

# Mixed Moderate Thermophilic Bioleaching of Cu, Mo and Re from Molybdenite Concentrate: Effects of Silver Ion, Medium and Energy Sources

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## ABSTRACT

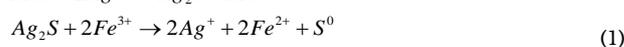
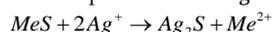
This study evaluates the effects of different additives, such as silver ion, as well as the effects of medium and energy sources on the efficiency of mixed moderate thermophilic bioleaching approach in extraction of Cu, Mo and Re from molybdenite concentrate containing 0.98% Cu, 1.56% Fe, 53.84% Mo, and 0.055% Re. Molybdenite was the major phase of Mo-bearing mineral and chalcopyrite, covellite and pyrite were distinguished as minor phases. Higher copper extraction was obtained in tests with silver additives in all types and quantities rather than tests without silver ion. Kinetic of copper dissolution varied in these experiments and depended on the types and amounts of silver, and other supplemented additives such as ferric ion. There was no clear difference in copper extraction by various culture media and 100% of Cu was dissolved after 30 days of treatment, using 50 mg/L of silver nitrate as additives. In the best condition and without silver additives, at most 60% of copper was extracted even in the presence of energy sources such as sulfur, ferrous and ferric ions. In the most effective test with initial pH 1.57, 50 mg/L silver nitrate, and 50 g/L ferric sulfate, 100% of copper was dissolved in less than a week with highest kinetics rate. Molybdenum and rhenium extraction had the same trends with redox potential graph. By increasing the redox potential to the 550-600mV, molybdenite started to dissolve, and finally, the extracted value of molybdenum and rhenium in the best condition were 2% and 9.53%, respectively.

**Keywords :** *Bioleaching, Nitrogen, Chalcopyrite bioleaching, Mo and Re extraction, Molybdenite concentrate, Silver catalysis*

## 1. Introduction

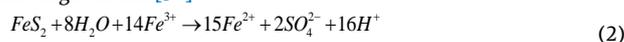
Bioleaching is based on the ability of different microorganisms including bacteria and fungi to transform solid compounds to extractable entities [1-10]. A bioleaching based process as an alternative method of hydrometallurgical processing of minerals for obtaining metals offers a number of advantages compared to the conventional methods. These include low operating costs, minimizing the volume of chemical and/or biological sludge to be handled and its high efficiency in detoxifying effluents [11-15]. Two mechanisms of bacterial action have been suggested: (i) a contact mechanism, wherein the bacterial attack on the mineral surface and its oxidation through enzymatic reactions take place; and (ii) a non-contact mechanism, wherein the bacteria regenerate the oxidizing agent of the mineral by means  $Fe^{2+}$  to  $Fe^{3+}$  oxidation via thiosulfate or polysulfide methods which depends on the type of minerals [16-18]. In fact, with these mechanisms, bacteria play the role of catalyst and increase dissolution rate of sulfide minerals [19]. In the bio-dissolution mechanism, passivating layers such as jarosite and sulfur commonly form on the sulfide mineral surface and consequently hinder the complete dissolution of sulfide minerals during the bioleaching treatment. Several processes have been considered to remove the passivating layer, such as elevated temperature, the use of more efficient sulfur-oxidizing microorganisms, and the addition of catalytic ions. The first two approaches have a potential application on bioleaching by combining high temperatures with the use of thermoacidophilic sulfur-oxidizing archaea [20-23]. Among catalytic ions, silver is of special interest, because it has been proven effective and

selective in enhancing Cu dissolution through the biological oxidation of chalcopyrite [24-26]. The positive catalytic effect of silver ions on the bioleaching of chalcopyrite in shake flasks and column bioreactors has been reported in different studies [24-34]. Silver ions participate in dissolution process according to the following reactions [35]:

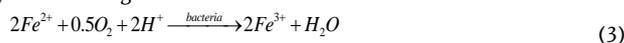


where MeS signifies sulfide minerals. Ferrous ions ( $Fe^{2+}$ ) are re-oxidized to ferric ions ( $Fe^{3+}$ ) in the presence of iron-oxidizing bacteria and complete the dissolution cycle.

The previous studies showed that ferric ions produced during iron-sulfur oxidizing bacterial activity have a great influence on dissolution of sulfide minerals [36, 37]. Therefore, almost all culture media that are used in the biooxidation/bioleaching processes include a source of iron in the form of sulfate ( $FeSO_4 \cdot 7H_2O$ ). However, adding high amounts of iron sulfate to industrial bioleaching systems is commercially impossible. Accordingly, the possibility of replacement of iron sulfate with pyrite has been investigated. Pyrite produces Fe (II) during the following reaction [38]:



Ferrous ions are re-oxidized to ferric ions in the presence of bacteria by the following reaction:



This study evaluates the effects of different additives such as silver ions, as well as the effects of medium and energy sources on the

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efficiency of mixed moderate thermophilic bioleaching approach for extraction of Cu, Mo and Re from molybdenite concentrate produced in the Sarcheshmeh Copper Complex (Iran). Silver-catalyzed bioleaching was evaluated with mixed moderate thermophilic cultures in shake flask. Several independent variables were tested, most importantly the silver ion types (nitrate, sulfate, chloride and pure) and their concentrations, initial pH, types of medium (9K, 0.9K, HP(A), Norris(B), Leathen(C), D1, D2, and E), and energy sources (Fe<sup>2+</sup> and S). In addition, the ability of replacing FeSO<sub>4</sub>·7H<sub>2</sub>O with pyrite was investigated, as well. Removal of Cu was carried out to purify the Mo-concentrate in the first step, and dissolution behaviors of Mo and Re were comprehensively investigated in the mixed moderate bioleaching experiments.

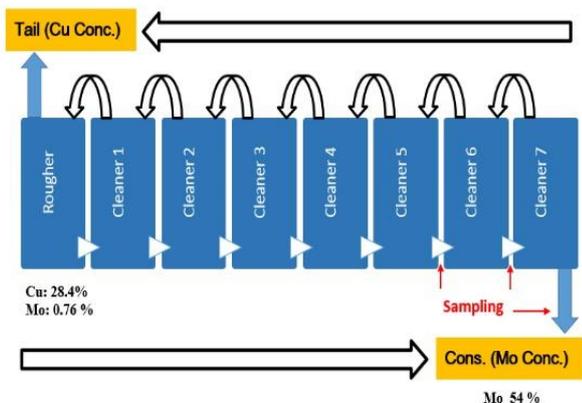
**2. Materials and Methods**

**2.1. Molybdenite concentrate and characterization studies**

A representative sample of molybdenite concentrate from the Sarcheshmeh Copper Complex of Iran with a size distribution of 90 wt. % less than 38 μm was used for bioleaching tests. The d<sub>50</sub> of the sample was determined to be 33 μm using cyclosizer equipment. Molybdenite was separated from copper bearing minerals, using a rougher and sequential seven flotation cells as cleaner in four series bank with capacity of approximately 20 t/d (final concentrate). The chemical analysis of 5<sup>th</sup>, 6<sup>th</sup> and final concentrate are presented in Table 1. As shown, there is 0.98% Cu in 5<sup>th</sup> cleaner sample which shows the highest amount of the copper content among the three final cleaners. Therefore, the representative sample obtained from 5<sup>th</sup> cleaner was used in the bioleaching tests. It should be noted that molybdenite is the preferred host mineral for rhenium. Table 1 shows the rhenium content of molybdenite concentrates in different stages of flotation circuit. The amount of rhenium in molybdenite concentrate produced by the Sarcheshmeh Copper Complex is relatively high [39, 40]. The schematic flotation process and sampling places in the circuit is illustrated in Fig. 1.

