

# Biooxidation of Chalcopyrite by Iron and/or Sulfur Oxidizing Bacteria Isolated In Armenia

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**ABSTRACT:** Bioleaching is based on the abilities of acidophilic bacteria and archaea to oxidize sulfide minerals. Bioleaching of the main copper mineral chalcopyrite ( $\text{CuFeS}_2$ ) by new isolated thermotolerant *Acidithiobacillus* sp. 13Zn and *Leptospirillum ferriphilum* CC has been studied. Experiments have been carried out in shake flasks. Data obtained showed that *Acidithiobacillus* sp. 13Zn actively oxidized chalcopyrite. It has been revealed that bioleaching of chalcopyrite more efficiently occurred at pH 2.0, and 10% of pulp density. Maximum yield of copper and total iron reached for the size range of - 63 - +45  $\mu\text{m}$ . It has been shown that bioleaching of chalcopyrite by *Acidithiobacillus* sp. 13Zn significantly increased in mixed culture with *L.ferriphilum* CC. In bioleaching of chalcopyrite more efficiently was mixed culture of *Acidithiobacillus* sp. 13Zn and sulfur-oxidizing bacteria *Acidithiobacillus albertensis* SO-2. Tolerance of *Acidithiobacillus* sp. 13Zn to copper and zinc much more higher than that of *L.ferriphilum* CC.

**KEYWORDS:** Thermotolerant iron oxidizing bacteria, Bioleaching of chalcopyrite, Mixed cultures.

## I. INTRODUCTION

Pyrite ( $\text{FeS}_2$ ) and chalcopyrite ( $\text{CuFeS}_2$ ) are the most abundant sulfide minerals. It is currently estimated that 70% of the world copper reserves now occur in chalcopyrite deposits (Waltling, 2006). However chalcopyrite is the most refractory mineral to chemical and biological leaching resulting in low dissolution rates. The main reason for low dissolution rate of chalcopyrite is formation of passive layer on mineral surface. Reaction products of bioleaching such as elemental sulfur and iron-hydroxide (jarosite) precipitate on the mineral surface and hinder its further dissolution by limiting the flow of bacteria and reaction products to and from the mineral surface (Dopson et al., 2003; Fu et al., 2008; Johnson, 2014; Rawlings et al, 1999).

It is known that the most important bacteria in commercial bioleaching processes are iron- and sulfur- oxidizing bacteria *Acidithiobacillus ferrooxidans*, sulfur-oxidizing *Acidithiobacillus thiooxidans* or *Acidithiobacillus caldus* and iron-oxidizing *Leptospirillum* spp. bacteria (Coram, Rawlings, 2002; Okibe et al, 2003). More over the dominant bacteria in the bioleaching processes operated at 45-50° C are *Leptospirillum ferriphilum* and *At.caldus* (Johnson, 2014; Johnson et al, 2005; Rawlings, Johnson, 2007; Sand et al, 1992; Schippers, Sand, 1999).

The objective of the current study is to investigate bioleaching of chalcopyrite by pure and mixed cultures of new isolated *Acidithiobacillus* sp. 13Zn, *L.ferriphilum* CC and *At.albertensis* as well as the influence of physicochemical factors.

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## II. MATERIALS AND METHODS

### 2.1 Culture preparation

*Acidithiobacillus* sp. 13Zn, *L.ferriphilum* CC and *At.albertensis* SO-2 isolated from bioleaching pulp of zinc and copper concentrates (Vardanyan et al, 2015) as well as their mixed cultures were used for bioleaching of chalcopyrite of Shamlugh ore deposit (Armenia). The bacteria were grown on Mackintosh medium containing  $\text{Fe}^{2+}$  or  $\text{S}^0$  as an energy source (Vardanyan, et al, 2015). In the logarithmic phase of growth the cells were collected by centrifugation at 6000g 10 min. Biomass collected were washed with acidified Mackintosh medium (Mackintosh,1978) without  $\text{Fe}^{2+}$  and resuspended in the same medium.

