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Optimization of Chalcopyrite Bioleaching using Two Different Adaptation Methods: Top-Down and Bottom-Up

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Abstract

Optimization of the effective parameters in the copper bioleaching of chalcopyrite concentrates (CuFeS2) is studied by moderately thermoacidophilic microorganisms. The microorganisms with extensive metabolic properties are used in two different ways: 'top-down' and 'bottom-up'. The bioleaching experiments are performed based on the parameters of silver, activated charcoal, concentrate type (Sarcheshmeh and Miduk), and a type of bacteria. By regrinding the concentrate particles down to 10 μm, bottom-up consortium, 500 ppm silver, and 3 g/L of coal, more than 97% of the copper from the Miduk chalcopyrite concentrate is recovered within 12 days. The final recovery of the control test without the microbes is 35%. The performance of the bottom-up method is significantly better than the top-down one. The moderate thermophiles have an important role in copper biomining.

1. Introduction

Chalcopyrite ($CuFeS_2$) represents the most abundant sulfide copper ore mineral, and thus has become the most important copper source worldwide. These facts have encouraged intensive research works in order to understand and to improve copper extraction from chalcopyrite [1]. The most important problems are the incomplete dissolution of chalcopyrite and low dissolution rate. Various metal ions have been used as catalyst y for the dissolution of chalcopyrite in an acidic medium but none of them, except silver, has been effective in the chemical and biological dissolution of chalcopyrite [2, 3]. When silver ions are used, the sulfur layer formed has a higher electrical conductivity in addition to a greater porosity, and thus facilitates the transfer of electrons and ions to and from the chalcopyrite surface [2, 3]. Moreover, the increase in chalcopyrite leaching in the presence of activated carbon is due to the galvanic effect between activated carbon and chalcopyrite. Activated carbon is thermodynamically much more stable than chalcopyrite, and can form galvanic couplings. Activated carbon acts as a cathode against the mineral chalcopyrite [3- 8]. The bioleaching process for sulfide minerals is affected by the choice of microorganism, pH, redox potential, and temperature, the choice of microorganism being one of the most important factors. Many published reports have indicated that mixed cultures are more efficient and more robust in oxidizing sulfide minerals than pure cultures [6-8]. However, the bioleaching system has many microbial niches, among which more than 14 genera and 33 species are distributed [9, 10]. Thus determining how to develop and optimize microbial consortia for bioleaching is a big challenge. Two contrasting approaches have been suggested and investigated in order to determine whether it would be possibe to produce an ideal or optimized consortium of bioleaching

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micro-organisms for a given process or substrate [11, 12].

Two completely different methods called 'top-down' and 'bottom-up' have been proposed to create a favorable microbial consortium for bioleaching (Figure 1). In the 'bottom- up' method, mixed cultures containing species of acidophilus from their natural environmental samples or specific cultures of the same strains are used as inoculum for the treatment of sulfide ores. In this method, a small number of species will remain, while undesirable microorganisms will be eliminated in the bioleaching process. Using this method, many researchers have obtained powerful and effective cultures to a pulp density of 12% [9, 13-15]. In the ‘top-down’ method, a sustainable, strong, and effective biochemical consortium must be created based on the environmental samples. The physiological and ecological characteristics of the various species of the consortium such as the ability to oxidize sulfur, ferrous iron for autotrophic, and heterotrophic growth are complement to each other. Temperature, pH, physiology of the species, and other factors such as the presence of toxic metals and ions are the influential factors in the designed consortium. The top down method is easy to apply to the stirred-tank consortium development. It is also likely that more than one combination of microorganisms, once fully adapted, is likely to be equally efficient at bio-oxidizing a particular mineral. Although a lot of preparative work is required, the logically designed ‘bottom up’ method has the advantage that only the beneficial microorganisms are required to be included. There is a danger that since the mineral bio-oxidation processes are not sterile, it may be difficult to prevent nuisance microorganisms from invading the consortium [9, 11]. Wang *et al*. (2014) have studied a moderately thermophilic mixed bacterial culture for bioleaching of the chalcopyrite concentrate by the two methods of 'top-down' and 'bottom-up', reporting that these methods are effective on bioleaching in 20% pulp density. Previous reports have indicated that the cultures prepared on the basis of the bottom-up approach are more efficient in increasing mineral oxidation [16-18]. Zhang *et al*. (2009) have demonstrated that a culture containing mixotrophs and autotrophs is far more effective in promoting the chalcopyrite leaching than a mixed culture containing any combination of two or three of the four bacteria *Acidithiobacillus caldus, Leptospirillum ferriphilum, Ferroplasma thermophilum*, and *Sulfobacillus spp*. [19]. The stepwise bioleaching method (mesophiles, moderately thermophiles, and thermophiles, in order) has a significant effect on increasing the copper recovery from chalcopyrite [2]. The type of microorganisms has also shown a significant impact on the leaching behavior of chalcopyrite [6, 9, 20]. The aim of the present work was to improve the performance of two types of thermoacidophiles using the two different methods of ‘top-down’ and ‘bottom-up’. Also the effective parameters for considering copper extraction from different chalcopyrite concentrates were studied.