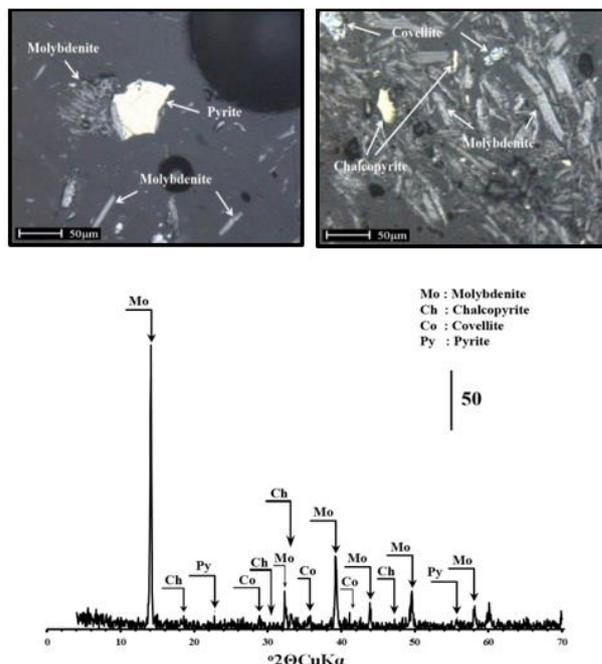
**Table 1.** Mineralogical (volume %) and chemical (weight %) analyses of molybdenite concentrate in three cleaners.

	Assay	5 <sup>th</sup> Cleaner	6 <sup>th</sup> Cleaner	7 <sup>th</sup> Cleaner
<b>Mineralogical</b>	CuS (Covellite)	0.3	0.3	0.0
	CuFeS <sub>2</sub> (Chalcopyrite)	2.1	1.8	1.3
	FeS <sub>2</sub> (Pyrite)	2	1.9	1.7
	MoS <sub>2</sub> (Molybdenite)	89.8	90.9	91.5
	Total	94.2	94.9	94.5
<b>Chemical</b>	Cu (%)	0.98	0.91	0.44
	Fe (%)	1.56	1.43	1.17
	Mo (%)	53.84	54.00	54.88
	Re (ppm)	550	650	700
	Total (%)	56.38	56.34	56.49



**Fig. 1.** The schematic flotation process and sampling places in the circuit.

Semi quantitative X-ray diffraction (SQXRD) technique was used to define the main and trace minerals in the sample. X-ray powder diffraction patterns were obtained using a Siemens D-500 diffractometer with Ni-filtered Cu–Kα radiation, and a goniometer speed of 1° 2θ/min. The diffraction profiles with a 0.01 precision of d-spacing measurements were conducted from 5° to 70° (2θ). The results of the mineralogical studies revealed that molybdenite is the major phase of the Mo bearing minerals. Also, mineralogical study of the sample showed molybdenite (MoS<sub>2</sub>) as the main mineral phase, chalcopyrite (CuFeS<sub>2</sub>) and covellite (CuS) as copper bearing minerals, and pyrite (FeS<sub>2</sub>) as the minor phase which are presented in Fig. 2. As it can be seen, chalcopyrite and pyrite can be distinguished easily in the molybdenite concentrate as unwanted minerals. These minerals are liberated from molybdenite in this size fraction, but in some cases, Cu bearing minerals and also pyrite are interlocked with molybdenite.



**Fig. 2.** Mineralogical observations and XRD graph of Molybdenite concentrate with chalcopyrite, covellite and pyrite minerals as impurities.

SEM-EDAX analysis was carried out for better detection of major and minor minerals in the molybdenite concentrate. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray Analyses (EDAX) were conducted on polished sections and grain samples. These samples were gold coated to obtain electron conductive surfaces. The examination of internal structure of the samples, distribution of mineral phases, and the choice of areas for EDAX-analyses were performed in a scanning electron microscope using the back scattered electrons (BSE) technique. The results confirmed the mineralogical studies (data and figures are not provided).

Dissolved metals were analyzed by inductively-coupled plasma emission spectrometry (ICP-ES) (Varian 735-ES, Agilent Technologies, and Ultima 2C, HORIBA Jobin Yvon). Scanning electron microscopy (SEM) and energy dispersive X-ray (EDAX) analyses (Philips XL 30 SEM) were conducted on solid residues from the bioleaching experiments. The samples were air dried followed by mounting on stubs and Au coating before examination. Mineralogical characterization of the sample was also performed using an optical polarizing microscope (Leitz, ORTHOLUX II Pol-BK).

**2.2. Microorganisms and media**

A mixed moderate thermophilic culture (45°C) was originally received from Mintek (Johannesburg, South Africa). According to the Mintek’s report, this culture was mainly composed of sulfur-oxidizing bacteria with a lower proportion of iron-oxidizing microorganisms. This

culture contained *Acidithiobacillus caldus*, *Leptospirillum ferriphilum*, *Sulfobacillus* sp., and *Ferroplasma* sp. [41, 42].

For this study, the cultures were initially grown with 10% chalcopyrite concentrate as an energy source. To establish resistance of microorganisms to molybdenum, the microorganisms were grown on molybdenite concentrate in 9K medium containing (per liter of distilled water) 3.0 g (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.5 g MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.1 g KCl, 0.5 g K<sub>2</sub>HPO<sub>4</sub>, 0.01 g Ca(NO<sub>3</sub>)<sub>2</sub>, 44.22 g FeSO<sub>4</sub>·7H<sub>2</sub>O and 10g sulfur; the pH was adjusted to 1.8 with H<sub>2</sub>SO<sub>4</sub> [43]. The cultures were gradually adapted over a three months period to grow on molybdenite through stepwise increase of up to 10% solid concentration.

Different mineral salt media formulations were used in this study (Table 2). The formulations have been pooled and modified from various literature sources, including 9K from Silverman and Lundgren (1959) and the others modified from McCready et al. (1986), Lizama and Suzuki (1988), Gómez et al. (1996), and Massinaie et al. (2006). Descriptions of additional media formulations can be found, for example, in González-Toril et al. (2006) [43-48]. The formulation E was specifically designed for the bioleaching tests, with phosphate supplied as H<sub>3</sub>PO<sub>4</sub> instead of K-phosphate. Phosphoric acid is relatively inexpensive as a bulk chemical and its use may help to reduce K-jarosite precipitation.

Table 2. Different mineral salt media formulations.

Mineral salts	Concentration (g/l)								
	9K	0.9K	A	B	C	D1	D2	E	
(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	3.0	0.3	0.2	0.4	0.2	0.06	0.01	3.0	
MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5	0.05	0.2	0.4	0.2	0.06	0.01	--	
K <sub>2</sub> HPO <sub>4</sub>	0.5	0.05	0.27	0.1	0.2	0.02	0.01	--	
KCl	0.1	0.01	0.2	--	--	0.02	0.01	0.1	
Ca(NO <sub>3</sub> ) <sub>2</sub> ·H <sub>2</sub> O	0.01	0.001	--	--	--	--	--	--	
H <sub>3</sub> PO <sub>4</sub>	--	--	--	--	--	--	--	0.5	

### 2.3. Bioleaching experiments

The experiments were designed to address the amenability of the Cu-

bearing molybdenite to the bioleaching process to minimize the Cu content of concentrate and enhance the extraction of molybdenum and rhenium. Specific formulations of nutrient solutions [26], silver catalytic effect, and the use of moderate thermophiles at elevated temperatures were tested in the research. Bioleaching experiments were performed in 250 mL Erlenmeyer flasks incubated at a constant temperature of 45°C in an orbital shaker agitating at 150 rpm for a month. Each flask contained 85mL of different media and their corresponding additives such as sulfur, ferrous sulfate, ferric sulfate, pyrite and silver ion, according to each case. Also, 15 mL of an active microbial culture, previously adapted was added to all tests. Abiotic control tests received 15mL of formaldehyde 10% V/V as biocide reagent instead of the inoculum. Solid concentration was 6% in all bioleaching experiments. Also, duplicate tests (randomly) were performed to check the precision of the experiments. The redox potential was measured using a Pt combination redox electrode with an Ag/AgCl reference electrode. pH was monitored using a gel-filled combination pH probe with an Ag/AgCl reference electrode. Water loss during evaporation was compensated by adding distilled water. At intervals, redox potential and pH were measured directly in the culture flasks and the suspensions were sampled for microscopic cell counts after ~1 min of settling. Microscopic cell counts were determined using a Neubauer chamber with a depth of 1/50mm and area of 1/400 mm<sup>2</sup>. For analysis of metals, the suspended solids were allowed to settle for 30 min before sampling the solution phase. Samples were filtered (0.45 μm) for analysis of dissolved copper, molybdenum, rhenium and total dissolved iron (total Fe<sub>d</sub>). Sample volumes were replaced with sterile mineral salt solution. The final results were corrected for the dilution effect caused by partial replacement of the nutrient solution. The conditions of moderate thermophilic bioleaching experiments are presented in Table 3. These conditions were selected according to the preliminary chemical and biochemical leaching tests (data are not shown).

