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 9.98% Cu, 15.56% Fe, 53.84% Mo, and 0.095% Re. Molybdenite was the major phase of Mo-bearing mineral and chalcopryite, covellite and pyrite were distinguished as minor phases. Higher copper extraction was observed 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 157, 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, Chalcopryite 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 Fe²⁺ to Fe³⁺ 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 chalcopryite [24-26]. The positive catalytic effect of silver ions on the bioleaching of chalcopryite 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]:

\[ \text{MeS} + 2\text{Ag}^+ \rightarrow \text{Ag}_2\text{S} + \text{Me}^+ \]
\[ \text{Ag}_2\text{S} + 2\text{Fe}^{3+} \rightarrow 2\text{Ag}^{+} + 2\text{Fe}^{2+} + \text{S} \]  

(1)

where MeS signifies sulfide minerals. Ferrous ions (Fe²⁺) are re-oxidized to ferric ions (Fe³⁺) 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 (FeSO₄·7H₂O). 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]:

\[ \text{FeS}_2 + 8\text{H}_2\text{O} \rightarrow 14\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 16\text{H}^+ \]  

(2)

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

\[ 2\text{Fe}^{2+} + 2\text{H}^+ \xrightarrow{\text{bacteria}} 2\text{Fe}^{3+} + \text{H}_2\text{O} \]  

(3)

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 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 (Fe²⁺ and S). In addition, the ability of replacing FeSO₄·7H₂O 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 dₘₜₐₜ 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 5th, 6th and final concentrate are presented in Table 1. As shown, there is 0.98% Cu in 5th cleaner sample which shows the highest amount of the copper content among the three final cleaners. Therefore, the representative sample obtained from 5th 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 Figure 1.

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

<table>
<thead>
<tr>
<th>Assay</th>
<th>5th Cleaner</th>
<th>6th Cleaner</th>
<th>7th Cleaner</th>
</tr>
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<tbody>
<tr>
<td>CuS (Covellite)</td>
<td>0.3</td>
<td>0.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Cu₉Fe₅S₄ (Chalcopyrite)</td>
<td>2.1</td>
<td>1.8</td>
<td>1.3</td>
</tr>
<tr>
<td>FeS₂ (Pyrite)</td>
<td>2</td>
<td>1.9</td>
<td>1.7</td>
</tr>
<tr>
<td>MoS₂ (Molybdenite)</td>
<td>89.8</td>
<td>90.9</td>
<td>91.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>94.2</strong></td>
<td><strong>94.9</strong></td>
<td><strong>94.5</strong></td>
</tr>
<tr>
<td>Cu (%)</td>
<td>0.98</td>
<td>0.91</td>
<td>0.64</td>
</tr>
<tr>
<td>Fe (%)</td>
<td>1.56</td>
<td>1.43</td>
<td>1.17</td>
</tr>
<tr>
<td>Mo (%)</td>
<td>53.84</td>
<td>54.00</td>
<td>54.88</td>
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<tr>
<td>Re (ppm)</td>
<td>550</td>
<td>650</td>
<td>700</td>
</tr>
<tr>
<td><strong>Total (%)</strong></td>
<td><strong>56.38</strong></td>
<td><strong>56.34</strong></td>
<td><strong>56.49</strong></td>
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</tbody>
</table>

Figure 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 ferripilum,
**Sulfobacillus sp.** and **Ferroplasma sp.** [41, 42].

For this study, the cultures were initially grown with 10% chalcopirite 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 \((NH_4)_2SO_4\), 0.5 g MgSO_4·7H_2O, 0.1 g KCl, 0.5 g K_HPO_4, 0.01 g Ca(NO_3)_2, 44.22 g FeSO_4·7H_2O and 10g sulfur; the pH was adjusted to 1.8 with H_2SO_4 [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 K from Silverman and Lundgren (1959) and the others modified from McCready et al. (1986), Lizama and Suzuki (1988), Gómez et al. (1996), and Massinaia et al. (2006). Descriptions of additional media formulations can be found, for example, in Gonzalez-Toril et al. (2006) [43-48]. The formulation E was specifically designed for the bioleaching tests, with phosphate supplied as HPO_4 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.

<table>
<thead>
<tr>
<th>Mineral salts</th>
<th>Concentration (g/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9K</td>
<td>0.9K A B C D1 D2 E</td>
</tr>
<tr>
<td>((NH_4)_2SO_4)</td>
<td>3.0 0.3 0.2 0.4 0.2 0.06 0.01 3.0</td>
</tr>
<tr>
<td>MgSO_4·7H_2O</td>
<td>0.5 0.05 0.2 0.4 0.2 0.06 0.01  --</td>
</tr>
<tr>
<td>K_HPO_4</td>
<td>0.5 0.05 0.27 0.1 0.2 0.02 0.01  --</td>
</tr>
<tr>
<td>KCl</td>
<td>0.01 0.01 0.2  --  0.02 0.01 0.1  --</td>
</tr>
<tr>
<td>Cu(NO_3)_2·H_2O</td>
<td>0.01 0.001  --  --  --  0.01 0.1  --</td>
</tr>
<tr>
<td>H_2PO_4</td>
<td>--  --  --  --  --  --  --  0.5</td>
</tr>
</tbody>
</table>

#### 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 serum bottles incubated at a constant temperature of 45°C in an orbital shaker agitating at 150 rpm for a month. Each flask contained 85 mL 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 15 mL 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 measured 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 1/100mm and area of 1/400 mm². 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 Fe₅). 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.