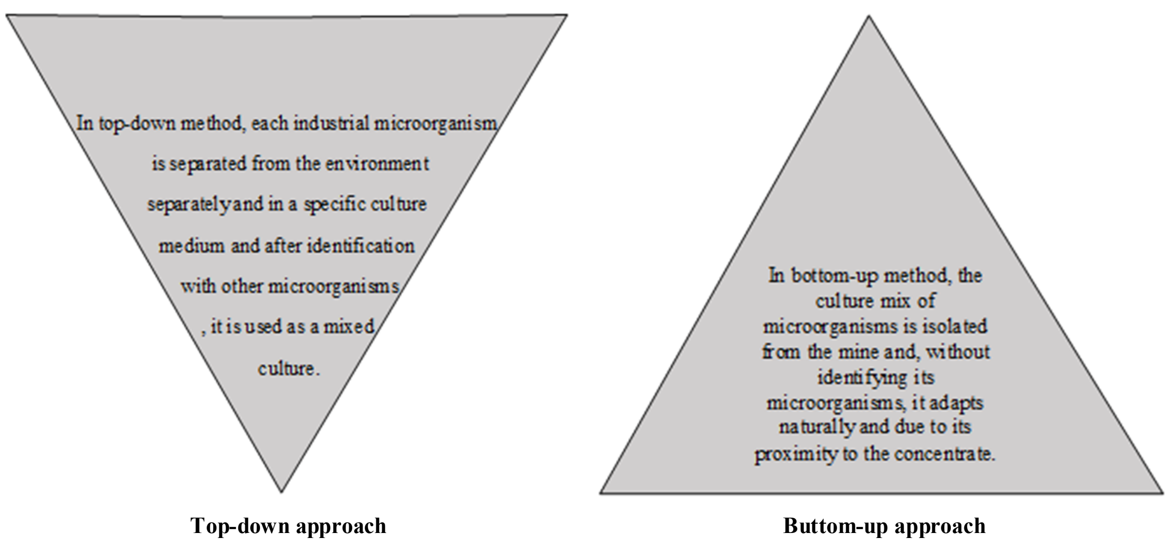

Figure 1. Top-down approach and Bottom-up approach.

2. Materials and Methods

2.1. Sampling of copper concentrate

Sampling of concentrate was performed from the concentrate plant of the two mines (Sarcheshmeh and Miduk, in Kerman, Iran) at 48-hour intervals for one month. The samples were called concentrate 1: Sarcheshmeh, and concentrate 2: Miduk. After drying, these samples were mixed and homogenized and passed through the riffle (divider) for several times in order to obtain a final representative sample through successive quadruple division.