Table 3. The condition of moderate thermophilic bioleaching experiments.

	Test No.	Medium	P.D (%)	IP (%)	pH	Silver nitrate (mg/L)	Sulfur (g/L)	Ferrous sulfate (g/L)	Ferric sulfate (g/L)	Pyrite (g/L)	Silver sulfate (mg/L)	Silver chloride (mg/L)	Pure Ag (mg/L)
Group 1	1	9k	6	15	1.9	50	0	0	0	0	0	0	0
	2	D1	6	15	1.9	50	0	0	0	0	0	0	0
	3	D2	6	15	1.9	50	0	0	0	0	0	0	0
	4	HP (A)	6	15	1.9	50	0	0	0	0	0	0	0
	5	Norris (B)	6	15	1.9	50	0	0	0	0	0	0	0
	6	Leathen (C)	6	15	1.9	50	0	0	0	0	0	0	0
	7	0.9k	6	15	1.9	50	0	0	0	0	0	0	0
	8	E	6	15	1.9	50	0	0	0	0	0	0	0
Group 2	9	9k	6	15	1.9	0	10	50	0	0	0	0	0
	10	9k	6	15	1.9	0	5	25	0	0	0	0	0
	11	9k	6	15	1.64	0	0	0	50	0	0	0	0
	12	9k	6	15	1.82	0	0	0	25	0	0	0	0
	13	9k	6	15	1.67	0	10	50	0	0	0	0	0
	14	9k	6	15	1.85	0	5	25	25	0	0	0	0
	15	9k	6	15	1.9	0	0	0	0	5	0	0	0
	16	9k	6	15	1.9	0	0	0	0	10	0	0	0
	17	9k	6	0	1.9	0	0	0	0	0	0	0	0
	18	9k	6	0	1.65	0	0	0	50	0	0	0	0
	19	9k	6	0	1.86	0	0	0	25	0	0	0	0
Group 3	20	9k	6	15	1.9	0	0	0	0	0	40	0	0
	21	9k	6	15	1.9	0	0	0	0	0	80	0	0
	22	9k	6	15	1.9	0	0	0	0	0	0	40	0
	23	9k	6	15	1.9	0	0	0	0	0	0	80	0
	24	9k	6	15	1.9	0	0	0	0	0	0	0	40
	25	9k	6	15	1.9	0	0	0	0	0	0	0	80
G-4	26	9k	6	15	1.57	50	0	0	50	0	0	0	0
	27	9k	6	15	1.4	0	10	50	50	0	0	0	0
	28	9k	6	15	1.75	0	0	0	25	10	0	0	0

- P.D: Pulp density, IP: Inoculation percent

### 3. Results and Discussion

#### 3.1. pH and ORP

A total of 28 bioleaching tests were conducted according to the specific condition presented in Table 3. The corresponding changes in pH and redox potential are shown in Fig. 3 (A-F). Most of the bioleaching experiments were started with initial pH 1.9 except tests 11 to 14, 18, 19, and 26 to 28. In these tests, the pH decreased as a result of the Fe(III) addition. As shown, pH of the solutions were decreased slightly by the end of the process. The pH of tests 9 and 10 with just sulfur and ferrous sulfate as additives, were decreased more sharply than that of other experiments. It is obvious that pH changed in the first two weeks of treatment, and after that it remained constant with a slight fluctuation by the end of the process. Also, the types of medium had no influential effects on the acid producing mechanism.

The redox potential changed in different trends compare to the pH graphs. In some experiments (tests 9, 10, 13, 14, and 27), the redox potential was drastically increased in first 15 days of treatment because of ferrous iron oxidation by moderate thermophilic microorganisms to

600mV and after that, leveled off. In tests with ferric sulfate as additive, the redox potential was decreased in first two days and after that increased with low gradient to the end of the process. Pyrite addition in some tests (tests 15, 16 and 28) had a little effect on the bioleaching efficiency and oxidation process in which the redox potential increased to 450 mV in optimum condition. Furthermore, by adding silver component to the solution in all types and quantities, the redox potential dramatically decreased only in 2-3 days to less than 400mV and leveled off at the end of the process. The silver ion has two different effects in the bioleaching mechanism. In this situation, electron transition is easily carried out and also the bacterial population decreases because of the toxicity effect of the silver ion on the bacterial activities and metabolism. By comparing the obtained results from pH, redox potential graphs and bacterial population, silver ion has more detrimental effect on the moderate thermophilic microorganism compared to the mesophilic bacteria [26]. Also, in abiotic experiments (tests 17 to 19), pH of the solution were reduced and redox potential were increased slightly which could be because of the bacterial contamination.

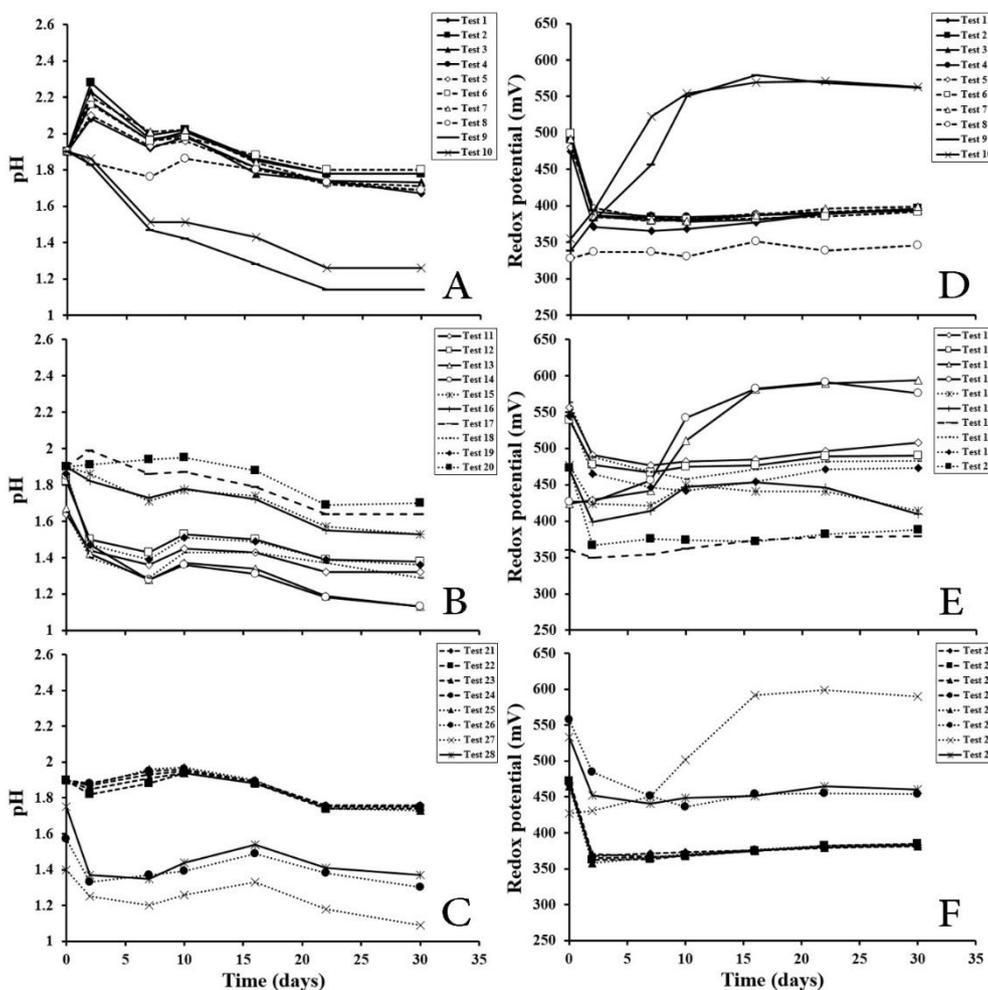


Fig. 3. Changes of pH and redox potential over time in the moderate thermophilic bioleaching tests.

