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Investigating Possibility of Replacing Some Chemical Reagents used in Sulfide Copper Flotation with Halophilic Bacteria

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Abstract

Flotation is the most important method for processing sulfide copper ores. Due to the high cost and environmental hazards caused by the chemical reagents used in this process (collectors, frothers, pH regulators, depressants, etc.), the possibility of replacing all these reagents or at least some of them are of special importance through environmentally friendly methods such as bio-flotation using halophilic bacteria. These bacteria have the ability of growth and proliferation in salty media and relatively neutral pHs such as sea salty water. In this research work, the four types of halophilic bacteria *Halobacillus sp.*, *Alkalibacillus almallahensis*, *Marinobacter sp.*, and *Alkalibacillus sp.* are studied to replace frothers (MIBC and F7240), depressant (sodium metabisulfite), and pH regulator (lime) in sulfide copper flotation using a Denver laboratory flotation cell. The results obtained indicate that each of the four types of bacteria mentioned above along with collectors (gasoil, Z11, and C7240) as the only chemical reagents (bio-flotation + collector) can depress pyrite better than the bacteria-free mode (flotation + all chemical reagents). Iron recovery in tailings in the standard flotation test is 46.8%, which is, respectively, increased to 91.9%, 74.5%, 70.3%, and 76.9% using the halophilic bacteria of *Halobacillus sp.*, *Alkalibacillus almallahensis*, *Marinobacter sp.*, and *Alkalibacillus sp.* On the other hand, the recovery of chalcopyrite using the bio-flotation method is lower than its recovery using the flotation method. Copper recovery in the concentrate in the standard flotation test is 89.1%, which is reached to 58.8%, 71.4%, 62.5%, and 69.4%, respectively, using the above bacteria in the bio-flotation method.

1. Introduction

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 compounds, called microorganisms, are a potential alternative to chemicals that are derived from petroleum [1]. 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 and temperature. In addition, sources of microorganisms are easily accessible and reproducible. As a result, the production process will have lower costs in many cases [1]. Microorganisms (such as bacteria, algae, fungi, and yeasts) can be used for the bio-flotation process due to their abundance in nature [2, 3]. Due to the ease of proliferation of bacteria to any desired number, the bio-flotation process is economically viable. For example, some hydrophobic bacteria have been widely used as collectors because they facilitate the adsorption of minerals to a gas-liquid interface [4, 5]. In 1981, Björn *et al.* investigated the hydrophobicity of bacteria as an important factor in their initial adhesion at the air-water interface. The results obtained revealed a positive correlation between the degree of enrichment of bacteria at the surface and their hydrophobicity [6].

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Bio-flotation of minerals is a new and effective method for separating and concentrating valuable minerals from other minerals. Due to the decrease in the grade of ores and the more complicated way of separating valuable minerals from them, in order for the flotation process to be carried out efficiently, inevitably, the dosage of chemical reagents used in this process has increased between 2% and 3% annually. Since the chemical reagents used in the flotation process cannot be recycled, and also due to the toxicity of some of these reagents [7], replacing them with environmentally friendly and recyclable microorganisms such as halophilic bacteria is of particular importance. In a research work in 1985, Solozhenkin and Lyubavina showed that by modifying the surface properties of cerussite by bacteria, the efficiency of flotation increased by 20-25% [8]. In 1991, James investigated the charge properties of microbial cell surfaces. The results showed that the cell surface of all microorganisms carried a negative charge caused by phosphate, carboxylate, and sulfate groups in the cell wall and capsular macromolecules [9]. In 1993, Ohmura *et al*. investigated the selective adhesion of the *Thiobacillus ferrooxidans* bacterium to pyrite. The results showed that *Escherichia coli* bacteria tended to adhere to more hydrophobic minerals by hydrophobic interaction, while *T. ferrooxidans* selectively adhered to iron containing minerals such as pyrite and chalcopyrite. Ferrous ion inhibited the selective adhesion of *T. ferrooxidans* to pyrite competitively, while ferric ion scarcely inhibited such adhesion [10]. Zheng *et al*. in 2001 studied the adhesion of both *Bacillus subtilis* and *Mycobacterium phlei* bacteria onto dolomite and apatite, and also their effect on dolomite depression in anionic flotation. In that study, the adhesion of the above bacteria to dolomite and apatite was investigated by sorption measurements and scanning electron microscopy (SEM). It was found that both *B. subtilis* and *M. phlei* adhered to dolomite more readily than onto apatite at acidic and near neutral pH values [11]. In 2003, Subramanian *et al*. conducted studies on surface modification of sulfide minerals using biological reagents. Bio-flotation and bio-flocculation studies on a synthetic mixture of galena and sphalerite demonstrated that galena could be selectively depressed or flocculated from sphalerite under appropriate conditions [12]. In 2003, Mesquita *et al*. investigated the interaction of a hydrophobic bacterial strain in a hematite-quartz flotation system. The micro-flotation tests with mineral mixtures showed that through biotreatment, it became possible to float the hematite and depress the quartz particles [13]. Patra and Natarajan in 2004 investigated microbially induced flocculation and flotation for the separation of chalcopyrite from quartz and calcite. In that research work, cells and metabolic products of the *Bacillus polymyxa* bacterium were successfully used in flocculation and flotation to remove chalcopyrite from quartz and calcite with the aim of environmental protection and ore beneficiation [14]. 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; however, did not change the floatability of chalcopyrite [15]. In 2008, Botero *et al*. investigated the effect of flotation of calcite and magnesite using *Rhodococcus opacus* bacteria. The recovery of bio-flotation for magnesite (for the concentration of R. opacus 200 ppm at a pH of about 5) and calcite (for the concentration of R. opacus 220 ppm at a pH of about 7) was about 93% and 55%, respectively [16]. In 2013, Merma *et al*. investigated the basic aspects of apatite and quartz flotation using the Rhodococcus opacus bacterium as a biological reagent. The results suggested that the bacterial adhesion onto the mineral surfaces was predominantly specific [17]. In 2013, Yang *et al*. investigated the flocculation and flotation response of the *Rhodococcus erythropolis* bacterium to pure minerals in hematite ores. In that study, the mentioned bacterium was evaluated as a collector for hematite flotation. The ability of that bacterium to collect hematite was stronger than its ability to collect quartz, kaolinite, and apatite [18]. In 2014, El-Midany and Abdel-Khalek investigated the reduction of sulfur and coal ash using *Bacillus subtilis* and *Paenibacillus polymyxa* bacteria. In that study, coal–bacteria interaction was investigated using adsorption kinetics, adsorption isotherm, Fourier Transform-InfraRed (FT-IR), and zeta potential. The bio-flotation results indicated that *B. subtilis* was better than *P. polymyxa* for reducing both sulfur and ash content [19]. In 2016, Edy Sanwani *et al*. studied the bio-flotation process. They investigated the interaction of bacteria-minerals for compatible, sustainable, and eco-friendly mineral processing using two bacteria, *Bacillus pumilus* SKC-2 and *Alicyclobacillus ferrooxydans* SKC/SAA-2. These

