Effects of Copper Solvent Extraction Reagents on metabolism and Leaching Efficiency of Bioleaching Bacteria

Hao Zhang\textsuperscript{1,*}, Xiaorong Liu\textsuperscript{1,2}, Chang Su\textsuperscript{1} and Yan Liu\textsuperscript{1}

\textsuperscript{1} School of Materials Science and Engineering, Shanghai Institute of Technology, Shanghai, 201418, PR China

\textsuperscript{2} School of Environmental Science and Engineering, Donghua University, Shanghai, 201620, PR China

*Corresponding author’s e-mail: 986401919@qq.com

Abstract. In the process of copper biohydrometallurgy, a small amount of copper extractant organic phase will remain in raffinate by entrainment of dissolving and emulsion droplets. Copper extraction organic phase can poison the bacteria and further affect the bioleaching process. The effects of organic extractants containing ketoximes and oximes on the metabolism of Acidithiobacillus ferrooxidans (A.ferrooxidans) and its bioleaching efficiency were studied. The results showed that the presence of organic phase inhibited bacterial metabolism and reduced its leaching activity.

1. Introduction

Compared with the traditional metallurgical method, copper biohydrometallurgy is a new way, which is more suitable for leaching low-grade and difficult-to-treat copper ore, and has advantage of mild reaction, simple process, low energy consumption and environmental protection\textsuperscript{[1]}. Modern copper hydrometallurgy technology generally includes bioleaching, solvent extraction, electrowinning and other processes, the basic process as shown in figure 1\textsuperscript{[2]}.

![Figure 1. Schematic diagram of bioleaching process.](image)

1.1. Effects of organic extractant on bioleaching process

The organic phase cannot be completely separated from the aqueous phase before the raffinate is recycled back to the heap leaching stage. Microorganisms used in bioleaching are usually sensitive to
organic solvents, but there are few studies on the negative effects of copper extraction on bacterial activity. The copper extraction organic phase usually consists of extractant and kerosene, and some extractant also contain a modifier. Extractants are usually highly surface active substances, and aldoxime and ketoxime are their functional components, which can promote the formation of microemulsion entrained droplets\[3\]. In addition, a small amount of solvent extraction organic substance may also be present in the raffinate in dissolved form. Olefin and aromatic hydrocarbon in kerosene will degrade gradually due to acid, oxidation and radiation in the process of use, resulting in surface active substances. The surface active substances can reduce the surface tension of the liquid drops and increase the stability of the liquid drops, especially the fine liquid drops, so that the clarification effect is deteriorated and the entrainment amount is increased. When the raffinate carrying the entrained organic phase and dissolved organic substance is returned to the bioleaching process, it will contaminate the bioleaching system and inhibit the metabolism of the bioleaching bacteria\[4\].

The research of different researchers was carried out by directly adding different doses and different concentrations of copper extraction organic substances to the bacterial growth environment\[5\]. Liu\[6\] et al. simulated the extraction process to study the effect of it on bioleaching process. It is found that the process variables in the extraction process, such as the concentration of LIX984N in the organic phase, the stirring speed during the extraction and stripping process, determine the content of the organic phase in the raffinate, and the final inhibition of the metabolism of the leaching bacteria. When the concentration of LIX984N in organic phase reached 15% v/v, the ability of A.ferrooxidans bacteria to oxidize Fe$^{2+}$ decreased by nearly 75%. When the concentration of LIX984N was 10% v/v, the extraction ratio of Cu decreased by about 20%. When mixed with fresh organic phase and 300 rpm speed, the strongest inhibition is achieved. A.ferrooxidans cells in 9K medium have normal cell wall and cell plasmalemma, but A.ferrooxidans bacteria growing in the contaminated medium, cell morphology changes, and cell membrane breaks\[4\].

This paper aims to study the effects of the lost organic substance on the metabolism of the leaching bacteria. It is hoped that the key problems of low efficiency of heap bioleaching will be interpreted from different angles, so as to lay a theoretical and experimental basis for improving the efficiency and the quality of bioleaching process.

### 1.2. Research method

#### 1.2.1. Experimental materials

The experimental strains, Acidithiobacillus ferrooxidansATCC23270, were provided by the Biomembrane Center of Duisburg-Essen University, Germany. MAC culture medium: (NH$_4$)$_2$SO$_4$, 0.132 g/L; CaCl$_2$•2H$_2$O, 0.147 g/L; MgCl$_2$•6H$_2$O, 0.025 g/L; KH$_2$PO$_4$, 0.027 g/L. Energy: 10g/L S$^0$. Chalcopyrite concentrate from Jiangxi Dexing copper industry group, is the product of chalcopyrite flotation.

Bacterial culture and leaching process were carried out in ZHWY-2102 double-layer constant temperature culture oscillator (Shanghai Zhicheng) at a constant temperature of 30 °C and a rotation speed of 180 rpm.

