Journal of Mining and Environment (JME) Vol. 13, No. 4, 2022, 1119-1138

Shahrood University of Technology

Journal of Mining and Environment (JME)

Journal homepage: www.jme.shahroodut.ac.ir

Iranian Society of Mining Engineering (IRSME)

Bio-flotation of Chalcopyrite using Halophilic Bacteria Separately and Their Combination as Pyrite bio-Depressant

Amirmohammad Nasrollahzadeh[1], Mohammad Jahani Chegeni[1], Ahmad Moghooeinejad[2], and Zahra Manafi [2]

1. Faculty of Mining, Petroleum & Geophysics Engineering, Shahrood University of Technology, Shahrood, Iran
2. Research and Development Center of Sarcheshmeh Copper Complex, Rafsanjan, Iran

Article Info

Received 30 September 2022
Received in Revised form 26 November 2022
Accepted 23 November 2022
Published online 20 November 2022

DOI:10.22044/jme.2022.12313.2234

Keywords

Bioflotation
Halophilic bacteria
Combination of bacteria
Chalcopyrite flotation
Pyrite depression

Abstract

Due to the increasing consumption of lime in the flotation process to increase the pH of the system and create an alkaline environment, as well as its gradual increase in cost, the attention of researchers has been drawn to perform flotation operations in a neutral environment. Halophilic bacteria have the potential to replace flotation reducers such as lime because flotation can be done with their help at neutral pH as well. Also, due to the buffer effect of sea water, which is the chosen medium for bio-flotation, the use of bio-flotation method reduces the use of drinking water, and also reduces the consumption of chemicals. In this research work, five types of halophilic bacteria are studied for pyrite bio-depression and chalcopyrite flotation. Bio-flotation experiments are conducted using Hallimond tubes, and the bacteria Halobacillus sp., Alkalibacillus almallahensis, and Alkalibacillus sp. had better performance in pyrite depression and chalcopyrite flotation than other bacteria. The recovery of pyrite depression when using them was 30.9, 30.3, and 34.0 %, respectively, and the recovery of chalcopyrite flotation by them was equal to 52.9, 68.6, and 55.7, respectively, which indicates the high selectivity of these bacteria in flotation. In addition to the above tests, the effect of the combination of these three types of bacteria on pyrite depression and chalcopyrite flotation was also studied. The results obtained indicate that in the combination (mix) test of all three types of bacteria (33.3% of each type), pyrite was depressed better than other tests, and its recovery was 27.5%, which was lower than the single bacteria tests. Also, the effect of the combination of these three types of bacteria on the flotation of chalcopyrite is investigated, and its recovery was 72.6%, which was higher than the single bacteria tests. On the other hand, considering that the recovery of chalcopyrite in the three-bacteria combination tests was is higher than the single-bacteria and two-bacteria tests, it can be concluded that the combination of all three bacteria can cause a better synergism and improve their performance in micro-flotation tests.

1. Introduction

Recent advances in biotechnology, in addition to the growth of hydrometallurgical processes, have also had significant effects on the processing of minerals. These effects have appeared more than anything else in bio-flotation. The cause of these effects can be related to the abilities of the biological science. Microorganisms and their products are readily available. Therefore, they can be produced easily and at a minimal cost, as *in situ*, and they are harmless in many cases. As a result, unlike the synthetic chemical reagents, their entry into nature will not have a negative impact on the environment. In addition, with recent advances in genetic science, it has been possible to train microorganisms to achieve more favorable selectivity [1]. Today, the ability of some bacteria to produce surface-active compounds is well known. During the last two decades, especially with the spread of concerns regarding the preservation of the environment, attention to the production and study of these natural products has been very impressive. These surface-active

Corresponding author: m.jahani1983@gmail.com (M. Jahani Chegeni)

compounds, called microorganisms, are a potential alternative to chemicals that are derived from petroleum. The most important advantages of microorganisms are low toxicity, better compatibility with the environment, natural degradability, and preservation of properties in a wide range of pH values and temperatures. In addition, the sources of microorganisms are easily accessible and reproducible. As a result, the production process will have lower costs in many cases [2].

Halophilic bacteria are a group of bacteria that are adapted to grow in extreme conditions such as high salt concentrations. These conditions are usually achieved due to the creation of severe osmotic shocks and high concentrations of chloride ions, which are harmful to the growth of bacteria. Halophilic bacteria are also known to produce extracellular polymeric substances (EPSs) with interesting properties such as emulsifying or antibacterial properties. So far, halophiles have been used to produce bioplastic polyhydroxyalkanoates (PHAs), actins, enzymes, and bio-surfactants. There are increasing effects for the development of halophiles into a low-cost infrastructure for bio-treatment with the benefits of low energy, less freshwater consumption, low fixed capital investment, and continuous production [3]. For growth in a hypersaline environment, the main adaptation mechanism to prevent the diffusion of NaCl into the cells is the accumulation of inorganic ions (mainly KCl) to balance the osmotic pressure. This mechanism is mainly used by aerobic halophilic bacteria and some anaerobic halophilic bacteria [4, 5]. On the other hand, most halophilic bacteria accumulate water-soluble organic compounds with low molecular weight, called compatible solutes or osmolytes, to maintain low intracellular salt concentration [6-8]. Compatible solutes can also act as stabilizers for biological structures, allowing cells to adapt not only to salts but also to heat, desiccation, cold or even freezing conditions [9]. As a result, they allow the halophile to grow at a pH of about 10 and at a temperature of more than 50 $^{\circ}$C [10]. Many halophilic bacteria accumulate ectoine or hydroxyectoine as the predominant compatible solute. Other compatible intracellular solutes include amino acids, glycine betaine, and other osmotic solutes accumulated in small amounts [11-13].

Pyrite is the most abundant and important iron sulfide mineral, which has a negative effect on the selectivity of copper flotation. Pyrite is considered as a waste mineral, and is usually associated with valuable minerals such as galena, sphalerite, and chalcopyrite, and dealing with it in selective flotation is an important issue because pyrite, due to its high hydrophobicity or activation by copper or lead ions from other minerals, is easily floated and transferred to the concentrate of other minerals. The presence of pyrite in the concentrate of other minerals reduces the grade of the concentrate and lowers its economic value. Therefore, it is necessary to use depressants and microorganisms to selectively depress pyrite and prevent it from entering the flotation concentrate. As a result, the role of halophilic bacteria is with the biological reduction of pyrite as a reagent in replacing lime as a depressing agent, and the flotation of pyrite will be significantly reduced depending on the halophilic bacteria used [14].

Chalcopyrite is a valuable mineral that must be floated with the use of halophilic bacteria; it is possible to witness a significant decrease in sedimentation and an increase in its flotation [14]. In 2000, Sharma *et al*. investigated the bio-flotation of sulfide minerals in the presence of heterotrophic and chemolithotrophic bacteria, and concluded that pyrite flotation was reduced by using xanthate collector, while this collector had no effect on the chalcopyrite flotation [15]. This work was done using optical and infrared spectroscopic studies and according to their flotation responses in the presence of *Thiobacillus ferrooxidans* and *Paentbacillus polymyxa* cells compatible with chalcopyrite [15]. In 2003, Hosseini Tabatabai *et al*. investigated the feasibility of bio-flotation of Sarcheshemeh copper sulfur ore, and they found that the pyrite mineral contained in the ore was depressed by using the bacterium *Thiobacillus ferrooxidans* and xanthate collector, while the bacterium in question had no effect on the flotation of chalcopyrite and other copper sulfide minerals [16]. In 2004, Kolahdoozan *et al.* investigated the bio-flotation of Sarcheshmeh copper sulfide ore, and studied the use of *Acidithiobacillus ferrooxidan* bacteria in the flotation of Sarcheshmeh low-grade copper sulfide ore. The results showed that the recovery of pyrite in the presence of bacteria was 50% less than when there were no bacteria; however, the recovery of chalcopyrite remained unchanged [17]. In 2005, Hosseini *et al*. investigated the bio-flotation of Sarcheshmeh copper ore using *Thiobacillus ferrooxidans* bacteria. The results showed that the recovery of pyrite in the presence of bacteria (*Thiobacillus ferrooxidan*) was 50% lower than in the absence of any bacteria, which indicated the reducing effect of bacteria on pyrite. It was also

concluded that the use of *Thiobacillus ferrooxidans* reduced the recovery of pyrite but did not change the floatability of chalcopyrite [18]. The obtained result was similar to Kolahdoozan *et al*. [17]. In 2008, Botero *et al*. investigated the effect of flotation of calcite and magnesite using *Rhodococcus opacus* bacteria. The results of bio-flotation for magnesite and calcite were about 93% for *R. opacus* concentration of 200 ppm at pH of about 5% and 55% for *R. opacus* concentration of 220 ppm at pH of about 7, respectively. Using the thermodynamic method, the results showed that the total Gibbs free energy of adhesion of this bacterium on magnesite was more negative than the calcite system. Therefore, this approach predicts that the affinity of this bacterium for magnesite is higher than calcite [19]. In 2011, Govender and Gericke investigated extracellular polymeric substances (EPSs) from bioleaching systems and their application in bio-flotation [20]. Analysis of EPS extracted from different bioleach systems showed that EPS was mainly composed of carbohydrates, proteins, and uronic acids. In micro-flotation experiments using free EPS, 77% chalcopyrite recovery was achieved when chalcopyrite was floated alone. Also, 70% recovery of chalcopyrite was obtained during the separation of a mixture of pure chalcopyrite and pure pyrite. In general, the obtained results showed that the flotation of chalcopyrite could be significantly increased in the presence of EPS extracted from the bioleaching operation [20]. In 2011, Khoshdast *et al*. investigated the possibility of flotation of copper ores using rhamnolipid bio-surfactants as frother. They found that the biosurfactant had a negative effect on the flotation of copper and molybdenum minerals so that the efficiency of the operation decreased with the increase in the concentration of the bio-surfactant. It is worth mentioning that biological surfactants have a positive effect on the flotation of iron-containing minerals (pyrite, hematite and limonite). This effect is due to the ability of bio-surfactant to carry heavy particles and possible positive interaction on their hydrophobicity [21]. In 2016, Kim *et al*. investigated malachite bio-flotation from complex systems using *Rhodococcus opacus* bacteria. In that study, for the first time, the effect of bacterial growth phase on malachite flotation in a well-controlled Hallimond tube system was investigated. The results of the experiments showed that the bacteria in the stationary phase floated twice more than the bacteria in the middle exponential phase. In general, both phases showed recovery and grade of more than 90%. This trend was consistent with the classical DLVO interaction energy profiles, which showed the relative magnitude of adhesion forces between the ore and the attached cell under equal hydrodynamic conditions [22]. In 2017, Olivera *et al*. investigated the fundamental aspects of hematite bio-flotation using a Gram-positive strain with the isoelectric point (IEP) of hematite shifted after biomass interaction. This showed that the bacterial cells were attached to the mineral surface. In addition, bacterial adhesion was higher at acidic pH, indicating an electrostatic attraction between the mineral surface and biomass in this pH range. Micro-flotation experiments were performed in a modified Hallimond tube by achieving the maximum hematite flotation of 86.83% at pH = 6 [23]. In 2017, Kim *et al*. addressed the feasibility of selective flotation of copper oxide minerals using *Rhodococcus opacus* bacteria. The results showed that the values of recovery and grade of malachite for finer stones (recovery = 29.15% and grade = 5.17%) were much higher than their values for coarse stones (recovery = 1.34% and grade = 3.06%), which was probably due to the difference in the degree of freedom of malachite. Also, a further comparison of the result of fine ore with the result of the conventional process (i.e. sulfidation following xanthate absorption) showed that the selective separation of malachite in bio-flotation was much higher than the conventional process [24]. In 2022, Abedi Ashkavandi *et al*., in a research for the first time, studied the effect of *Bacillus licheniformis* bacteria and its metabolites for the selective flotation of barite from quartz. Bio-flotation experiments showed that recovery of barite up to 87% was possible at pH = 3 with the help of *Bacillus licheniformis* bacteria [25]. In 2020, Simões *et al*. addressed the electro-flotation of fine and ultrafine particles of an itabirite iron ore using a bio-surfactant extracted from *Rhodococcus opacus* bacterium; the recovery of iron using this method was about 83% [26]. In a 2021 study, El-Sayed *et al*. used *Bacillus cereus* bacterium to enhance gold flotation in the presence of potassium butyl xanthate (PBX) as a collector and pine oil as a frother, and achieved a 95% gold recovery. Also, they found that pH could strengthen or weaken the bio-flotation of gold [27]. Pineda and Godoy in 2019, in a research work, by studying the bio-oxidation of pyrite in Colombian coal using *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* bacteria, found that in the presence of bacterial cells, the oxidation of pyrite in experiments containing 60 mg/L of cysteine, 8.18% increased [28]. In 2021, Çelik *et*

