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Thermoacidophilic bacteria isolated from Sarcheshmeh low-grade copper ore in chalcopyrite bioleaching from mineral tailing

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ABSTRACT

This research has focused on isolating and identifying different thermoacidophilic bacteria from a Sarcheshmeh low-grade copper ore and evaluating their ability to copper bioleaching from the mineral tailing. After the isolation of the bacteria, molecular identification was carried out based on the 16S rRNA gene sequences and drawing the phylogenetic tree. Then, the effect of the pH, pulp density, and composition of the media on the copper bioleaching was determined using the identified bacteria. The isolated strain (*Strain SCM1*) belonged to *Delftia acidovorans* with a 95.73% of identity. The optimal condition for the copper bioleaching was reported in a medium consisting of sulfur (10 g/L), glucose (10 g/L), yeast extract (2 g/L), and mineral tailing (5% wt/vol) at the pH of 2.00 at 50°C. Under this condition, the highest amount of copper (83%) was bioleached. It proves that the lately isolated strain can be effectively employed in the copper bioleaching process.

Keywords: *Bioleaching, Copper, Mineral tailing, Thermoacidophilic bacteria, 16S rRNA*

1. Introduction

The mining of metals generates considerable amounts of waste materials. These wastes generally have little economic value, making their exploitation unprofitable, though they often have the potential to pose a long-term threat and cause damage to the environment. Mine wastes vary depending on their physical and chemical composition, the type of mining, and the way the minerals are processed. Millions of tons of ore are processed every year by the mining industry, of which over 95% is disposed of in the form of waste rocks and mine tailings. The mine tailings may contain base transition metals, such as iron, copper, nickel, and zinc, in relatively high concentrations, and also occasionally precious metals such as gold and silver, in the form of minerals (and native metals) that have not been separated by the froth flotation [1, 2].

Mines have a severely acidic environment with a special bacterial population. Sometimes, this acidic environment can assist human activities to better exploit the mines. Bioleaching is one of the processes in which insoluble metal compounds are converted to soluble forms through biological oxidation by microorganisms [3, 4]. The materials remaining in the process of isolating valuable parts from non-valuable components are called tailing [5]. Over time, mineral resources will be finished and mineral tailings, of which the main part is a low-grade copper ore, acquire value.

Sarcheshmeh copper mine is the most important copper mine in Iran, which has 690 million tons of mineral tailings with a copper average grade of 0.22wt%. Since the chemical extraction of metals from these tailings is not affordable; therefore, it is necessary to use an appropriate strategy for the extraction. Currently, bioleaching technology as a simple process has drawn significant attention, due to its abundant advantage, including mild reaction, low energy consumption, low cost, being environmentally friendly, and being suitable for low-grade mine tailings and residues [6–8]. In recent years, scientists have suggested various microorganisms using molecular phylogenetic techniques to study the relationships between all bacteria. 16S rRNA is a universal molecular marker gene in bacteria that identifies relationships between all bacteria. The identification and use of these microorganisms are vital for the mining industry [9].

Thermoacidophilic microorganisms are a group of microorganisms taking part in the bioleaching processes [10]. They are lithoautotrophic and usually resistant to heavy metals. They like acidic conditions with optimal pH of 1.00 to 3.00. Their copper bioleaching rate is more than the rate of the mesophiles. A high amount of heat is produced in the bioleaching processes due to the exothermic reactions of sulfide oxidation. The production of this heat increases the bioleaching temperature from 50 °C to 80 °C. Possibly, this increase in temperature inhibits the growth and activity of the mesophiles. On the other hand, the increased temperature enhances the rate of bioleaching, which is carried out by thermoacidophilic bacteria. Thus, it seems that thermoacidophilic bacteria have a better performance in converting insoluble copper to its soluble forms [11, 12].