results indicated that the changes of pyrite surface properties were clearly as the results of bacterial action, likely serving as both bio-collector or bio-frother and depressant that would be very applicable for flotation processes [20]. 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 [21]. In 2020, Simões *et al*. addressed the electro-flotation of fine and ultrafine particles of an itabirite iron ore using a biosurfactant extracted from *Rhodococcus opacus* bacterium; the recovery of iron using this method was about 83% [22]. 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 [23]. In 2021, Çelik *et al*. investigated the effect of biosurfactant collection obtained from *Bacillus subtilis* bacteria on the flotation of calcite mineral; h the recovery of calcite through bio-flotation was about 80% [24]. In 2022, Ashkavandi *et al*., in a research work 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].

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. There are increasing effects for the development of halophiles into a low-cost infrastructure for biotreatment with the benefits of low energy, less freshwater consumption, low fixed capital investment, and continuous production [26]. For growth in 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 [27, 28]. 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 [29-31]. 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 [32]. As a result, they allow the halophile to grow at a pH of about 10 and at a temperature of more than 50 °C [33]. 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 [34-36]. Recently, in 2022, Nasrollahzadeh *et al*. investigated the flotation of chalcopyrite and the depression of pyrite using halophilic bacteria individually and as a combination of bacteria. The results showed that the combination of bacteria could synergize and improve their performance in chalcopyrite flotation and pyrite depression [37].

The purpose of this research work is to investigate the possibility of replacing all the chemical reagents used in the flotation process of sulfide copper minerals or at least some of them by environmentally friendly methods such as bio-flotation. Due to the high cost and environmental hazards caused by these reagents, the possibility of replacing them with halophilic bacteria, which have the ability to grow and survive in salty media such as sea salty water, and utilization of the bio-flotation method is investigated. For this purpose, four types of halophilic bacteria are studied to replace the frothers (MIBC and F7240), depressant (sodium metabisulfite), pH regulator (lime), and collectors (gasoil, Z11, and C7240). Among the advantages of this research work and its innovations compared to the past research works, the following can be mentioned: It is possible to reuse halophilic bacteria (recycling them) in the flotation process, while chemical reagents are disposable and it is not possible to reuse them (recycle) in the flotation process. Also halophilic bacteria are completely environmentally friendly, and the environmental hazards caused by them are very little while common chemical reagents in flotation cause very serious and sometimes irreparable damage to the environment.

2. Materials and methods

2.1. Sampling from mine for bacterial culture

In order to extract halophilic bacteria, soil was collected from three places of Sarcheshmeh copper mine (mine pit and mine floor) at a depth of 2 cm in places where the surface of the soil was saline and brackish. Also the required amount of soil from

all three points along with 0.5 L of mine water for cultivation of halophilic bacteria were collected. In total, 11 samples were prepared and named from 0 to 10. Samples 0–3 included soils collected from different parts of Sarchesmeh copper mine. Sample 4 was prepared from the combination of samples 0–3. Also sample 5 included mine spring water. On the other hand, sample 6 was taken from the bottom of the mine. Also samples 7 and 8 were prepared from the combination of bacteria from samples 0–6 along with 50 and 25 g of chalcopyrite, respectively. In addition, sample 9 was also prepared from the combination of bacteria from samples 0–6 along with 50 g of pyrite. It is worth mentioning that the reason for adding chalcopyrite and pyrite to samples 7, 8, and 9 was to observe the effect of the produced bacteria on them. In samples 0–9, 200 mg of culture medium was added to the samples. Also sample 10 was prepared from the combination of bacteria from samples 0–9 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 10, which are a mixture of two or more halophilic bacteria, are called (mix culture). In this research work, sample 10 was used to purify four types of halophilic bacteria.

2.2. Cultivation of halophilic bacteria

The results of cultivation of halophilic bacteria can be seen in Table 1. The chemicals required to make the culture medium (DSMZ_Medium514) of halophilic bacteria are as follows (Table 2):

Table 1. 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
0	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

Table 2. Chemicals required to make culture medium of halophilic bacteria (DSMZ, medium 514) [38].

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

Halophilic bacteria used for flotation tests, cultivation conditions (using DSMZ_Medium514 and DSMZ_Medium514b culture media), strain type, method of preparation, country of origin, and the date of their first sampling could be seen in Table 3. It is worth mentioning that Medium514b has 17.5 g/L agar compared to Medium514.

Table 3. Halophilic bacteria used for flotation tests [39].

Bacteria name	Cultivation conditions	Strain designation	Isolated from	Country of origin	Date of sampling
Halobacillus sp.	Medium 514 , 30 ˚C	MA17	N.A.	Unknown	Before 1990/07/23
Marinobacter sp.	Medium 514 , 28 ˚C	KGB22	Eastern sea water	South Korea	2004/05
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 sp.	Medium 514b, 37 ˚C + 100 g/L NaCl + 17.5 g/L agar)	YIM98829	Sediment soil	China	Unknown

The method of purifying halophilic bacteria is that after making the required four types of bacteria cultures (Table 3), using the bacteria in sample 10, 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 can be seen in Table 1. 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, i.e. the pH suitable for the growth of halophilic bacteria in medium 514 (secondary pH), pH regulators, NaOH, and H_2SO_4 were used (Table 1). In addition, Eh values were measured for all 11 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 1. It is worth mentioning that the speed of the incubator for all 11 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. 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 1).