### 2.2 Bioleaching experiments

Chalcopyrite ( $\text{CuFeS}_2$ ) from Shamlugh ore deposit in Armenia ground to particle size 43-125  $\mu\text{m}$  were used for leaching experiments. Mineral grains were placed into 250 ml Erlenmeyer flasks, moistened with distilled water and sterilized. Then 50 ml Mackintosh medium without  $\text{Fe}^{2+}$ , pH 2.0 adjusted by  $\text{H}_2\text{SO}_4$  and bacterial suspension ( $10^8$  cells/ml) were added to the flasks. The bioleaching experiments were carried out at 37° C in the periodic mode and shaking conditions (180 rpm/min.). Mackintosh medium was added regularly to the flasks to compensate evaporation losses. Control experiments were carried out without inoculum. The pulp density was calculated as chalcopyrite mass ratio to the volume of the medium.

### 2.3 Optimal conditions of bioleaching

Bioleaching of chalcopyrite was studied at pH 1.4 - 2.2, in the range of concentration of mineral from 2 to 15 % and 45 - 120  $\mu\text{m}$  of particle size. Sampling was performed at 24 h intervals and pH was analyzed. Copper, total iron, ferric Fe(III) and ferrous (Fe(II)) ions concentration in the bioleached solutions were determined by atomic absorption spectrometer AAS 1N (Germany).

### 2.4 Influence of copper and zinc

Influence of copper and zinc on oxidation of iron by *Acidithiobacillus* sp. 13Zn and *L.ferriphilum* CC was studied in Mackintosh medium with 100 mM ferrous iron (Fe(II)) in the range of concentration from 10 to 250 mM. The data reported in the text are from the average of duplicate experiments with  $\pm 2\%$  variation.

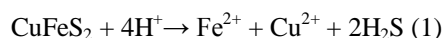
## III. EXPERIMENTAL RESULTS

### 3.1 Bioleaching of chalcopyrite, effect of pH

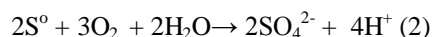
In our previous work it was shown that new isolated iron- and sulfur- oxidizing bacteria *Acidithiobacillus* sp. 13Zn with optimal temperature for growth of 35°C posses high activity of bioleaching of pyrite (Vardanyan, et al, 2015). In this work *Acidithiobacillus* sp. 13Zn was tested for bioleaching of chalcopyrite. The influence of physico-chemical factors on bioleaching of chalcopyrite was studied.

In order to determine the effect of acidification on bioleaching of chalcopyrite by *Acidithiobacillus* sp. 13Zn the experiments were carried out at initial pH from 1.4 to 2.2 (Fig.1).

At the beginning of the experiment the increase of pH of the bioleaching solution was observed. According to mechanism of bioleaching of sulfide minerals the increase in pH occurs due to consumption of acid during the protonic attack of the chalcopyrite (Eq.1):



After 4-7 days pH started to decrease because of the oxidation of elemental sulfur by *Acidithiobacillus* sp. 13Zn (Eq.2):



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Simultaneously to pH decrease, the extraction of copper and iron from chalcopyrite increased. As shown in Figure 1, the increase of initial pH from 1.4 to 2.0 resulted in enhancing of extraction of copper and total iron about 3 times. However, further increase of pH leads to decrease of extracted copper and iron from chalcopyrite. Thus, optimal initial pH for growth of bacteria and bioleaching of copper and iron is 2.0.

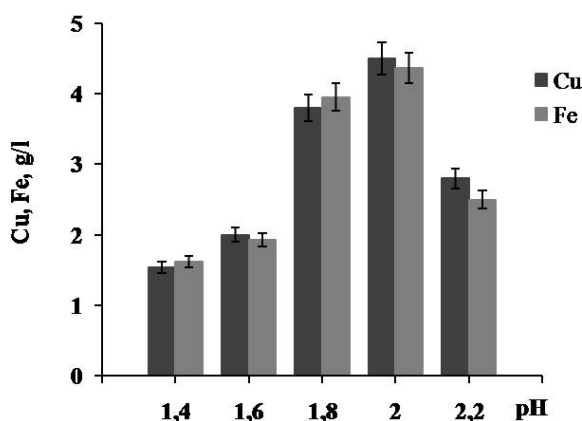


Fig. 1. Effect of initial pH on extraction of copper and iron from chalcopyrite by *Acidithiobacillus* sp.13Zn (PD - 4%, t - 35°C, 180 rpm, duration -16 days)

### 3.2 Effect of concentration of CuFeS<sub>2</sub>

Studies carried out showed that extraction of copper from chalcopyrite enhanced by increasing concentration of CuFeS<sub>2</sub> from 2 to 10% (Fig.2). However, at 15% of chalcopyrite bioleaching of copper by strain 13Zn decreased. Maximum extraction of copper and iron by *Acidithiobacillus* sp. 13Zn occurred at 10% of chalcopyrite (Fig.2).