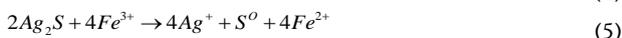
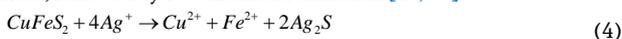
#### 3.2. Copper dissolution

Copper extraction in the moderate thermophilic bioleaching processes is demonstrated in Fig. 4 (A-C). Moderate thermophilic bioleaching eliminated copper from molybdenite concentrate with different recoveries based on the test conditions and the additives types. As it can be seen, copper extraction was enhanced by the end of the process. The maximum copper dissolution was achieved in the tests with

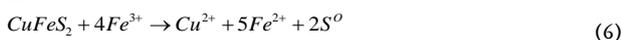
silver additives in all types and quantities (in optimum range). But the kinetics of copper extraction varied in these experiments based on the types and amounts of silver ions, and other supplemented additives such as ferric ion added in the solution. There was no clear difference in copper extraction between the first seven tests (tests 1-7) with various culture media, using 50mg/L of silver nitrate as additives. In these conditions, 100% of Cu was dissolved in after 30 days of treatment. This confirmed the previous results in which different types of medium had no meaningful effects on the rate of copper dissolution in the mesophilic

conditions [26]. Also, a low copper recovery was observed in the test 8 with medium E (3 g/l  $(\text{NH}_4)_2\text{SO}_4$ , 0.01 g/l KCl, 0.5 g/l concentration of  $\text{H}_3\text{PO}_4$ ) which might be due to the neutralization effect of silver or formation of inactive silver components in the solution in presence of phosphoric acid or chloride ion. In the best condition and without silver additives, 60% of copper was eliminated even in presence of sulfur, ferrous and ferric ions in the processes (tests 9, 10, 13, 14, and 27). In test 26 (initial pH 1.57, 50mg/L silver nitrate, and 50g/L ferric sulfate), 100% of copper was extracted in less than a week with highest kinetics rate.

As already mentioned, the literature survey on the silver catalyst mechanism in the bioleaching process show, among the catalytic ions, silver is of special interest because it has been proven to be effective and selective in enhancing Cu dissolution through the biological oxidation of chalcopyrite [24-26]. The positive catalytic effect of silver ions on the bacterial leaching of chalcopyrite has been demonstrated in shake flasks and column bioreactors [24-34]. The mechanism of silver catalysis, which was proposed by Miller et al. (1981) and Miller and Portillo (1981), is based on the reaction of  $\text{Ag}^+$  to form Ag-sulfide on chalcopyrite surface, followed by  $\text{Fe}^{3+}$ -mediated oxidation [49, 50]:



Ideally, silver recirculates between the solution and solid phase reactions and the overall sum of the chalcopyrite reaction yields elemental S:



Elemental Ag can also form during the process [51, 52].

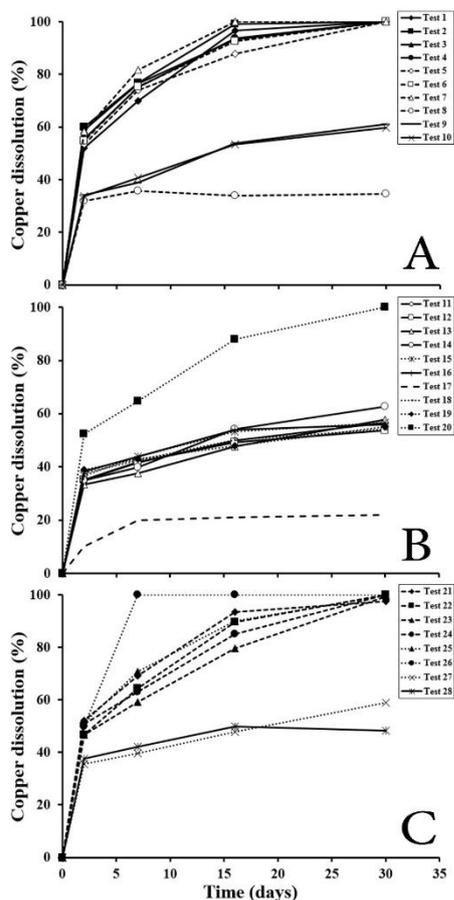
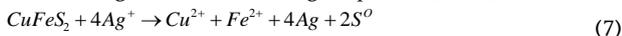


Fig. 4. Copper recovery in the moderate thermophilic bioleaching process.

The formation of  $\text{Ag}_2\text{S}$ -Ag film on chalcopyrite surface modifies the sulfur layer and increases its conductivity and accelerates the electron transfer through the oxidant. The presence of  $\text{Fe}^{3+}$  and its regeneration

from  $\text{Fe}^{2+}$  emphasize the critical role of iron-oxidizing bacteria such as *Acidithiobacillus ferrooxidans* in the silver-catalyzed bioleaching of chalcopyrite [24, 29-31 and 53]. The rate-controlling step during the process may be the reaction of silver ion with chalcopyrite, but this is subject to speculation. The mechanism of the process proposed by Hiroyoshi et al. (2002) applies to lower potentials and involves the formation of  $\text{Ag}_2\text{S}$  and  $\text{Cu}_2\text{S}$  by the prior reduction of chalcopyrite with ferrous ion and evolution of  $\text{H}_2\text{S}$  [54]. In this case, the silver sulfide is not formed through reaction (1) but by the reaction of silver ions with  $\text{H}_2\text{S}$ . The principal pursuit of the silver-catalyzed chalcopyrite process is to create the optimal conditions for the formation of a stable galvanic couple interaction between  $\text{CuFeS}_2$  and  $\text{Ag}_2\text{S}$ . In this way, the effectiveness of the catalysis relies on silver availability (applicable to both chemical and biological processes) and silver toxicity (applicable only to the biological process). Silver is toxic to all microorganisms; iron oxidation by *A. ferrooxidans* is inhibited by 0.1 ppm of  $\text{Ag}^+$  [55]. Silver availability in solution is mainly affected by formation of precipitates during the bioleaching and mineralogical composition of the ore. Ferric ion in the leach solution is subjected to hydrolysis leading to Fe(III)-hydroxysulfates. In sulfate-rich solutions these precipitate either as schwertmannite or, in presence of monovalent cations, as jarosites. Silver can precipitate as argentojarosite with an ideal formula of  $\text{AgFe}_3(\text{SO}_4)_2(\text{OH})_6$ . Argentojarosite lacks the catalytic effect that  $\text{Ag}^+$  has on chalcopyrite leaching. Silver additionally precipitated as  $\text{AgCl}$  because 9 K medium contains 0.13 mM  $\text{Cl}^-$  (solubility product  $K_{\text{sp}} \text{AgCl } 1.77 \times 10^{-10}$ ). Thus, mapping Ag is not specific to acanthite as it also includes  $\text{AgCl}$  and  $\text{Ag}$ -jarosite precipitates. The actual concentration of silver dissolved in the leach solution is difficult to predict because of the recirculation of silver species between the solid and solution phases and concurrent precipitation of  $\text{AgCl}$  and silver- jarosite. Secondary sulfides such as  $\text{CuS}$  and  $\text{Cu}_2\text{S}$  can also sequester  $\text{Ag}^+$  and their roles in the distribution of Ag in the solid phase are generally unknown. Covellite was present in trace levels in the sample and was more readily oxidized to soluble products than chalcopyrite, but their differential dissolution was not dissected from the dissolved copper data. The silver catalytic effect on copper dissolution from chalcopyrite that was demonstrated in this research is in agreement with previous reports [29-31, 52, 53 and 56]. Silver resistance in acidophiles and toxicity in bioleaching systems has not been elucidated to date. In general, silver has a long history of biocide use and the best known molecular mechanisms of resistance to silver in gram negative bacteria are based on efflux systems involving ATPases [57].