al. investigated the effect of bio-surfactant collection obtained from *Bacillus subtilis* bacteria on the flotation of calcite mineral; the recovery of calcite through bio-flotation was about 80% [29]. In 2020, Consuegra *et al*. investigated halophilic bacteria as pyrite bio-depressants in copper and molybdenum bio-flotation, and by using five types of halophilic bacteria as potential biohazard reducers of pyrite, found that the selective separation of pyrite was greatly increased in the presence of *Halobacillus* bacteria. They also found that these types of bacteria had interesting properties such as emulsification. They found that another useful feature of these bacteria was their inherent ability to grow in salty environments such as seawater. The results of their research work showed that the biological recovery of pyrite (gangue mineral) using halophilic bacteria as a substitute agent for lime and reducer of pyrite micro-flotation had reached from about 68% to 10% depending on the bacteria used. The results of their research work showed that the biological recovery of pyrite (gangue mineral) by using one of the halophilic bacteria as a substitute agent for lime and reducer of pyrite micro-flotation had reached from about 68% to 10%. Also, the recovery of chalcopyrite (valuable mineral) using another halophilic bacterium had increased from 40% to 54% [2]. However, in the research work conducted by the authors of this article, there was a significant difference with reference [2], and it is that the three types of bacteria used simultaneously had the ability to depress pyrite and float chalcopyrite. The use of sea water as processed water in order to reduce the consumption and costs of using fresh water in the flotation operation of minerals in Las Luces copper, and molybdenum plant in Taltal (Chile) was investigated by Moreno *et al*. and favorable results were obtained. Therefore, mines like Sierra Gorda in the Atacama Desert use sea water to float minerals [30].

The aim of the present research work is the bio-flotation of chalcopyrite using halophilic bacteria separately and their combination as pyrite bio-depressants. To achieve this goal, bio-flotation tests were performed in control mode (no bacteria) with sodium isopropyl xanthate (SIPX) collector using Hallimond tube. Then bio-flotation tests were performed using five types of halophilic bacteria as pyrite biological depressants, and it was found that three bacteria *Alkalibacillus almallahensis*, *Halobacillus sp.*, and *Alkalibacillus sp.* had a better performance in pyrite depression than other bacteria. Finally, by combining (mixing) these three types of bacteria (with different percentages of each of them), the effect of their combination on both chalcopyrite flotation recovery and pyrite depression recovery was also studied. Also, the recoveries of chalcopyrite flotation and pyrite depression were investigated and their values were compared with each other and with other modes, i.e. bacteria-free and single-bacteria modes.

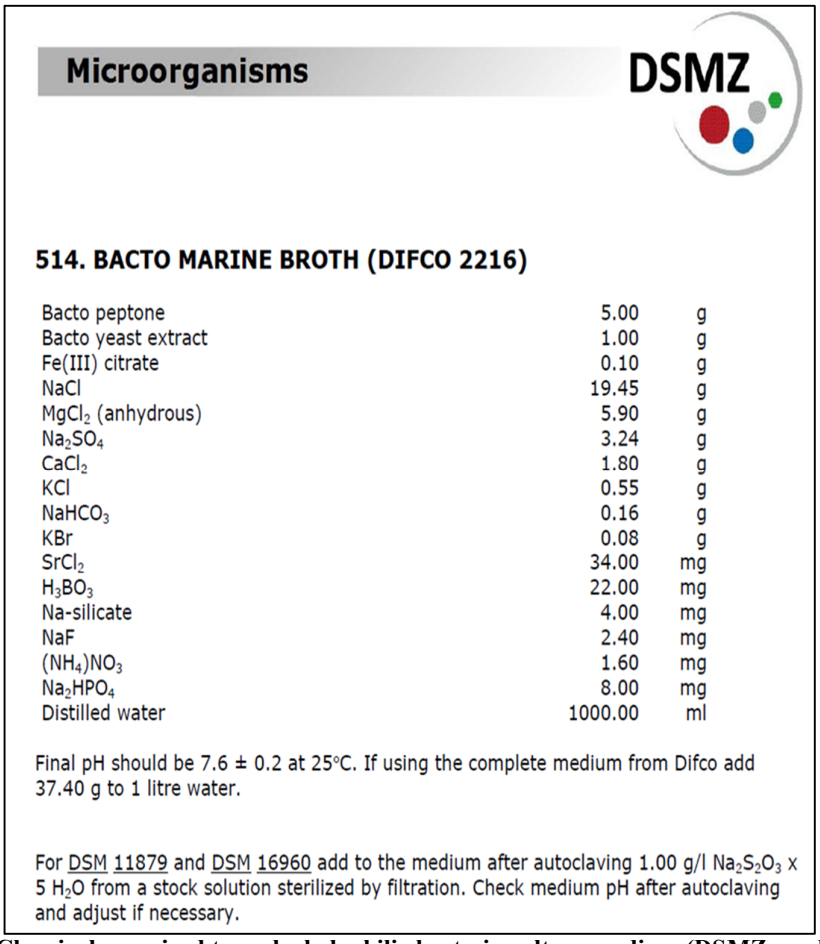
2. Materials and Methods

2.1. Taking samples from mine for bacterium cultivation

In order to extract halophilic bacteria, four one-kilogram samples of soil were collected from three points of Sarchesmeh copper mine with coordinates 29.950048, 55.879050, and 0.5 liters of mine water were also collected.

2.2. Cultivation of halophilic bacteria

The chemicals required to make the culture medium (DSMZ_Medium514) of halophilic bacteria are as follows (Figure 1):



Microorganisms DSMZ

514. BACTO MARINE BROTH (DIFCO 2216)

Bacto peptone	5.00	g
Bacto yeast extract	1.00	g
Fe(III) citrate	0.10	g
NaCl	19.45	g
$MgCl_2$ (anhydrous)	5.90	g
Na_2SO_4	3.24	g
$CaCl_2$	1.80	g
KCl	0.55	g
$NaHCO_3$	0.16	g
KBr	0.08	g
$SrCl_2$	34.00	mg
H_3BO_3	22.00	mg
Na-silicate	4.00	mg
NaF	2.40	mg
$(NH_4)NO_3$	1.60	mg
Na_2HPO_4	8.00	mg
Distilled water	1000.00	ml

Final pH should be 7.6 ± 0.2 at 25°C. If using the complete medium from Difco add 37.40 g to 1 litre water.

For DSM 11879 and DSM 16960 add to the medium after autoclaving 1.00 g/l $Na_2S_2O_3$ x 5 H_2O from a stock solution sterilized by filtration. Check medium pH after autoclaving and adjust if necessary.

Figure 1. Chemicals required to make halophilic bacteria culture medium (DSMZ, medium 514).

As seen in Figure 1, the pH of the culture medium should be set to approximately neutral and around 7.6 ± 0.2. Also, the growth temperature of bacteria varies between 30 and 37 °C and the time required for their growth was between 18 and 48 hours. It is worth mentioning that an optical microscope was used to count the number of bacteria, and the autoclave device of the Biohydrometallurgical Laboratory of Sarcheshmeh Copper Complex of Kerman was used to sterilize the culture media.

2.3. Determining type and strain of halophilic bacteria

Halophilic bacteria used for micro-flotation tests, cultivation conditions (using DSMZ_Medium514 and DSMZ_Medium514b culture media), stain type, method of preparation, country of origin, and the date of their first sampling could be seen in Table 1. It is worth mentioning that Medium514b has 17.5 g/L Agar compared to Medium514.

Table 1. Halophilic bacteria used for micro-flotation tests.

Bacteria name	Cultivation conditions	Strain designation	Isolated from	Country of origin	Date of sampling
Halobacillus sp.	Medium 514 , 30 °C	MA17	Unknown	Unknown	Before 1990/07/23
Marinobacter sp.	Medium 514 , 30 °C	CAB	Marine sediment	France	Before 1997/11/28
Alkalibacillus almallahensis	Medium 514 + 80 g/L NaCl, pH 8.0, 30 °C	S1LM8	Sediment from an inland solar saltern	Spain	2009/11/6
Alkalibacillus salilacus	Medium 514 + 8% NaCl + $MnSO_4$, 30 °C	BH163	Salt lake soil	China	2002/08
Alkalibacillus sp.	Medium 514b, 37 °C + 100 g/L NaCl + 17.5 g/L Agar)	YIM98829	Sediment soil	China	Unknown

2.4. Mineral samples

In this research work, two samples were used to perform bio-flotation tests. The first sample was an almost pure pyrite sample that was obtained from Sarchesmeh copper mine, which was used to investigate the recovery of pyrite depression. The second sample contained chalcopyrite, which was taken from the crushed soil sample from the conveyor belt No. 13 of Sarcheshmeh copper concentration plant in the amount of 5 kg to determine the flotation rate of chalcopyrite. The specifications of these samples could be seen in Tables 2 and 3. First, the samples were crushed by a laboratory jaw crusher, and the pyrite and chalcopyrite samples were ground with a laboratory ball mill of Sarcheshmeh Copper Complex pilot plant and reached -200 mesh (-75 µm) in size. Due to rapid oxidation of the surface of pyrite and chalcopyrite minerals, the samples were washed with 6 M HCl and distilled water and dried at 30 °C before being used in the micro-flotation tests.

Table 2. Characteristics of first sample used for pyrite depression.

Name/type of mineral	Chemical formula	Amount in sample (%)
Pyrite	FeS_2	68.024
Hematite	Fe_2O_3	0.810
Chalcopyrite	$CuFeS_2$	0.058
Metal minerals	-	68.892
Non-metallic minerals	-	31.090
Oxide minerals	-	0.018
Total	-	100

Table 3. Specifications of second sample used for chalcopyrite flotation.