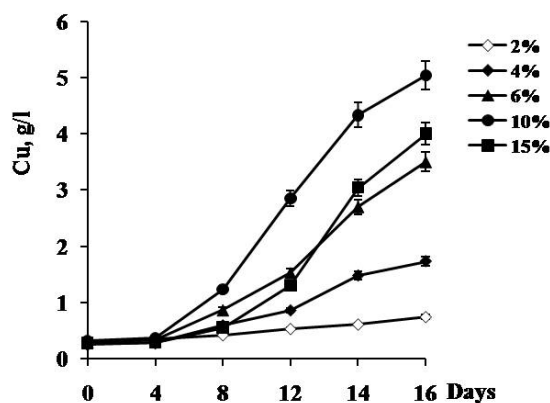


Fig. 2. Effect of concentration of chalcopyrite on dynamics of bioleaching of iron by *Acidithiobacillus* sp. 13Zn (pH 1.7; t -35°C)

The same patterns were observed for the bioleaching of iron from chalcopyrite depending on its concentration by *Acidithiobacillus* sp. 13Zn (Fig. 3). Thus, maximum extraction of copper and iron from chalcopyrite by *Acidithiobacillus* sp. 13Zn occurred at 10% of chalcopyrite.

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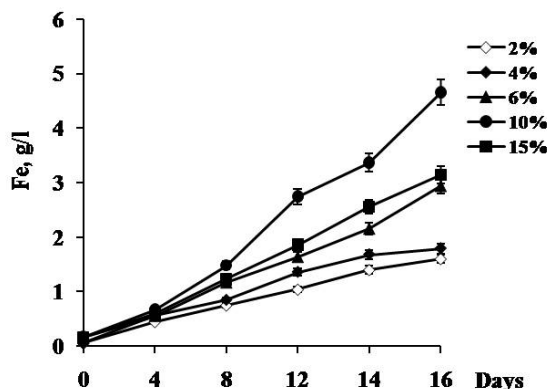


Fig. 3. Effect of concentration of chalcopyrite on dynamics of bioleaching of copper by *Acidithiobacillus* sp. 13Zn (pH 1.7; t - 35°C)

### 3.3 Effect of particle size

As shown in Fig. 4, the rates of copper and iron dissolution increased with decreasing particle size from +125  $\mu\text{m}$  to size fraction of + 45  $\mu\text{m}$ . Maximum yield of copper (0.7g/l) and total iron (1.23g/l) reached for the size range of + 45  $\mu\text{m}$  after 13 days of bioleaching of chalcopyrite by *Acidithiobacillus* 13Zn (Fig. 4). The explanation for this increase is that smaller particle size has more surface area, enhancing metal dissolution.

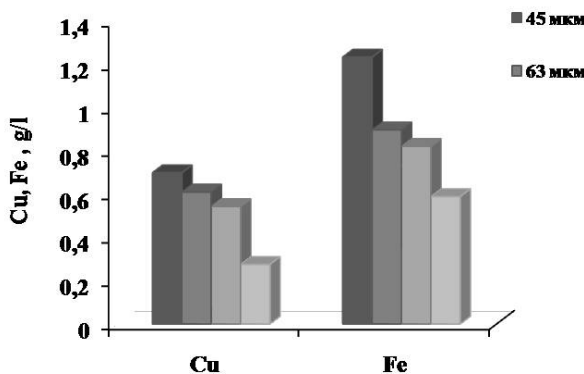


Fig. 4. Effect of particle size on bioleaching of copper and iron from  $\text{CuFeS}_2$  by *Acidithiobacillus* sp.13Zn (PD - 4%, pH 1.8; t - 35°C, 180 rpm, duration -16 days)