The influence of type and quantity of silver ion on copper dissolution was investigated by running seven experiments with similar tests conditions. In seven days of treatment, 80mg/L of silver in pure and solid form had the best results with 70.66% Cu recovery (test 25). In contrast, 96.56% of copper was dissolved using 50 mg/L of silver nitrate during 16 days of treatment (test 1). In addition, the lowest amount of copper extraction was observed in test 23 with 80mg/L of silver chloride as additive in both 7 and 16 days of bioleaching process compared to other six experiments, respectively. It seems that the presence of chloride ion had negative effects on the bioleaching process and reduced its efficiency (Fig. 5).

### 3.3. Molybdenum dissolution

Molybdenum extraction from concentrate in the moderate thermophilic bioleaching process is demonstrated in Fig. 6 (A-C) as one of the main purposes of this study. Molybdenite started to dissolve after 10 days of treatment in some tests (tests 9, 10, 13, 14 and 27). As the graphs show, molybdenum dissolution has the same trends with redox potential changes. By increasing redox potential to the 550-600mV, molybdenite started to dissolve and reached to 2% (test 27) in the optimal condition. There were maximum amounts of sulfur, ferrous and ferric ions in the solution and pH was 1.4 in this circumstance (test 27). The sole ferric iron could dissolve very low amounts of molybdenite and had detrimental effects on the microorganism growth compared to the ferrous ion.

Olson and Clark (2008) showed that Mo extraction from high purity

molybdenite was initially faster with rates decreasing after about 60% Mo was extracted from sample 1 (P<sub>90</sub> 2.9 μm) and 20% from sample 2 (P<sub>90</sub> 36 μm) [58]. Extraction of Mo ultimately was about 85% from sample 1 and 30% from sample 2 after 100 days of treatment. Solution ORP remained high (>900 mV) even during the later stages of these tests. Bioleaching of Mo from all these molybdenite samples initially followed shrinking particle kinetics. The subsequent slowing of leaching rates may indicate formation of a surface layer. Wadsworth (1979) found an Mo (IV) oxy-sulfide surface layer formed during the molybdenite leaching at “low” voltages (less than about 0.7 V) [59]. Molybdenite leaching kinetics in a chloride system was initially parabolic, becoming linear when a fixed thickness of the diffusion layer boundary was attained. An extended lag was observed in flasks containing molybdenite sample 1 before iron was biooxidized and Mo bioleaching began (40 days). This lag period was shorter with molybdenite sample 2 (15 days).

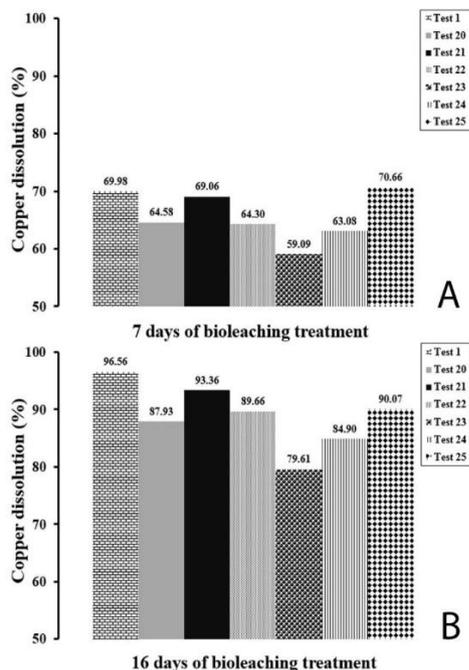


Fig. 5. Copper dissolution after 7 and 16 days of treatment in the presence of silver ion.

In this study, the molybdenum concentration in the solutions after 30 days of moderate thermophilic bioleaching is presented in Fig. 7. Dissolved molybdenum in test 27 reached to 648.45 mg/L after 30 day of treatment. It is concluded that higher redox potential (>500 mV, Ag/AgCl) and a bioleaching period of more than a month are required to start dissolving the molybdenite particles.

3.4. Total iron concentration

The total iron concentration in different solutions is presented in Fig. 8 (A-C). Iron concentration was decreased in solution by the end of the first 10 days of treatment and leveled off at the end. It can be due to iron precipitation as jarosite or other hydroxide forms. The jarosite precipitation on the particle surface during bioleaching tests was confirmed by XRD and SEM/ EDAX methods (Fig. 9).

3.5. Bacterial counts

The bacterial population at the end of experiments is demonstrated in Fig. 10. The highest bacterial population was observed in test 14 (5g/L sulfur, 25g/L ferrous sulfate and 25 g/L ferric sulfate). Because of contamination, the bacterial cells in low amounts were observed in abiotic condition (tests 18 and 19). As mentioned before, bacterial growth vitally depends on the energy sources, and in each test with ferrous iron and sulfur, the bacteria had a large population. In contrast, bacterial counts decreased drastically in every test with silver in any type and quantity. The initial population of microorganism was

approximately 10<sup>7</sup> cells/ml.

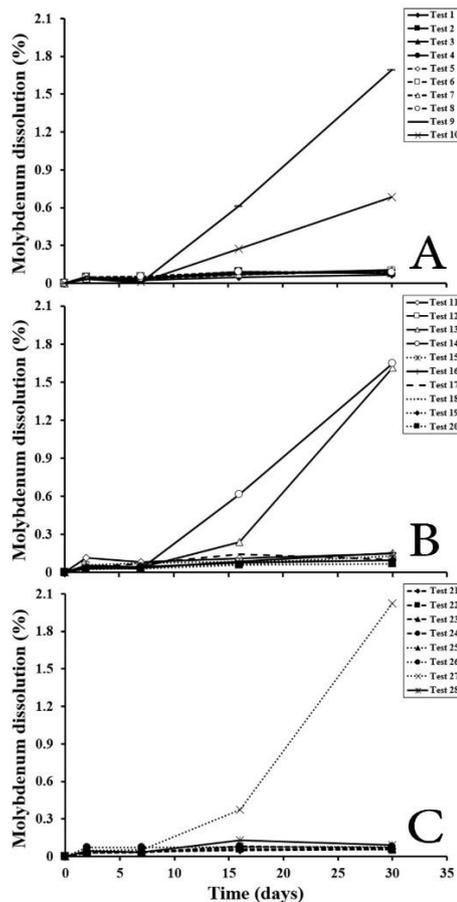


Fig. 6. Molybdenum recovery in the moderate thermophilic bioleaching process.

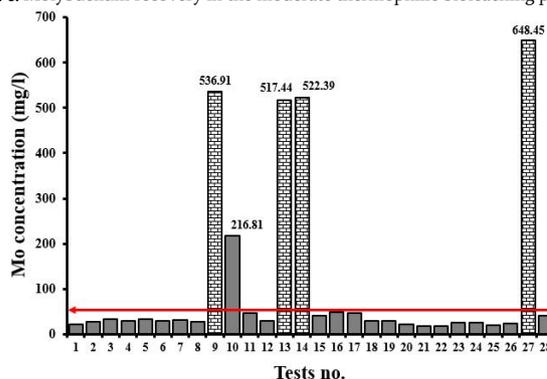


Fig. 7. Molybdenum concentration in the solutions after 30 days of treatment.