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<th>Test No.</th>
<th>Medium</th>
<th>P.D (%)</th>
<th>IP (%)</th>
<th>pH</th>
<th>Silver nitrate (mg/L)</th>
<th>Sulfur (g/L)</th>
<th>Ferrous sulphate (g/L)</th>
<th>Ferric sulphate (g/L)</th>
<th>Pyrite (g/L)</th>
<th>Silver sulphate (mg/L)</th>
<th>Silver chloride (mg/L)</th>
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</table>

* 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 Figure 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.

3.2 Copper dissolution

Copper extraction in the moderate thermophilic bioleaching processes is demonstrated in Figures 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 (NH₄)₂SO₄, 0.01 g/l KCl, 0.5 g/l concentration of H₃PO₄) 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 Ag⁺ to form Ag-sulfide on chalcopyrite surface, followed by Fe³⁺-mediated oxidation [49, 50]:

\[
\begin{align*}
\text{CuFeS₂} + 4\text{Ag}^+ & \rightarrow \text{Cu}^{2+} + \text{Fe}^{2+} + 2\text{Ag}_2\text{S} & (4) \\
2\text{Ag}_2\text{S} + 4\text{Fe}^{2+} & \rightarrow 4\text{Ag}^+ + S^{2-} + 4\text{Fe}^{2+} & (5)
\end{align*}
\]

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

\[
\begin{align*}
\text{CuFeS₂} + 4\text{Ag}^+ + 4\text{Fe}^{2+} & \rightarrow \text{Cu}^{2+} + 5\text{Fe}^{3+} + 2\text{S}^{2-} & (6)
\end{align*}
\]

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

\[
\begin{align*}
\text{CuFeS₂} + 4\text{Ag}^+ & \rightarrow \text{Cu}^{2+} + \text{Fe}^{3+} + 4\text{Ag} + 2\text{S}^{2-} & (7)
\end{align*}
\]

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

The formation of Ag₂S-Ag film on chalcopyrite surface modifies the sulfur layer and increases its conductivity and accelerates the electron transfer through the oxidant. The presence of Fe³⁺ and its regeneration from Fe²⁺ 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 Ag₂S and Cu₂S by the prior reduction of chalcopyrite with ferrous ion and evolution of H₂S [54]. In this case, the silver sulfide is not formed through reaction (1) but by the reaction of silver ions with H₂S.

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 Cu₂FeS₄ and Ag₂S. 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 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 Ag₈Fe₆(SO₄)₄(OH)₄. Argentojarosite lacks the catalytic effect that Ag⁺ has on chalcopyrite leaching. Silver additionally precipitated as AgCl because 9 K medium contains 0.13 mm Cl⁻ (solubility product Kₛₐ₃Cl = 1.77x10⁻¹⁰). Thus, mapping Ag⁺ is not specific to acanthite as it also includes AgCl and 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 AgCl and silver-jarosite. Secondary sulfides such as Cu₅S and Cu₅S can also sequester 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, 96.36% of copper was dissolved using 50mg/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 one of the main purposes of this study. Molybdicenite started to dissolve after 10 days of treatment in some tests (tests 9, 10, 13, 14 and 27). As the graphs show, molybdicenite dissolution has the same trends with redox potential changes. By increasing redox potential to the 550-600mV, molybdicenite 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 14 in this circumstance (test 27).

The sole ferric iron could dissolve very low amounts of molybdite 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 (P90 2.9 μm) and 20% from sample 2 (P90 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).

![Figure 5. Copper dissolution after 7 and 16 days of treatment in the presence of silver ion.](image)

In this study, the molybdenum concentration in the solutions after 30 days of moderate thermophilic bioleaching is presented in Figure 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 Figure 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 (Figure 9).

### 3.5. Bacterial counts

The bacterial population at the end of experiments is demonstrated in Figure 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 10⁷ cells/ml.

![Figure 6. Molybdenum recovery in the moderate thermophilic bioleaching process.](image)

Moreover, rhenium extraction at the end of the experiments is presented in Figure 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.

![Figure 7. Molybdenum concentration in the solutions after 30 days of treatment.](image)
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 (Figure 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\(_2\)S is continually dissolved through the oxidizing action of Fe\(^{3+}\), followed by re-precipitation on chalcopyrite surface (Figure 13). However, silver may also precipitate in the jarosite fraction as argentojarosite and become thereby irreversibly unavailable as a catalytic ion.
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 50mg/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 according to each test condition, and the dissolution behavior of Cu, Mo, Re and Fe from molybdenite concentrate using mix mesophilic microorganism in shake flask. Transactions of Nonferrous Metals Society of China 23, 219-230.

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REFERENCES

microbiological leaching of finely ground chalcopyrite-containing ore materials in shake flasks. Hydrometallurgy 24, 219-236.