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CHEMICAL OXIDATION INTEGRATED INTO BIOLEACHING OF PYRITE AND CHALCOPYRITE USING IMMOBILIZED BIOMASS

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Abstract

Chemical oxidation of pyrite and chalcopyrite by ferric sulfate ($\text{Fe}_2(\text{SO}_4)_3$) solution and biogenic ferric iron obtained by mixed culture of isolated thermotolerant *Acidithiobacillus* sp. 13Zn and *Leptospirillum ferriphilum* CC immobilized on natural carriers-zeolite and shungite was studied. Oxidation rate of sulfide minerals was estimated by the decrease of Fe^{3+} (oxidant) and increase of Fe^{2+} ions in the solution. It was revealed that chemical oxidation of chalcopyrite by biogenic ferric iron occurred 2-3 times more intensively than that by $\text{Fe}_2(\text{SO}_4)_3$ solution. Pyrite oxidation rate by biogenic ferric iron was twice higher than that by chemical ferric iron solution. It was shown that the treatment of pyrite and chalcopyrite by biogenic ferric iron allows to increase on average 1.5 - 2 times the bioleaching of iron from pyrite and iron and copper from chalcopyrite by the associations of iron and sulfur oxidizing bacteria.

Key words: *Acidithiobacillus* sp. 13Zn, biogenic ferric iron, chalcopyrite, chemical oxidation, immobilized biomass, pyrite

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1. Introduction

Currently, it is accepted that there are two mechanisms for bioleaching of sulfide minerals: indirect “contact” and “non-contact” (Tributsch, 2001). According to indirect “contact” mechanism, the process of bioleaching occurs in the microenvironment - the reaction space between the surface of cell wall and mineral filled with extracellular polymeric substances (EPS) (Pace et al., 2005; Sand and Gehrke, 2006; Telegdi et al., 1998). Herneir et al. (2006) suppose that the complex of Fe (II) with glucuronic acid is unstable that allows Fe(II) ions to move freely within the EPS. Diffusing into the outer membrane of bacteria, Fe(II) can be oxidized by cell enzyme system and re-enter into the reduction-oxidation cycle (Herneir et al., 2006).

In case of “non-contact” mechanism, the mineral bioleaching is occurred in the liquid phase

through Fe (III) ions generated by bacteria. From this viewpoint the intensity of bioleaching is mainly determined by the regeneration of oxidizer – Fe (III) (Crundwell, 2003; Sand et al., 2001). Thus, the reduction of activity of leaching liquors or their bioregeneration is considered to be important for the development of efficient technology for the leaching of non-ferrous metals from minerals. At present, regeneration of ferric ion in operating leaching technologies realized by chemical methods, mainly by chlorine, ozone, hydrogen peroxide, makes the process more complicated and cost inefficient (http://www.khoamoitruonghue.edu.vn/courses/EnvTech/Fe_and_Mn_removal.pdf)

The use of chemolithotrophic microorganisms (CM) allows to simplify the process of regeneration of leaching solutions and significantly decrease its cost as microorganisms are considered to be unexhausted catalysts. On the other hand, to increase the intensity

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of bioregeneration of Fe(III), the method of immobilization of bacterial cells on porous solid carriers can be used. Immobilization of iron-oxidizing bacteria *At. ferrooxidans* and *L. ferrooxidans* on solid carriers allows to significantly increase the conversion rate of $\text{Fe}^{2+}/\text{Fe}^{3+}$ due to the concentration of bacterial cells (Armentia and Webb, 1992; Jaisankar and Modak, 2009; Nematy and Webb, 1996; Nikolov and Karamanev, 1992; Wood et al., 2001). Simultaneously, immobilization ensures long-term use of bacterial biomass (Gomez and Cantero, 1998; Ginsburg et al., 2009).

The process of immobilization of *At. ferrooxidans* is based on a number of factors, such as surface properties of adsorbent (Absolom et al., 1983), zeta potential (West et al., 1998), surface tension or moisture (Becker, 1998) and composition of nutrient media (An and Freidman, 1998; Li and Logan, 2004).