Name/type of mineral	Chemical formula	Amount in sample (%)
Chalcopyrite	$CuFeS_2$	0.656
Pyrite	FeS_2	14.093
Molybdenite	MoS_2	0.008
Sphalerite	ZnS	0.526
Hematite	Fe_2O_3	0.249
Magnetite	Fe_3O_4	0.314
Covellite	CuS	0.021
Metal minerals	-	15.867
Non-metallic minerals	-	84.098
Oxide minerals	-	0.035
Total	-	100

2.5. How to perform bio-flotation tests

Bio-flotation experiments were performed using a Hallimond tube with 160 mL of distilled water. 1.5 g of sample in -200 mesh fraction was used for the experiments. Sodium isopropyl xanthate (SIPX) or Z11 was used as a collector and the combination of bacteria as a frother and depressant (Figure 2). Then 10 mL of bacteria were prepared from special culture medium, and 1.5 grams of mineral was added to the Hallimond tube at 300 rpm; this process lasted for 5 minutes. Then SIPX solution (0.1% (volume/weight), 0.24 mL) was added to the system, and aeration was performed for 5 minutes with a flow rate of 2-4 NL/min by dry air. In the next step, the concentrate and tailing were collected, and before measuring their recovery and grade by mineralogical and XRD methods, they were filtered and dried by filter paper and oven device. Bacteria-free flotation tests were performed as control tests (first stage), and all tests were repeated as many times as necessary (ten times) because at least 3 grams of minerals were required to perform XRD and mineralogical analyses. Then single bacteria tests (second stage) using five types of halophilic bacteria *Halobacillus sp.*, *Marinobacter sp.*, *Alkalibacillus almallahensis*, *Alkalibacillus salilacus*, and *Alkalibacillus sp.* as biodepressants of pyrite and using Hallimond tube; the bacteria *Halobacillus sp.*, *Alkalibacillus almallahensis*, and *Alkalibacillus sp.* had a better performance than the other two bacteria. For this reason, only these three types of bacteria were used in the combined (mix) tests (third stage). In the third stage, the combined tests were performed with four different combinations of the three mentioned bacteria as what follows. First, a combination of all three types of bacteria was used in the same proportion (33.3%) to perform the tests. Then in other cases,

only the combination of two types of the above three types of bacteria was used, and the tests were performed with an equal proportion (50%) of each of them.

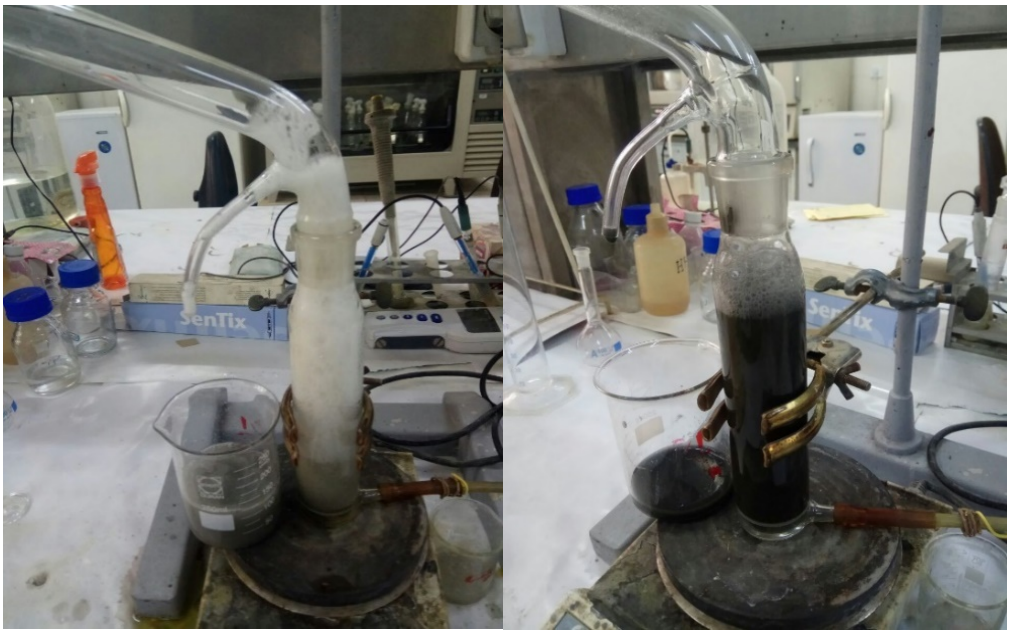


Figure 2. Micro-flotation experiments using Hallimond tube; chalcopyrite (right) and pyrite (left).

3. Results and Discussion

3.1. Results of growth of halophilic bacteria in measured pHs and Ehs

The results of cultivation of halophilic bacteria could be seen in Table 2. In total, eleven samples were prepared and named from zero to ten. Samples number zero to three included soil collected from different parts of Sarchesmeh copper mine. Sample number four was prepared from the combination of samples number zero to three. Also, sample number five included mine spring water. On the other hand, sample number six was taken from the bottom of the mine. Also, samples number seven and eight were prepared from the combination of bacteria from samples zero to six along with 50 and 25 grams of chalcopyrite, respectively. In addition, sample number nine was also prepared from the combination of bacteria from samples zero to six along with 50 grams of pyrite. It is worth mentioning that the reason for adding chalcopyrite and pyrite to samples number seven, eight, and nine was to observe the effect of the produced bacteria on them. In samples zero to nine, 200 mg of culture medium was added to the samples. Also, sample number ten was prepared from the combination of bacteria from samples number zero to nine along with 250 mg of culture medium in order to determine and isolate the type and strain of halophilic bacteria. It is worth mentioning that the bacteria of sample number ten, which are a mixture of two or more halophilic bacteria, are called (mix culture). In this research work, sample number ten was used to purify five types of halophilic bacteria. The method of purifying halophilic bacteria is that after making the required five types of bacteria cultures (Table 1), using the bacteria in sample number ten, the bacteria in this sample were added to each culture medium using a sampler. After a period of 24 to 48 hours, when the bacteria reached the desired growth, the grown bacteria were again added to their new culture medium using a sampler and this process was repeated 2 to 3 times; this repetition operation is called sub-culture. Also, the initial and secondary weights of the sample could be seen in Table 4. The initial weight included the weight of the prepared sample, the culture medium (medium 514), and the weight of laboratory Erlenmeyer before placing the samples in the incubator. It is worth noting that after placing the samples in the incubator due to its high temperature, the volume of the samples decreases slightly, and by adding distilled water, their volume is almost brought to the initial value (secondary weight). In the same way, in order to bring the initial pH of the medium to 7.6 ± 0.2, that is, the pH suitable for the growth of halophilic bacteria in medium 514 (secondary pH), pH regulators, NaOH, and H_2SO_4 were used (Table 4). In addition,

the Eh values were measured for all eleven mentioned samples. On the other hand, in order to count the number of bacteria, according to the design of the optical microscope slide used in the form of four-by-four grids (sixteen grids), the average number of bacteria in each grid was counted, and their results could also be seen in Table 4. It is worth mentioning that the speed of the incubator for all eleven mentioned samples was 130 rpm, and its temperature was 30 °C.

Different microorganisms have different sensitivities to the oxidation and reduction potential of different culture media. This factor is defined as the ability of a substance to gain or lose electrons. In general, when an element or chemical compound loses electrons, it is said to be oxidized, and on the contrary, the element or compound that receives electrons is reduced. Therefore, a substance that easily loses electrons is considered a good reductant (with a lower Eh), and a substance that easily gains electrons is a good oxidizer (with a higher Eh). When an electron is transferred from one object to another, a potential difference is created between them, which can be measured in millivolts (mV). In general, the more chemical compounds are oxidized; the higher their electrical potential will be, and on the contrary, the reduction of a substance causes its electrical potential to decrease in the same proportion. Therefore, according to the reduction property of halophile bacteria (lower Eh), in samples 7, 8, 9, and 10, the number of halophile bacteria in these samples has increased compared to other cases (Table 4).

Table 4. Results of cultivation of halophilic bacteria.

Sample number	Initial weight (g)	Secondary weight (g)	Initial pH	Secondary pH	pH regulator type	Volume of pH regulator (mL)	Eh (mV)	Number of bacteria
Zero	372.31	373.57	6.80	7.64	NaOH	1.07	140	6×16
1	519.30	523.65	5.94	7.59	NaOH	2.60	353	9×16
2	505.59	509.68	6.49	7.68	NaOH	0.78	329	10×16
3	489.51	494.13	6.64	7.50	NaOH	0.33	279	10×16
4	734.10	742.32	7.03	7.58	NaOH	0.21	249	12×16
5	424.94	428.06	8.45	7.80	H_2SO_4	0.06	85	15×16
6	548.43	553.16	7.68	7.68	-	-	153	3×16
7	514.09	519.76	7.79	7.79	-	-	-32	18×16
8	451.41	455.00	7.96	7.45	H_2SO_4	0.04	-76	14×16
9	405.13	407.93	7.91	7.68	H_2SO_4	0.04	-214	20×16
10	467.12	473.93	7.77	7.77	-	-	-232	25×16

Figures 3 and 4 show, respectively, the changes in pH and Eh resulting from the growth of bacteria in the soil of samples number zero to nine. As seen in Figure 3, after 13 days of bacteria cultivation, the initial pH values in Table 4 were obtained, and it was tried to return the pH values to the corresponding values on day zero (secondary pH in Table 4) to form sample number 10. Also, in Figure 4, Eh values measured on the thirteenth day are reported in Table 4. It is worth mentioning that sample number ten was the basis for the continuation of the research conducted in this study and was used for purification of five types of bacteria (Figures 5 and 6). Figures 5 and 6, respectively, show the changes in pH and Eh resulting from the growth of five halophilic bacteria studied in this research work with time. As it can be seen, in Figure 5, with the passage of time, the pH of the culture medium of *Halobacillus sp.* has increased from about 2.6 to about 4.8 after one day. After four days, it has decreased again to about 2.6. Then on the seventh day, it reached about 8.2, on the tenth day, it reached about 6.3, and on the thirteenth day, it reached about 8.2 again. As it can be seen, the pH is increasing and decreasing alternately. There is almost a similar trend for other bacteria. In general, there is no clear trend between the pH of the culture medium and the preparation time. As it is clear in Figure 6, and according to the previously mentioned content, i.e. the reduction properties of halophilic bacteria, Eh values have decreased drastically with the passage of time. For example, in the case of *Halobacillus sp.* bacteria, the Eh value was around 160 mV on the 0th day, which reached -320 mV on the 13th day, which is a significant decrease. Other bacteria have shown a completely similar trend.

