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The effect of microwave irradiation on operational parameters of copper flotation concentrate bioleaching by irradiated mesophilic bacteria

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Previous bioleaching experiments carried out on Sarcheshmeh copper concentrate samples revealed that the optimum bioleaching operational condition are as temperature 32°C, pH 1.8 and agitation rate 125 rpm. A control bioleaching test was carried out using this optimum operational condition and the obtained copper recovery was 34.2% after 30 days which was in conjunction with previous researches. This study was carried out with the aim of investigating the effect of microwave irradiation on the operational parameters affecting copper sulfide concentrate bioleaching. With this regard, three commonly used mesophilic bacteria in bioleaching of sulfidic copper concentrate (*Acidithiobacillus ferrooxidan, Leptospirillum ferrooxidan and Acidithiobacillus thiooxidan*) were treated by microwave irradiation. Microwave irradiation were carried out for 5, 10, 15 and 20 s and the optimum irradiation times were chosen based on oxidation rate test results. The oxidation rate test results showed that optimized time of microwave irradiation for *Acidithiobacillus ferrooxidan, Leptospirillum ferrooxidan and Acidithiobacillus thiooxidan* were 10, 15 and 10 s respectively. A mixed culture of optimum irradiated bacteria were used in bioleaching experiments. The optimization of bioleaching operational parameters was carried out on initial pH (1.2, 1.5, 1.8 and 2), temperature (32, 38, 44 and 50 °C) and agitation rate (125, 150, 175 and 200 rpm). Using microwave treated bacteria and based on copper extraction results, it was concluded that optimum bioleaching condition is changed to pH 1.5, temperature 44°C and agitation rate 125 rpm and maximum copper recovery obtained under this optimum operational condition for microwave treated mixed culture reached to 44.36% after 30 days. The bioleaching rate was also enhanced using microwave treated mixed cultures, so that the copper recovery was reached to 34.36% after 12 days.

Keywords: Bioleaching, Copper Concentrate, Microwave irradiation, Mesophilic Bacteria

INTRODUCTION

With considering commercial and environmental disadvantages of pyro-metallurgical processes, many works were established with the aim of developing a hydrometallurgical process for treating chalcopyrite concentrate [1,2]. Despite of successfully implementation of bio-hydrometallurgical processes for treating refractory gold ores and the majority of secondary copper sulfides [3,4], bioleaching of chalcopyrite concentrates which is the most important copper-bearing mineral is still a promising method [5-7].

Using *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* which are commonly used mesophilic bacteria in bioleaching of copper sulfides [8-11], the rate and amount of copper recovery from chalcopyrite concentrate is very low. Accordingly, some efforts including over grinding, addition of catalysts, potential control and microorganism adaptation have been done with the aim of enhancing copper recovery [7, 13,14].

Since the adaptation processes are time consuming, multi stage and low efficient methods [15], applying some of physical and chemical methods in order to change bacterial activity properties were investigated by authors [15-19]. Microwave irradiation is a physical method which can affect bacterial activity through changing oxidation strength of bacteria. This theory is investigated by comparison of oxidation rates obtained by irradiated bacteria in this study. Furthermore, as a new research area, bioleaching of copper concentrate sample using optimum irradiated bacteria were carried out and the changes in bioleaching operational condition were investigated.

EXPERIMENTAL

Ore concentrate

Bioleaching experiments were carried out on a sulfidic flotation concentrate representative sample from Sarcheshmeh Copper Mine (Kerman, Iran). Fig. 1 is demonstrated the XRD pattern of the sample and chalcopyrite and pyrite as major and minor minerals, respectively. The D80 of the sample was 45 μm. The chemical composition of the sample is given in Table. 1.