It has been revealed that the increase of temperature promotes the growth of free-living cells and oxidation of Fe (II) (Gomez and Cantero, 1998). However, unlike free-growing cells, the effect of the temperature on the immobilized cells of *At. ferrooxidans* is weaker (Nikolov and Karamanev, 1992). It is assumed that this is due to the physiological changes in the EPS of fixed cells, the nature and mechanism of which are not yet known (Gomez et al., 2000; Nikolov and Karamanev, 1992). Immobilization results in the increase of the oxidation rate of Fe (II) due to the enhancement of metabolic activity of *At. ferrooxidans* (Nemati and Webb, 1996, 1997; Wood et al., 2001; Zhong et al., 2004).

The immobilization mostly depends on the pH of the environment. The studies have shown that immobilization of *At. ferrooxidans* is 2 to 8 times more efficient at pH 2.0 as compared to pH 1.7 and 1.4, respectively (Gomez et al., 2000). It is assumed that under low pH conditions, the generation of jarosite is rapidly reduced. As the biofilm consists of the cells of *At. ferrooxidans* attached to the porous surface of jarosite, the biomass of immobilized cells on the carrier decreases at low pH ($\text{pH} < 2.0$). Thus, the temperature and pH may be used to control the processes of immobilization and biomass generation in the bioreactor (Nemati and Webb, 1997).

In this study for the first time natural inorganic carriers zeolite and shungite have been used for immobilization of the mixed culture of iron oxidizing bacteria *Acidithiobacillus* sp.13Zn and *L. ferriphilum*. Previously, the immobilization of *L. ferriphilum* CC was successfully implemented on the mentioned carriers (Vardanyan et al., 2013). It was shown that bacteria immobilized on zeolite and shungite might be prospective for regeneration of oxidant (Fe^{3+}) in bioleaching processes.

The objective of this paper is to study comparative activities of ferric sulfate ($\text{Fe}_2(\text{SO}_4)_3$) solution and biogenic ferric iron obtained by the isolated thermotolerant bacteria on chemical oxidation of pyrite and chalcopyrite. The studies will allow to reveal optimal conditions for the regeneration of leaching liquors. Based on the obtained results the

efficient integrated bioleaching technology for the extraction of non-ferrous and precious metals will be developed.

2. Material and methods

2.1. Microorganisms and media

In this study thermotolerant iron oxidizing CM *Acidithiobacillus* sp.13Zn (Stepanyan, 2016), *L. ferriphilum* CC (Vardanyan et al., 2013) and sulfur oxidizing bacteria *Acidithiobacillus albertensis* SO-2 (Vardanyan and Vardanyan, 2014) isolated in Armenia were used. For the growth of bacteria, Mackintosh medium (Mackintosh, 1978) with ferrous iron or sulfur as a source of energy was used.

2.2. Immobilization of microorganisms on natural carriers

For immobilization of iron oxidizing bacteria *Acidithiobacillus* sp.13Zn and *L. ferriphilum* CC natural inorganic carriers such as zeolite, shungite and activated carbon were tested. The mentioned carriers were provided by the Department of Mineralogy of Yerevan State University. The main features of the carriers are presented below.

Armenian natural zeolite (Clinoptilolite): chemical formula - $(\text{Na}, \text{K}, \text{Ca}) (\text{AlSi}_5\text{O}_{12}) \times 6\text{H}_2\text{O}$, bulk density-980 kg/m^3 , porosity - 60-63%, specific surface - $(50-65) \times 10^{-3} \text{ m}^2/\text{kg}$, Ion Exchange Capacity by Ca^{++} , K^+ , Na^+ mg eqv on 100g- 90-150.

Brilliant shungite (Karelsky Region, Russia): Carbon content 94 %, density 2.25 – 2.84 g/cm^3 , porosity 0.5 – 5 %.

Birch activated carbon BAU-A: Grains of black color, adsorption activity on iodine 60 %, total volume on water - no less than 1.6 cm^3/g , bulk density - no more than 240 g/dm^3 , mass fraction of ash - no more than 6.0 %, mass fraction of moisture - no more than 10 %.