3.6. Rhenium dissolution

Moreover, rhenium extraction at the end of the experiments is presented in Fig. 11. Dissolution of rhenium from molybdenite concentrate depends on the redox potential and consequently on molybdenum dissolution rate. By increasing the molybdenum extraction in the bioleaching process, rhenium dissolution increases, as well. So, it was confirmed that rhenium is located in the molybdenite lattice and in order to liberate rhenium, dissolution of molybdenite particles is required (data are not shown). In addition, these two elements (Mo and Re) have the same dissolution behaviour in the chemical and bioleaching approaches. Maximum dissolution of rhenium occurred in test 27 with 9.53% recovery.

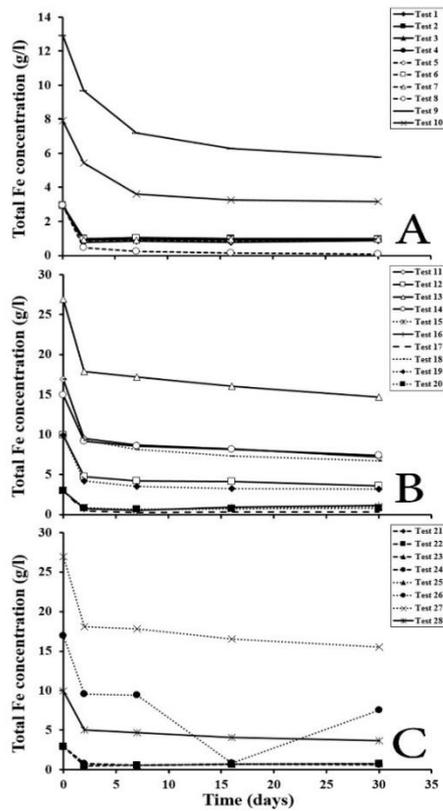


Fig. 8. The total iron concentration in the solution of the experiments.

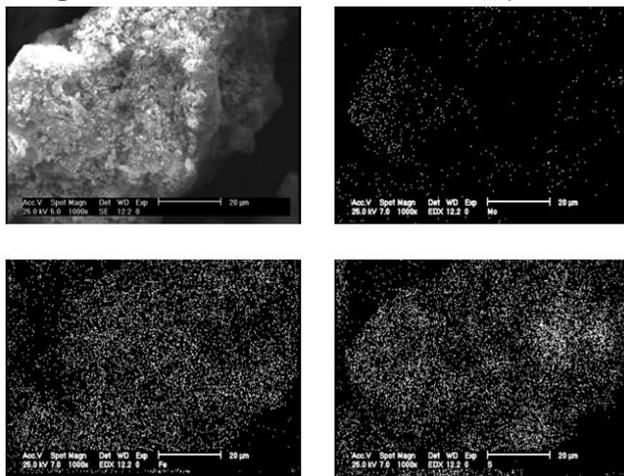


Fig. 9. SEM and EDAX analyses for molybdenite residue after bioleaching treatment.

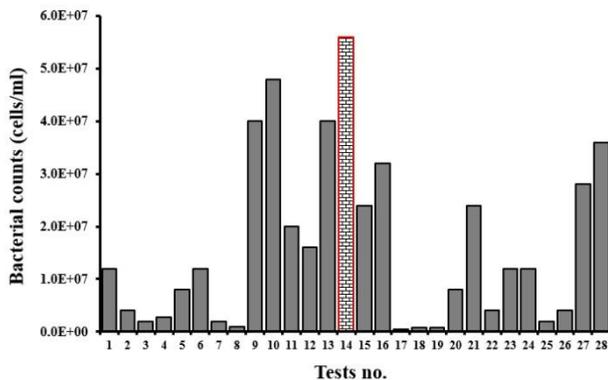


Fig. 10. The bacterial population at the end of the experiments.

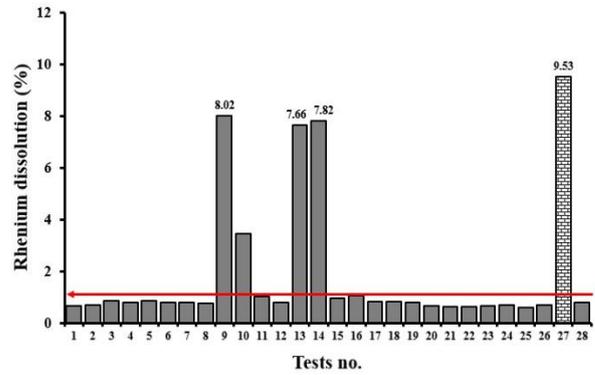


Fig. 11. Rhenium recovery at the end of the experiments.

### 3.7. Ag species formation

In addition, precipitation of silver on chalcopyrite surface was confirmed by SEM and EDAX mapping of Ag with ion microprobe (Fig. 12). Ag-precipitates were associated with chalcopyrite and pyrite grains, but they were not detected on molybdenite surfaces. Part of silver precipitates were dispersed in a random fashion and some were in form of clusters. The enhancing effect of  $Ag^+$  is attributed to precipitation of Ag-sulfide on chalcopyrite surface, establishing galvanic coupling which enhances anodic dissolution of chalcopyrite. This is a catalytic effect as  $Ag_2S$  is continually dissolved through the oxidizing action of  $Fe^{3+}$ , followed by re-precipitation on chalcopyrite surface (Fig. 13). However, silver may also precipitate in the jarosite fraction as argentojarosite and become thereby irreversibly unavailable as a catalytic ion.

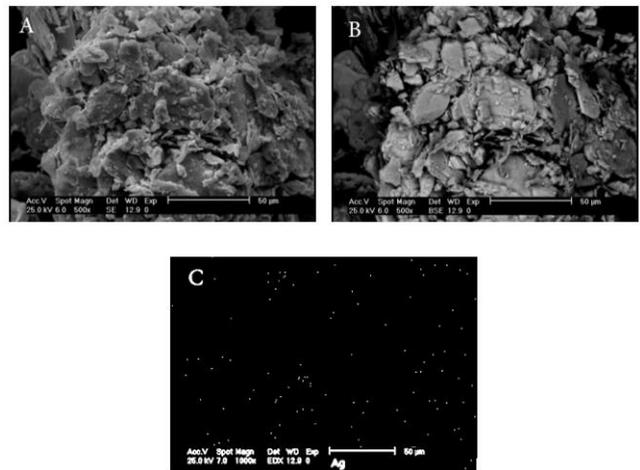


Fig. 12. SEM micrographs (A and B) and the corresponding EDAX mapping of Ag (C) of residual solids from the bioleaching of molybdenite concentrate in the presence of Ag; contact time 30 days.

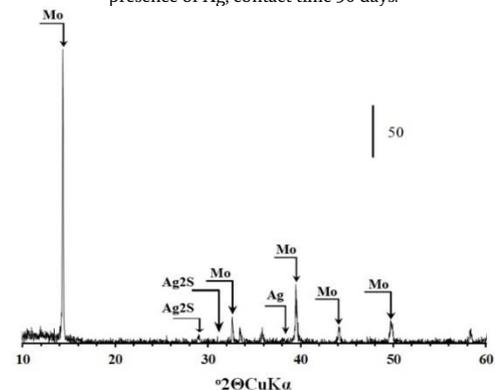


Fig. 13. Formation of silver and silver bearing components during mixed moderate bioleaching test (XRD).