2.2. Microorganisms

The thermoacidophilic microorganisms were isolated from the acid mine drainages (AMDs) and ores of different mines [9]. The microorganisms were evaluated in the bioleaching experiments using two different methods, 'bottom-up' and 'top-down'. Consortium#1 was a mixture of cultured bacteria, and was isolated directly from the ore and concentrates ('bottom-up' method). Consortium#2 was isolated and identified in its specific culture medium ('top-down' method) [9].

2.3. Chemical and mineralogical analysis

After preparing the representative sample by the systematic row division method from the Sarcheshmeh and Midouk copper mines, they were chemically and mineralogically analyzed. The chemical composition of the samples was determined by X-ray fluorescence (XRF, Phillips model). Mineralogy study was performed in order to investigate the mineralogical composition of the solid samples by polished sections and by polarizing microscopy (Leica DMLP).

2.4. Identification of microorganisms

In order to perform the 'top-down' method, the isolated microorganisms were identified, and then a mixed culture of these microorganisms called consortium #2 was prepared. Detection of these microorganisms was performed using the PCR (polymerase chain reaction) method [3, 9].

2.5. Experimental design

In order to study the effects of different parameters, the interactions of these parameters, and also to reduce the number of experiments, the design of experiments (DOE) method was conducted using the DX7 software. At this stage, four parameters of activated charcoal, silver, type of bacteria (consortium#1: direct bacteria taken from the environment and consortium#2: isolated bacteria), and type of concentrate (concentrate 1: Sarcheshmeh and concentrate 2: Miduk) in two levels were tested to evaluate the behavior of the concentrate bioleaching with the basic parameters involved in the copper dissolution (Table 1).

Table 1. Factors and levels of DOE.

Parameter	Levels	
	-	+
Silver (g/t of concentrate)	0	500
Active carbon (AC)	0	3
Concentrate	Concentrate 1	Concentrate 2
Type of bacteria	Consortium#1	Consortium#2

2.6 Bioleaching tests

These experiments were performed in 500 mL Erlenmeyer flasks with a final content of 200 mL for an optimal aeration. 20 g of the concentrate (10% solid) and 20 mL of the bacterial medium (10% inoculation) were added to 180 mL of the bacterial culture medium (Table 2). In case of different additives (depending on the DOE), the materials were added to the Erlenmeyer flasks, and after adjusting the pH value of the medium to 1, they were placed in a shaking incubator with a temperature of 50 ºC and 125 rpm for the desired time. In order to compare the leaching and bioleaching tests, an experiment with similar conditions and with the best results from the bioleaching tests but without a bacterial consortium was designed. In order to inactivate the native microbes in the concentrate, 5% (volume/volume) of 2% thymol solution in ethanol was added to the leaching medium as an anti-bacterial [3].

The pH value of the medium was monitored daily, and if the pH increased, it was re-adjusted to the desired value with sulfuric acid. The Metrom 826 and Metteroldo devices were used in order to measure pH and ORP, respectively. A platinum electrode with Ag/AgCl reference (+207 mV SHE) was used to measure the oxidation reduction potential (ORP). Eh was also examined. The concentration of bacteria was evaluated on a daily basis under an optical microscope (Zeiss, Axioskop 40) using a blood cell counting chambers [21, 22]. Finally, the analysis of the dissolved and solid copper and iron was conducted.

Table 2. Salts used in preparation of culture medium 10NPK.

10 NPK culture media	g/l
$MgSO_4.7H_2O$	0.16
K_2HPO_4	0.4
KCl	0.1
$FeSO_4.7H_2O$	50
Distilled water	1000
pH	1

3. Results

3.1. Individual particle analysis

The results of the chemical analysis and mineralogy of the concentrate of the Sarcheshmeh and Miduk mines are shown in Tables 3 and 4. All the secondary sulfides in the Sarcheshmeh concentrate were more than those of Miduk, except for chalcocite.

Table 3. Mineralogical analysis.