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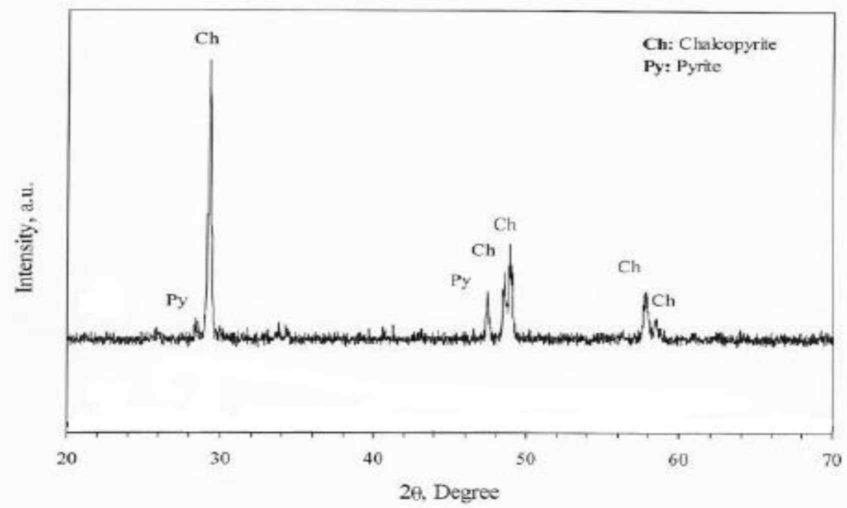

Fig. 1. XRD patterns of the concentrate.

Table 1. Chemical composition of flotation concentrate

Elements	**Cu**	**Fe**	**S**	**Si**	**Al**	**Zn**	**K**	**Ca**
Wt. %	24.1	29.34	34.06	7.63	1.93	0.96	0.79	0.41

Bacterial specimen

Acidithiobacillus ferrooxidans, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*, as three main iron and sulfur oxidizing bacteria were used in experiments. These strains were previously isolated from the Sarcheshmeh Copper Mine.

These strains were grown on a 9K liquid medium containing 3.0 g/L $(NH_4)_2SO_4$, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.63 g/L K_2HPO_4, 0.1 g/L KCl, 0.014 g/L $Ca(NO_3)_2$. For *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*, 9 gr and 15 gr of ferrous sulfate was added as energy source, respectively. Elemental sulfur was added for *Acidithiobacillus thiooxidans* (2 gr).

Growth condition were set according to previous experiments which have been done by other authors [5, 7, 12, 20]. Cultures were incubated in 500 ml Erlenmeyer flasks and in a temperature-controlled shaker. Flasks were contained 200 ml of the medium and 10% (v/v) inoculums and culturing were carried out at 125 rpm with a constant temperature of 32 °C. The initial pH for *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* were adjusted by sulfuric acid to 1.8, 2, 1.6, respectively. The control and adjustment of the pH were carried out daily by sulfuric acid addition. Bacterial growth was monitored by daily measurements of ORP values.

Irradiation

Three bacterial strains were subjected to microwave irradiation in the logarithmic growth phase. Irradiation circumstance was as a petri dish containing bacterial suspension placed into a microwave oven (power: 600 W, frequency: 2450MHz) and treated for duration times of 5, 10, 15 and 20 s. After termination of irradiation, the cultivation of remained bacterial population commenced with the optimum conditions of growth. Then, cultured strains were used in oxidation rate measuring experiments.

For oxidation rate measuring, 30 g/l and 50 g/l of ferrous sulfate were added to medium for *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*. For *Acidithiobacillus thiooxidans*, 10 g/l of elemental sulfur were added to medium. During oxidation rate measuring experiments, the concentrations of ferrous iron and elemental sulfur were measured at given intervals and the oxidation rates were calculated based on measured concentrations.

The irradiated strains which posse best oxidation activity on Fe^{2+} (for *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*) and on S^0 (for *Acidithiobacillus thiooxidans*) during oxidation rate tests, were used in preparation of a mixed culture with the addition ratio of 40% (v/v) *Acidithiobacillus ferrooxidans*, 40% (v/v) *Leptospirillum ferrooxidans*and 20% (v/v) *Acidithiobacillus thiooxidans*. This mixed culture was used in bioleaching optimization tests.

Bioleaching of sulfidic copper concentrate

At the beginning of bioleaching experiments and as a control test, bioleaching test using a mixed culture of un-irradiated strains were carried out. This test were carried out with applying the optimum bioleaching operational condition obtained from previous bioleaching experiments carried out on Sarcheshmeh copper concentrate samples. These optimum operational condition are as temperature= 32°C, pH= 1.8 and agitation rate= 125 rpm. The duration of bioleaching tests were 30 days.

With considering oxidation rate tests result, bioleaching experiments using irradiated mixed culture were carried out under same operational condition with un-irradiated mixed culture. The aim of this test was investigating the effect of microwave irradiation on copper recovery.