#### 4. Conclusions

Mixed moderate thermophilic bioleaching experiments were applied on molybdenite concentrate. This approach successfully removed impurities such as copper (chalcopyrite and covellite) from the concentrate. Different additives such as sulfur, ferrous sulfate, ferric sulfate, pyrite and silver ion, were added according to each test condition, and the dissolution behavior of Cu, Mo, Re and Fe were comprehensively investigated. The maximum copper dissolution was achieved in the tests with silver additives. Also, there is no clear difference in the copper extraction between tests with various culture media. In these experiments, 100% of Cu was dissolved after 30 days of treatment, using 50mg/L of silver nitrate as additives. In the best condition of experiment without silver additives, 60% of copper was extracted even in presence of sulfur, ferrous and ferric ions. Also, 100% of copper was extracted in less than a week with highest kinetic rate in test with initial pH 1.57, 50 mg/L of silver nitrate, and 50g/L of ferric sulfate. In seven days of treatment, 80mg/L of silver in pure and solid form had the best results with 70.66% copper recovery. In contrast, 96.56% of copper was dissolved using 50 mg/L of silver nitrate during 16 days of treatment. In addition, the lowest amount of copper dissolution was observed in test with 80mg/L of silver chloride as additive in both 7 and 16 days of bioleaching process compared to other similar experiments. It is concluded that the presence of chloride ion had a negative effect on the bioleaching process and reduced its removal efficiency. Also, molybdenum dissolution had the same trend with redox potential graph. By increasing redox potential to the 550-600mV, molybdenite started to dissolve and reached to 2% in the best condition. Dissolution of Re from the molybdenite concentrate depends directly on the redox potential and molybdenum dissolution. As the molybdenum dissolution increases in the bioleaching process, the rhenium dissolution increases, as well. Maximum rhenium dissolution was occurred by 9.53%.

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#### REFERENCES

- [1] Santhiya, D., Ting, Y.P., 2006. Use of adapted *Aspergillus niger* in the bioleaching of spent refinery processing catalyst. *Journal of Biotechnology* 121, 62-74.
- [2] Brierley, C.L., 2008. How will biomining be applied in future? *Transactions of Nonferrous Metals Society of China* 18, 1302-1310.
- [3] Beolchini, F., Fonti, V., Ferella, F., Vegliò, F., 2010. Metal recovery from spent refinery catalysts by means of biotechnological strategies. *Journal of Hazardous Materials* 178, 529-534.
- [4] Pradhan, D., Kim, D.J., Ahn, J.G., Chaudhury, G.R., Lee, S.W., 2010. Kinetics and statistical behavior of metals dissolution from spent petroleum catalyst using acidophilic iron oxidizing bacteria. *Journal of Industrial and Engineering Chemistry* 16, 866-871.
- [5] Pradhan, D., Patra, A.K., Kim, D.-J., Chung, H.-S., Lee, S.-W., 2013. A novel sequential process of bioleaching and chemical leaching for dissolving Ni, V, and Mo from spent petroleum refinery catalyst. *Hydrometallurgy* 131-132, 114-119.
- [6] Amiri, F., Yaghmaei, S., Mousavi, S.M., Sheiban, S., 2011. Recovery of metals from spent refinery hydrocracking catalyst using adapted *Aspergillus niger*. *Hydrometallurgy* 109, 65-71.
- [7] Gholami, R.M., Borghei, S.M., Mousavi, S.M., 2011. Bacterial leaching of a spent Mo-Co-Ni refinery catalyst using *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. *Hydrometallurgy* 106, 26-31.
- [8] Gholami, R.M., Mousavi, S.M., Borghei, S.M., 2012. Process optimization and modeling of heavy metals extraction from a molybdenum rich spent catalyst by *Aspergillus niger* using response surface methodology. *Journal of Industrial and Engineering Chemistry* 18, 218-224.
- [9] Abdollahi, H., Manafi, Z., Shafaei, S.Z., Noaparast, M., Manafi moorkani, N., 2013a. Removal of copper from molybdenite concentrate by mesophilic and extreme thermophilic microorganisms. *International Journal of Mining Science and Technology* 23(6), 827-834.
- [10] Abdollahi, H., Shafaei, S. Z., Noaparast, M., Manafi, Z., Aslan, N., 2013b. Bio-dissolution of Cu, Mo and Re from molybdenite concentrate using mix mesophilic microorganism in shake flask. *Transactions of Nonferrous Metals Society of China* 23, 219-230.
- [11] Brierley, J.A., Brierley, C.L., 2001. Present and future commercial applications of biohydrometallurgy. *Hydrometallurgy* 59, 233-239.
- [12] Gericke, M., Pinches, A., Van Rooyen, J.V., 2001. Bioleaching of a chalcopyrite concentrate using an extremely thermophile culture. *International Journal of Mineral Processing* 62, 243-255.
- [13] Petersen, J., Dixon, J.G., Timmins, M., Ruitenberg, R., 2001. Batch reactor studies of the leaching of a pyrite/chalcopyrite concentrate using thermophilic bacteria. In: Ciminelli, V.S.T., Garcia Jr., O. (Eds.), *Biohydrometallurgy: Fundamentals, Technology and Sustainable Development-Part A*. Elsevier, Amsterdam, pp. 526-533.
- [14] Olson, G.J., Brierley, J.A., Brierley C.L., 2003. Bioleaching review part B: Progress in bioleaching: applications of microbial processes by the minerals industries. *Applied Microbiology and Biotechnology* 63, 249-257.
- [15] Akcil, A., Deveci, H., 2010. Mineral biotechnology of sulphides. In: Jain, S., Khan, A., Rai, M.K. (Eds.), *Geomicrobiology*. Science Publishers, Enfield, New Hampshire, 101-137.
- [16] Schippers, A., Sand, W., 1999. Bacterial leaching of metal sulfide proceeds by two indirect mechanisms via thiosulfate or via polysulfide and sulfur. *Applied and Environmental Microbiology* 65, 319-321.
- [17] Tributsch, H., 2001. Direct versus indirect bioleaching. *Hydrometallurgy* 59, 177-185.
- [18] Xingyu, L., Biao, W., Bowei, C., Jiankang, W., Renman, R., Guocheng, Y., Dianzuo, W., 2010. Bioleaching of chalcocite started at different pH: Response of the microbial community to environmental stress and leaching kinetics. *Hydrometallurgy* 103, 1-6.
- [19] Vargas, T., Davis-Belmar, C.S., Cárcamo, C., 2014. Biological and chemical control in copper bioleaching processes: When inoculation would be of any benefit?. *Hydrometallurgy* 150, 290-298.
- [20] Clark, D.A., and Norris, P.R., 1996. Oxidation of mineral sulphides by thermophilic microorganisms. *Minerals Engineering* 9, 1119-1125.
- [21] Gericke, M., Pinches, A., 1999. Bioleaching of copper sulphide concentrate using extreme thermophilic bacteria. *Minerals Engineering* 12, 893-904.
- [22] Plumb, J.J., Mc Sweeney, N.J., P.D. Franzmann, P.D., 2008. Growth and activity of pure and mixed bioleaching strains on low grade chalcopyrite ore. *Minerals Engineering* 21, 93-99.
- [23] Norris, P.R., Burton, N.P., Clark, D.A. 2013. Mineral sulfide concentrates leaching in high temperature bioreactors. *Minerals Engineering* 48, 10-19.
- [24] Ahonen, L., Tuovinen, O.H., 1990a. Catalytic effects of silver in the