Thermoacidophilic bacteria are used to dissolve sulfide minerals such as chalcopyrite. Dissolution of chalcopyrite by microorganisms is done by both direct and indirect mechanisms. In the direct mechanism, contact is made between bacteria and chalcopyrite, and using enzymes secreted by bacteria, chalcopyrite is oxidized to copper sulfate (Eq. 1). The indirect mechanism is performed by two paths of thiosulfate and

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polysulfide. Iron is converted by bacteria to ferric iron, which is a strong oxidizing agent for chalcopyrite (Eq. 2-5), but in this method, due to the deposition of iron compounds such as jarosite (Eq. 6), the surface of chalcopyrite is inactivated and the speed Copper dissolution decreases [13].

$$2CuFeS_2 + 17/2O_2 + 2H^+ \rightarrow 2Cu^{2+} + 2Fe^{3+} + 4SO4^{2-} + H_2O \quad (1)$$
$$CuFeS_2 + O_2 + 4H^+ \rightarrow Cu^{2+} + Fe^{2+} + 2S^0 + 2H_2O \quad (2)$$
$$4Fe^{2+} + O_2 + 4H^+ \rightarrow 4Fe^{3+} + 2H_2O \quad (3)$$
$$CuFeS_2 + 4Fe^{3+} \rightarrow Cu^{2+} + 5Fe^{2+} + 2S^0 \quad (4)$$
$$2S^0 + 2H_2O + 3O_2 \rightarrow 2SO4^{2-} + 4H^+ \quad (5)$$
$$M^+ + 3Fe^{3+} + 2SO_4^{2-} + 6H_2O \rightarrow MFe_3(SO_4)_2(OH)_6 + 6H^+ \quad (6)$$

Sarcheshmeh copper mine is one of the largest mines in the world containing significant amounts of iron and copper sulfides. It can be a suitable habitat for many strains of sulfide oxidizing bacteria. As a result, the isolation of different thermoacidophilic bacteria with maximum sulfide oxidation ability can be effective in achieving maximum bioleaching efficiency. Also, the use of sulfur-oxidizing bacteria in the bioleaching process can be effective in reducing the environmental pollution of sulfur in mines. Moreover, copper bioleaching could be better performed by indigenous strains compared to standard ones, due to their adaptation to metal sulfides and leaching conditions. Consequently, the main objectives of the present study were:

1. The isolation and identification of a different native group of thermoacidophilic bacteria from a Sarcheshmeh low-grade copper ore with a high capacity for bioleaching.
2. The determination of environmental, biological, and physicochemical factors that affect the bioleaching ability and therefore, the rate of the metal extraction.

2. Materials and Methods

2.1. Sampling

Sampling was carried out on 10 different areas of a mixture of the Sarcheshmeh low-grade copper ore, sulfide, and oxide along with an acid mine drainage from Kerman province, Iran. The samples were transported to the lab in a sterile condition, maintained at 4 °C. The mineral tailing was ground into particles with a size finer than 70 µm. Afterward, the initial sample of the low-grade copper ore was chemically analyzed using X-ray fluorescence (XRF) (Philips, model PW 1480) and X-ray diffraction (XRD) (Bruker, model D8 advance).

2.2. Experimental

All the chemicals used in this study, their formulas, purity percentages, and manufacturing companies are listed in Table 1.