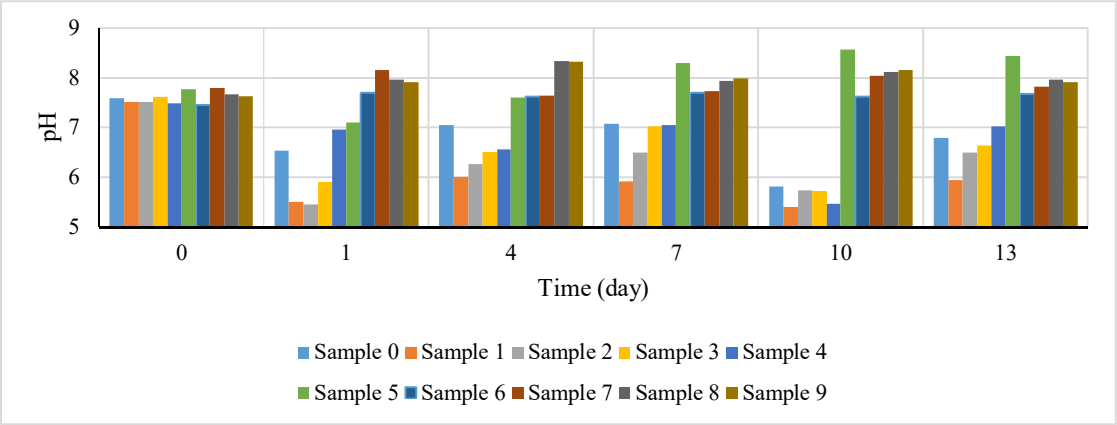


Figure 3. pH changes resulting from growth of present bacteria in soil of samples zero to nine.

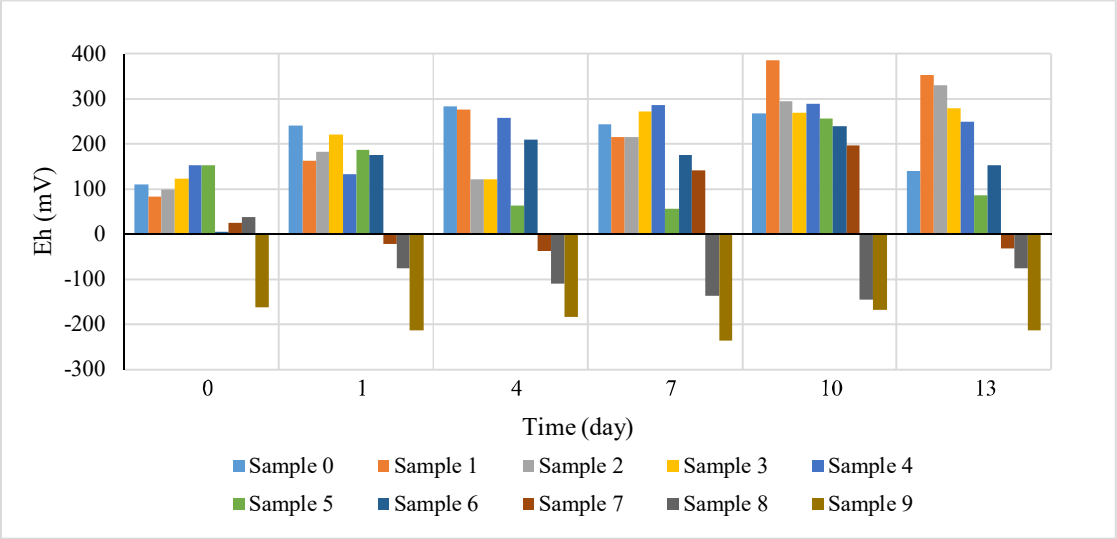


Figure 4. Eh changes resulting from growth of present bacteria in soil of samples zero to nine.

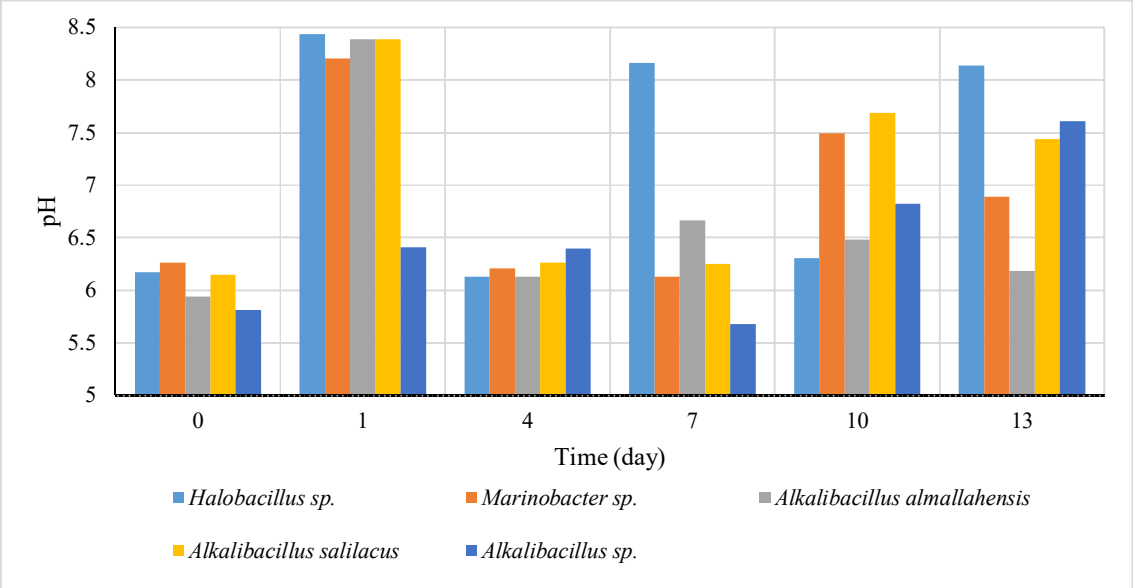


Figure 5. pH changes resulting from growth of halophilic bacteria over time.

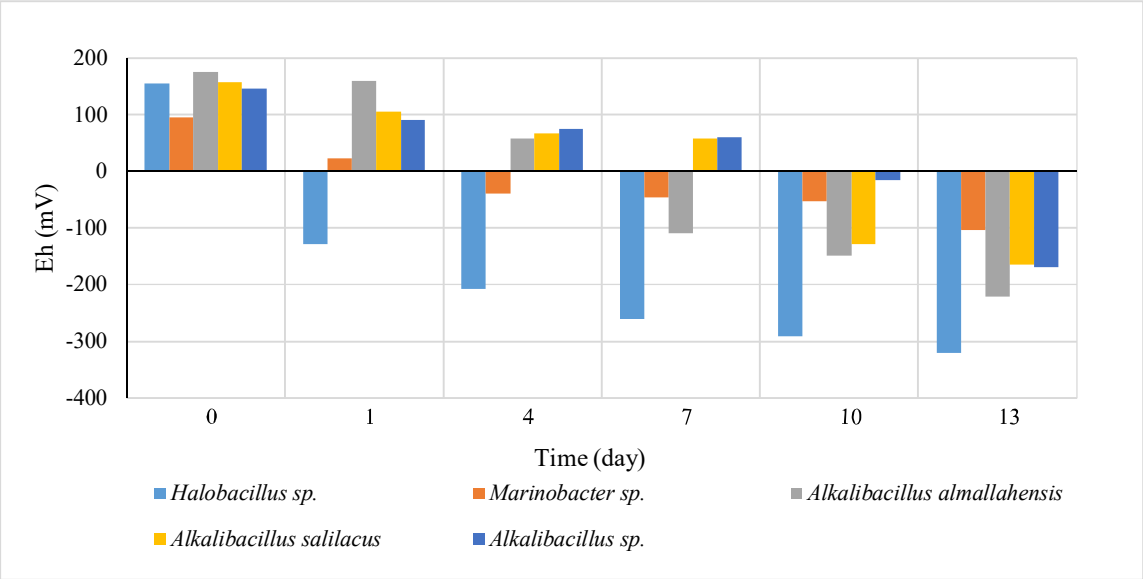


Figure 5. Eh changes resulting from growth of halophilic bacteria over time.

3.2. Parameters affecting pyrite depression and chalcopyrite flotation by halophilic bacteria

According to the conditions of performing micro-flotation experiments of pyrite and chalcopyrite by five types of halophilic bacteria (Table 1), the only variable factor was the type of bacteria in Hallimond tube. Also, considering that halophilic bacteria could replace lime, flotation tests were performed at almost neutral pH (7–8). In addition, the concentration and volume of Z11 (SIPX) collector used for micro-flotation tests were equal to 1 g/L and 0.24 mL for all tests, respectively. In addition, the volume of bacteria added in single bacteria micro-flotation tests was 10 mL, and in the bacteria-free tests, it was zero. Also, the volume of distilled water added to the Hallimond tube was 160 cc, the mass of the dry mineral added inside it was 1.5 g, the stirring speed was 300 rpm, and the flow rate of the air blown into the Hallimond tube was between 2 and 4 nanoliters per minute (NL/min) in all tests (Table 5). As it could be seen in Table 5, a total of 12 tests were performed for the bacteria-free and single-bacteria modes (five types of bacteria) for pyrite depression and chalcopyrite flotation.

Table 5. Tests performed for bacteria-free and single-bacteria modes (five types of bacteria) for pyrite depression and chalcopyrite flotation.

Test number	Bacteria name	Mineral type
1	No bacteria	Pyrite
2	*Halobacillus sp.*	Pyrite
3	*Alkalibacillus almallahensis*	Pyrite
4	*Alkalibacillus salilacus*	Pyrite
5	*Alkalibacillus sp.*	Pyrite
6	*Marinobacter sp.*	Pyrite
7	No bacteria	Chalcopyrite
8	*Halobacillus sp.*	Chalcopyrite
9	*Alkalibacillus almallahensis*	Chalcopyrite
10	*Alkalibacillus sp.*	Chalcopyrite
11	*Alkalibacillus salilacus*	Chalcopyrite
12	*Marinobacter sp.*	Chalcopyrite

3.3. Recoveries obtained from bacteria-free and single-bacteria micro-flotation tests for pyrite depression

According to the volume of Hallimond tube, only about 1.5 grams of mineral could be introduced into it. Also, due to the fact that at least 3 grams of minerals were required to perform mineralogical analyses, all tests in the bacteria-free and single-bacteria modes were repeated in the same conditions (ten times). In Table 6, the data obtained from the process of pyrite micro-flotation using the mineralogical method can be seen. As it can be seen, in test number 1 (bacteria-free mode), the tailing grade is lower than other tests (single bacteria tests) (37.175%). On the contrary, the concentration grade in this test is higher than that of single bacteria modes (97.275%). In tests 2 to 6 (single bacteria modes), the highest tailing grade is related to test number 6 and *Alkalibacillus sp.* bacterium (63.176 %). Also, the lowest concentration grade is related to this test (80.491%), which indicates the proper performance of this bacterium in pyrite depression. Also in Figure 7, pyrite depression recovery from micro-flotation tests can be seen. As it can be seen, the pyrite recovery in the bacteria-free mode is more than the single bacteria modes (77.4 %), which shows that the bacteria had the ability to depress pyrite. The depression recovery of pyrite by *Alkalibacillus almallahensis* bacterium was higher than other bacteria. Adhesion to the pyrite surface by hydrophobic bacteria such as *Halobacillus sp.*, *Marinobacter sp.*, and *Alkalibacillus almallahensis* was observed with a good trend, which is in accordance with the research done by Consuegra et al. and Pérez-Davó *et al*. [2, 31]. Hydrophobic bacteria tend to stick better to surfaces and accumulate more than hydrophilic bacteria because hydrophilic bacteria tend to disperse, and also do not work well in sticking to surfaces. However, due to being hydrophilic and being 100% surfactant, *Alkalibacillus sp.* bacterium showed a good performance in the pyrite depression, which is in accordance with the research conducted by Wiegel and Mesbah [32]. Also, *Alkalibacillus salilacus* bacterium had a relatively good performance in pyrite depression due to its hydrophilic property, which is in accordance with the research conducted by Samaei-Nouroozi *et al*. [33]. Compared to the hydrophilic bacteria, which tend to disperse in solutions with high ionic strength, hydrophobic bacteria accumulate at the air-water interface. This offers a plausible mechanism for further depression of pyrite by hydrophobic bacteria, because competition for the air-water interface occurs between the bacterium and the modified mineral. It cannot be concluded that this mechanism alone is selective for pyrite [34]. Another plausible explanation, using current understanding of the importance of pyrite surface oxidation for the adhesion of xanthate salts, is that adhesion of bacteria prevents oxidation of the mineral surface. Therefore, the adhesion of bacteria can reduce the amount of oxidizing sites, and thus the floatability of pyrite in this way [36, 35].