2.3. Preparation of mineral samples

At first, the amount of sample required for crushing and grinding was prepared from the input feed to the concentration plant 1 of Sarchesmeh Copper Complex. The specifications of this sample could be seen in Table 4. Next, using a jaw crusher (with a fixed opening), initial crushing was performed on the sample, and in the next step, using a 10 mesh (2 mm) vibrating screen, separation was done on the minerals because to work on plant feed, the particle size must be 100% smaller than 10 mesh. Also the remaining materials were collected on the screens and crushed again by a jaw crusher (with a variable opening). On the other hand, in order to bring all minerals to the size of 10 mesh, all the mentioned steps were repeated. To perform flotation tests, a 4.3-L Denver laboratory flotation cell with 28% solids percentage was used. In the next step, after mixing and homogenization, the samples were divided into 1469-g ones using a riffle splitter. The required crushing time to reach the size of 80% passing through the 74-micron screen (P_{80}) (cyclone overflow size or flotation unit feed) was 19 minutes and 15 seconds.

Table 4. Specifications of the sample used for flotation tests.

Name/type of mineral	Chemical formula	Amount in sample (%)
Chalcopyrite	$CuFeS_2$	0.994
Pyrite	FeS_2	11.829
Molybdenite	MoS_2	0.022
Sphalerite	ZnS	0.054
Hematite	Fe_2O_3	0.424
Chalcocite	Cu_2S	0.161
Covellite	CuS	0.011
Metal minerals	-	13.495
Non-metallic minerals	-	86.435
Oxide minerals	-	0.070
Total	-	100

2.4. How to perform flotation and bio-flotation tests

In order to perform flotation tests, one test was performed with the standard conditions of Sarcheshmeh copper plant and four other tests were performed using halophilic bacteria as a depressant. In the standard bacteria-free flotation test, all chemical reagents (collectors, frothers, depressants, and pH regulators) were used. However, in single-bacteria bio-flotation tests, only collectors were used, and the possibility of replacing frothers and depressants by bacteria was investigated. Also in the standard bacteria-free flotation test, the pulp pH should be around 11.8, which is done by adding lime to the flotation cell. However, there is no requirement to add lime in single-bacteria bio-flotation tests because halophilic bacteria have the ability to survive, grow and reproduce at almost neutral pH (7–8) (Table 5). In Table 5, the names of the bacteria used, their volume (mL), and the collector dosage in the bio-flotation tests, as well as the medium pH, the collector, frother, and depressant dosages in the flotation test (plant standard conditions) are visible. It is worth noting that all the tests were performed using the Denver flotation cell of the pilot plant of the Sarchesmeh Copper Complex with a stirring speed of 1400 rpm and a retention time of 12 minutes (Figure 1). In addition, every 10 seconds, frothing operation from the cell was performed. Bio-flotation tests were also performed with an experimental amount of 268.75 mL of bacteria along with the mentioned collectors at neutral pH (Figure 1). Then the concentrate and Tailings were collected, filtered, and dried. Afterwards, they were sent for mineralogical analysis.

Table 5. Comparison of operational conditions of standard flotation and bio-flotation tests.

Test type	Bacteria name	Medium pH	Volume of bacteria added to flotation cell (mL)	Chemical dosage (g/t)					
				Collector name			Frother name		Depressant name
				C7240	Z11	Gasoil	MIBC	F7240	Sodium metabisulfite
Flotation	No bacteria	11.8	-	25	15	13	15	15	200
Bio-flotation	*Halobacillus sp.*	7–8	268.75	25	15	13	-	-	-
Bio-flotation	*Alkalibacillus almallahensis*	7–8	268.75	25	15	13	-	-	-
Bio-flotation	*Marinobacter sp.*	7–8	268.75	25	15	13	-	-	-
Bio-flotation	*Alkalibacillus sp.*	7–8	268.75	25	15	13	-	-	-

Figure 1. Sarcheshmeh flotation test using DENVER flotation cell left) standard flotation; right) bio-flotation.

3. Results and Discussions

3.1. pH and Eh changes resulting from growth of halophilic bacteria over time

Figures 2 and 3 show, respectively, the changes in pH and Eh resulting from the growth of bacteria in the soil of samples 0–9. As seen in Figure 2, after 13 days of bacteria cultivation, the initial pH values in Table 1 were obtained, and it was tried to return the pH values to the corresponding values on day 0 (secondary pH in Table 1) to form sample number 10. Also in Figure 3, Eh values measured on the 13th day are reported in Table 1. It is worth mentioning that sample 10 was the basis for the continuation of the research work conducted in this study, and was used for purification of four types of bacteria (Figures 4 and 5). Figures 4 and 5, respectively, show the changes in pH and Eh resulting from the growth of four halophilic bacteria studied in this research work with time. As it can be seen, in Figure 4, with the passage of time, the pH of the culture medium of *Halobacillus sp.* has increased from about 6.2 to about 8.4 after one day. After four days, it has decreased again to about 6.2. Then on the 7th day, it reached about 8.2, on the 10th day, it reached about 6.3, and on the 13th day, it reached about 8.2 once more. 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 5, 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 155 mV on the 0th day, which reached -321 mV on the 13th day, which is a significant decrease. Other bacteria have shown a completely similar trend.