Immobilization was performed according to the following procedure: 10g of each carrier was placed in 500 mL flask containing 200 mL Makintosh medium and 5 mL mixed culture of *Acidithiobacillus* sp.13Zn and *L. ferriphilum* CC in logarithmic phase of the growth. The flasks were shaken at 180 rpm on an orbital shaker at 35°C. In case of vessels stirring was performed through blowing. Ferrous iron oxidation by immobilized cells was monitoring by measuring its concentration. When ferrous iron was oxidized completely, the media were replaced with 200 mL fresh media. The procedure was continued several times until ferrous iron oxidation rate was stable.

The number of bacterial cells was determined using Thoma Chamber and the method of the most probable number (MPN). Obtained results were compared with standard McCrady's Table (Gerhardt et al., 1981). The number of adhered cells was determined as the difference between the number of inoculated cells and the cells remaining in the medium after immobilization.

2.3. Chemical and biogenic ferric iron (Fe^{3+}) solutions

As chemical ferric iron, $Fe_2(SO_4)_3$ salt solution was used. Biogenic ferric iron was obtained by the oxidation of ferrous iron ($Fe(II)$) by the biomass of newly isolated thermotolerant *Acidithiobacillus* sp. 13Zn and *L. ferriphilum* immobilized on zeolite and shungite.

2.4. Chemical oxidation of pyrite and chalcopyrite

Pyrite containing 43.8% Fe, 49% S and chalcopyrite containing 30.2% Cu, 29.7% Fe, 38% S from Shamlugh ore deposit (Armenia) were used. The minerals were ground to a size fraction of between 45-65 μm and sterilized at 112°C for 20 min.

Chemical oxidation of pyrite and chalcopyrite was carried out in 250 mL Erlenmeyer flasks containing 100 mL of Mackintosh medium, 5 and 10% of minerals and chemical and biogenic Fe^{3+} in concentration of 5 g/L. The experiments were performed under shaking conditions on rotary shaker (180 rpm) at 40°C. The rate of chemical oxidation was evaluated by either the increase of ferrous iron ($Fe(II)$) or decrease of ferric iron ($Fe(III)$) in the leaching solution. The amount of extracted iron from pyrite was calculated as the difference between total iron and added initial ferric iron concentrations (oxidant). Sampling was performed with an hour interval and ferric and ferrous ions were analyzed. The amount of copper and total iron in the leaching solution was determined by atomic absorption spectrometer AAS 1N (Carl Zeiss, Germany). Ferrous (Fe^{2+}) and ferric ions (Fe^{3+}) were determined complexometrically by titration with EDTA (Karavayko et al., 1989). The intensity of chemical oxidation was evaluated by the increase of $Fe(II)$ ions concentration in the leaching solution. The amount of extracted iron from pyrite was calculated as a difference between total iron and added initial ferric iron concentration.

2.5. Bioleaching of pyrite and chalcopyrite treated by ferric iron

For bioleaching of treated and untreated pyrite the association of iron oxidizing bacteria *Acidithiobacillus* sp. 13Zn and *L. ferriphilum* CC was used. Bioleaching of chalcopyrite was carried out by

the association of *Acidithiobacillus* sp. 13Zn, *L. ferriphilum* CC and sulfur oxidizing bacteria *At. albertensis* SO-2. The bioleaching experiments were carried out in 250 mL flasks in periodic mode of cultivation on a shaker (180 rpm) at 35°C.

The intensity of bioleaching of minerals was evaluated by the increase of copper and ferrous ions concentrations in the leaching solution. Sampling was performed with a 72 hour intervals and copper, total iron, ferric $Fe(III)$ and ferrous ($Fe(II)$) ions were analyzed. Ferric $Fe(III)$ and ferrous ($Fe(II)$) ions were determined complexometrically by EDTA. Total iron and copper were determined by atomic absorption spectrometer AAS 1N (Germany). The experiments were performed in triplicate. The data obtained were analyzed statistically by Excel.

3. Results and discussion

3.1. Immobilization of microorganisms

The studies have shown that the iron oxidation rate by the immobilized cells of *Acidithiobacillus* sp. 13Zn on zeolite, carbon and shungite in comparison with free-living cells increases about 2.3, 2 and 1.8 times, respectively. The most suitable carrier for *L. ferriphilum* CC is carbon followed by natural zeolite and shungite (Table 1).