Mine	Weight percentage (%wt)							
	Sphalerite ZnS	Molybdenite MoS_2	Pyrite FeS_2	Bornite Su_5FeS_4	Natural copper -Cu-N	Chalcopyrite $CuFeS_2$	Covellite CuS	Chalcocite Cu_2S
Sarcheshmeh	1.538	0.329	3.372	0.000	0.000	49.440	4.537	2.02
Miduk	0.00	0.245	4.987	1.461	0.000	29.179	1.468	19.671

Table 4. Chemical analysis of concentrates from different mines.

Mine	Combination (%)			
	Mo	Fe	CuO	Cu
Sarcheshme	0.197	30.23	0.044	22.25
Miduk	0.147	27.96	3.23	30.94

3.2. Bacterial identification

The strains included *Sulfobacillus thermosulfidooxidance* KMM3, *Sulfobacillus acidophilus* strains KMM26, KMS10, KMS22 and KMSU23, and *Sulfovacillus benefaciens* strain (KMM25, KMSU32), identified using the PCR method. Their phylogenetic tree is shown in Figures 2 and 3. While marked in red, the phylogenetic tree of the Sulfobacillus strains is plotted by considering the neighbor-joining method and the bootstrap coefficient value of 100.

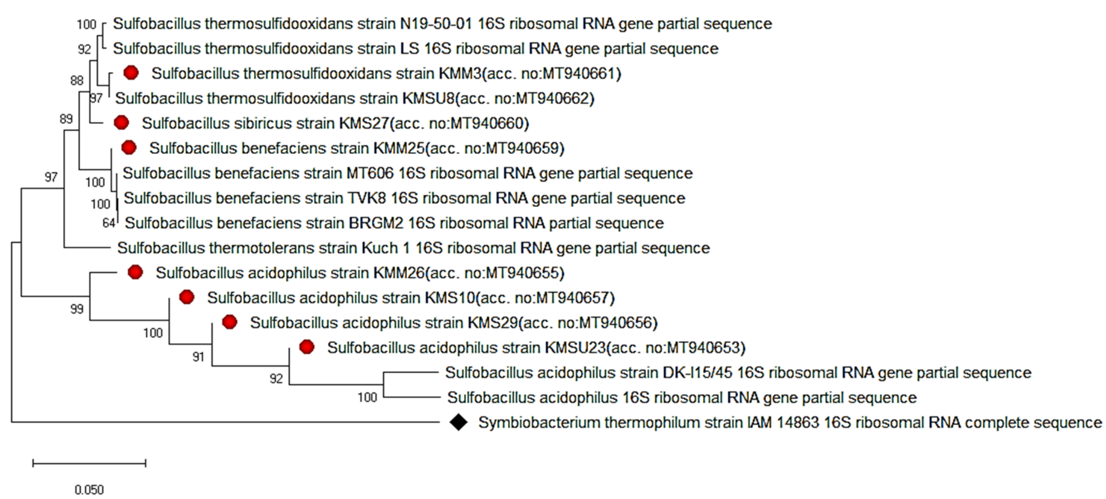

Figure 2. Phylogenetic tree of Sulfobacillus strains using neighbor-joining method and bootstrap coefficient of 100.

3.3. DOE with 4 full factorials

The results of the parameters of silver, activated carbon, type of concentrate (Sarcheshmeh and miduk), and type of bacteria (consortium#1 and 2) are shown in Table 5. The bacterial control tests were also performed for the two concentrates with a recovery between 25% and 27%. Comparison of the final dissolution in different bioleaching experiments according to DOE is shown in Figure 4.

