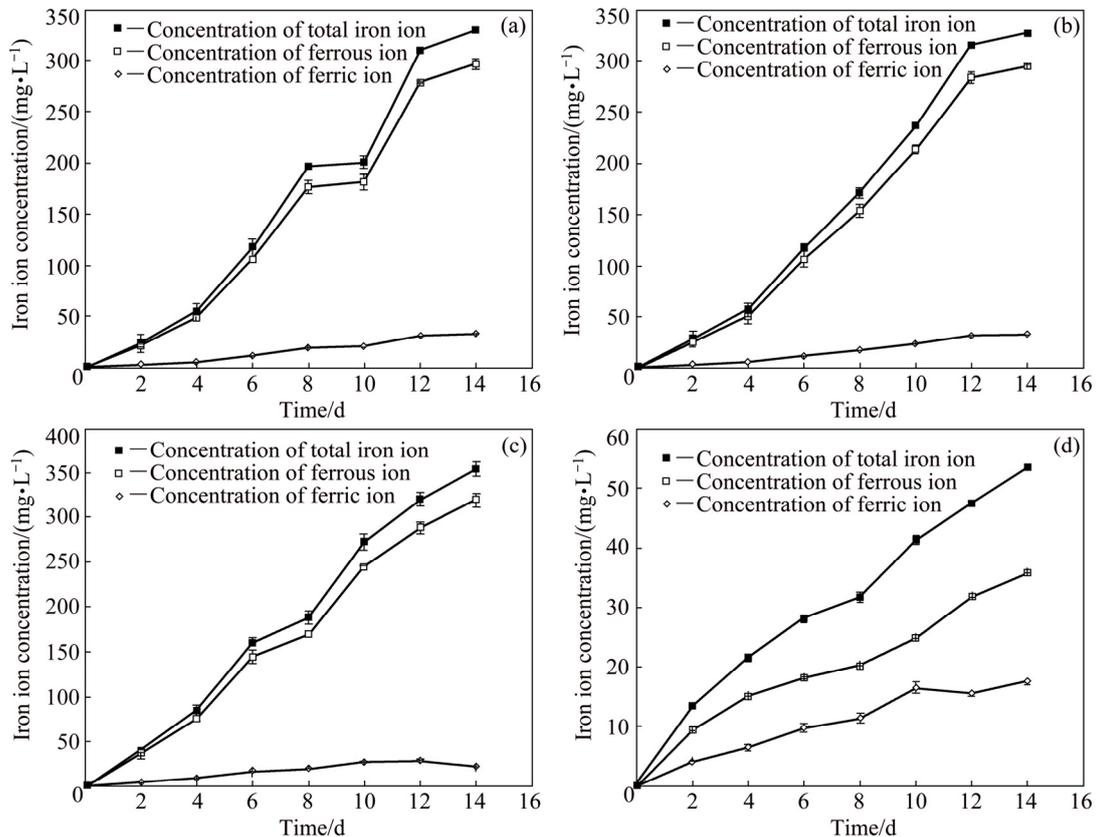


Fig. 2 Concentrations of iron ion of *A. brierleyi* (a), *S. metallicus* (b), mixed community (c) and control group (d)

leaching of chalcopyrite by the mixed archaea system was slowed, and thus the leaching rate of copper ions of the mixed archaea system was higher than that of the single-archaea system.

3.2 Analysis of microbial communities during mixed-archaea leaching process

Figure 3(a) shows the strains in the leaching system on the second day of the leaching process. As for the planktonic archaea in solution and those adsorbed on the surface of the chalcopyrite, the predominant strain was