The solvent extractants (SE) used in this experiment were LIX84-ICNS and LIX860N-I, which were supplied by BASF (Shanghai) company, Germany. The molecular structures of the different hydroximes are as follows:

| Table 1. The molecular structures of LIX860N-I and LIX84-ICNS. |
|-----------------|-----------------|
| LIX860N-I       | LIX84-ICNS      |
1.2.2. Test method. In order to achieve our research, we mixed pure MAC culture medium with organic phase containing 10 %v/v LIX860N-I and LIX84-ICNS respectively at 400 rpm for 10 min, took that raffinate as the culture medium polluted by the organic phase, and measured the raffinate in a sterile conical flask.

S powder (10 g/L) is respectively added to the culture medium using S⁰ as energy source, and chalcopyrite concentrate (2 g) of 0.18-0.425 mm grade is respectively added to the culture medium using chalcopyrite concentrate as energy source. Weigh. Place in an oscillation incubator and culturing at 180 rpm. Parallel experiments were conducted with pollution-free culture as control. Follow-up monitoring of growth.

2. Effects of organic extractant on metabolism and leaching activity of A. ferrooxidans cells

2.1. Effects of copper extractant on bacterial metabolism

S⁰ was used as energy source to cultivate A. ferrooxidans. Then inoculated with 10% v/v bacterial fluid with a concentration of 3.4×10⁷ cells/ml. The effects of copper extraction organic substances on the growth of A. ferrooxidans are shown in figures 2 and 3.

![Figure 2](image1.png)  
**Figure 2.** These two figures have been placed side-by-side to save space. Justify the caption.

According to figure 2, under normal culture conditions, A. ferrooxidans bacteria first entered the exponential growth stage on the 3.5 days. Then, under the influence of LIX860N-I organic phase on the 4.5 day, and under the influence of LIX84-ICNS organic phase on the sixth day, they entered the exponential growth period.

It can be seen that organic substance can inhibit the growth of A. ferrooxidans bacteria. The inhibitory effect of LIX84-ICNS on A. ferrooxidans bacteria was more obvious than that of LIX860N-I.

Davis method[7] was used to calculate the hydrophilic balance of organic compounds. For every additional carbon in the lipid chain in the surface group, HLB⁰ decreases by 0.475. Quaternary carbon
is surrounded by tetrahedron composed of four surrounding carbon atoms and has no contribution to oil- or water-affinity.

\[
\text{HLB}^D(\text{LIX84-ICNS}) = \text{HLB}^D(-\text{CH}_2-) + 9\text{HLB}^D(-\text{CH}_2-) = 6.6 + 9(-0.475) = 2.325
\] (1)

\[
\text{HLB}^D(\text{LIX860N-I}) = \text{HLB}^D(-\text{CH}_2-) + 12\text{HLB}^D(-\text{CH}_2-) = 6.6 + 12(-0.475) = 0.9
\] (2)

It can be seen that LIX860N-I is more hydrophobic. When sulfur powder is used as energy, it is easy to adsorb on the surface of S powder and accumulate the powder of S. The free concentration of LIX860N-I decreased more than that of LIX84-ICNS, so the toxicity was less. Though, the aldehyde oxime group of LIX860N-I is more active.

\[
2\text{S}^0 + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + 2\text{SO}_4^{2-} \text{ (under the effect of bacteria)}
\] (3)

*A. ferrooxidans* bacteria use the sulfur energy as the acid consumption process (formula 3). So the pH keeps going down (figure 3).

### 2.2. Effect of copper extraction organic substance on copper leaching activity of *A. ferrooxidans*

In order to study the effect of organic substance on the leaching activity of *A. ferrooxidans* bacteria, the changes of the parameters of the leaching process of copper concentrate bioleaching under the influence of 10% v/v LIX860 organic phase were investigated. The condition of comparison experiment was that there was no organic pollution. The pure bacteria solution with a concentration of \(3.6 \times 10^7\) cells/ml was inoculated at 10% v/v. The results are figure 4-7.

![Figure 4. Suspension cell numbers of *A. ferrooxidans* in bioleaching process of chalcopyrite concentrate under influence of LIX860N-I.](image)

![Figure 5. pH curve of *A. ferrooxidans* culture medium in bioleaching process of chalcopyrite concentrate under influence of LIX860N-I.](image)
2.2.1. Analysis of A.ferrooxidans bacterial leaching process without pollution. According to figure 4, the adaptation period was 50 days after inoculation, and the adaptation period was longer because the bacteria had not been domesticated. Days 50-72 were exponential growth period, days 72-78 were the stable period, and the period after 78 days was the decline phase. The indirect leaching and direct leaching of A.ferrooxidans bacteria were:

- **Direct leaching:**
  \[ 4\text{CuFeS}_2 + 4\text{H}^+ + 17\text{O}_2 \rightarrow 4\text{Cu}^{2+} + 4\text{Fe}^{3+} + 8\text{SO}_4^{2-} + 2\text{H}_2\text{O} \] (under the effect of bacteria) \hspace{1cm} (4)