Table 6. Data from micro-flotation tests of pyrite depression by mineralogical method in bacteria-free and single-bacteria modes.

Test number	Bacteria name	Tailing weight (g)	Concentration weight (g)	Tailing grade (%)	Concentration grade (%)	Recovery (%)
1	No bacteria	5.88	7.70	37.175	97.275	77.4
2	*Halobacillus sp.*	10.77	3.67	62.833	82.471	30.9
3	*Marinobacter sp.*	9.78	4.88	59.808	85.075	41.5
4	*Alkalibacillus almallahensis*	11.21	3.52	62.388	86.391	30.3
5	*Alkalibacillus salilacus*	9.66	4.95	58.947	86.441	42.9
6	*Alkalibacillus sp.*	10.41	4.20	63.176	80.491	34.0

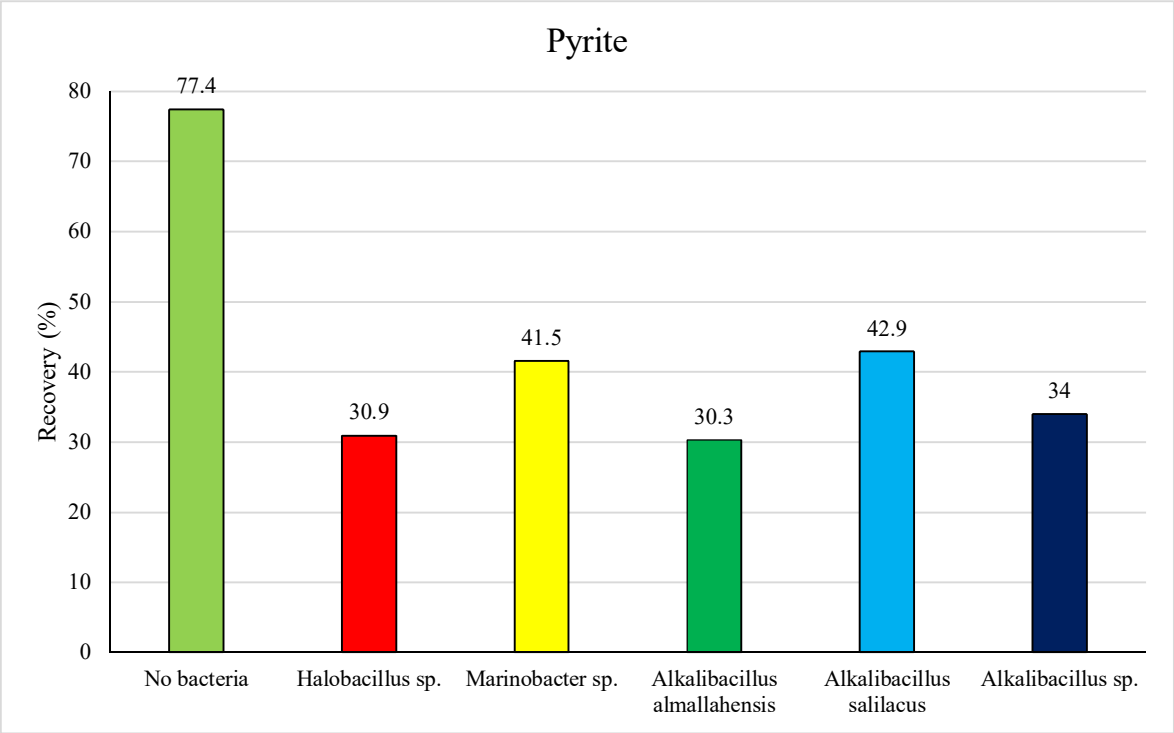


Figure 7. Pyrite depression recovery from bacterial-free and mono-bacterial micro-flotation tests.

3.4. Recoveries obtained from bacteria-free and single-bacteria micro-flotation tests for chalcopyrite flotation

The data obtained from the chalcopyrite micro-flotation process using the mineralogical method can be seen in Table 7. As it can be seen, in tests No. 3 and 5, which were performed using the bacteria *Marinobacter sp.* and *Alkalibacillus salilacus*, the concentration grade has decreased compared to the tailing grade, which indicates the unfavorable performance of these two bacteria in chalcopyrite flotation. However, in other modes, other bacteria have increased the concentration grade (tests 2, 4, and 6). It is noteworthy that in the bacteria-free mode (test 1) compared to other modes, the concentration grade has increased significantly. However, in this mode, the concentration weight is 2.90 grams, which is very small compared to other single bacteria modes. Therefore, to choose the right bacteria, recovery of chalcopyrite should be the basis of analysis. As it can be seen, in tests number 2, 4, and 6 in which the bacteria *Halobacillus sp.*, *Alkalibacillus almallahensis*, and *Alkalibacillus sp.* were used, the flotation recovery of chalcopyrite was higher than other modes. Also, in these tests, the concentration grade has also increased, which indicates the positive effect of these three types of bacteria on the flotation of chalcopyrite. Therefore, the combination of these three types of bacteria was used in the combined (mix) tests (Sections 3-5 to 3-7). In addition, the highest recovery of chalcopyrite was related to *Alkalibacillus almallahensis* bacterium with the value of 68.6 (Figure 8).

Table 7. Data from chalcopyrite micro-flotation tests by mineralogical method.

Test number	Bacteria name	Tailing weight (g)	Concentration weight (g)	Tailing grade (%)	Concentration grade (%)	Recovery (%)
1	No bacteria	8.03	2.90	0.78	1.61	42.7
2	*Halobacillus sp.*	6.76	5.06	0.62	0.93	52.9
3	*Marinobacter sp.*	6.22	6.81	0.96	0.89	50.4
4	*Alkalibacillus almallahensis*	5.95	7.16	0.54	0.98	68.6
5	*Alkalibacillus salilacus*	6.73	7.30	1.10	0.84	45.3
6	*Alkalibacillus sp.*	7.15	7.05	0.69	0.88	55.7

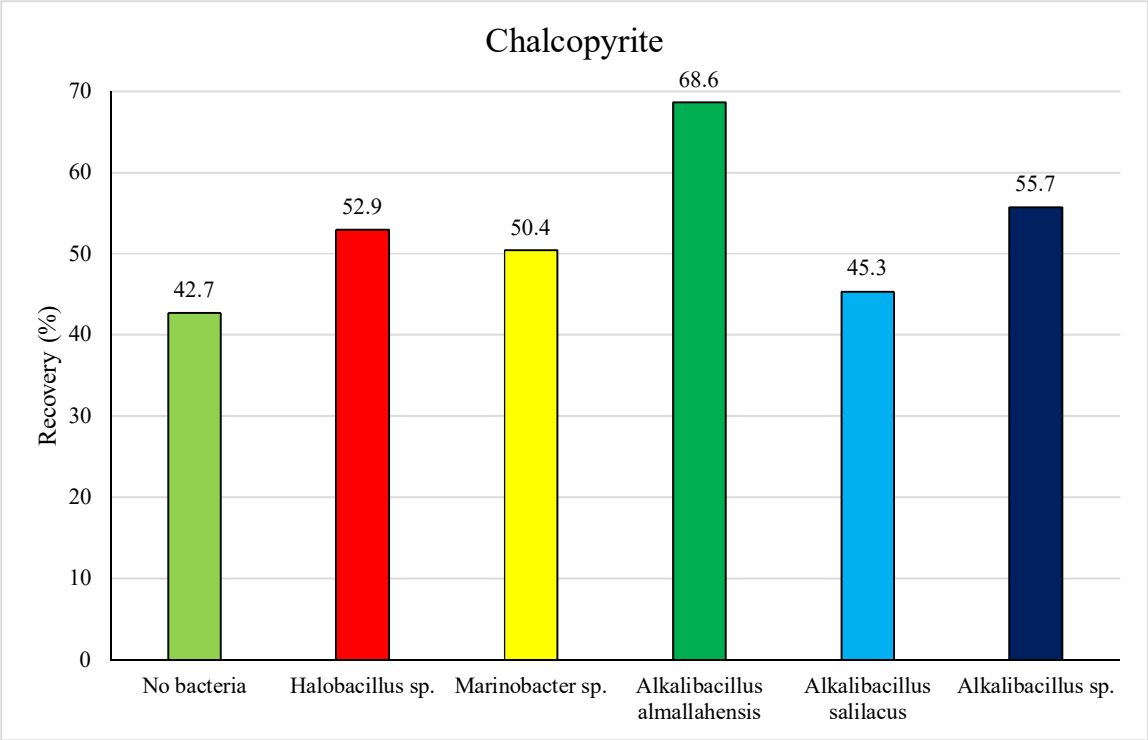


Figure 8. Chalcopyrite flotation recovery from bacteria-free and single-bacteria micro-flotation tests.

Figure 9 demonstrates the recoveries of pyrite depression and chalcopyrite flotation in bacteria-free and single-bacteria micro-flotation tests simultaneously. According to the shape, *Alkalibacillus almalallahensis* bacterium has both the ability to float chalcopyrite better and the ability to depress pyrite better than other bacteria, which is due to the hydrophobicity and greater tendency of this bacterium to stick to surfaces compared to other studied bacteria. On the other hand, it is observed that the selectivity of *Halobacillus sp.*, *Alkalibacillus almallahensis*, and *Alkalibacillus sp.* bacteria in chalcopyrite flotation and pyrite depression is higher than the control mode (no bacteria). It can also be concluded that bacteria of the *Alkalibacillus* family show a greater tendency to stick to the surface of chalcopyrite. This can lead to the hypothesis that there are some special surface features in the *Alkalibacillus* family that help their adhesion to the chalcopyrite surface compared to other halophilic bacteria.