2.3. Isolation of the thermoacidophilic bacteria

The isolation of the thermoacidophilic bacteria and the effect of different media on their bacterial cell growth were studied. As shown in Table 1, the standard 9K medium [14, 15] and the media of 9K1 (10 g/L of the elemental sulfur powder added to 9K), 9K2 (44 g/L of ferrous sulfate heptahydrate added to 9K), 9K3 (10 g/L of the elemental sulfur powder and 2 g/L of yeast extract added to 9K), 9k4 (10 g/L of the elemental sulfur powder, 2 g/L of yeast extract and 10 g/L of glucose added to 9K) and 9K5 (44 g/L of ferrous sulfate heptahydrate, 2 g/L of yeast extract and 10 g/L of glucose added to the 9K) were employed to isolate the thermoacidophilic bacteria. For this purpose, 100 mL of each of the 9K, 9K1, 9K2, and 9K5 media were separately mixed with 10 mL of a mixture of the low-grade copper ore sample and acid mine drainage sample at the pH of 2.50 in 300 mL Erlenmeyer flasks. The pH of the media was adjusted to 2.00 by adding a 0.5 M sulfuric acid solution. The mixtures were incubated at 50°C and 150 rpm for one week. Then, 200 µl of each mixture was transferred into a fresh growth medium and incubated in the same condition. This stage was repeated three times and finally, the cultures, in which the microbial growth was distinguishable based on the turbidity, were selected to isolate the pure strains of the sulfur and iron-oxidizing bacteria. To purify strains, a diluted (10^{-6} cell/mL) microbial suspension of each medium was prepared and transferred into the 9K, 9K1, 9K2, and 9K5 media. Then, the media were incubated at 50°C and 150 rpm for one week.

2.4. Molecular identification of the strain based on the sequence of 16S rRNA gene

2.4.1. Deoxyribonucleic acid (DNA) extraction

Genomic DNA extraction was carried out, using Mamur's method [16], and modified as follows. All the steps were performed at room temperature (25 ± 2 °C) unless otherwise mentioned. The bacterial cells were harvested by a centrifuge at 1000 × g for 5 minutes in a 2.0 ml microtube and transferred to 200 µl of a 1X Tris-EDTA buffer (TE buffer). Then, the sample was treated with 25 µl of sodium dodecyl sulfate (SDS) (10%), 25 µl of proteinase K (20 mg/mL), and 3 µl of a 10 mg/ml Ribonuclease (RNase) solution, consequently. The treated sample was vortex-mixed and incubated at 37 °C for 60 minutes. After that 90 µl of a 5 M NaCl solution was added to the sample and vortex-mixed. In the next step, 75 µl of lysozyme was added followed by vortex mixing and incubation at 65 °C for 20 minutes. A volume of phenol-chloroform-isoamyl alcohol with a ratio of 25: 24: 1 was added to the sample and centrifuged at 11500 × g for 15 minutes. The supernatant was transferred to a sterile tube. Again, a specific volume of phenol-chloroform-isoamyl alcohol with the ratio of 25: 24: 1 was added to the supernatant from the previous step, centrifuged at 11500 × g for 10 minutes, and transferred to a sterile tube. Nucleic acid was precipitated by adding 600 µl of cold isopropanol to 1 mL of the solution from the previous stage and incubated at -20 °C for 20 minutes followed by centrifuge trials at 11500 × g for 15 minutes. Then, the pellets from the centrifuge were rinsed with 70% ethanol. In the end, the pellets were dissolved in 50 µl of a TE buffer and stored at -20 °C for further analysis. The verification of the DNA extraction was performed by EV234 consort Suisse electrophoresis using a 0.7% agarose gel.

2.4.2. 16S rRNA amplification

The PCR, in a 0.2 mL PCR tube, was performed into 50 µl of a solution containing 5 µl of a 10 x PCR buffer, 3 µl of an $MgCl_2$ buffer at 25 mM, 1 µl of dNTP at 10 mM, 1 µl of each of the two primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) at 20 µM, 0.5 µl of a 0.5 U/µl Taq DNA polymerase, 0.5 µl of DNA and 38 µl of distilled water. The optimum condition for the PCR cycling was: initial denaturation at 95 ºC for one minute. The process was followed by 30 cycles of denaturation at 94 ºC for 60 seconds, annealing at 57 ºC for 30 seconds, and extension at 72 ºC for 60 seconds. The final extension was done at 72 ºC for 5 minutes. In the end, the PCR product was confirmed by a 1.5% agarose gel electrophoresis, and the replicated DNA was sent to "Topaz Gene" for sequencing [17].