After observation of the positive effect of microwave irradiation, optimization tests were carried out using irradiated mixed culture. The optimization of bioleaching operational parameters was carried out on initial pH (1.2, 1.5, 1.8 and 2), temperature (32, 38, 44 and 50 °C) and agitation rate (125, 150, 175 and 200 rpm). The initial pH was adjusted to 1.8 using sulfuric acid as needed and all experiment were carried out in triplicate.

Analysis

The oxidation rates of ferrous iron for *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* were determined by potassium dichromate titration and the oxidation rates of S^0 for *Acidithiobacillus thiooxidans* were measured by weighing the non-oxidized sulfur. Atomic absorption spectrometry (AAS) was applied for copper concentration analysis. The pH measurements were carried out by a Mettler Toledo pH meter (model MP120) and the ORPs were measured using a Metrohm pH/Eh meter (model 827).

RESULTS AND DISCUSSION

Optimization for microwave irradiation time

Optimization of microwave irradiation on *Acidithiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* were carried out through measuring of the ferrous oxidation rate. Ferrous oxidation rate is calculated via the following equation:

$$Ferrous\ oxidation\ rate\ \% = \left(1 - \frac{[Fe_n^{2+}]}{[Fe_0^{2+}]}\right) \times 100 \qquad (1)$$

Where $[Fe^{2+}{}_0]$ is the concentration of Fe^{2+} in the medium at the beginning of culturing and $[Fe^{2+}{}_n]$ is the concentration of Fe^{2+} in the medium after n-hour culturing.

The effect of irradiation on *Acidithiobacillus thiooxidans* is determined through the measuring of elemental sulfur oxidation rate. Oxidation rate is calculated via the following equation:

$$Sulfur\ oxidation\ rate\ \% = \left(1 - \frac{[S_0^0]-[S_n^0]}{[S_0^0]}\right) \times 100 \qquad (2)$$

Where $[S^0{}_0]$ and $[S^0{}_n]$ are the amount of non-oxidized elemental sulfur in the medium at the initial and after n-hour culturing, respectively.

Irradiation on Acidithiobacillus thiooxidans

Elemental sulfur oxidation rate for un-irradiated *Acidithiobacillus thiooxidans* was reached to 38.6% after 56 h of culturing. The effect of microwave irradiation on oxidation strength of this strain is showed in Fig. 2. As is showed, the optimum microwave irradiation time for this strain is 10 s, where the oxidation ratio after 56 h of culturing reached to 45.4%. *Acidithiobacillus thiooxidans* is highly sensitive to microwave irradiation and with increasing irradiation time to 15 and 20 s, the oxidation strength was dramatically reduced.

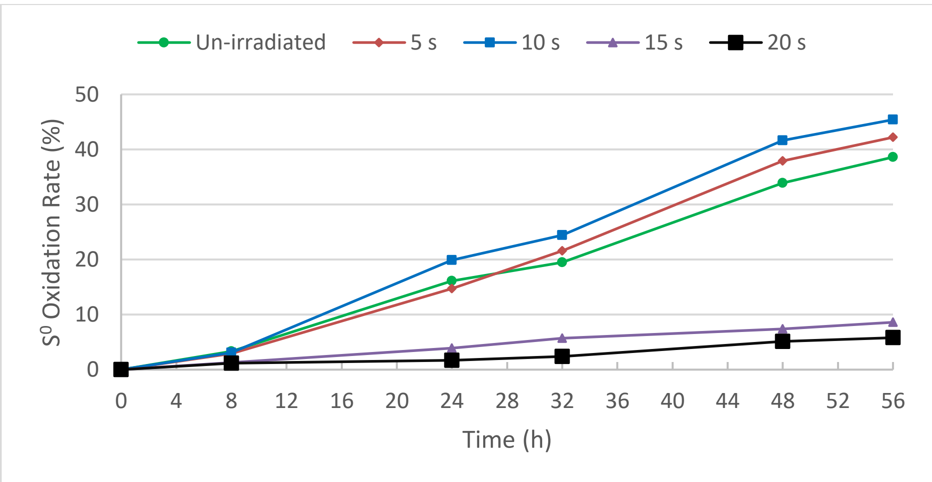

Fig. 2. Effect of microwave irradiation on oxidation strength of Acidithiobacillus thiooxidans

Irradiation on Acidithiobacillus ferrooxidans

Ferrous iron oxidation rate using un-irradiated *Acidithiobacillus ferrooxidans* after 56 h of culturing was reached to 70.26%. As is demonstrated in Fig. 3, the optimum microwave irradiation time for *Acidithiobacillus ferrooxidans* was 10 s and with increasing irradiation time to 15 s, the oxidation rate was decreased. With increasing irradiation time to 20 s, the oxidation rate was dramatically decreased. Maximum oxidation rate (79.90%) was achieved after 56 h of culturing and is 9.64% higher than the oxidation rate of un-irradiated strain.