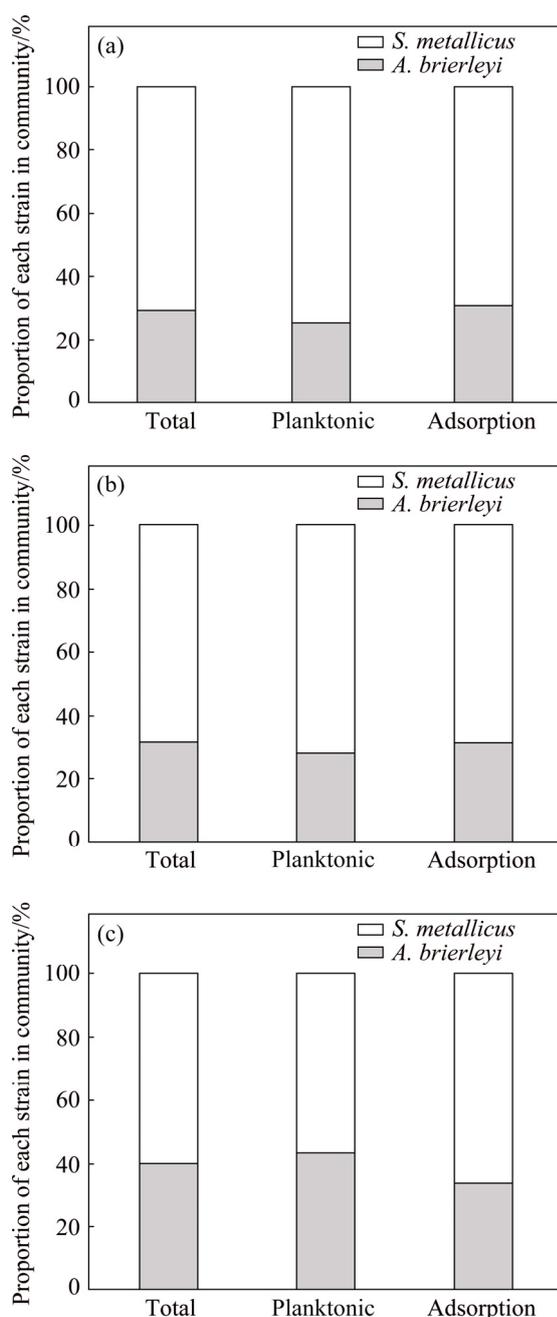


Fig. 3 Population structure of chalcopyrite leachate from mixed-archaea system including total community, planktonic community, and community adsorbed on surface of minerals at different time: (a) Day 2; (b) Day 8; (c) Day 14

S. metallicus, while *A. brierleyi* was less abundant. This is because *S. metallicus* has a comparatively high ability to oxidize ferrous ion in low- Fe^{2+} -concentration systems. Comparison of planktonic and adsorbed populations revealed that the proportion of *S. metallicus* in the planktonic archaea population was approximately 5.28% higher than that in the adsorbed microbial population. As shown in Fig. 3(b), in the middle of the leaching process (day 8), changes were observed in the microbial communities. Although *S. metallicus* was still a dominant species, the proportions of *A. brierleyi* were increased by 2.6% and 0.53% of the planktonic and adsorbed populations, respectively, while the proportion of *S. metallicus* was decreased by the same percentage. Figure 3(c) shows the microbial community structure at the end stage of the leaching process (day 14). Although the predominant microbial population was still *S. metallicus* (56.74% of planktonic and 66% of adsorbed), the percentage of *A. brierleyi* increased significantly by 15.39% (planktonic) and 2.87% (adsorbed). Throughout the leaching process, *A. brierleyi* increased, likely because of its strong sulfur oxidation activity. Additionally, instability of *S. metallicus* in the leaching environment may have caused its proportion in the leaching system to decrease.

3.3 Adsorption of chalcopyrite by microorganisms

Langmuir curves of each separate strain and the mixed community on chalcopyrite are shown in Fig. 4. Based on the adsorption data, the reference parameters of the isothermal adsorption curve model were obtained by linear regression using Eq. (1) [28] as follows:

$$\frac{1}{C_A} = \frac{1}{K_A} \times \frac{1}{C_{Am}} \times \frac{1}{C_L} + \frac{1}{C_{Am}} \quad (1)$$

where C_A denotes the amount of microorganisms adsorbed per unit mass of chalcopyrite when the absorption balance is reached, K_A represents the

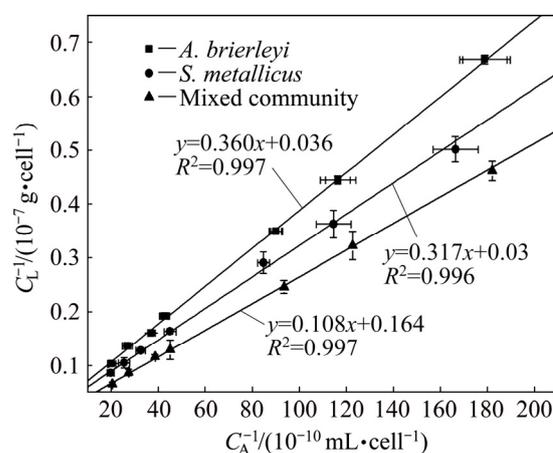


Fig. 4 Langmuir curves of *A. brierleyi*, *S. metallicus*, and mixed community adsorbed on chalcopyrite surface

adsorption equilibrium constant, C_L is the amount of planktonic microbial mass in the solution, and C_{Am} represents the ability of unit chalcopyrite to adsorb the maximum cell number. Next, K_A and C_{Am} were obtained by plotting C_L/C_A against C_A .