### 3.4 Bioleaching by mixed cultures

The comparison of leaching of chalcopyrite by pure and mixed cultures of *Acidithiobacillus* sp. 13Zn, iron-oxidizing *L.ferriphilum* CC and sulfur-oxidizing *At.albertensis* SO-2 at 37°C were studied. As shown in Figure 5 and Figure 6 mixed cultures of new isolated thermotolerant *Acidithiobacillus* sp. 13Zn and iron-oxidizing *L.ferriphilum* CC or sulfur-oxidizing *At.albertensis* SO-2 oxidize chalcopyrite more actively than pure culture of *Acidithiobacillus* sp. 13Zn. Thus, the extraction of copper from chalcopyrite by *Acidithiobacillus* sp. 13Zn enhances by about 1.3 and 1.2 times in the presence of *L.ferriphilum* CC and *At.albertensis* SO-2 respectively (Fig.5).

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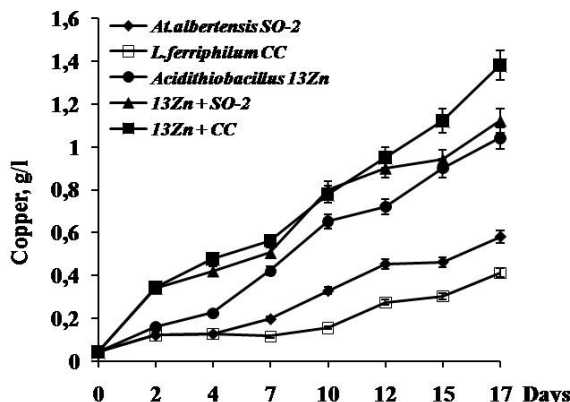


Fig. 5. Bioleaching of copper from chalcopyrite by pure and mixed cultures of isolated *Acidithiobacillus* sp. 13Zn, iron- oxidizing *L.ferriphilum* CC and sulfur- oxidizing *At.albertensis* SO-2 (pH 1.8; t - 35°C, CuFeS<sub>2</sub> - 4%, 180 rpm)

Data presented in Figure 6 indicated that in bioleaching of iron from chalcopyrite more actively was the association of *Acidithiobacillus* sp. 13Zn with *At.albertensis* SO-2. Thus, the presence of *At.albertensis* SO-2 in the mixed culture increases the dissolution of iron from chalcopyrite about 1.3. In case of mixed culture of *Acidithiobacillus* sp. 13Zn with *L.ferriphilum* CC extraction of total iron from chalcopyrite enhances only 1.2 times (Fig. 6).

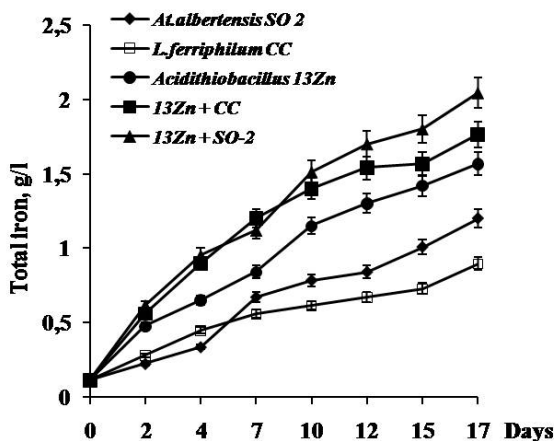


Fig. 6. Bioleaching of iron from chalcopyrite by pure and mixed cultures of isolated *Acidithiobacillus* sp. 13Zn, *L.ferriphilum* CC and *At.albertensis* SO-2 (pH 1.8; t - 35°C CuFeS<sub>2</sub> - 4%, 180 rpm)

It is concluded, that the presence of *L.ferriphilum* CC in mixed cultures resulted in rapid oxidation of ferrous ion (Fe (II)) and regeneration of oxidative agent – Fe (III), which in its turn accelerates chalcopyrite oxidation. However in bioleaching of chalcopyrite more efficiently was mixed culture of *Acidithiobacillus* sp. 13Zn and sulfur-oxidizing bacteria *At.albertensis* SO-2. It can be explained that *At.albertensis* SO-2 in the mixed culture contributed in sulfur oxidation into sulfuric acid which prevented the formation of jarosite as well as passivation sulfur layer on the chalcopyrite surface.