Differences for carrier preference between *Acidithiobacillus* sp. 13Zn and *L. ferriphilum* can be explained by chemical composition of EPS produced by bacteria that mediate their adhesion on mineral and carrier surface (Harneit et al., 2006; Sand and Gehrke, 2006) as well as surface properties of carriers (Absolom et al., 1983).

To obtain biogenic ferric iron, the association of *Acidithiobacillus* sp.13Zn and *L. ferriphilum* CC immobilized on zeolite or shungite was used. Immobilization process was carried out at 37°C in a stirring mode for 10 - 15 days to achieve the maximum activity of iron oxidation (Fig. 1).

3.2. Chemical oxidation of pyrite and chalcopyrite

For chemical oxidation of pyrite, ferric sulfate ($(Fe_2(SO_4)_3 \times 9H_2O)$ salt solution and biogenic ferric iron obtained by mixed culture of *Acidithiobacillus* sp. 13Zn and *L. ferriphilum* immobilized on zeolite and shungite, were used. The initial concentration of ferric iron ($Fe(III)$) in both solutions was 5 g/L.

Table 1. Oxidation of ferrous iron by free-living and immobilized cells of *Acidithiobacillus* sp.13Zn and *L. ferriphilum* CC

Cells	<i>Acidithiobacillus</i> sp. 13Zn			<i>L. ferriphilum</i> CC		
	Oxidized Fe^{2+} , g/L in 48 h	Oxidation rate, g/L h	Adhered cell number, cells/mL	Oxidized Fe^{2+} , g/L in 48 h	Oxidation rate, g/L h	Adhered cell number, cells/mL
Free-living cells	1.8	0.038	-	0.62	0.013	
Cells Zeolite	4.5	0.09	6.2×10^7	1.34	0.028	1.2×10^6
Cells immobilized on shungite	3.4	0.07	5.26×10^7	0.86	0.018	2.6×10^6
Cells immobilized on activated carbon	3.6	0.075	5.8×10^6	2.42	0.05	5.0×10^6



Fig. 1. Immobilization of *Acidithiobacillus* sp. 13 Zn and *L. ferriphilum* CC on zeolite (37°C, stirring through blowing)

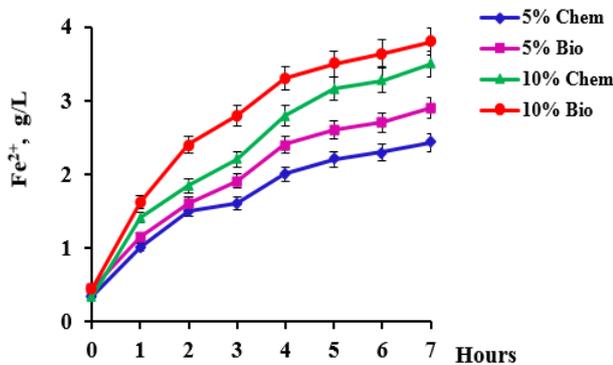


Fig. 2. Generation of Fe^{2+} during chemical oxidation of pyrite by solution of $Fe_2(SO_4)_3$ and biogenic ferric iron at 5 and 10% PD (initial concentration of Fe^{3+} - 5 g/L, pH 1.8, 40°C)

Chemical oxidation of pyrite was carried out under shaking conditions (180 rpm) at 5 and 10% pulp

density (PD) and 40°C. The intensity of chemical leaching was evaluated by the increase of Fe(II) ions concentration in the leaching solution (Fig. 2). As it can be seen from Fig. 3, the amount of iron leached from pyrite in the case of biogenic ferric iron is larger than in the case of $Fe_2(SO_4)_3$ solution (chemical solution). Besides, this regularity was observed at both 5 and 10% of the tested pulp densities (Figs. 3 a, b).

It should be noted that rapid extraction of iron was observed in the first 4 h, then the process was gradually slowed down. The maximum rate of iron extraction (2.4 - 2.5 g/L h) by chemical oxidation of pyrite was observed in the first hour and gradually declined to 0.2 - 0.3 g /L h for 6 hrs (Fig. 4). Direct dependence of iron extraction rate on the pulp density was also observed. Thus, the maximum rate of iron extraction at 10% pulp density was 2.52 g/L h and 1.6 times higher than that observed at 5% pulp density (1.4 g/L h) (Fig. 4).