- microbiological leaching of finely ground chalcopyrite-containing ore materials in shake flasks. *Hydrometallurgy* 24, 219-236.
- [25] Ahonen, L., Tuovinen, O.H., 1990b. Silver catalysis of the bacterial leaching chalcopyrite-containing ore material in column reactors. *Minerals Engineering* 3, 437-445.
- [26] Abdollahi, H., Shafaei, S.Z., Noaparast, M., Manafi, Z., Niemelä, Seppo I., Tuovinen, Olli H., 2014. Mesophilic and thermophilic bioleaching of copper from a chalcopyrite-containing molybdenite concentrate. *International Journal of Mineral Processing* 128, 25-32.
- [27] Hu, Y., Qiu, G., Wang, J., Wang, D., 2002. The effect of silver-bearing catalysts on bioleaching of chalcopyrite. *Hydrometallurgy* 64, 81-88.
- [28] Juarez, A.L., Arenas, N.G., Santillan, R.E.R., 2006. Electrochemical behavior of massive chalcopyrite bioleached electrodes in presence of silver at 35 °C. *Hydrometallurgy* 83, 63-68.
- [29] Muñoz, J.A., Dreisinger, D.B., Cooper, W.C., Young, S.K., 2007a. Silver-catalyzed bioleaching of low-grade copper ores. Part I: Shake flasks tests. *Hydrometallurgy* 88, 3-8.
- [30] Muñoz, J.A., Dreisinger, D.B., Cooper, W.C., Young, S.K., 2007b. Silver-catalyzed bioleaching of low-grade copper ores. Part II: Stirred tank tests. *Hydrometallurgy* 88, 19-34.
- [31] Muñoz, J.A., Dreisinger, D.B., Cooper, W.C., Young, S.K., 2007c. Silver-catalyzed bioleaching of low-grade copper ores. Part III: Column reactors. *Hydrometallurgy* 88, 35-51.
- [32] Chen, S., Lin, J., 2009. Enhancement of metal bioleaching from contaminated sediment using silver ion. *Journal of Hazardous Materials* 161, 893-899.
- [33] Córdoba, E.M., Muñoz, J.A., Blázquez, M.L., González, F., Ballester, A., 2009. Comparative kinetic study of the silver-catalyzed chalcopyrite leaching at 35 and 68 °C. *International Journal of Mineral Processing* 92, 137-149.
- [34] Feng, S., Yang, H., Gao, K., Yang, J., Liu, T., Zhang, L., Wang, W., 2013. A novel and highly efficient system for chalcopyrite bioleaching by mixed strains of *Acidithiobacillus*. *Bioresource Technology* 129, 456-462.
- [35] Wang, M., Zhang, Y., Deng, T., Wang, K., 2004. Kinetic modeling for the bacterial leaching of chalcopyrite catalyzed by silver ions. *Minerals Engineering* 17, 943-947.
- [36] Sand, W., Gehrke, T., Jozsa, P.G., Shippers, A., 2001. (Bio) chemistry of bacterial leaching-direct vs. indirect bioleaching. *Hydrometallurgy* 59, 159-175.
- [37] Deveci, H., Akcil, A., Alp, I., 2004. Bioleaching of complex zinc sulphides using mesophilic and thermophilic bacteria: comparative importance of pH and iron. *Hydrometallurgy* 73, 293-303.
- [38] Leahy, M.J., Schwarz, M.P., 2009. Modelling jarosite precipitation in isothermal chalcopyrite bioleaching columns. *Hydrometallurgy* 98, 181-191.
- [39] Habashi, F. (Ed.), 1997. *Handbook of Extractive Metallurgy*, vol. 3, Wiley-VCH, pp. 1492.
- [40] Askari Zamani, M.A., Hiroiyoshi, N., Tsunekawa, M., Vaghar, R., Oliazadeh, M., 2005. Bioleaching of Sarcheshmeh molybdenite concentrates for extraction of rhenium. *Hydrometallurgy* 80, 23-31.
- [41] Ahmadi, A., Schaffie, M., Manafi, Z., Ranjbar, M., 2010. Electrochemical bioleaching of high grade chalcopyrite flotation concentrates in a stirred bioreactor. *Hydrometallurgy* 104, 99-105.
- [42] Vakylabad, A.B., Schaffie, M., Ranjbar, M., Manafi, Z., Darezereshki, E., 2012. Bioprocessing of copper from combined smelter dust and flotation concentrate: a comparative study on the stirred tank and airlift reactors. *Journal of Hazardous Materials* 241-242, 197-206.
- [43] Silverman, M.P. and Lundgren, D.S., 1959. Studies of the chemoautotrophic iron bacterium *Ferrobacillus ferrooxidans*. An improved medium and a harvesting procedure for securing high cells yields. *Journal of Bacteriology* 77, 642-647.
- [44] McCready, R.G.L., Wadden, D., Marchbank, A., 1986. Nutrient requirements for the in-place leaching of uranium by *Thiobacillus ferrooxidans*. *Hydrometallurgy* 17, 61-71.
- [45] Lizama, H., Suzuki, I., 1988. Bacterial leaching of a sulphide ore by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, I. Shake flask studies. *Biotechnology and Bioengineering* 32, 110-116.
- [46] Gómez, C., Blázquez, M.L., Ballester, A., 1996. Bioleaching of a Spanish complex sulphide ore bulk concentrate. *Minerals Engineering* 12, 93-106.
- [47] Massinaie, M., Oliazadeh, M., Seyed Bagheri, A., 2006. Biological copper extraction from melting furnaces dust of Sarcheshmeh copper mine. *International Journal of Mineral Processing* 81, 58-62.
- [48] González-Toril, E., Gómez, F., Malki, M., Amils, R., 2006. The isolation and study of acidophilic microorganisms. *Methods in Microbiology* 35, 471-510.
- [49] Miller, J.D., McDonough, P.J., Portillo, H.Q., 1981. Electrochemistry in silver-catalyzed ferric sulfate leaching of chalcopyrite. In: Keaton, M.C. (Ed.), *Process and Fundamental Consideration of Selected Hydrometallurgical Systems*. SME-AIME, New York, 327-338.
- [50] Miller, J.D., Portillo, H.Q., 1981. Silver catalysis in ferric sulphate leaching of chalcopyrite. In: Laskowski, J.(Ed.), *13th International Mineral Processing Congress*, Warsaw, 1979. Elsevier, Amsterdam, 851-898.
- [51] Muñoz, J.A., Gómez, C., Ballester, A., Blázquez, M.L., González, F., 1998. Electrochemical behavior of chalcopyrite in the presence of silver and *Sulfolobus* bacteria. *Journal of Applied Electrochemistry* 28, 49-56.
- [52] Muñoz, J.A., Dreisinger, D.B., Cooper, W.C., Young, S.K., 2008. Interaction of silver ions with sulphide minerals with special emphasis on the chalcopyrite/pyrite galvanic couple. *Canadian Metallurgical Quarterly* 47, 259-268.
- [53] Sato, H., Nakazawa, H., Kudo, Y., 2000. Effect of silver chloride on the bioleaching of chalcopyrite concentrate. *International Journal of Mineral Processing* 59, 17-24.
- [54] Hiroiyoshi, N., Arai, M., Miki, H., Tsunekawa, M., Hirajima, T., 2002. A new reaction model for the catalytic effect of silver ions on chalcopyrite leaching in sulfuric acid solutions. *Hydrometallurgy* 63, 257-267.
- [55] Tuovinen, O.H., Puhakka, J., Hiltunen, P., Dolan, K.M., 1985. Silver toxicity to ferrous ion and pyrite oxidation and its alleviation by yeast extract in cultures of *Thiobacillus ferrooxidans*. *Biotechnology Letters* 7, 389-394.
- [56] Cancho, L., Blázquez, M.L., Ballester, A., González, F., Muñoz, J.A., 2007. Bioleaching of a chalcopyrite concentrate with moderate thermophilic microorganisms in a continuous reactor system. *Hydrometallurgy* 87, 100-111.
- [57] Franke, S., 2007. Microbiology of the toxic noble metal silver. In: Nies, D.H., Silver, S. (Eds.), *Molecular Microbiology of Heavy Metals*. Springer-Verlag, Berlin, pp. 343-355.
- [58] Olson, G.J., Clark, T.R., 2008. Bioleaching of molybdenite. *Hydrometallurgy* 93, 10-15.
- [59] Wadsworth, M.E. 1979. Hydrometallurgical processes. In: *Rate Processes of Extractive Metallurgy* (H. Y. Sohn, M. E. Wadsworth, eds.), p. 133-241. Plenum Press, New York.