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### 3.5 Tolerance to copper

The influence of copper and zinc on oxidation of iron by *Acidithiobacillus* sp. 13Zn and *L.ferriphilum* CC was studied in the range of concentration from 10 to 250 mM. It was shown that iron oxidation by *L.ferriphilum* CC is inhibited about 50% both in the presence of copper and zinc in concentration of 10 mM, while inhibition of iron oxidation by *Acidithiobacillus* sp. 13Zn reached 45% only at 100 mM copper (Table 1). No inhibition of iron oxidation by *Acidithiobacillus* sp. 13Zn was observed up to 150 mM Zn in the medium. However, in concentration of 200 and 250 mM zinc inhibited iron oxidation by *Acidithiobacillus* sp. 13Zn 82 and 84%, respectively (Table 1).

Table 1. The influence of copper and zinc on oxidation of iron by *Acidithiobacillus* sp. 13Zn and *L.ferriphilum* CC (for 48 hour of cultivation)

| <i>Acidithiobacillus</i> sp. 13Zn |                          |               | <i>L.ferrooxidans</i> CC |               | <i>Acidithiobacillus</i> sp. 13Zn |                         |               | <i>L.ferrooxidans</i> CC |               |
|-----------------------------------|--------------------------|---------------|--------------------------|---------------|-----------------------------------|-------------------------|---------------|--------------------------|---------------|
| In the presence of Cu             |                          |               |                          |               | In the presence of Zn             |                         |               |                          |               |
| Cu, mM                            | Iron oxidized, g/l, 48 h | Inhibition, % | Iron oxidized, g/l, 42h  | Inhibition, % | Zn, mM                            | Iron oxidized, g/l, 48h | Inhibition, % | Iron oxidized, g/l, 48h  | Inhibition, % |
| 0                                 | 2.95                     | 0             | 2.44                     | 0             | 0                                 | 1.4                     | 0             | 2.44                     | 0             |
| 10                                | 3.53                     | 12.5          | 1.29                     | 47.1          | 10                                | 1.87                    | 0             | 1.23                     | 49.4          |
| 25                                | 3.5                      | 12.0          | 0.95                     | 60.9          | 20                                | 1.87                    | 0             | 1.12                     | 54.0          |
| 50                                | 2.44                     | 17.4          | 0.95                     | 60.9          | 50                                | 1.82                    | 0             | 1.0                      | 58.6          |
| 100                               | 1.62                     | 44.9          | 0.87                     | 64.3          | 100                               | 1.62                    | 0             | 0.95                     | 60.9          |
| 150                               | -                        | -             | 0.82                     | 66.3          | 150                               | 1.57                    | 0             | 0.89                     | 63.2          |
| 200                               | 0.73                     | 75.3          | 0.67                     | 72.4          | 200                               | 0.25                    | 82.0          | 0.84                     | 65.5          |
| 250                               | 0.59                     | 80.1          | 0.62                     | 74.7          | 250                               | 0.22                    | 84.0          | -                        |               |

Thus, tolerance of *Acidithiobacillus* sp. 13Zn to copper was much higher than that of *L.ferriphilum* CC. Zinc inhibited iron oxidation by *Acidithiobacillus* sp. 13Zn at concentration of 200 mM and more.

### IV. CONCLUSION

*Acidithiobacillus* sp. 13Zn isolated from bioleaching pulp of zinc concentrate showed high activity of chalcopyrite oxidation. It has been revealed that bioleaching of chalcopyrite by *Acidithiobacillus* sp. 13Zn more efficiently occurred at pH 2.0, and 10% of pulp density. Maximum yield of copper and total iron reached for the size range of - 63 - +45  $\mu$ m. Data obtained indicated that the efficiency of *Acidithiobacillus* sp. 13Zn in chalcopyrite oxidation significantly increased in mixed culture with iron- oxidizing *L.ferriphilum* CC, isolated from bioleaching pulp of copper concentrate. However in bioleaching of chalcopyrite more efficiently was mixed culture of *Acidithiobacillus* sp. 13Zn and sulfur-oxidizing bacteria *At.albertensis* SO-2. It can be explained that *At.albertensis* SO-2 in the mixed culture contributed in sulfur oxidation into sulfuric acid which prevented the formation of jarosite as well as passivation sulfur layer on the chalcopyrite surface. It was shown that tolerance of *Acidithiobacillus* sp. 13Zn to copper and zinc much higher than that of *L.ferriphilum* CC.

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