The studies of several authors (Fomchenko and Biryukov, 2009; Muravev et al., 2009) have revealed that culture liquid containing ferric iron obtained by biooxidation of ferrous iron is a more active oxidizer for pyrite than the solution of $Fe_2(SO_4)_3 \cdot 9H_2O$. It is also shown that preliminary chemical leaching of pyrite concentrates by culture liquid containing ferric iron compounds increases the rate and depth of the subsequent biooxidation of pyrite. The results of the chemical oxidation of chalcopyrite are presented in Fig. 5 and Table 2.

According to the data presented, similar to pyrite the leaching of chalcopyrite by Fe^{3+} of bacterial origin as compared to chemical solution of Fe^{3+} proceeds more effectively regardless of pulp density. Thus, during leaching of chalcopyrite with Fe^{3+} of bacterial origin 2-3 times more copper passes into the medium than in the case of chemical solution of Fe^{3+} at 5% pulp density. The amount of extracted iron during leaching of chalcopyrite by Fe^{3+} of bacterial origin was twice higher than in the case of chemical solution of Fe^{3+} (Table 2, Fig. 5a).

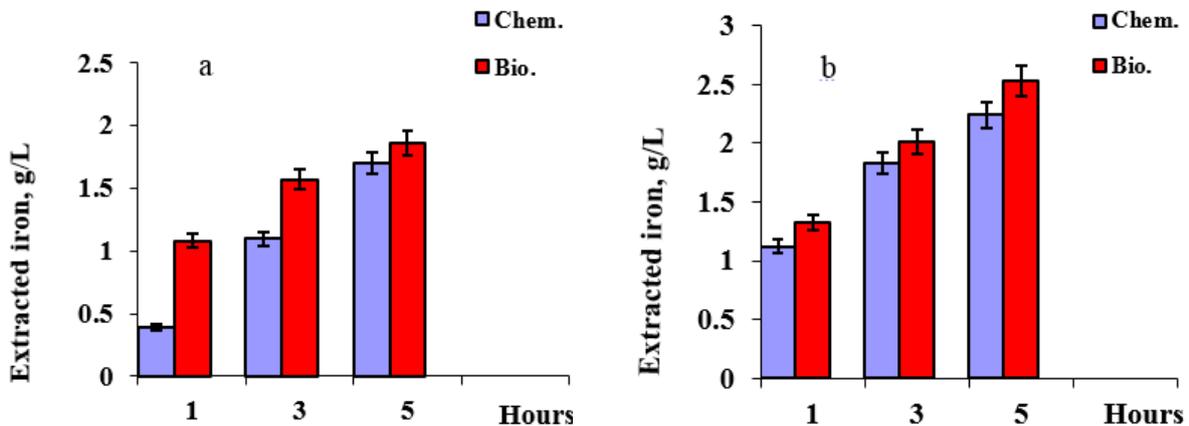


Fig. 3. Extraction of iron during chemical oxidation of pyrite at 5 % (a) and 10 % (b) pulp densities (PD) (initial concentration of Fe^{3+} - 5 g/L, pH 1.8, 40°C)

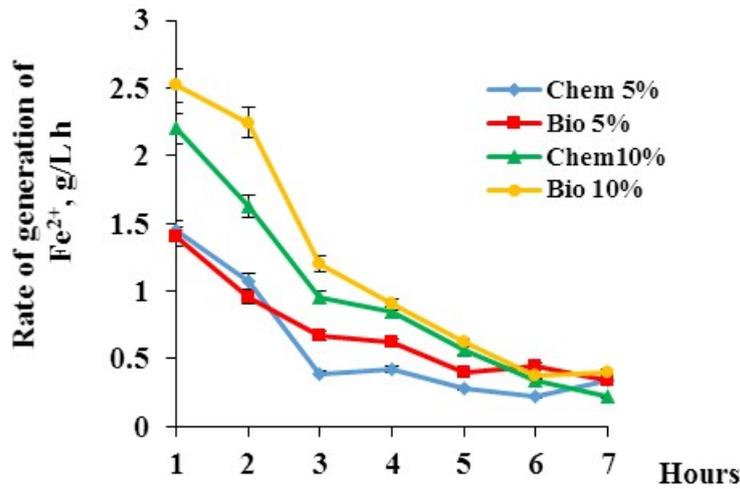


Fig. 4. Rate of generation of Fe²⁺ during chemical oxidation of pyrite at different pulp densities (initial concentration of Fe³⁺ - 5 g/L, pH 1.8, 40°C)