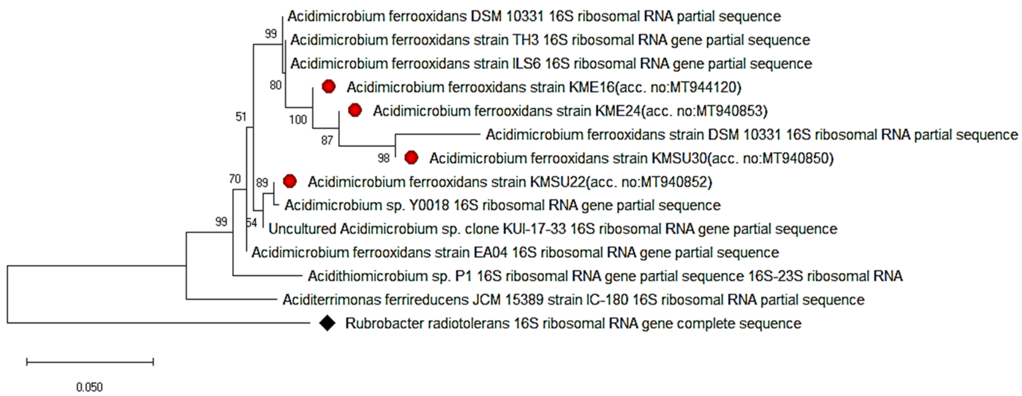

Figure 3. Phylogenetic tree of acidic microbiome strains using neighbor-joining method and boot strap coefficient of 100.

Table 5. DOE full factorial design with 4 effective factors at various levels.

Factor 1 A: Ag^+ g/t	Factor 2 B: Activated Carbon (AC), g/l	Factor 3 C: type Concentrate	Factor 4 D: Inoculum	esponse Recovery Cu (%)
0	0	Concentrate 1	Consortium#1	36.95
500	0	Concentrate 1	Consortium#1	90.87
0	3	Concentrate 1	Consortium#1	86
500	3	Concentrate 1	Consortium#1	95.42
0	0	Concentrate 2	Consortium#1	55.04
500	0	Concentrate 2	Consortium#1	99.01
0	3	Concentrate 2	Consortium#1	86.4
500	3	Concentrate 2	Consortium#1	97.04
0	0	Concentrate 1	Consortium#2	35.04
500	0	Concentrate 1	Consortium#2	90.43
0	3	Concentrate 1	Consortium#2	85.23
500	3	Concentrate 1	Consortium#2	93.07
0	0	Concentrate 2	Consortium#2	54.95
500	0	Concentrate 2	Consortium#2	91
0	3	Concentrate 2	Consortium#2	85.41
500	3	Concentrate 2	Consortium#2	94.61
250	1.5	Concentrate 1	Consortium#1	91.79
250	1.5	Concentrate 2	Consortium#2	93.18
250	1.5	Concentrate 1	Consortium#1	91.05
250	1.5	Concentrate 2	Consortium#2	93.01

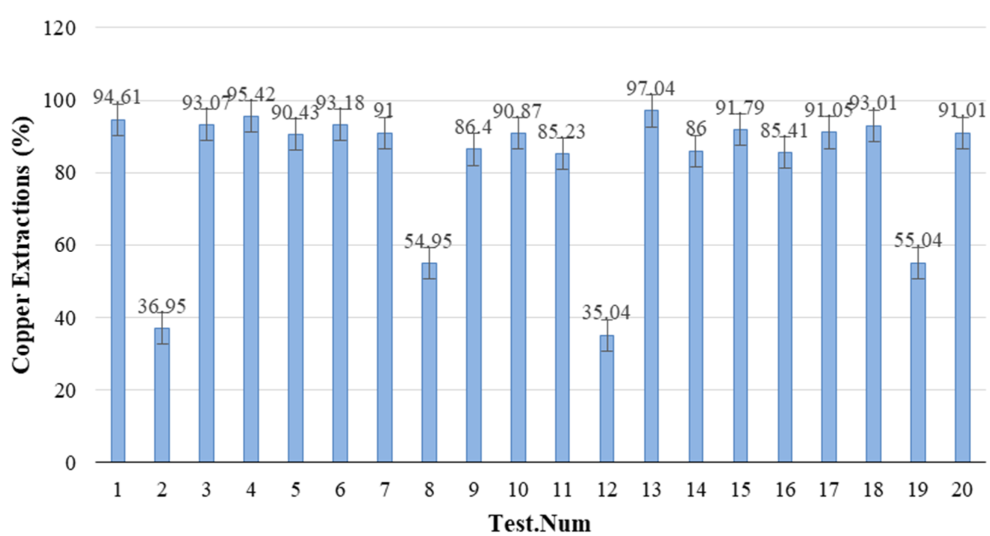

Figure 4. Comparison of final dissolution in different bioleaching experiments according to DOE.