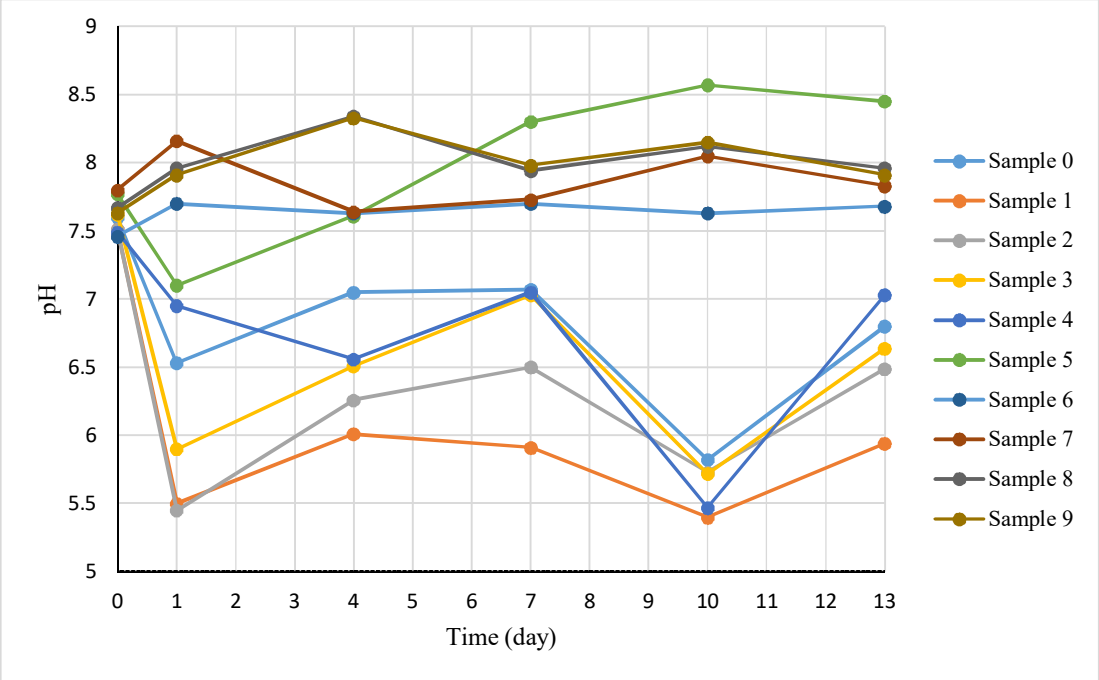

Figure 2. pH changes resulting from the growth of present bacteria in the soil of samples 0–9.

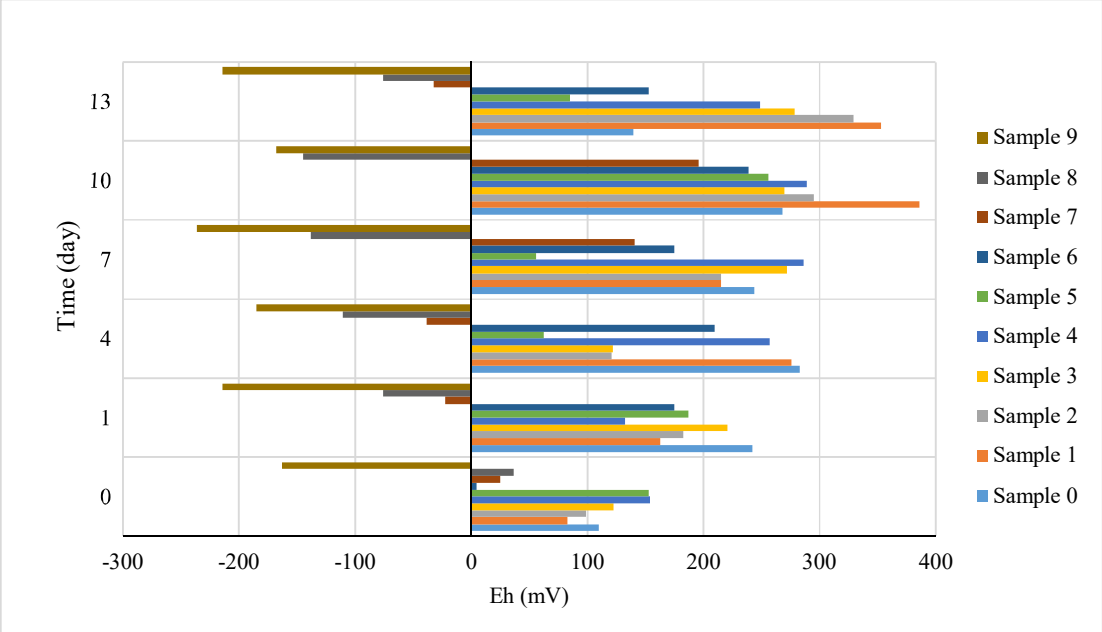

Figure 3. Eh changes resulting from the growth of present bacteria in the soil of samples 0–9.

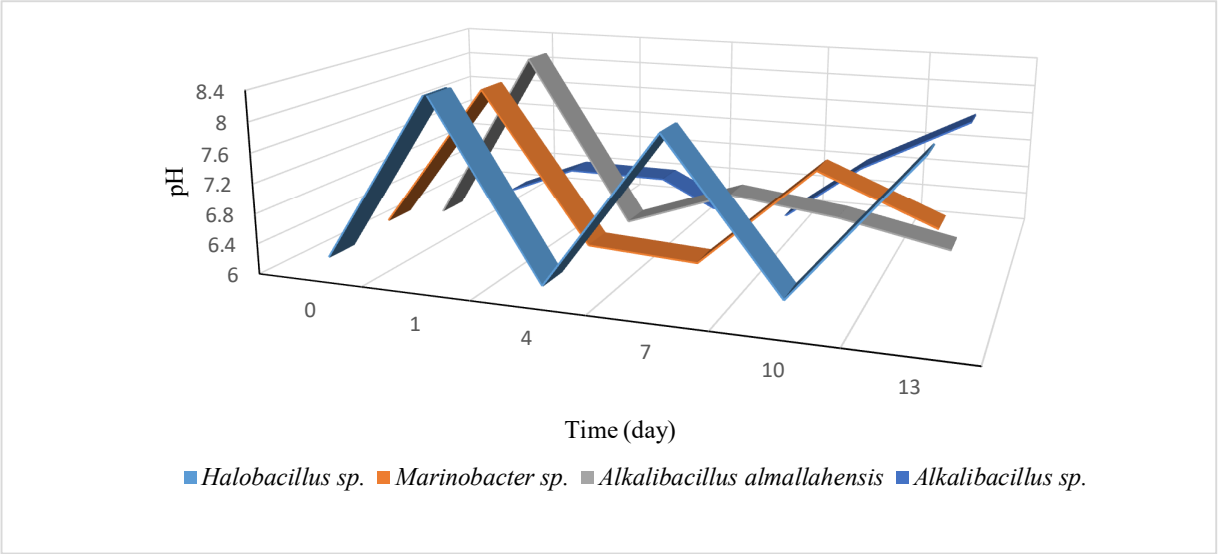

Figure 4. pH changes resulting from the growth of halophilic bacteria over time.