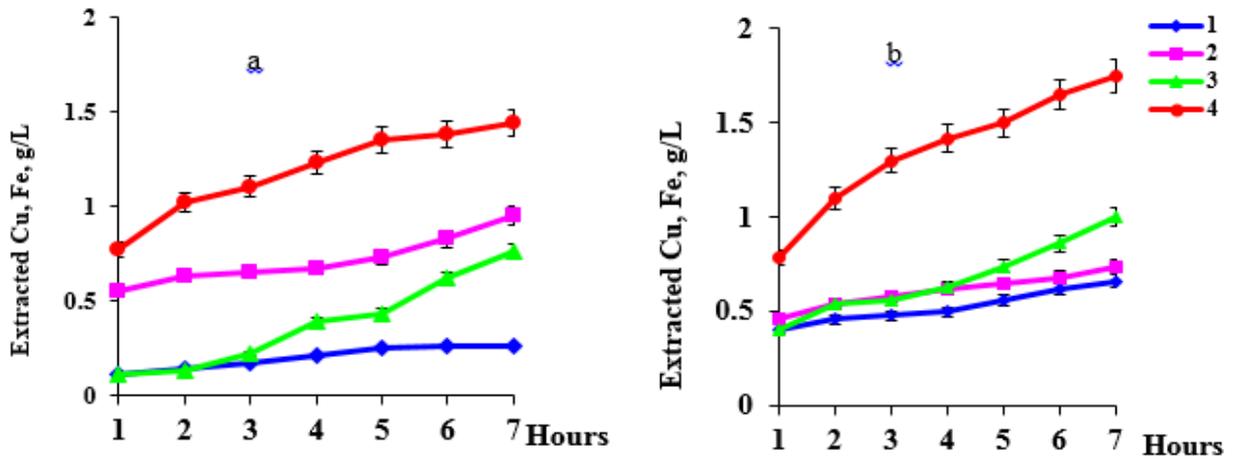


Fig. 5. Extraction of copper (1, 2) and iron (3, 4) during leaching of chalcopyrite by solution of Fe₂(SO₄)₃ (1, 3) and biogenic ferric iron (2, 4) at 5% (a) and 10% (b) PD (initial concentration of Fe³⁺ 5 g/L, pH 1.8, 40°C)

Table 2. Extraction of copper and iron during chemical oxidation of chalcopyrite

PD, %		Extraction of iron for 4h		Extraction of copper for 4h	
		g/L	%	g/L	%
5	Chem.	0.780	5.3	0.210	1.4
5	Bio.	1.232	8.3	0.650	4.3
10	Chem.	0.628	2.1	0.560	1.8
10	Bio.	1.434	4.8	0.670	2.0

The same pattern was observed at 10% pulp density, though the amount of leached copper was only 1.2 times higher. As a result, within 4 hours 4.3 and 2.0% of copper was leached from chalcopyrite when Fe³⁺ iron of bacterial origin was used and 1.4 and 1.8% in the case of chemical solution of Fe³⁺, respectively, at 5 and 10% pulp density (Fig. 5b, Table 2).

Oxidation of chalcopyrite by ferric iron can be presented by the following reaction:



Proceeding from this reaction some authors have concluded that “passivation” of chalcopyrite is

due to the formation of a layer of sulphur on the surface of mineral (Munoz, 1979). Another researchers have proposed that the passivating layer consists of jarosite that precipitate on the surface of the chalcopyrite particles thus preventing its further oxidation (Ahmadi et al., 2012; Cordoba, 2008; Yu et al., 2011). They have studied the effect of iron ions on the dissolution of chalcopyrite at low and high potential, and found that although Fe (III) ions are responsible for the oxidation of chalcopyrite, Fe (II) has an important role in controlling the formation and precipitation of the jarosite.