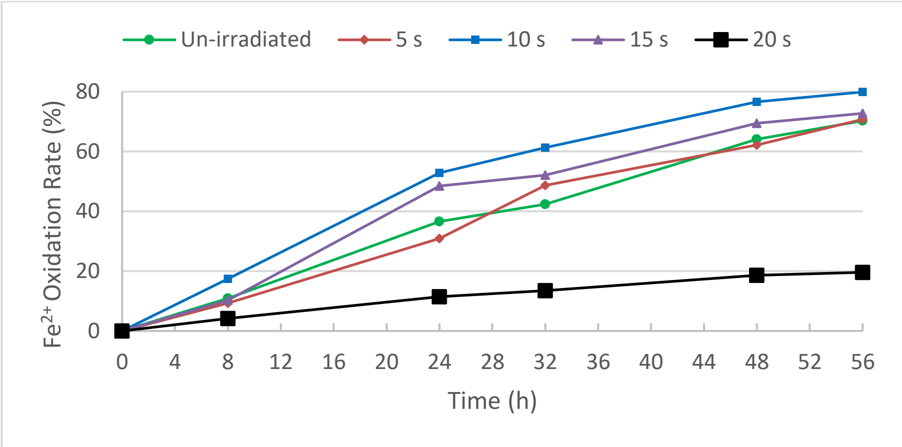

Fig. 3. Effect of microwave irradiation on oxidation strength of *Acidithiobacillus ferrooxidans*.

The considerable increase (about 20%) observed after 32 h culturing, using optimally irradiated *Acidithiobacillus ferrooxidans* in comparison with un-irradiated strain, indicates that the effect of microwave irradiation is more sensible at the middle stages of culturing

Irradiation on Leptospirillum ferrooxidans

Considering slower growth rate of *Leptospirillum ferrooxidans* during the initial stages of a mix batch culture in comparison with *Acidithiobacillus ferrooxidans* [21], oxidation rate measuring for this strain were carried out for 72 h.

Ferrous iron oxidation rate using un-irradiated *Leptospirillum ferrooxidans*after 72 h of culturing was reached to 74.87%.

The effect of microwave irradiation on oxidation strength of this strain is showed in Fig. 4. As is showed, the optimum microwave irradiation time for this strain is 15 s, where the oxidation ratio after 72 h of culturing reached to 79.96%. With increasing irradiation time to 20 s, the oxidation rate was decreased. The maximum increase of oxidation rate for optimally irradiated strain was obtained after 48 h and was about 13%.

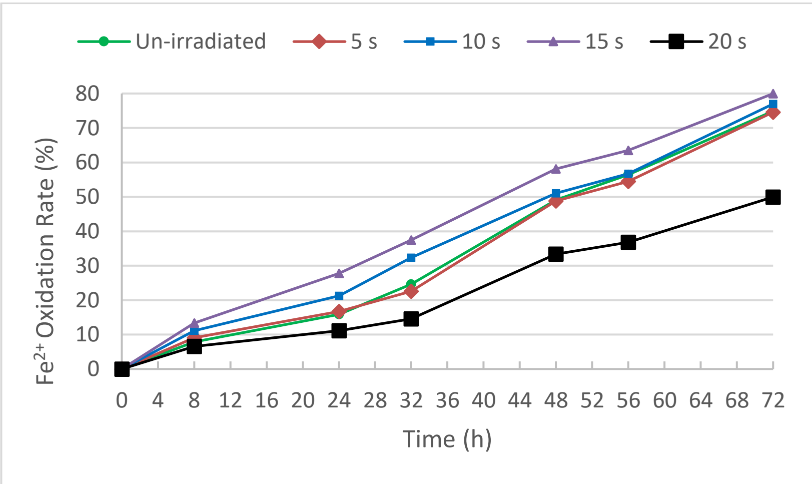

Fig. 4. Effect of microwave irradiation on oxidation strength of Leptospirillum ferrooxidans