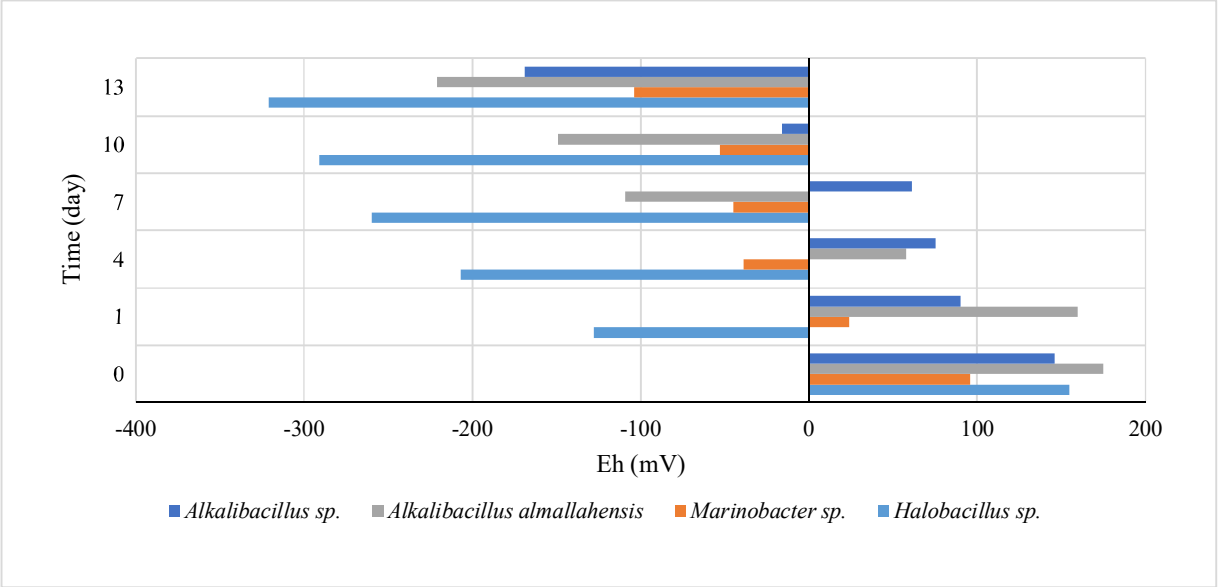

Figure 5. Eh changes resulting from the growth of halophilic bacteria over time.

3.2. Pyrite depression recovery

The data obtained from pyrite depression flotation tests by mineralogical method in standard bacteria-free flotation and single-bacteria bio-flotation modes is as described in Table 6. As it can be seen, in test number 1 (standard mode), the Fe grade in tailings is lower than other tests (single-bacteria tests) (3.17%). On the contrary, the Fe grade in feed at this test is almost higher than that of single-bacteria modes (5.93%). In tests 2 to 5 (single-bacteria modes), the highest Fe grade in tailings is related to test 2 and the *Halobacillus sp.* bacterium (4.93%). On the other hand, the lowest Fe grade in Feed is related to this test with a value of 5.24%, which indicates the proper performance of this bacterium in pyrite depression. Also in Figure 6, Fe recovery in tailings resulting from standard bacteria-free flotation and single-bacteria bio-flotation tests can be observed. As it can be seen, Fe recovery in tailings in the bacteria-free mode is lower than other single-bacteria modes (46.8%), which indicates the ability of halophilic bacteria in the more depression of pyrite. Also from Figure 6, it can be understood the extent of pyrite depression by the *Halobacillus sp.* bacterium is more than other bacteria and Fe recovery in tailings by this bacterium is about 91.9%, which is around 45.1% higher than the bacteria-free test. Therefore, the above bacterium has had a successful

performance in pyrite depression. In general, it can be said that halophilic bacteria can well replace the industrial depressants used in the flotation process such as sodium metabisulfite ($Na_2S_2O_5$), and if they are used to depress pyrite, there is no requirement to add any depressants to the flotation cell. Also due to the growth and proliferation of these bacteria in relatively neutral pHs (7–8), there is no requirement to add pH regulators such as lime to the system. In other words, the use of halophilic bacteria together with the collector alone has the ability to depress pyrite effectively.

Adhesion to the pyrite surface by hydrophobic bacteria such as *Halobacillus sp.*, *Marinobacter sp.*, and *Alkalibacillus almallahensis* bacteria was done properly, which is in accordance with the research works conducted by Consuegra *et al*. and Pérez-Davó *et al*. [40, 41]. Hydrophobic bacteria tend to stick better to surfaces, and accumulate more than hydrophilic bacteria because hydrophilic bacteria usually tend to disperse and also do not perform well in sticking to surfaces. However, *Alkalibacillus sp.* bacterium, due to being hydrophilic and 100% surfactant, surprisingly showed a good performance in pyrite depression, which is in accordance with the research work conducted by Mesbah and Wiegel [42]. Also compared to hydrophilic bacteria, which tend to disperse in solutions with high ionic strength, hydrophobic bacteria accumulate at the interface between air and water. This provides a plausible mechanism for further depression of pyrite by hydrophobic bacteria, as competition for the air-water interface occurs between the bacteria and the modified mineral. However, it is still not possible to conclude that this mechanism is alone selective for pyrite [43]. Another plausible explanation, using the current understanding of the importance of pyrite surface oxidation for xanthate salt adhesion, is that bacteria adhesion prevents oxidation of the mineral surface, so bacteria adhesion can reduce the number of oxidizing sites and thus pyrite floatability [44, 45].