- **Indirect leaching:**
  \[ 4\text{Fe}^{3+} + \text{CuFeS}_2 \rightarrow 5\text{Fe}^{2+} + 2\text{S}^0 + \text{Cu}^{2+} \] \hspace{1cm} (5)
  \[ 4\text{Fe}^{2+} + 4\text{H}^+ + \text{O}_2 \rightarrow 4\text{Fe}^{3+} + 2\text{H}_2\text{O} \] (under the effect of bacteria) \hspace{1cm} (6)
  \[ 2\text{S}^0 + 3\text{O}_2 + 2\text{H}_2\text{O} \rightarrow 4\text{H}^+ + 2\text{SO}_4^{2-} \] (under the effect of bacteria) \hspace{1cm} (7)

In the leaching process, the alkaline components in the ore consumed acid, while the A.ferrooxidans bacteria used the ferrous ion energy as the acid consumption process, and the sulfur energy was used as the acid producing process. So the pH of the solution fluctuated up and down (figure 5). The rapid oxidation of ferrous ions corresponded to the rapid growth of free cell concentration (figure 4, figure 7). At about 50 days, the growth of bacteria entered exponential phase, and ferrous ions in leaching solution were rapidly consumed. When the suspension cells began to enter the exponential growth period, the rate of potential decline slowed down rapidly (figure 6), and \( \frac{c(\text{Fe}^{3+})}{c(\text{Fe}^{2+})} \) was rapidly increased (figure 7). A.ferrooxidans had good indirect leaching activity and strong oxidation capacity. The extracted ferrous energy was then oxidized and utilized, and the concentration of trivalent iron ions increased. The concentration of ferrous ion in the later stage of leaching was close to 0, and the total iron content increased slowly.

2.2.2. A.ferrooxidans bacterial leaching analysis under the influence of LIX860N-I. Under the influence of organic phase containing LIX860N-I, the growth process of A.ferrooxidans bacteria was similar to that without pollution, and could be divided into delay period, exponential growth period, stable period and decline period. On the first 2 days after inoculation, the concentration of suspended A.ferrooxidans cells in the two conditions decreased as the bacteria adsorbed on the ore surface. Under the influence of 10%LIX860N-I organic phase, the concentration of suspended cells was close to 0, and remained low after that. After sixty-fifth day, it entered exponential growth. The delay period was longer than that of the A.ferrooxidans strain growing in the pollution-free environment for 15 days.

![Figure 6](image_url)  
**Figure 6.** Potentials of A.ferrooxidans culture medium in bioleaching process of chalcopyrite concentrate under influence of LIX860N-I.

![Figure 7](image_url)  
**Figure 7.** \( \frac{c(\text{Fe}^{3+})}{c(\text{Fe}^{2+})} \) ratio of A.ferrooxidans culture medium in bioleaching process of chalcopyrite under influence of LIX860N-I.
The exponential growth period was from day 65 to day 75, days 75-80 was the stable period, and the suspended cell concentration reached the maximum at seventy-eighth day, then decreased.

LIX860N-I inhibited the leaching of chalcopyrite concentrate by *A. ferrooxidans*, and had the same effect on the metabolism of *A. ferrooxidans* with $S^0$ as energy sources.

In the short time growth, the toxicity to bacteria was smaller than that of LIX84-ICNS. But in the long time leaching process of chalcopyrite concentrate, LIX860N-I is easy to combine with copper and iron on the surface of sulfide ore, reducing the concentration of redox reactant and reaction microzone in the mechanism of direct leaching of bacteria. This reduction made the toxicity to bacteria prolonged for a long time, resulted in the inhibition of the leaching process and greatly prolonged the leaching process.

The ferrous ions in both groups were rapidly oxidized at the beginning of exponential cell growth, and then remained near zero.

### 3. Conclusions

When $S$ was used as energy source, the metabolism of *A. ferrooxidans* was inhibited by organic extractant, and the lag period was prolonged. Organic extractant also inhibited the leaching activity of *A. ferrooxidans* from chalcopyrite concentrate. Under the influence of 10% v/v LIX860N-I extraction organic phase, *A. ferrooxidans* delayed 15 days to enter the exponential growth period. The inhibitory degree of organic phase containing 10% v/v LIX84-ICNS on cells metabolism was greater than that of the organic phase containing 10% v/v LIX860N-I, indicating that the toxicity of LIX84-ICNS to *A. ferrooxidans* bacteria was larger than that of LIX860N-I.

### Acknowledgments

This paper is completed under the guidance and care of the tutor Professor Liu Xiaorong. Mrs. Liu is very careful to guide and modify my experiment process and thesis. From Mrs. Liu, I learned that academic research should be very serious and unambiguous. We should extensively dabble in relevant domestic and foreign related literature, accumulate basic knowledge, understand and master the latest scientific research results, and deepen the interest in my own specialty. I express my great gratitude to her here for her immense help.

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