Optimization of bioleaching parameters

According to oxidation tests result, a mixed culture of optimally irradiated strains were prepared and used in bioleaching experiments. The aim of these experiments was to determine the effect of microwave irradiation on bioleaching operational parameters including initial pH, agitation rate and temperature. Therefore, first a control test using un-irradiated mixed culture were carried out and the copper recovery obtained after 30 days was 34.2%. Then, another test using a mixed culture made by optimally irradiated strains (ratio of 2:2:1 for *Acidithiobacillus ferrooxidans*:*Leptospirillum ferrooxidans*and:*Acidithiobacillus thiooxidans*) were carried out under same operational condition of control test and the coppery recovery after 30 days increased to 37.21%. This increase is related to improved oxidation rate of the bacterial culture achieved by microwave irradiation.

Optimizing initial pH

Fig. 5 shows the copper recovery during the initial pH optimization tests while the other bioleaching condition, i.e. temperature 32°C and agitation rate 125 rpm were constant. As is demonstrated, with increasing pH from 1.8 to 2.2, copper recovery was fell to less than 15%, which is due to acidophilic nature of mixed culture. Copper recovery was increased by decreasing pH to 1.5 and was reached to 42.12% after 30 days, which was 7.92% higher than recovery obtained at the pH 1.8. The increase in copper recovery with decreasing pH to 1.2 is negligible and the pH 1.5 was chosen as optimum pH. It is also noteworthy that, the copper recoveries obtained by optimum pH after 18 and 15 days are higher than copper recoveries obtained at pH 1.8 and un-irradiated culture after 30 days, respectively.

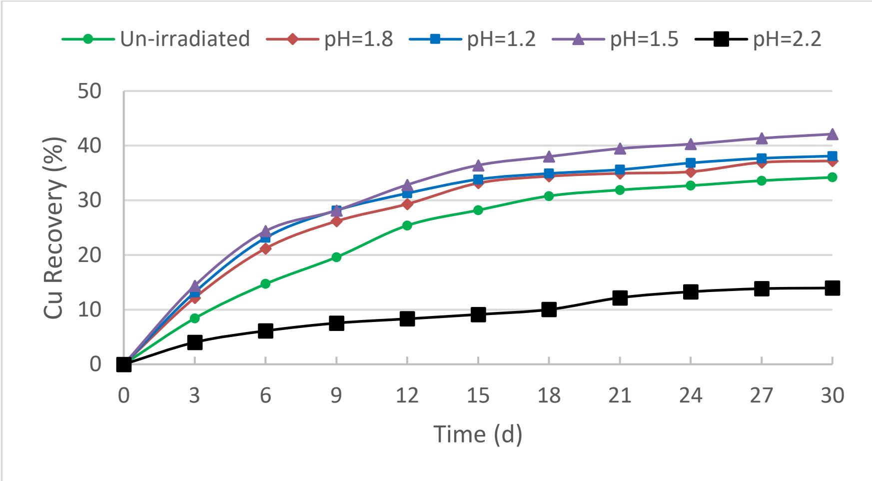

Fig. 5. Effect of initial pH changes on copper recovery using optimally irradiated mesophilic mixed culture (temperature= 32°C and agitation rate= 125 rpm)

Optimizing temperature

Fig. 6 shows the copper recovery during the temperature optimization tests while the other bioleaching condition, i.e. initial pH 1.8 and agitation rate 125 rpm were constant. Maximum copper recovery was obtained by optimally irradiated culture at temperature 44°C after 30 days. This recovery was about 3 and 6 percentages higher than recoveries obtained by irradiated culture at 32°C and by un-irradiated culture, respectively. As can be seen, enhanced activity of irradiated cultures, due to microwave irradiation, result in higher copper extraction kinetic at the initial stage of extraction. Furthermore, copper extraction at 44°C after 18 days was more than what achieved at 32°C after 30 days, which could be due to positive effect of increased temperature on extraction rate. The main result of these experiments was that optimally irradiated bacteria will be able to tolerate higher temperatures.