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

Test number	Test type	Bacteria name	T (Tailings weight) (g)	F (Feed weight) (g)	t (Fe grade in tailings) (%)	f (Fe grade in feed) (%)	R = Tt/Ff (Fe recovery in tailings) (%)
1	Standard flotation	No bacteria	1267.0	1447.4	3.17	5.93	46.8
2	Bio-flotation	*Halobacillus sp.*	1417.6	1451.8	4.93	5.24	91.9
3	Bio-flotation	*Alkalibacillus almallahensis*	1372.3	1453.2	4.51	5.72	74.5
4	Bio-flotation	*Marinobacter sp.*	1352.0	1455.8	4.72	6.23	70.3
5	Bio-flotation	*Alkalibacillus sp.*	1377.0	1452.0	4.40	5.43	76.9

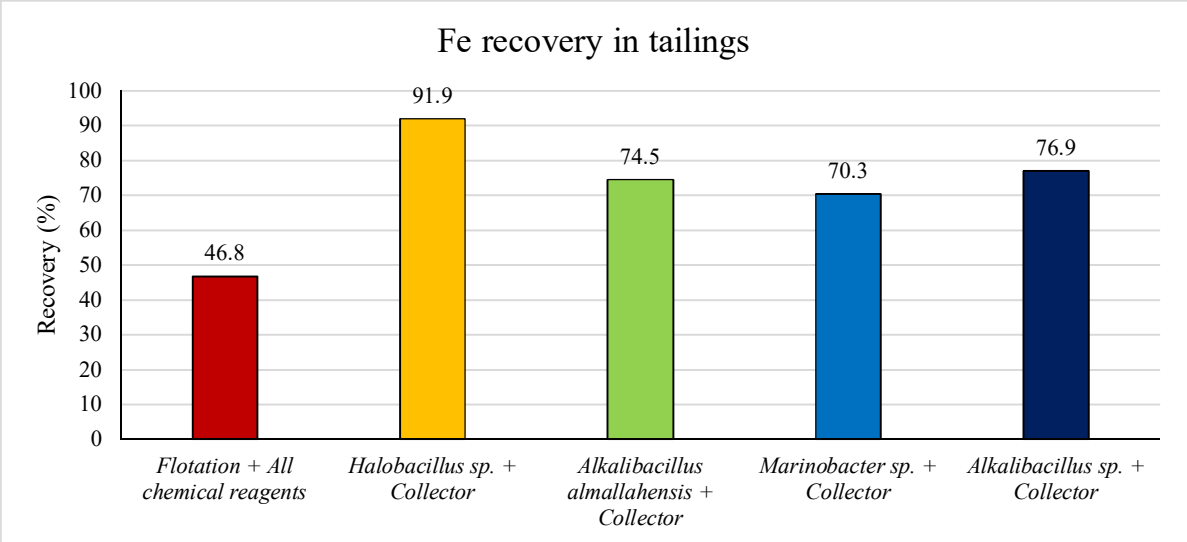

Figure 6. Fe recovery in tailings resulting from standard bacteria-free flotation and single-bacteria bio-flotation tests.

3.3. Chalcopyrite flotation recovery in standard bacteria-free flotation and single-bacteria bio-flotation modes

The data obtained from chalcopyrite flotation tests by mineralogical method in standard bacteria-free flotation and single-bacteria bio-flotation modes can be seen in Table 7. As it can be seen, the Cu grades in concentrate at all single-bacteria bio-flotation tests (tests 2 to 5) have higher values than the Cu grade in concentrate at the standard bacteria-free flotation test 1 (4.02%). However, due to the fact that the weight of the concentrate in the standard flotation test (180.4 g) is more than that of the single-bacteria modes, the Cu recovery in this test was higher than the single-bacteria modes (89.1%). Also the highest Cu grade in concentrate at bio-flotation tests was related to test 2 and *Halobacillus sp.* bacterium with a value of 10.04%, which has increased about 6.02% compared to the standard test. In addition, due to the fact that *Alkalibacillus almalallahensis* bacterium had a higher concentrate weight than *Halobacillus sp.* bacterium (80.9 g), the Cu flotation recovery in concentrate when using this bacterium was 71.4%, which was higher than other single-bacteria tests. However, it was about 17.7% lower than the standard flotation test. Figure 7 demonstrates the flotation recovery of Cu in concentrate obtained from standard bacteria-free flotation and single-bacteria bio-flotation tests. As it can be seen, the flotation recovery of Cu in concentrate at the standard flotation test (89.1%) is higher than in the single-bacteria bio-flotation tests. Therefore, it can be concluded that halophilic bacteria can replace the industrial frothers used in the chalcopyrite flotation process to a relatively acceptable extent. However, the problem is that the frothers used in this research work (MIBC and F7240) cause the death of halophilic bacteria. Therefore, it was not possible to use them together with bacteria in the flotation cell. It is suggested by finding frothers that do not cause problems for them and do not cause their destruction, the performance of these bacteria is improved in the flotation of chalcopyrite which is the subject of future research by the authors of this article. In general, it can be said that halophilic bacteria can completely replace chemical reagents such as depressants and pH regulators. Nevertheless, they can replace the frothers to an acceptable extent; however, they cannot fully fulfill the role of the frother. It is worth mentioning that, when using halophilic bacteria, adding collectors to the flotation cell is inevitable and halophilic bacteria cannot float chalcopyrite without the help of collectors.

Table 7. Data obtained from chalcopyrite flotation tests by mineralogical method in standard bacteria-free flotation and single-bacteria bio-flotation modes.