3.4. Analysis of variance (ANOVA)

According to the ANOVA results (Table 6), the effects of all 4 parameters were significant in bioleaching. The presence of silver had the greatest effect. In this DOE, the activated charcoal also showed a significant effect with more than 50% improvement in the recovery. Thus activated charcoal in concentrate 1 with the presence and absence of silver recovered 95.42% and 86%, respectively, and in concentrate 2, with the presence and absence of silver showed 97.04% and 86.4% recovery, respectively. Due to the index interaction between silver and activated carbon, the simultaneous presence of two effective parameters could significantly increase the recovery of copper from the concentrate.

3.5. Redox potential and pH

The result of the variations in pH and ORP are shown in Figures 5 and 6.

Table 6. ANOVA of DOE, significance of additives (silver, AC) in bioleaching.

Source	Sum of squares	df	Mean square	F value	p-value Prob > F	Sum of squares
Model	6522.94	10	652.29	32.19	0.0006	Significant
A- Silver ion (Ag+)	2981.98	1	2981.98	147.18	< 0.0001	Significant
B- Activated carbon	1977.80	1	1977.80	97.61	0.0002	Significant
AB	1300.14	1	1300.14	64.17	0.0005	Significant
Curvature	549.89	4	137.47	6.78	0.0297	Significant
Residual	101.31	5	20.26			
Cor Total	7174.13	19				

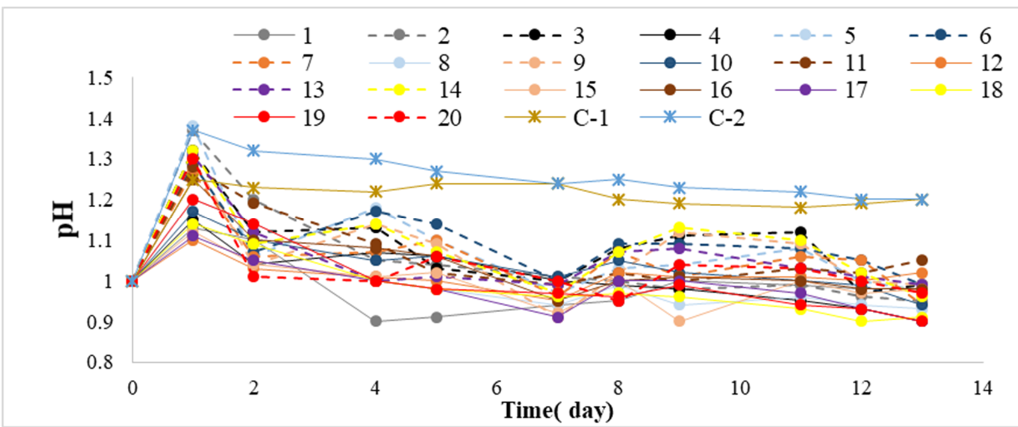

Figure 5. pH changes in various bioleaching experiments.