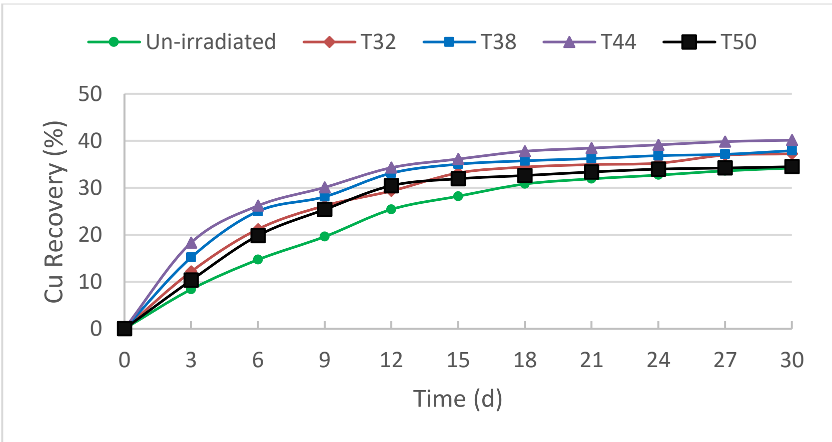

Fig. 6. Effect of temperature changes on copper recovery using optimally irradiated mesophilic mixed culture (initial pH= 1.8 and agitation rate= 125 rpm)

Optimizing agitation rate

Fig. 7 shows the copper recovery during the agitation rate optimization tests while the other bioleaching condition, i.e. initial pH 1.8 and temperature 32 °C were constant. With increasing agitation rate from 125 to 175 rpm, no remarkable change in copper recovery was observed. Increase the agitation rate to 200 rpm led to sharp drop of copper recovery. This could be related to remarkable bacterial population loss due to higher sear force created by higher agitation rate. Therefore, it can be concluded that, increasing agitation rate had no positive effect on copper recovery by optimally irradiated culture.

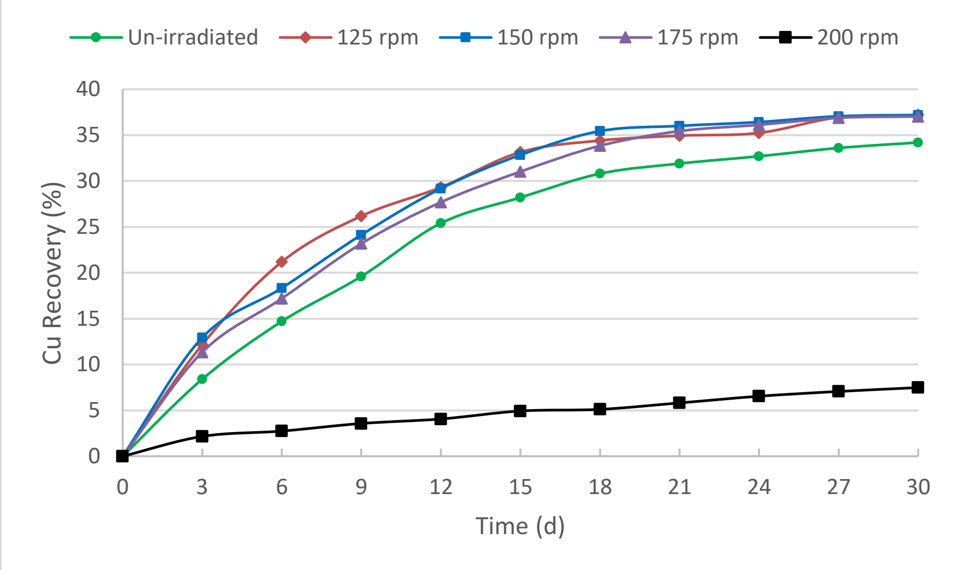

Fig. 7. Effect of agitation rate changes on copper recovery using optimally irradiated mesophilic mixed culture (initial pH= 1.8 and temperature= 125 rpm).

Bioleaching test under optimized condition

The operational condition including initial pH (1.2, 1.5, 1.8 and 2), temperature (32, 38, 44 and 50 °C) and agitation rate (125, 150, 175 and 200 rpm) were optimized for bioleaching using optimally irradiated mixed culture. Under optimum operational condition (initial pH=1.5, temperature=44 °C, agitation rate=125 rpm) maximum copper recovery reached to 44.36%. This recovery was 10.16% higher than the recovery obtained by control test with un-irradiated mixed culture. This recovery was also 7.15% higher than the recovery obtained by optimally irradiated mixed culture with the same operational condition as control test.