Test number	Test type	Bacteria name	F (Feed weight) (g)	C (Concentrate weight) (g)	f (Cu grade in feed) (%)	c (Cu grade in concentrate) (%)	R = Cc/Ff (Cu Recovery in concentrate) (%)
1	Standard flotation	No bacteria	1447.4	180.4	0.56	4.02	89.1
2	Bio-flotation	*Halobacillus sp.*	1451.8	34.2	0.40	10.04	58.8
3	Bio-flotation	*Alkalibacillus almallahensis*	1453.2	80.9	0.63	8.03	71.4
4	Bio-flotation	*Marinobacter sp.*	1455.8	103.8	0.62	5.42	62.5
5	Bio-flotation	*Alkalibacillus sp.*	1452.0	75.0	0.56	7.48	69.4

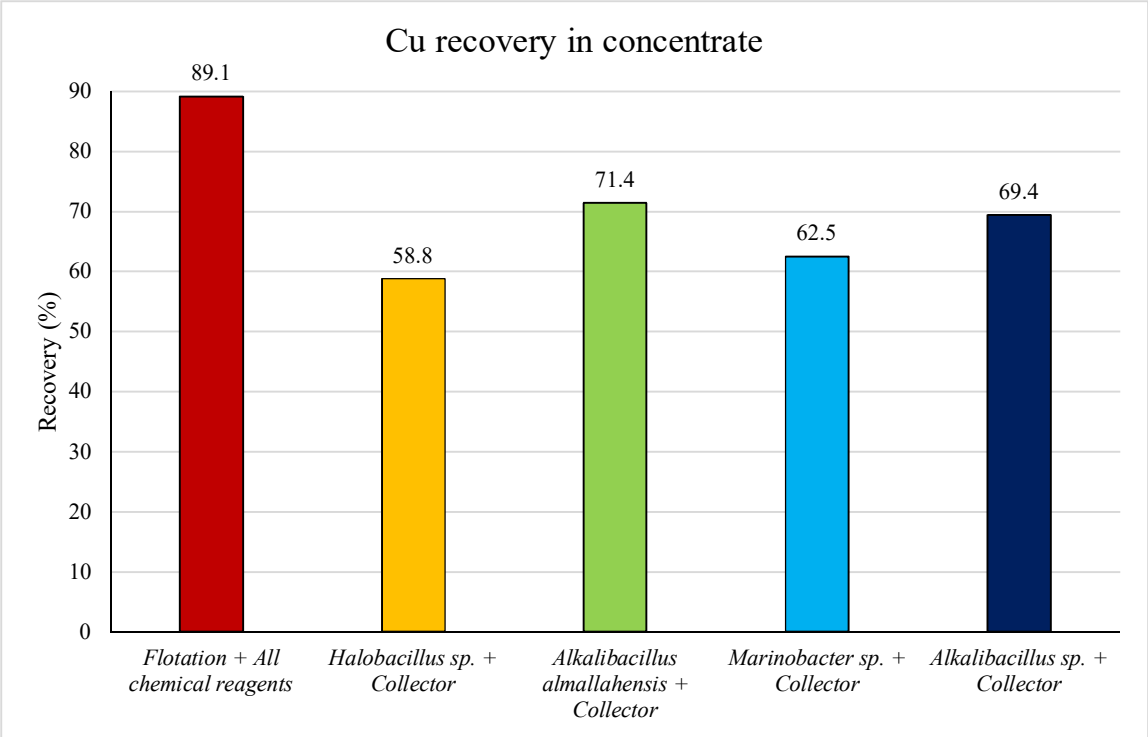

Figure 7. Cu recovery in concentrate resulting from standard bacteria-free flotation and single-bacteria bio-flotation tests.

Figure 8 illustrates Fe recovery in tailings and Cu recovery in concentrate at standard bacteria-free flotation and single-bacteria bio-flotation tests simultaneously. As it can be seen, the *Halobacillus sp.* bacterium showed the best performance in pyrite depression compared to other bacteria and the standard bacteria-free test. On the other hand, *Alkalibacillus almalallahensis* had the best performance in chalcopyrite flotation compared to other bacteria. However, compared to the standard test, recovery of chalcopyrite flotation by this bacterium was lower.

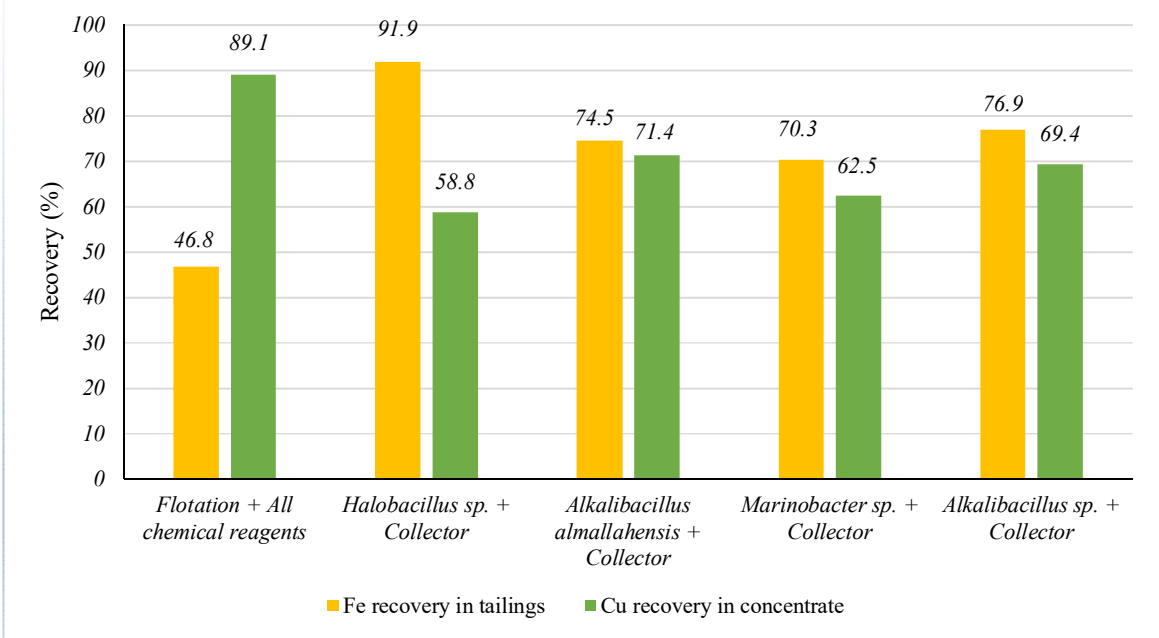

Figure 8. Fe recovery in tailings and Cu recovery in concentrate at standard bacteria-free flotation and single-bacteria bio-flotation tests.