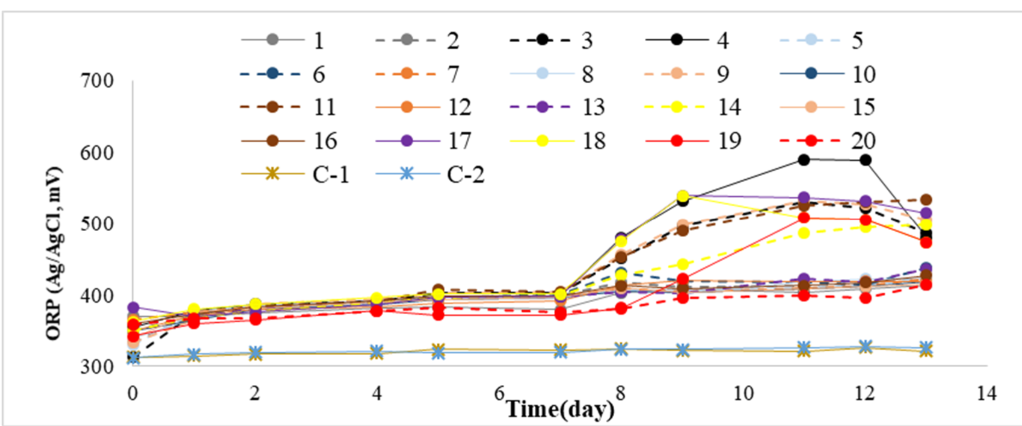

Figure 6. ORP changes in various bioleaching experiments.

4. Discussion

According to the results obtained, the Miduk concentrate contained 30.94% copper and 27.96% iron, while the Sarcheshmeh concentrate contained 22.25% copper and 30.23% iron. The content of oxide copper in the Miduk and

Sarcheshmeh copper mines was 3.23% and 0.44%, respectively. Based on the results of the mineralogical study, the main valuable minerals in the Miduk concentrate were chalcopyrite (29.179%), covellite (1.468%), and chalcocite (19.671%), while the amount of chalcopyrite (49.440%) was dominant, and covellite (4.537%) and chalcocite (2.102%) were in minor amounts in the Sarcheshmeh concentrate. In Miduk and Sarcheshmeh, the most important gangue was pyrite 4.987% and 3.372% interval.

The study of mineralogical results showed that copper was mainly present in the concentrate in the form of primary sulfide compounds, namely chalcopyrite. Therefore, the bioleaching process will face many problems such as very slow kinetics, poor extraction, long leaching time, and passivation of chalcopyrite. Therefore, according to the available literature, it was predicted that thermoacidophiles could be suitable for the bioleaching tests [5, 6, 9].

Continuous decreasing changes in the pH value in the presence of microorganisms indicate their activity and acid production in the bioleaching environment (Figure 5). Also continuous incremental changes in ORP (Ag/AgCl, mV) in the presence of microorganisms compared to their absence indicate a continuous production of oxidized iron ferric by the micoorganisms (Figure 6).

Moderate thermophilic microorganisms are more efficient than mesophiles at higher temperatures. They have higher rates of leaching due to a higher temperature tolerance, higher heavy metal tolerance, and better metabolic properties. They also have the ability to overcome the issues in bioleaching of the recalcitrant sulfides such as chalcopyrite but using the potential of extremely thermophilic bacteria in the temperature range of 60-84 $^{\circ}C$ will not be economical in the industry, because providing the temperatures above 70 degrees is too expensive. It imposes on the industry in terms of energy supply and in terms of equipment corrosion. Also the nutritional needs of extreme thermoacidophiles are more complex than those of the mesophilic and moderately thermoacidophilic microorganisms. Also environmental fluctuations such as temperature changes will cause a sharp decline in their numbers. In addition, the lack of peptidoglycans in moderate thermophilic bacteria caused them to be more sensitive to the environmental conditions and high solid content than mesophiles. This is another problem of the industrial application of these microorganisms [9, 6, 23]. As a comparison between the three classes of mesophilic, moderately thermophilic, and extremely thermophilic microbes, the commercial use of moderately thermophilic bacteria at 45 $^{\circ}C$ to 50 $^{\circ}C$ will have a high potential for the extraction of metals from mineral sulfides with economic considerations. The two completely opposite methods called 'top-down' and 'bottom-up' were used to create a microbial consortium for the bioleaching process.

The results obtained showed that the performance of consortium#1, which was cultured with a mixture of microorganisms directly isolated from the ore and concentrate (‘bottom-up’ method), was better than that of consortium#2, which was cultured with a mixture of isolated bacteria (‘top-down’ method). The main reason was the adaptability of the bacteria in consortium#1 with harsh environmental conditions, while the bank deposited microbes of consortium#2 was isolated after a period of culture in their specific media.