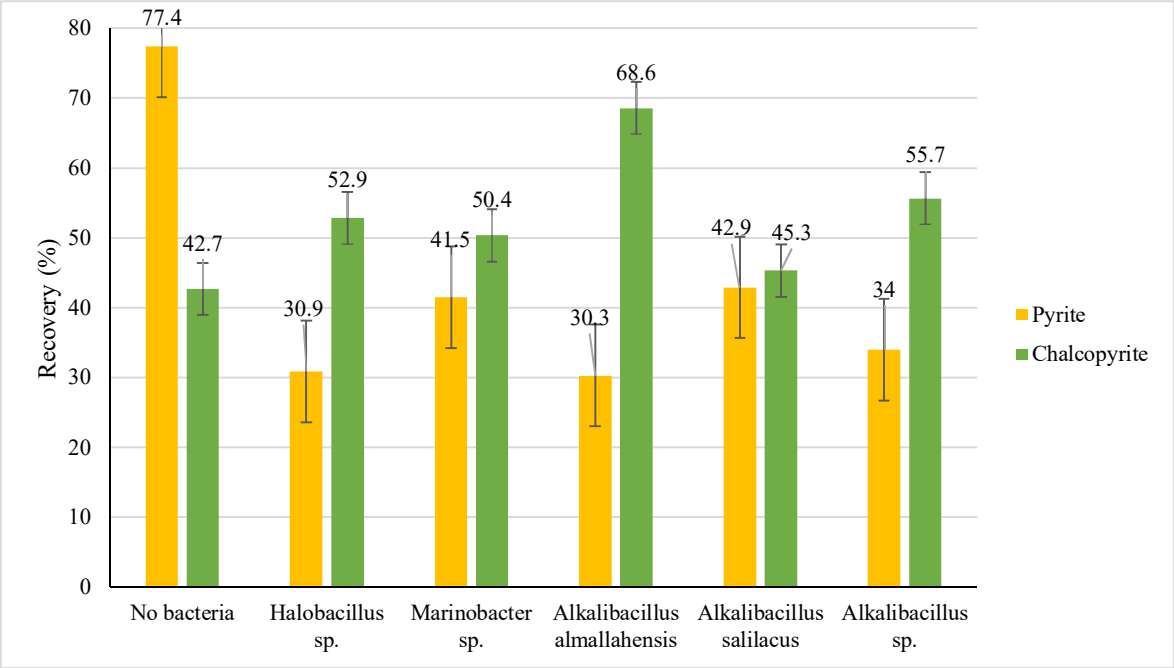


Figure 9. Pyrite depression and chalcopyrite flotation recoveries in bacteria-free and mono-bacterial micro-flotation tests.

3.5. Effect of combination of *Halobacillus sp.*, *Alkalibacillus almallahensis*, and *Alkalibacillus sp.* bacteria on pyrite depression and chalcopyrite flotation

Considering the proper performance of *Halobacillus sp.*, *Alkalibacillus almallahensis*, and *Alkalibacillus sp.* bacteria in pyrite depression and chalcopyrite flotation in single-bacteria tests (Sections 3.3 and 3.4), it was decided to use the combination (mix) of these three types of bacteria with different proportions in order to identify their synergistic effects. Therefore, as it can be seen in Table 8, in four other separate tests, these bacteria were mixed with each other in different proportions. In the combined tests No. 1, 2, and 3, only the combination of two types of bacteria was used in an equal amount (50% of each of them). However, in the combined test number 4, the combination of all three types of bacteria was used in an equal amount (33.3% of each of them).

Table 8. Percentage of *Halobacillus sp.*, *Alkalibacillus almallahensis*, and *Alkalibacillus sp.* bacteria in combined (mix) tests.

Bacteria name	Amount of bacteria (%)			
	Test 1	Test 2	Test 3	Test 4
Halobacillus sp.	0	50	50	33.3
Alkalibacillus sp.	50	50	0	33.3
Alkalibacillus almallahensis	50	0	50	33.3

3.6. Recoveries obtained from combined micro-flotation tests for pyrite depression

Table 9 shows the data obtained from combined micro-flotation tests for pyrite depression using the tests designed in Table 8. As it can be seen, in all four tests, the concentration grade has decreased compared to the tailing grade, which indicates the pyrite depression by the combination of these bacteria. Also, the concentration weight has decreased significantly compared to the tailing weight in all four tests. According to the pyrite recovery in the bacteria-free mode (77.4%), it can be seen that in all combined tests, the pyrite recovery has decreased significantly, which indicates the proper performance of the combination of bacteria in pyrite depression. The lowest recovery of pyrite is related to test number 4 (combination of all three types of bacteria) by 27.5%. As a result, it can be said that by combining all three types of bacteria, their performance and synergism for pyrite depression has improved

compared to other modes (Figure 10). It can also be concluded that the combination of hydrophilic (*Alkalibacillus sp.*) and hydrophobic (*Alkalibacillus almallahensis* and *Halobacillus sp.*) halophile bacteria can have a more positive effect on the pyrite depression process.

Table 9. Data obtained from combined micro-flotation tests for pyrite depression using tests designed in Table 8.

Test number	Bacteria name	Tailing weight (g)	Concentration weight (g)	Tailing grade (%)	Concentration grade (%)	Recovery (%)
1	*Alkalibacillus almallahensis + Alkalibacillus sp.*	7.56	3.60	56.57	53.67	31.1
2	*Alkalibacillus almallahensis + Halobacillus sp.*	7.47	3.17	55.70	52.49	28.6
3	*Alkalibacillus sp. + Halobacillus sp.*	7.43	3.41	53.32	50.92	30.5
4	Mix of all	8.23	3.59	57.42	49.86	27.5

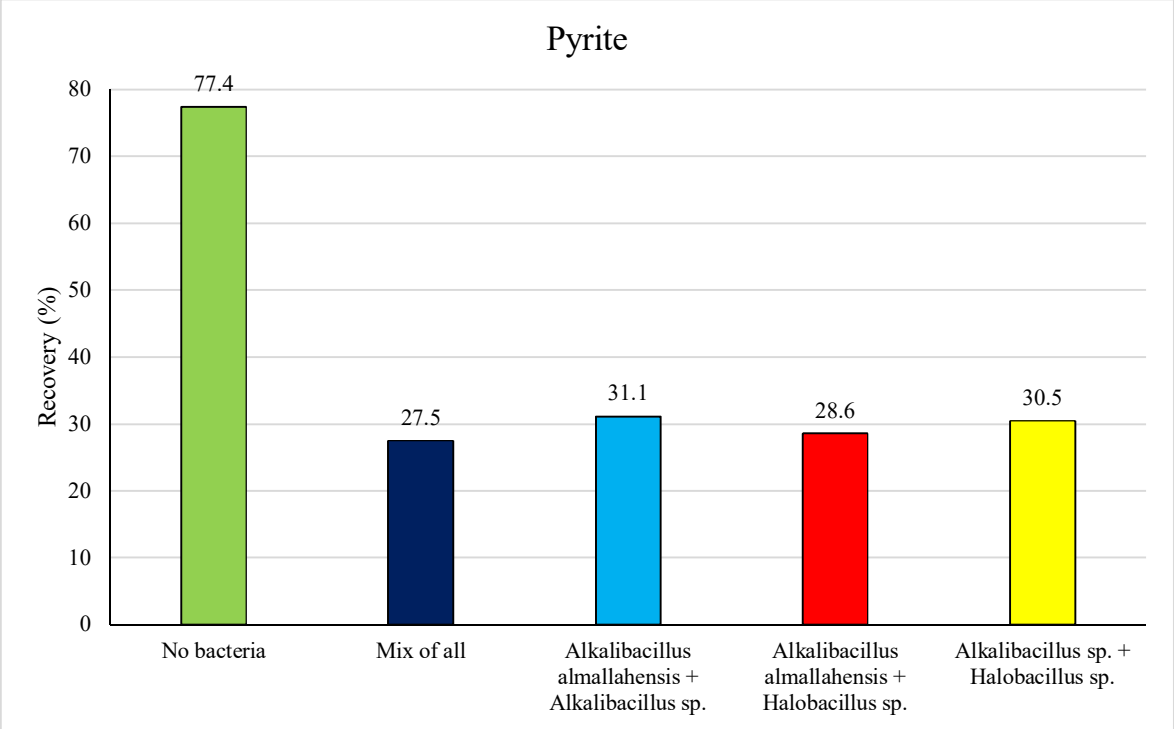


Figure 10. Comparison of pyrite depression recovery obtained from micro-flotation tests in bacteria-free and bacteria-combined modes.

3.7. Recoveries obtained from combined micro-flotation tests for chalcopyrite flotation

Table 10 shows the data from combined micro-flotation tests for chalcopyrite flotation using the tests designed in Table 8. As it can be seen, in all four tests, the concentration grade has increased significantly compared to the tailing grade, which indicates the proper flotation of chalcopyrite by the combination of these bacteria. Also, the concentration weight has increased in all four tests compared to the tailing weight. Regarding the chalcopyrite recovery in the bacteria-free mode (42.7%), it can be seen that in all combined tests, the chalcopyrite recovery has increased significantly, which indicates the proper performance of the combination of bacteria in the chalcopyrite flotation. The highest recovery of chalcopyrite is related to test number 4 (combination of all three types of bacteria) with the value of 72.6%. As a result, it can be said that by the combination of all three types of bacteria, their performance and synergism for chalcopyrite flotation has improved compared to other modes (Figure 11). It can also be concluded that the combination of hydrophilic (*Alkalibacillus sp.*) and hydrophobic (*Alkalibacillus almallahensis* and *Halobacillus sp.*) halophile bacteria can have a more positive effect on the flotation process of chalcopyrite. Due to the hydrophilicity and being

100% surfactant of *Alkalibacillus sp.*, as a frother in the flotation of chalcopyrite; this bio-surfactant showed good frothing properties such as reducing the surface tension of water, suitable froth height and stability of the froth, which is in accordance with the research work conducted by Banat *et al.* [37]. It is worth mentioning that this bio-surfactant has the following advantages compared to conventional chemical reagents: less toxicity, good performance at different pHs and temperatures, and less adverse environmental effects [37].

Table 10. Data obtained from combined micro-flotation tests for chalcopyrite flotation using tests designed in Table 8.