4. Conclusions

In this research work, Fe recovery in tailings and Cu recovery in concentrate in two modes, i.e. bacteria-free (standard flotation) and single-bacteria (bio-flotation) modes using four types of halophilic bacteria *Halobacillus sp.*, *Alkalibacillus almallahensis*, *Marinobacter sp.*, and *Alkalibacillus sp.* were investigated in the flotation of copper sulfide using a Denver laboratory flotation cell. Also the possibility of replacing the chemical reagents used in the industrial flotation process of chalcopyrite such as frothers (MIBC and F7240), depressants (sodium metabisulfite), pH regulators (lime), and collectors (gasoil, Z11, and C7240) with halophilic bacteria was studied. The results indicated that each of the above four types of bacteria along with the mentioned collectors as the only chemical reagents (bio-flotation + collector) were able to depress pyrite better than the bacteria-free mode (flotation + all chemical reagents). However, the recovery of chalcopyrite using the bio-flotation method was lower than its recovery using the flotation method.

The following results were also obtained:

- ✓ Halophilic bacteria were able to replace the industrial depressants used in the process of pyrite depression such as sodium metabisulfite ($Na_2S_2O_5$). Therefore, if these bacteria are used, there is no requirement to use other depressants in the flotation cell.
- ✓ Due to the proper growth and proliferation of halophilic bacteria in relatively neutral media (pH = 7–8), they were able to replace the pH regulators used in industry such as lime. Therefore, in case of using these bacteria, there was no requirement to add pH regulator to the flotation cell for chalcopyrite flotation.
- ✓ To some extent, halophilic bacteria were able to play the role of frother in the chalcopyrite flotation process. However, considering that the frothers used in this research work (MIBC and F7240) caused the death of bacteria, it was not possible to use these frothers together with halophilic bacteria in the flotation cell. It is suggested by finding appropriate and compatible frothers with these bacteria their flotation performance is improved in the flotation of chalcopyrite.
- ✓ The use of collectors (gasoil, Z11 and C7240) together with halophilic bacteria was necessary because these bacteria alone and without the help of collectors did not succeed in recovering chalcopyrite.
- ✓ According to the research work conducted by Nasrollahzadeh *et al*. [37], it is suggested to use the combination of four halophilic bacteria used in this research work to investigate their improvement of performance and their synergism in chalcopyrite flotation and pyrite depression.

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بررسی امکان جایگزینی برخی از معرف‌های شیمیایی مورداستفاده در فلوتاسیون مس سولفیدی با باکتری‌های هالوفیل

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چکیده:

فلوتاسیون مهمترین روش برای فرآوری کانسنگ‌های مس سولفیدی می‌باشد. به دلیل پرهزینه بودن و مخاطرات زیست‌محیطی ناشی از معرف‌های شیمیایی مورداستفاده در این فرآیند (مانند کلکتورها، کف‌سازها، تنظیم‌کننده‌های pH، بازداشت‌کننده‌ها و ...)، امکان جایگزینی همه این معرف‌ها یا حداقل برخی از آن‌ها توسط روش‌های محیط‌زیست دوستی مانند بیوتکنولوژی از اهمیت ویژه‌ای برخوردار است. یکی از این روش‌ها، بیوفلوتاسیون با استفاده از باکتری‌های هالوفیل می‌باشد. این باکتری‌ها توانایی رشد و زنده ماندن در محیط‌های نمکی مانند آب شور دریا را دارند. همچنین برای تکثیر و رشد آن‌ها به pHهای بالای قلیایی نیازی نیست و در pHهای نسبتاً خنثی نیز قابلیت رشد و تکثیر دارند. در این تحقیق، چهار نوع باکتری هالوفیل *Halobacillus sp.*، *Alkalibacillus almallahensis*، *Marinobacter sp.* و *Alkalibacillus sp.* برای جایگزینی کف‌سازها (MIBC و F7240)، بازداشت‌کننده (متابی سولفید سدیم)، و تنظیم‌کننده pH (آهک) در فلوتاسیون مس سولفیدی با استفاده از سلول فلوتاسیون آزمایشگاهی دنور مورد مطالعه قرار گرفتند. نتایج به‌دست‌آمده نشان می‌دهد که هر یک از چهار نوع باکتری ذکر شده در بالا به همراه کلکتورها (گازوئیل، Z11 و C7240) به عنوان تنها معرف‌های شیمیایی (بیوفلوتاسیون + کلکتور) می‌توانند پیریت را بهتر از حالت بدون باکتری (فلوتاسیون + تمام معرف‌های شیمیایی) بازداشت کنند. بازیابی آهن در باطله آزمایش فلوتاسیون استاندارد ۴۶/۸ درصد است که با استفاده از باکتری‌های هالوفیل *Halobacillus sp.*، *Alkalibacillus almallahensis*، *Marinobacter sp.* و *Alkalibacillus sp.* به ترتیب به ۹۱/۹، ۷۴/۵، ۷۰/۳ و ۷۶/۹ درصد افزایش یافته است. از طرفی بازیابی کالکوپیریت با استفاده از روش بیوفلوتاسیون کمتر از بازیابی آن با روش فلوتاسیون است. بازیابی مس در کنسانتره در آزمایش فلوتاسیون استاندارد ۸۹/۱ درصد است که با استفاده از باکتری‌های فوق در روش بیوفلوتاسیون به ترتیب به ۵۸/۸، ۷۱/۴، ۶۲/۵ و ۶۹/۴ درصد می‌رسد.

کلمات کلیدی: فلوتاسیون مس سولفیدی، بیوفلوتاسیون، باکتری‌های هالوفیل، معرف‌های شیمیایی، پیریت و کالکوپیریت.