As is demonstrated in Fig. 8, due to the effects of microwave irradiation on oxidation strength of mesophilic strains, the rate of copper extraction with microwave irradiated mixed cultures (optimized and un-optimized) is higher than un-irradiated mixed culture. So, the copper recovery was reached to a comparable amount for un-irradiated, un-optimized and optimized mixed cultures after 30, 18 and 12 days, respectively.

The higher recoveries which are obtained using microwave irradiated cultures could be related to the enhanced oxidation rate of irradiated bacteria which means that, the extraction of copper before formation of passive layer could be more than ordinary bioleaching. Furthermore, irradiated mixed culture are able to tolerate higher temperature and can keep their activity at lower pH, which these are not favorable for formation of passivation layer [22-23].

CONCLUSION

The effects of microwave irradiations on operational condition of copper sulfide concentrate bioleaching by a mixed culture of commonly used mesophilic bacteria, including *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans*, were studied. The optimization of microwave irradiation was carried out by through measuring the oxidation rate of ferrous iron for iron oxidizing bacteria and elemental sulfur for sulfur oxidizing bacterium. Microwave irradiation under optimum time achieved 9.64%, 5.09% and 6.80% increase in oxidation rates for *Acidithiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *Acidithiobacillus thiooxidans*, respectively.

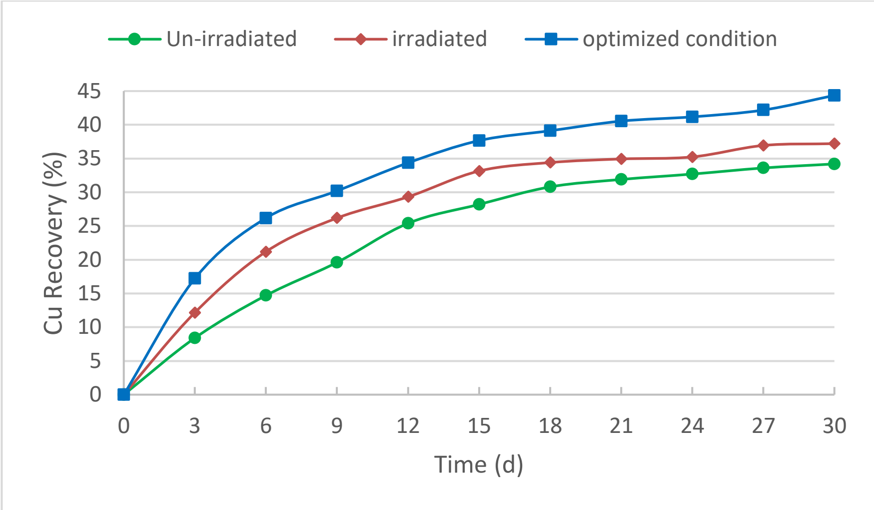

Fig. 8. Copper recovery by different mixed cultures; Un-irradiated: control test with un-irradiated mixed culture (initial pH 1.8, temperature 32 °C and agitation rate 125), Irradiated: test with optimally irradiated mixed culture with the same operational condition as control test, and Optimized condition: test with optimally irradiated mixed culture under optimized operational condition (initial pH 1.5, temperature 44 °C and agitation rate 125)

Using optimally irradiated mixed culture and due to the higher oxidation strength, the copper recoveries after 30 days bioleaching reached from 34.2% for un-irradiated mixed culture to 37.21%. Irradiated mixed culture also showed higher bioleaching rates and at the middle stages of bioleaching time reached to maximum copper recovery obtained by un-irradiated mixed culture.

The optimization experiments were carried out using optimally irradiated mixed culture on initial pH (1.2, 1.5, 1.8 and 2), temperature (32, 38, 44 and 50 °C) and agitation rate (125, 150, 175 and 200 rpm). Optimum condition which result in 44.36% of copper recovery after 30 days were as initial pH 1.5, temperature 44°C) and agitation rate 125 rpm. Under this condition, the copper recovery was reached to a comparable amount for un-irradiated, un-optimized and optimized mixed cultures after 30, 18 and 12 days, respectively.

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