2.4.3 Data analysis

The sequencing result was compared and blasted by the chromas pro software with the sequence recorded in the NCBI. Subsequently, the phylogenetic analysis of the selected strain was performed using the Clustal x, Bio Edit, and Mega6 software, and the phylogeny tree was generated by the Neighbor-joining method [18, 19].

2.5. Determination of the optimal growth conditions

To determine the optimal growth conditions of the isolated strain, and therefore, to achieve the highest rate of bioleaching, the effect of the composition of the media, pH, and temperature on bacterial growth was investigated. To obtain the maximum bacterial growth rate, a 10 vol% inoculum of the bacterial solutions, with a concentration of 1×10^7 cell/mL, was cultured in 100 ml of each of the 9K1, 9K3, 9K4 media at the pH of 1.00 to 3.00 and temperature (40-60 °C). Finally, the bacterial growth rate in each medium was measured by counting the number of alive and mobile bacterial cells using a Neubauer improved counting chamber [5].

Table 1. Mineral salt composition of the growth media (g/L).

Media Composition	9K	9K1	9K2	9K3	9K4	9K5	Purity	Company
$(NH_4)_2SO_4$	3.0	3.0	3.0	3.0	3.0	3.0	99.5 %≥	Merck/Germany
$MgSO_4.7H_2O$	0.5	0.5	0.5	0.5	0.5	0.5	99.5 %≥	Merck/Germany
K_2HPO_4	0.5	0.5	0.5	0.5	0.5	0.5	99.0 %≥	Merck/Germany
KCl	0.1	0.1	0.1	0.1	0.1	0.1	99.5 %≥	Merck/Germany
$Ca(NO_3)_2.4H_2O$	0.01	0.01	0.01	0.01	0.01	0.01	99.0 %≥	Merck/Germany
Distilled water	1000	1000	1000	1000	1000	1000	-	-
S	-	10	-	10	10	-	99.0 %≥	Merck/Germany
$FeSO_4.7H_2O$	-	-	44	-	-	44	99.5 %≥	Merck/Germany
Glucose	-	-	-	-	10	10	99.5 %≥	Sigma-Aldrich/USA
Yeast extract	-	-	-	2	2	2	-	Sigma-Aldrich/USA

2.6. Chalcopyrite bioleaching

The bioleaching of copper from the tailings was performed using the isolated strain. The effect of the contents of the modified 9K media (9K3 and 9K4 in Table 1) and the pulp density on the process was assessed. The bioleaching tests were performed in 300-mL Erlenmeyer flasks, of which each contains 100 mL of one of the 9K3 and 9K4 media, a 20 vol% inoculum of the bacterial solutions, with a concentration of 1×10^7 cell/mL, and copper tailings (5, 10, 15% wt/vol) incubated at 50°C and 150 rpm for 30 days. Every two days the pH was adjusted to 2.00 and the evaporated water from the medium was compensated by adding distilled water. After the sampling and filtration (0.45 µm), the concentration of the bioleached copper ion was measured on the 5th, 10th, 15th, 20th, 25th, and 30th days using an atomic absorption spectrometer (AAS) (Shimadzu, AA 6300).

The copper leaching efficiency was calculated using the following eq. 7 [20]:

$$\eta = \frac{C\times V}{\propto\times m} \times 100\% \quad (7)$$

where η is the copper leaching efficiency (%), C is the copper concentration in the leachate (g/mL), V is the leachate volume (mL), m is the mass of tailing (g) and α is the copper content in the copper tailing (wt %).

3. Results and discussions

3.1. Characterization of the tailing sample

The chemical analysis of the mineral tailing by X-ray fluorescence (XRF) is given in Table 2. The XRF analysis showed that the sample contained about 0.23 wt % of copper, 11.3 wt % of Fe_2O_3, and the predominant components were 43.5 wt % of SiO_2 and 22.2 wt % of Al_2O_3. The high amount of SiO_2 and Al_2O_3 might cause problems, in the copper extraction, which might have prevented the copper extraction [21]. In addition, X-ray diffraction (XRD) analysis was performed to determine the different ratios of each mineral phase in the mineral tailing sample. The minerals identified were quartz (SiO_2), albite ($NaAlSi_3O_8$), chalcopyrite ($CuFeS_2$), and magnetite (Fe_3O_4). Quartz and albite were the main minerals in the mineral tailing sample, which confirmed the XRF results. The XRD diffraction of a mineral tailing sample is shown in Fig. 1.