Test number	Bacteria name	Tailing weight (g)	Concentration weight (g)	Tailing grade (%)	Concentration grade (%)	Recovery (%)
1	*Alkalibacillus almallahensis + Alkalibacillus sp.*	4.79	4.80	0.78	1.10	58.4
2	*Alkalibacillus almallahensis + Halobacillus sp.*	3.71	5.64	0.72	1.10	69.7
3	*Alkalibacillus sp. + Halobacillus sp.*	4.41	5.25	0.72	1.13	65.2
4	Mix of all	3.73	5.61	0.66	1.17	72.6

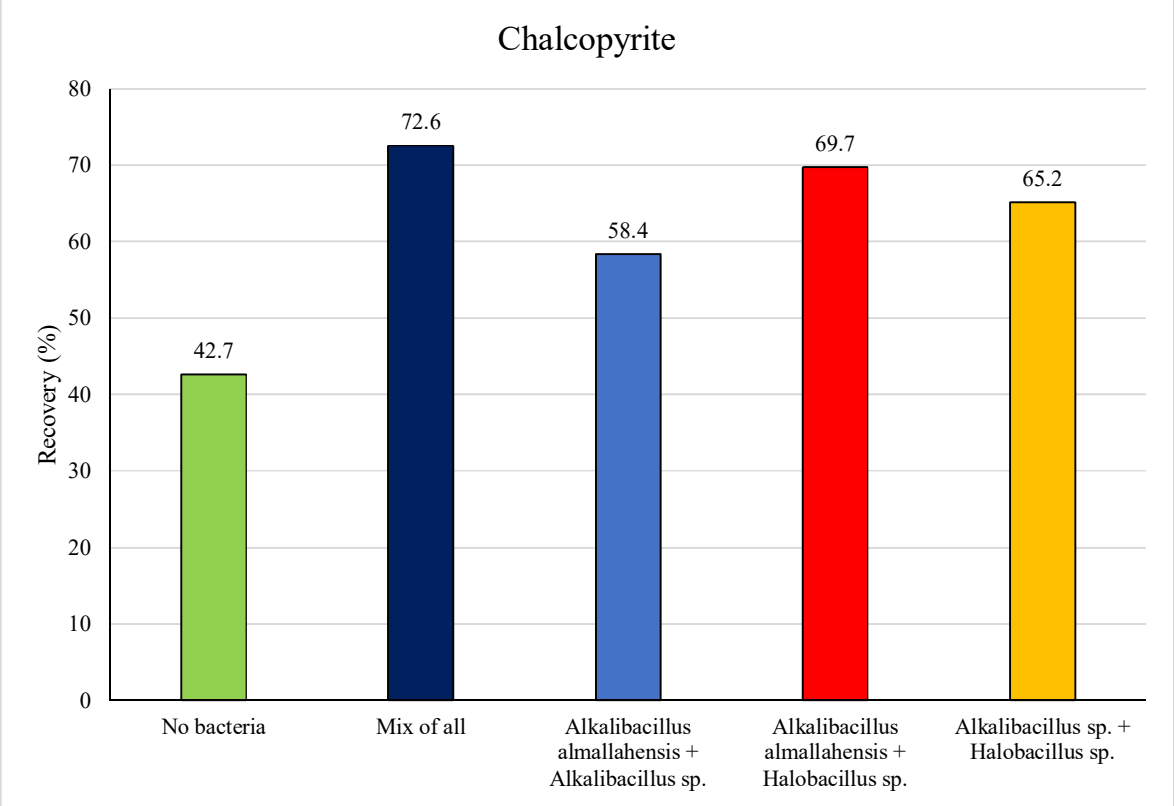


Figure 11. Comparison of chalcopyrite flotation recovery obtained from micro-flotation tests in bacteria-free and bacteria-combined modes.

Figure 12 compares the recoveries of pyrite depression and chalcopyrite flotation in the bacteria-free and bacteria-combined micro-flotation tests. According to the figure, the flotation recovery of pyrite in the combined tests was reduced compared to the control mode (no bacteria), which indicates the effective depression of pyrite by the combination of bacteria. On the contrary, the chalcopyrite flotation recovery in the combination tests increased compared to the bacteria-free mode, which indicates the proper flotation of chalcopyrite by the combination of bacteria. In other words, the combination of all three types of bacteria has both the ability to float chalcopyrite better and the ability to retain pyrite better than other modes. On the other hand, it is

observed that the selectivity of binary and ternary combination of bacteria in chalcopyrite flotation and pyrite depression is higher than the control mode (no bacteria).

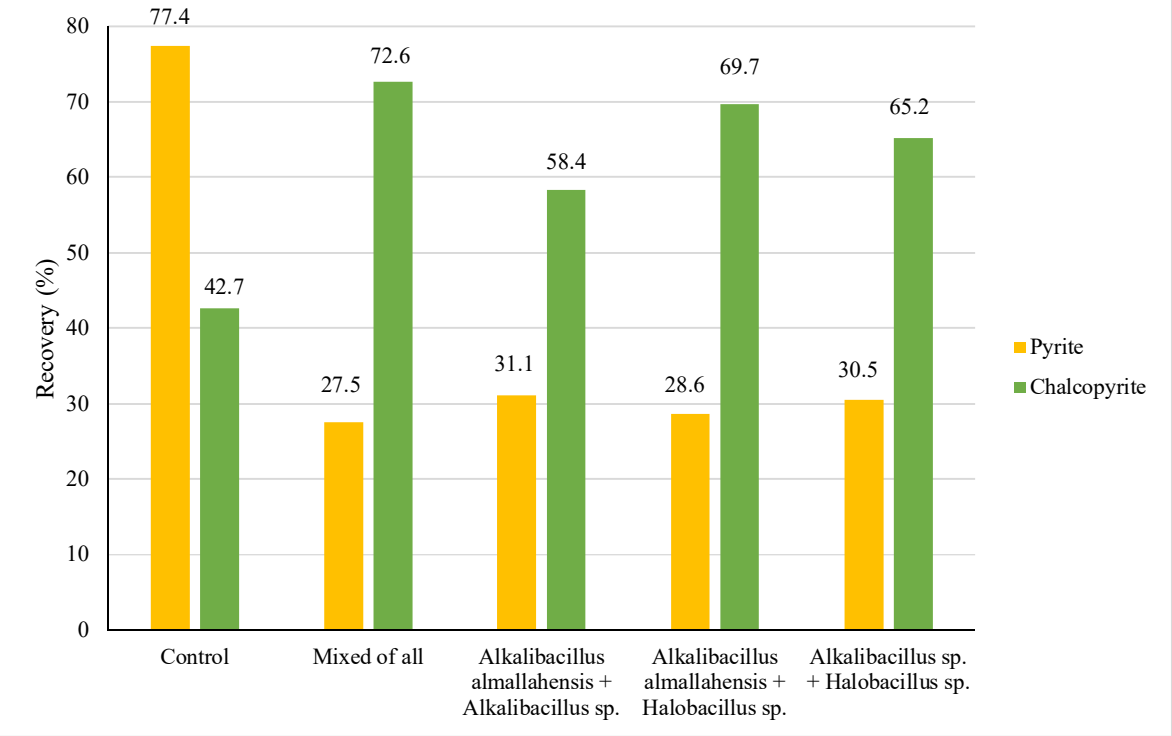


Figure 12. Pyrite depression and chalcopyrite flotation recoveries in bacteria-free and bacteria-combined micro-flotation tests.

4. Conclusions

In this research work, five types of halophile bacteria were studied as pyrite bio-depressant and for chalcopyrite flotation, among which three types of bacteria *Halobacillus sp.*, *Alkalibacillus almallahensis*, and *Alkalibacillus sp.* Had a better performance in pyrite depression and chalcopyrite flotation than the other bacteria. The pyrite recovery when using them was 30.9%, 30.3%, and 34.0%, respectively, and the chalcopyrite flotation recovery by them was equal to 52.9, 68.6, and 55.7, respectively, which indicated their high selectivity in flotation. Finally, by combining these three types of bacteria (with different percentages of each of them), the effect of combining these three types of bacteria on chalcopyrite flotation and pyrite depression was also studied. Also, the recoveries of chalcopyrite flotation and pyrite depression were investigated and their values were compared with each other and with other bacteria-free and single-bacteria modes. The obtained results showed that the combination of all three types of bacteria could depress pyrite better than other modes, and pyrite recovery was equal to 27.5%, which was less than single bacteria. Also, the effect of their combination on the chalcopyrite flotation was investigated and the recovery of chalcopyrite was obtained 72.6%, which was higher than single bacteria. As a result, the combination of all three bacteria caused better synergism and improved their performance in microflotation tests.

The following results were also obtained:

- ✓ The recovery of pyrite depression using the combination of all three types of bacteria decreased from 77.4% in the bacteria-free mode to 27.5%. Also, the chalcopyrite flotation recovery by using the combination of all three types of bacteria increased from 42.7% in the bacteria-free mode to 72.6%, which indicated the high selectivity of the three combined bacteria.
- ✓ The combination of hydrophilic and hydrophobic halophile bacteria can have a positive effect on the flotation process.
- ✓ Among the advantages of this research work compared to other similar research works conducted in the past, which were done using several types of halophilic bacteria, it could be mentioned that in the previous research works, the performance of one type of bacteria was successful in depressing pyrite, and the

performance of another bacteria was more effective for chalcopyrite flotation. However, none of the bacteria had the ability to depress pyrite and float chalcopyrite simultaneously. Nevertheless, in this research work, for the first time, the utilized bacteria, especially the *Alkalibacillus almallahensis* bacteria, had a successful performance in both pyrite depression and chalcopyrite flotation. Also, the combination of the hydrophilic bacterium (*Alkalibacillus sp.*) and the hydrophobic bacteria (*Alkalibacillus almallahensis* and *Halobacillus sp.*) demonstrated a remarkable performance compared to single bacteria.

Acknowledgments

The authors of the article sincerely thank and appreciate the cooperation of Sarcheshmeh Copper Complex research and development (R&D) center and its hydrometallurgy unit, as well as Shahrood University of Technology.

References

[1]. Houot, R. (1983). Beneficiation of iron-ore by flotation–review of industrial and potential applications. International Journal of Mineral Processing, 10, 183–204.

[2]. Consuegra, G. L., Kutschke, S., Rudolph, M. and Pollmann, K. (2020). Halophilic bacteria as potential pyrite bio-depressants in Cu-Mo bioflotation. Minerals Engineering, 145, 106062.

[3]. Yin, J., Chen, J.C., Wu, Q. and Chen, G.Q. (2015). Halophiles, coming stars for industrial biotechnology. Biotechnology Advances, 33, 7, 1433 – 1442.

[4]. Oren, A. (1999). Bioenergetic aspects of halophilism. Microbiology and Molecular Biology Reviews, 334–348.

[5]. Oren, A. (2002). Diversity of halophilic microorganisms: Environments, phylogeny, physiology, and applications. Journal of Industrial Microbiology & Biotechnology, 28, 56–63.

[6]. Oren, A. (2008) Microbial life at high salt concentrations: phylogenetic and metabolic diversity. Saline Systems, 4:2.

[7]. Quillaguaman, J., Guzman, H., Van-Thuoc, D. and Hatti-Kaul, R. (2010). Synthesis and production of polyhydroxyalkanoates by halophiles: current potential and future prospects. Applied Microbiology and Biotechnology, 85, 6, 1687–1696.

[8]. Roberts, M.F. (2005). Organic compatible solutes of halotolerant and halophilic microorganisms. Saline Systems, 1:5, 1–30.

[9]. Delgado-García, M., Valdivia-Urdiales, B., Aguilar-González, C.N., Contreras-Esquivel, J.C. and Rodríguez-Herrera, R. (2012). Halophilic hydrolases as a new tool for the biotechnological industries. J Sci Food Agric, 92, 2575–2580.

[10]. Hozzein, W.N., Reyad, A.M., Abdel Hameed, M.S. and Ali, M.I.A. (2013). Characterization of a new protease produced by a thermohaloalkali tolerant Halobacillus strain. Journal of Pure and Applied Microbiology, 7,509–515.

[11]. Louis, P. and Galinski, E.A. (1997). Characterization of genes for the biosynthesis of the compatible solute ectoine from Marinococcus halophilus and osmoregulated expression in Escherichia coli. Microbiology, 143, 1141–1149.