According to the another researchers’ data (Gusakov et al., 2011; Gusakov, 2012), the solutions of ferric sulfate (Fe₂(SO₄)₃) and biogenic ferric iron of

bacterial origin significantly differ by their ionic composition. It is revealed that unlike the chemical solution, in biogenic one ferric iron (Fe(III)) is connected with high-molecular organic compounds such as polysaccharides, able to form complexes with metal cations, including iron.

It is also shown that during the leaching of sulfide minerals by chemical solution ferric iron precipitates and iron concentration in leaching solution sharply reduces, which results in the decrease of its oxidation activity. In case of ferric iron of bacterial origin precipitation of Fe(III) does not occur, which results in the maintenance of oxidation activity at a high level.

3.3. Bioleaching of pyrite and chalcopyrite after treatment with biogenic ferric iron

A comparative study of microbiological leaching of untreated and treated with biogenic Fe³⁺ pyrite and chalcopyrite was carried out. For leaching of pyrite the mixed culture of *Acidithiobacillus* sp.13Zn with *L. ferriphilum* CC was used, for chalcopyrite – the mixed cultures of *Acidithiobacillus* sp.13Zn with *L. ferriphilum* CC and *At. albertensis* SO-2 were used. For bioleaching of pyrite the

association of iron oxidizing bacteria *Acidithiobacillus* sp. 13Zn and *L. ferriphilum* CC was used. Bioleaching of chalcopyrite was carried out by the association of *Acidithiobacillus* sp. 13Zn, *L. ferriphilum* CC and sulfur oxidizing bacteria *A. albertensis* SO-2 taking into consideration other author's research (Rawlings and Johnson, 2007; Watling et al., 2013; Zhou et al., 2009) as well as our previous research (Vardanyan et al., 2016). The results of bioleaching of pyrite are shown in Fig. 6.

As follows from the presented data, the amount of leached iron from pyrite treated with biogenic Fe³⁺ is about 1.3 times more than that of the untreated mineral. The extent of total iron extraction by the used mixed culture was 38.9% and 31.9% from both treated and untreated pyrite, respectively (Table 3). In general, after the chemical oxidation and subsequent bacterial leaching about 50% of iron from pyrite is extracted. The obtained results are in agreement with the research of other authors (Fomchenko and Biryukov, 2009). According to these studies, preliminary chemical leaching of pyrite concentrates by culture liquid, containing ferric iron compounds, increases the rate and depth of the subsequent biooxidation of sulfide mineral by moderate thermophilic bacteria.

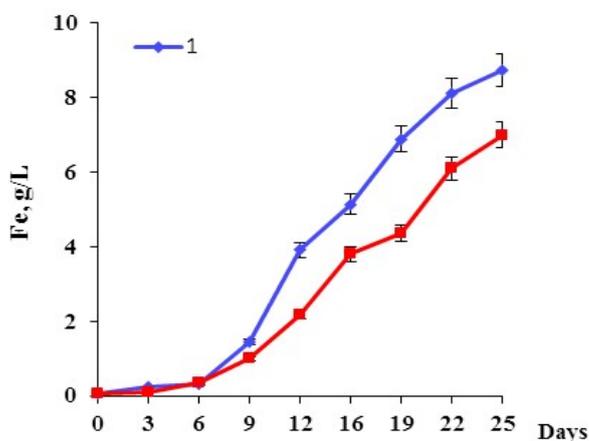


Fig. 6. Leaching of iron from treated (1) and untreated with biogenic Fe(III) (2) pyrite by the mixed culture of *Acidithiobacillus* sp.13Zn with *L. ferriphilum* CC (FeS₂ 5%, pH 1.8, 35°C)