3.2. Identification and characterization of the isolated strain (Strain SCM1)

The results of gram staining and microscopic observations of the *SCM1* strain showed gram-positive bacilli (Fig. 2). According to the results of microscopic observations from each of the culture media, the bacterial cell growth was observed in the media containing elemental sulfur (9K1, 9K3, and 9K4). Also, no bacterial cell growth was observed in the media containing ferrous sulfate (9K2 and 9K5) and the 9K medium. Thus, three media were excluded from the experiments. Also, Fig. 3 shows a phylogenetic tree based on the Neighbor-Joining method from 16S rRNA gene sequences. Based on this figure, the (*Strain SCM1*) belongs to *Delftia acidovorans* with 95.73% of identity. In a previous study, the *Delftia sp.* was isolated from the rice field soil with the ability of sulfur oxidation [22]. Also, previous studies have shown that this bacterium is suitable for gold biomineralization [23] and bioextraction [24]. The isolated strain (*Strain SCM1*) showed significant differences in morphological characteristics, cell growth environment conditions, and identity of less than 97% (95.73%) with *Delftia acidovorans*, indicating a significant difference in species or even genus level between this strain and Strains registered in the gene bank can be classified into a new species without DNA-DNA hybridization. Although analyzing the diversity of the thermoacidophilic bacteria and archaebacteria is problematic because those bacteria have rigid cell walls as compared to other bacterial cells [25], the identification and selection of the microorganisms are among the most important factors in the bioleaching process.

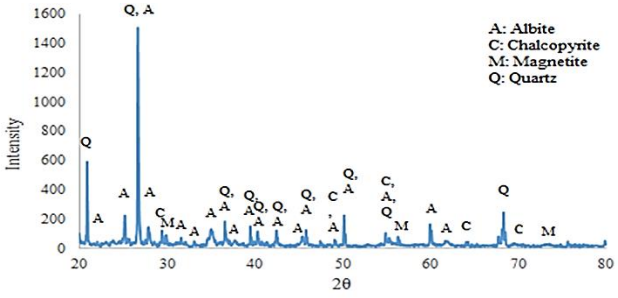

Fig. 1. XRD diffractogram of a mineral tailing sample collected from the Sarcheshmeh copper mine

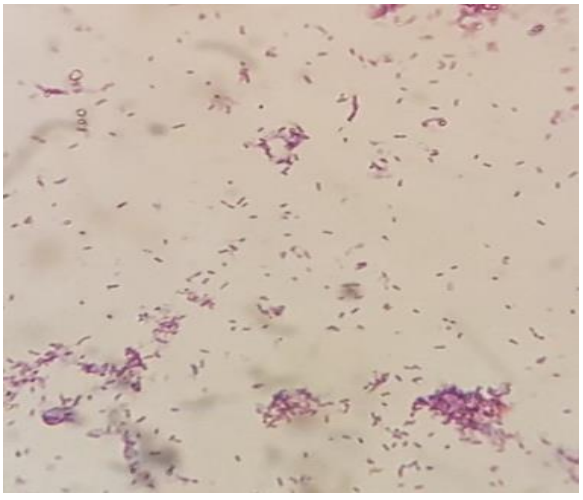

Fig. 2. Gram staining, the isolated (Strain *SCM1*) thermoacidophilic bacteria from a Sarcheshmeh low-grade copper ore.

Table 2. XRF results of a copper tailings sample collected from the Sarcheshmeh copper mine.