The similarity of the consortium#1 media to their natural environment and the maintenance of microbial relationships between the microorganisms such as biofilms, nutritional collaborations, and bacterial communications through a network of signals or chromosensing mechanism and population structure regulation are the most important factors that make it more successful. Compared with the consortiums, consortium#1, the case of consortium#2, and their separation from nutrient-rich environments can be a deterrent to a group of microorganisms or lead to the early growth of the proliferation of microorganisms compared to late growth ones. In other words, the lack of suitable biological and non-biological factors in the consortium environment are two reasons for its poorer performance. The effects of the parameters such as activated carbon and silver were evaluated. Yuehua *et al.* (2002) have demonstrated that the addition of silver is effective in dissolving copper from chalcopyrite with forming a layer of argentite on the mineral surface [24]. Argentite is converted to Ag^{+} again during the microbial process, and this cycle continues, during which the dissolution rate may increase by 25% to 75% [25]. Addition of carbon particles to the leaching process may improve the electrical conductivity of the sulfur insulation phase. They concluded that carbon particles quenched the solubility rate. The researchers have stated that carbon reduces the strength of the inhibitory film [26]. In the present work, the performance of activated carbon and silver in the bioleaching experiments was quite favorable. Silver ion alone increased the dissolution rate by more than 90%. Activated charcoal also worked well in the chalcopyrite dissolution. The results obtained showed a positive effect of up to 50%. Activated charcoal alone in concentrate 1 with the presence and absence of silver recovered 95.42% and 86% of copper respectively, and in concentrate 2, with the presence and absence of silver showed 97.04% and 86.4% recovery, respectively [26].

5. Conclusions

The Miduk concentrate had a higher copper extraction efficiency due to its lower chalcopyrite content. The bioleaching experiments in shaking flasks based on DOE (full factorial design) with the Miduk concentrate, 'bottom-up' method consortium, and silver 500 and 3 g/L coal, recovered more than 97% copper. The 'bottom-up' method showed a better performance for the biological dissolution of chalcopyrite concentrate. The silver ions and activated carbon could be used to prevent passivation of the chalcopyrite concentrate. The result of this literature gives useful insights into how a bacterial community of moderate thermophiles develops and interacts during adaptation and the bioleaching process, and also into how a mixed culture of microorganisms can be assembled and adapted for an optimal bioleaching of the chalcopyrite concentrate.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

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بهینه سازی بیولیچینگ کالکوپیریت با استفاده از دو روش سازگاری مختلف: بالا به پایین و پایین به بالا

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چکیده:

بهینه‌سازی پارامترهای مؤثر بیولیچینگ کنسانتره‌های کالکوپیریتی ($CuFeS_2$) مس، توسط میکروارگانیسم‌های ترمواسیدوفیل معتدل مورد مطالعه قرار گرفت. میکروارگانیسم‌ها با خواص متابولیک گسترده به دو روش مختلف «بالا به پایین» و «پایین به بالا» مورد استفاده قرار گرفتند. آزمایش های بیولیچینگ بر اساس پارامترهای نقره، زغال فعال، نوع کنسانتره (سرچشمه و میدوک) و نوعی باکتری انجام شد. با آسیاب مجدد ذرات کنسانتره تا ۱۰ میکرومتر، کنسرسیوم از پایین به بالا، ۵۰۰ پی پی ام نقره و ۳ گرم در لیتر زغال سنگ، بیش از ۹۷ درصد مس از کنسانتره کالکوپیریت میدوک ظرف ۱۲ روز بازیابی شد. بازیابی نهایی آزمایش کنترل بدون میکروب ۳۵ درصد بود. عملکرد روش از پایین به بالا به طور قابل توجهی بهتر از روش بالا به پایین می‌باشد. باکتری های ترموفیل معتدل نقش مهمی در بیولیچینگ مس دارند.

کلمات کلیدی: ترمواسیدوفیل های معتدل، از بالا به پایین، از پایین به بالا، کنسانتره کالکوپیریت.