[12]. Vargas, C., Argandoña, M., Reina-Bueno, M., Rodríguez-Moya, J., Fernández-Aunión, C. and Joaquín, J.N. (2008). Unravelling the adaptation responses to osmotic and temperature stress in Chromohalobacter salexigens, a bacterium with broad salinity tolerance. Saline Systems, 4:14.

[13]. Ventosa, A., Nieto, J.J. and Oren, A. (1998). Biology of moderately halophilic aerobic bacteria. Microbiology and Molecular Biology Reviews, 62, 2, 504–544.

[14]. Cohen, R. and Exerowa, D. (2007). Surface forces and properties of foam films from rhamnolipid biosurfactants. Advances in Colloid and Interface Science, 134–135, 24–34.

[15]. Sharma, P.K., Hanumantha Rao, K., Natarajan, K.A. and Forssberg, K.S.E. (2000). Bioflotation of sulphide minerals in the presence of heterotrophic and chemolitotrophic bacteria. In: Massacci, P. (Ed.), Proc. XXI International Mineral Processing Congress (IMPC), Developments in Mineral Processing, No. 13. Elsevier, B8a, pp. 93–103.

[16]. Hosseini Tabatabaei, R. (2003). Feasibility study of bioflotation of Sarcheshmeh copper sulfide ore. Master of Science Thesis in Mineral Processing, University of Tehran, In Persian.

[17]. Kolahdoozan, M., Tabatabaei, H., Oliazadeh, M., Noaparast, M., Tabatabaei, Y.S.M., Shahverdi, A.R., Eslami, A. and Manafi, Z. (2004). Bioflotation of Sarcheshmeh copper sulphide ore. Particle Size Enlargement in Mineral Processing, Proceedings of the 5th UBC-McGill Biennial International Symposium on Fundamentals of Mineral, COM 2004, August 22-25, 43rd Annual Conference of Metallurgists of CIM, August 22 - 25, 2004, Hamilton, Toronto, Canada.

[18]. Hosseini, T.R., Kolahdoozan, M., Tabatabaei, Y.S.M., Oliazadeh, M., Noaparast, M., Eslami, A., Manafi, Z. and Alfantazi, A. (2005). Bioflotation of Sarcheshmeh copper ore using Thiobacillus ferrooxidans bacteria. Minerals Engineering, 18, 371–374.

[19]. Botero, A.E.C., Torem, M.L. and de Mesquita, L.M.S. (2008). Surface chemistry fundamentals of

biosorption of Rhodococcus opacus and its effect in calcite and magnesite flotation. Minerals Engineering, 21, 83–92.

[20]. Govender, Y. and Gericke, M. (2011). Extracellular polymeric substances (EPS) from bioleaching systems and its application in bioflotation. Minerals Engineering, 24, 1122–1127.

[21]. Khoshdast, H. (2011). Investigating the possibility of flotation of copper ores using Rhamnolipid biosurfactants as frother. PhD dissertation in Mineral Processing, Shahid-Bahonar University of Kerman, In Persian.

[22]. Kim, G., Choi, J., Choi, S.Q., Song, Y. and Kim, H. (2016). Bioflotation of malachite from complex system using Rhodococcus opacus. International Mineral Processing Congress (IMPC), XXVIII International Mineral Processing Congress Proceedings.

[23]. Olivera, C.A.C., Merma, A.G., Puelles, J.G.S. and Torem, M.L. (2017). On the fundamentals aspects of hematite bioflotation using a Gram positive strain. Minerals Engineering, 106, 55 – 63.

[24]. Kim, G., Choi, J., Silva, R.A., Song, Y. and Kim, H. (2017). Feasibility of bench-scale selective bioflotation of copper oxide minerals using Rhodococcus opacus. Hydrometallurgy, 168, 94–102.

[25]. Abedi Ashkavandi, R., Azimi, E. and Raouf Hosseini, M. (2022). Bacillus licheniformis a potential bio-collector for barite-quartz selective separation. Minerals Engineering, 175, 107285.

[26]. Simões, C.R., Hacha, R.R., Merma, A.G. and Torem, M.L. (2020). On the recovery of hematite from an iron ore fine fraction by electroflotation using a biosurfactant. Minerals. 10 (12):1057.

[27]. El-Sayed, S., El-Shatoury, E.H., Abdel-Khalek, N.A., Abdel-Motelib, A. and Abdel Khalek, M.A. (2021). Influence of Bacillus cereus-Gold interaction on bio-flotation of gold in the presence of potassium butyl xanthate. Biointerface Research in Applied Chemistry. 11 (5): 13005–13018.

[28]. Pineda, G.A.C. and Godoy, M.A.M. (2019). Effect of Thiobacillus thiooxidans-cysteine interactions on pyrite biooxidation by Acidithiobacillus ferrooxidans in the presence of coal compounds. Brazilian Journal of Chemical Engineering. 36 (2): 681–692.

[29]. Çelik, P.A., Çakmak, H. and Öz Aksoy, D. (2021). Green bioflotation of calcite using surfactin as a collector. Journal of Dispersion Science and Technology, 1–11.

[30]. Moreno, P.A., Aral, H., Cuevas, J., Monardes, A., Adaro, M., Norgate, T. and Bruckard, W. (2011). The use of seawater as process water at Las Luces copper–molybdenum beneficiation plant in Taltal (Chile). Minerals Engineering, 24, 852–858.

[31]. Pérez-Davó, A., Aguilera, M., Ramos-Cormenzana, A. and Monteoliva-Sánchez, M. (2014). Alkalibacillus almallahensis sp. nov., a halophilic bacterium isolated from an inland solar saltern. International Journal of Systematic and Evolutionary Microbiology, 64, 2066–2071.

[32]. Mesbah, N.M. and Wiegel, J. (2014). Purification and biochemical characterization of halophilic, alkalithermophilic protease AbCP from Alkalibacillus sp. NM-Fa4. Journal of Molecular Catalysis B: Enzymatic, 105, 74–81.

[33]. Samaei-Nouroozi, A., Rezaei, S., Khoshnevis, N., Doosti, M., Hajihoseini, R., Khoshayand, M.R. and Faramarzi, M.A. (2015). Medium-based optimization of an organic solvent-tolerant extracellular lipase from the isolated halophilic Alkalibacillus salilacus. Extremophiles, 19, 5, 933 – 947.

[34]. Schäfer, A., Harms, H. and Zehnder, A.J.B. (1998). Bacterial accumulation at the air-water interface. Environmental Science & Technology. 32 (23): 3704–3712.

[35]. Tolley, W., Kotlyar, D. and Van Wagoner, R. (1996). Fundamental electrochemical studies of sulfide mineral flotation. Minerals Engineering, 9, 6, 603 – 637.

[36]. Moslemi, H. and Gharabaghi, M. (2017). A review on electrochemical behavior of pyrite in the froth flotation process. Journal of Industrial and Engineering Chemistry, 47, 1–18.

[37]. Banat, I. M., Makkar, R.S. and Cameotra, S.S. (2000). Potential commercial applications of microbial surfactants. Applied Microbiology and Biotechnology. 53 (5): 495–508.

بیوفلوتاسیون کالکوپیریت با استفاده از باکتری‌های هالوفیل به صورت مجزا و ترکیب آن‌ها به عنوان بازداشت‌کننده‌های زیستی پیریت

امیرمحمد نصرالله‌زاده بافتی[۱]، محمد جهانی چگنی[۱*]، احمد مغویی‌نژاد[۲] و زهرا منافی[۲]

۱- دانشکده مهندسی معدن، نفت و ژئوفیزیک، دانشگاه صنعتی شاهرود، ایران

۲- مرکز تحقیق و توسعه، مجتمع مس سرچشمه، رفسنجان، ایران

ارسال ۲۰۲۲/۰۹/۳۰، پذیرش ۲۰۲۲/۱۱/۲۳

* نویسنده مسئول مکاتبات: m.jahani@shahroodut.ac.ir

چکیده:

با توجه به افزایش روزافزون مصرف آهک در فرآیند فلوتاسیون برای افزایش pH سیستم و ایجاد محیط قلیایی و نیز گران شدن تدریجی آن، توجه محققان به سمت انجام عملیات فلوتاسیون در محیط خنثی جلب شده است. باکتری‌های هالوفیل پتانسیل جایگزینی برای کاهش‌دهنده‌های فلوتاسیون مانند آهک را دارند زیرا عمل فلوتاسیون با کمک آن‌ها در pH خنثی نیز قابل انجام است. همچنین به دلیل اثر بافر آب دریا، که واسطه انتخابی برای بیوفلوتاسیون است، استفاده از روش بیوفلوتاسیون باعث کاهش استفاده از آب آشامیدنی و همچنین کاهش مصرف مواد شیمیایی می‌شود. در این پژوهش، پنج نوع باکتری هالوفیل به عنوان بازداشت-کننده‌های زیستی پیریت و شناورکننده‌های کالکوپیریت مورد مطالعه قرار گرفتند. آزمایش‌های بیوفلوتاسیون با استفاده از لوله هالیموند انجام شدند که باکتری‌های *Halobacillus sp.*، *Alkalibacillus almallahensis* و *Alkalibacillus sp.* عملکرد بهتری در بازداشت پیریت و شناورسازی کالکوپیریت نسبت به سایر باکتری ها داشتند. بازیابی بازداشت پیریت هنگام استفاده از آن‌ها به ترتیب ۳۰/۹، ۳۰/۳، و ۳۴/۰ درصد و بازیابی شناورسازی کالکوپیریت توسط آن‌ها به ترتیب برابر با ۵۲/۹، ۶۸/۶، و ۵۵/۷ بود که نشان‌دهنده انتخاب‌پذیری بالای این باکتری‌ها در فلوتاسیون می‌باشد. علاوه بر آزمایش‌های فوق، تأثیر ترکیب این سه نوع باکتری بر بازداشت پیریت و شناورسازی کالکوپیریت نیز مورد مطالعه قرار گرفت. نتایج نشان دادند که ترکیب هر سه نوع باکتری (۳۳/۳ درصد از هر نوع) با هم توانستند پیریت را نسبت به سایر حالت‌ها بهتر بازداشت کنند و مقدار بازیابی پیریت ۲۷/۵ درصد به دست آمد که نسبت به باکتری‌های تک کمتر بود. همچنین تأثیر عملکرد ترکیب این سه نوع باکتری بر روی شناورسازی کالکوپیریت بررسی شد و بازیابی آن ۷۲/۶ درصد به دست آمد که نسبت به باکتری‌های تک بالاتر بود. از سوی دیگر با توجه به اینکه مقدار بازیابی کالکوپیریت در تست‌های ترکیب سه باکتری از تست‌های تک باکتری، و دو باکتری بیشتر بود، می‌توان نتیجه گرفت ترکیب هر سه باکتری می‌تواند باعث هم‌افزایی بهتر و بهبود عملکرد آن‌ها در تست‌های میکروفلوتاسیون شود.

کلمات کلیدی: بیوفلوتاسیون، باکتری‌های هالوفیل، ترکیب باکتری‌ها، شناورسازی کالکوپیریت، بازداشت پیریت.