Elements	Na_2O	MgO	Al_2O_3	SiO_2	P_2O_5	SO_3	K_2O	CaO	TiO_2
Wt %	0.924	2.452	22.299	43.562	0.180	14.051	3.165	0.791	0.556
Elements	V_2O_5	Cr_2O_3	MnO	Fe_2O_3	NiO	BaO	SrO	Y_2O_3	PbO
Wt %	<<	<<	0.313	11.306	-	-	-	-	<<
Elements	ZrO_2	Zn	Cl	Co	Cd	Mo	W	Cu	L.O.I
Wt %	<<	0.165	-	<<	-	-	-	0.235	-

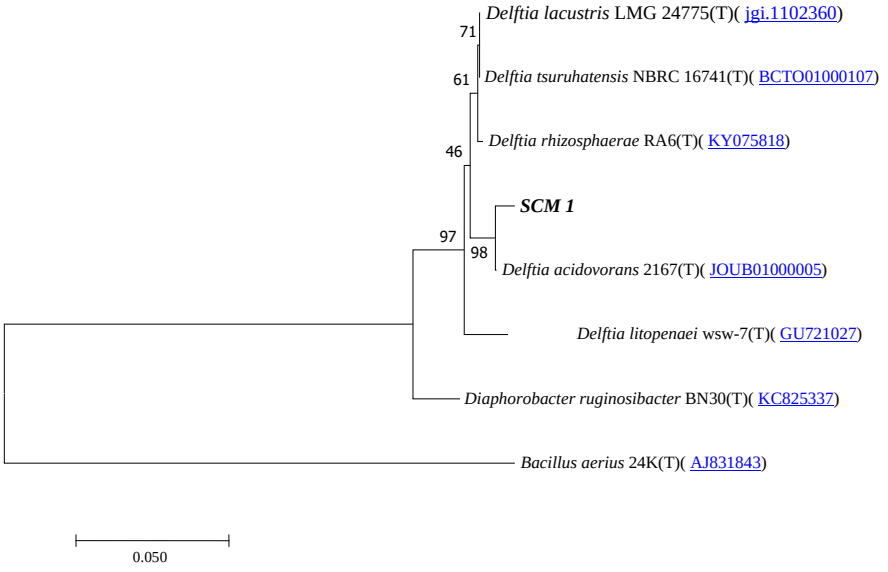

Fig. 3. Phylogenetic tree from the analysis of 16S rRNA genes sequences based on the Neighbor-joining method [18, 19].

3.3. The optimal growth conditions of the isolated strain (SCM1)

Previous reports have shown that the use of the thermoacidophilic [26] and sulfur-oxidizing bacteria [27] reinforces copper extraction. The leaching rate in a process using the thermoacidophilic bacteria is higher than that of the mesophilic bacteria due to the optimum growth temperature, the higher metal tolerance capacity, and the metabolic characteristics [28]. Fig. 4 shows the cell concentration of this strain in the 9K1, 9K3, and 9K4 media at different pH values (1.00 to 3.00) at 50°C. Based on this figure, the highest bacterial cell concentration was observed with the addition of glucose and yeast extract in the 9K3 and 9K4 media at a pH of 2.00. Also, the bacterial cell concentration decreased with every ±0.20 change in the pH value, starting from 2.00. Table 3 shows the cell concentration of this strain in the 9K1, 9K3, and 9K4 media at different temperatures (40-60 °C) at pH 2.00. According to this table, the highest rate of bacterial cell growth was observed at 50 °C in the 9K3 and 9K4 media. Therefore, the 9K3, and 9K4 media, with an initial pH of 2.00, and temperature of 50 °C, due to the highest bacterial cell concentration, were selected to investigate the copper bioleaching process. The effect of adding yeast extract on bacterial cell growth was also reported in previous studies [29]. In previous studies, *Delftia* was a motile and gram-negative bacillus with growth conditions of 3-37 ° C (optimum growth at 25 ° C), pH 5-10 (optimal growth at pH 6-7) was reported. Therefore, due to the difference in environmental growth conditions of the *Strain SCM1* and the percentage of low phylogenetic identity with *Delftia*, (*Strain SCM1*) may be a new strain [30].