The C_{Am} (6.113×10^{12} cell/g) of the mixed system was 2.18- and 1.86-fold C_{Am} of the two single-strain adsorption models, respectively, and the C_{Am} values of *A. brierleyi* and *S. metallicus* were 2.807×10^{12} , 3.287×10^{12} cell/g, respectively. The C_{Am} value of the mixed system approached the sum of the C_{Am} values of the two individual archaea (6.094×10^{12} cell/g). These findings indicated that the two strains had their own unique adsorption sites on the chalcopyrite surface, and nearly no common adsorption sites were observed. Therefore, the two strains produced no competitive adsorption in the mixed system. The K_A value of the same mixed system (1.97×10^{-9} mL/cell) was greater than that of the two strains when adsorbed separately (*A. brierleyi*: 0.98×10^{-9} mL/cell and *S. metallicus*: 0.96×10^{-9} mL/cell). According to previous studies, when the C_{Am} and K_A values of the mixed system are greater than the sum of the two single archaea, the promotion of adsorption occurs before the mixed archaea group.

The two strains in the mixed system influenced one another. Therefore, qPCR was conducted to measure the number of microorganisms that were adsorbed by each strain in the mixed system. As shown in Fig. 5, the adsorption rates of *A. brierleyi* and *S. metallicus* were 64% and 76.7%, respectively, when they were individually adsorbed to equilibrium. However, when the two strains in the mixed system were adsorbed in solution until equilibrium was reached, their initial adsorption rates were 66.01% and 77.59%, respectively. Therefore, the adsorption rates of both strains in the mixed system increased. This result indicated that when the mixed system was adsorbed on the surface of

the chalcopyrite, adsorption occurred between *A. brierleyi* and *S. metallicus*, and both showed increased adsorption rates on the mineral surface.

4 Conclusions

The results of this study showed that the leaching rate of chalcopyrite by *S. metallicus* was slightly higher than that of *A. brierleyi*. Additionally, the chalcopyrite leaching rate of a mixed system of both strains was much higher than that of the single strain. Community structure analysis during the leaching process demonstrated that *S. metallicus* was consistent in a predominant state within the community. However, the proportion of *A. brierleyi* in the community exhibited an increasing trend. Changes in the concentrations of copper in the leachate were due to the fast increase of the leaching rate of the mixed archaea than that of two single-archaea systems in the later stage of leaching. Langmuir parameter analysis revealed no competitive adsorption between the two thermophilic archaea. Moreover, qPCR confirmed that adsorption promotion occurred between *S. metallicus* and *A. brierleyi* in the mixed system.

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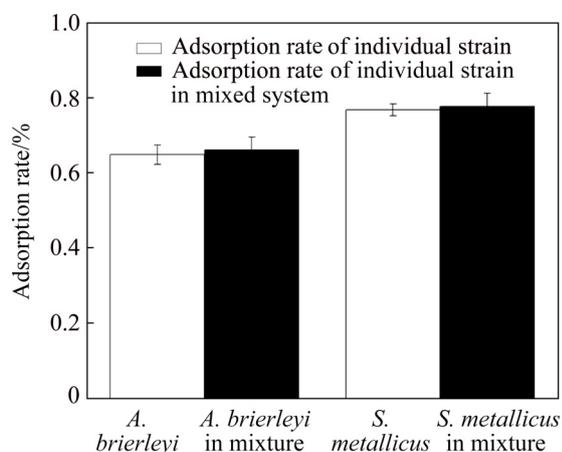


Fig. 5 Adsorption rates of individual strain and mixed system (Rates are numbers of microorganisms of various strains/mixture adsorbed to their respective initial addition numbers)

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两种极端嗜热古菌对黄铜矿的吸附和浸出行为

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摘要: 研究两株具有代表性的极端嗜热古菌 *A. brierleyi* 和 *S. metallicus* 及其混合物对黄铜矿的吸附和浸出行为。结果表明, *S. metallicus* 的铜浸出率略高于 *A. brierleyi* 的, 其中混合菌体系的铜浸出率最高。浸出过程中的群落结构分析表明, *S. metallicus* 在菌群中属于优势菌群; *A. brierleyi* 所占比例呈现上升趋势, 而这一上升趋势与黄铜矿在混合体系浸出后期的铜浓度比在单菌体系增长更快有关。Langmuir 参数分析得出两种古菌之间不存在竞争性吸附关系, 而 qPCR 结果显示, 在混合浸出的过程中, *S. metallicus* 和 *A. brierleyi* 之间会产生促进性吸附。本研究进一步阐明混合极端嗜热菌在矿物表面上所发生的吸附行为。

关键词: 极端嗜热古菌; *A. brierleyi*; *S. metallicus*; 黄铜矿; 吸附; 浸出

(Edited by Wei-ping CHEN)