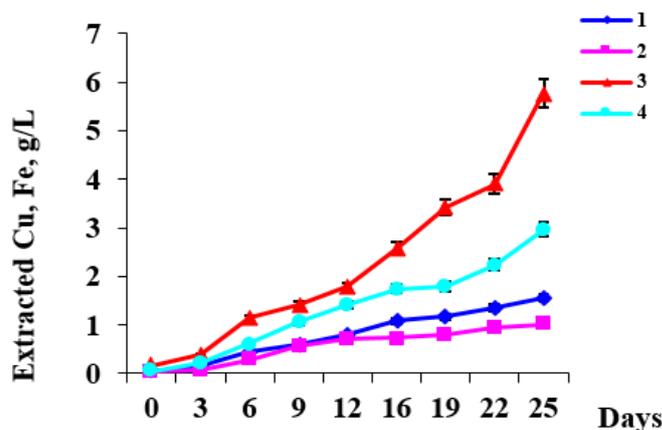


Fig 7. Leaching of copper (1, 2) and iron (3, 4) from treated (1, 3) and untreated with biogenic Fe(III) (2, 4) chalcopyrite by the mixed cultures of *Acidithiobacillus* sp.13Zn, *L. ferriphilum* CC and *At. albertensis* SO-2 (CuFeS₂ 5%, pH 1.8, 35°C)

Table 3. Extraction of copper and iron from untreated and treated pyrite and chalcopyrite by the mixed culture of *Acidithiobacillus* sp.13Zn, *L. ferriphilum* CC and *At. albertensis* SO-2 (for 25 days)

Mineral	Leached Cu, g/L	Extraction Cu, %	Leached Fe, g/L	Extraction Fe, %	Initial/final pH	Final ORP, mV
FeS ₂ , untreated	-	-	7.0	31.9	1.8 / 1.6	780
FeS ₂ , treated with Fe(III)	-	-	8.74	39.9	1.8 / 1.35	830
CuFeS ₂ , untreated	1.01	6.7	2.97	19.9	1.8 / 1.8	450
CuFeS ₂ , treated with Fe(III)	1.56	10.4	5.78	38.9	1.8 / 1.6	625

The results of bacterial leaching of chalcopyrite without pretreatment and after treatment with biogenic solution Fe(III) are shown in Fig. 7. As follows from the presented data, unlike untreated chalcopyrite, the treated one was leached more intensively. Thus, for 25 days of the experiment, from chalcopyrite treated with biogenic iron about 1.5 times more copper (1.56 g/L) was leached by the mixed cultures of *Acidithiobacillus* sp.13Zn with *L. ferriphilum* CC and *At. albertensis* SO-2 (Fig. 7) than that from the untreated mineral (1.01 g/L). In the same period the amount of leached iron was 5.8 g/L and 2.97 g/L from treated and untreated chalcopyrite, respectively (Fig. 7).

As a result, for 25 days of bioleaching from treated chalcopyrite 10.4% copper and 38.9% iron were leached (extracted) by the mixed culture of *Acidithiobacillus* sp. 13Zn with *L. ferriphilum* CC and *At. albertensis* SO-2, while from untreated mineral 6.3% and 31.9%, respectively (Table 3).

The intensity of chalcopyrite leaching also proved the changes in pH and ORP of the leaching medium. The more intensively the mineral is leached, the lower the final pH value and ORP of the leaching solution are (Table 3).

4. Conclusions

It can be concluded that mixed culture of *Acidithiobacillus* 13Zn and *L. ferrooxidans* CC immobilized on zeolite and shugite can be prospective for regeneration of oxidant (Fe³⁺) in the bioleaching processes. Results obtained show that during chemical leaching of pyrite and chalcopyrite by ferric iron Fe³⁺ of bacterial origin about twice more copper and iron pass into the medium than in case of chemical solution of Fe³⁺.

Treatment of pyrite and chalcopyrite with Fe(III) solution obtained by the immobilized mixed culture of *Acidithiobacillus* sp. 13Zn and *L. ferriphilum* allows to increase on average 1.5-2 times the extraction of iron and copper from chalcopyrite and iron from pyrite.

Thus, chemical oxidation of pyrite and chalcopyrite by biogenic ferric iron obtained by the immobilized biomass of CM integrated into biohydrometallurgical technology will allow to significantly increase the efficiency of subsequent bioleaching process and the extent of recovery of metals.

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