3.4. Effect of different parameters on the bioleaching of copper

Bioleaching is influenced by environmental, biological, and physicochemical factors that affect the rate of metal extraction. Optimal conditions of the pH, temperature, energy source, nutrients, and oxidation-reduction potential (ORP) positively affect the growth of the microorganisms involved in this process [31].

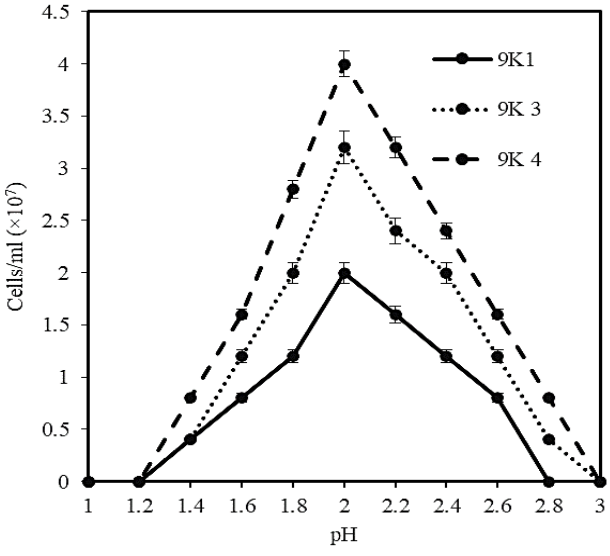

Fig. 4. Bacterial cell concentration (cell mL^{-1}) at different pH values in the 9K1; 9K3; 9K4 media

3.4.1. Effect of pH

The adjustment of the pH to around the acidic range is necessary to observe the maximal bacterial growth and to produce the metabolites required for the solubilizing of the metal compounds [32, 33]. The

Table 3. Bacterial cell concentration (cell mL^{-1}) at different temperatures at pH 2.00 in the 9K1; 9K3; 9K4 media

Media Temperature	9K1	9K3	9K4
40 °C	0	0	0
45 °C	0	0.4×10^7	1.2×10^7
50 °C	2×10^7	3.2×10^7	4×10^7
55 °C	0.4×10^7	0.8×10^7	1.6×10^7
60 °C	0	0	0

bacteria taking part in the metal extraction are active in acidic media (1.20 - 3.50) [34], and their activity decreases the pH of the growth medium. Fig. 5 shows the results of the pH alterations during 30 days of copper bioleaching in the 9K3 and 9K4 media. As can be seen in this figure, the pH increased to 2.38 on the 8th day of the bioleaching process and then reached the lowest value of 1.60 in the 9K3 medium with a pulp density of 5% wt/vol on the 30th day. Furthermore, Fig. 5b demonstrates that the pH increased to 2.28 on the 6th day and afterward reached the least value of 1.54 in the growth medium of 9K4 with a pulp density of 5% wt/vol on the last days of the bioleaching process. The results showed that the pH has increased on the initial days due to the alkaline nature of the sample. This reduces the growth and activity of the thermoacidophilic bacteria involved in the bioleaching process and delays the process of microbial dissolution. But over time and with a daily pH regulation at 2.00 (the pH value for the optimal bacterial cell concentration), the activity of the sulfur-oxidizing bacteria starts and the pH decreases due to the production of sulfuric acid [35]. The rate of the pH reduction in the 9K4 medium was greater than that in the 9K3 medium, due to the higher activity and growth of the bacteria. Also, the pH drop on the final days of the process reduces the growth of the bacteria involved in the bioleaching process. Therefore, the extraction of metals because of the acidic condition and the chemical reactions responsible for the growth of the bacteria reduces. These alterations in the pH value (increasing on the initial days and decreasing on the ending days) were also reported in the previous studies [36–38].

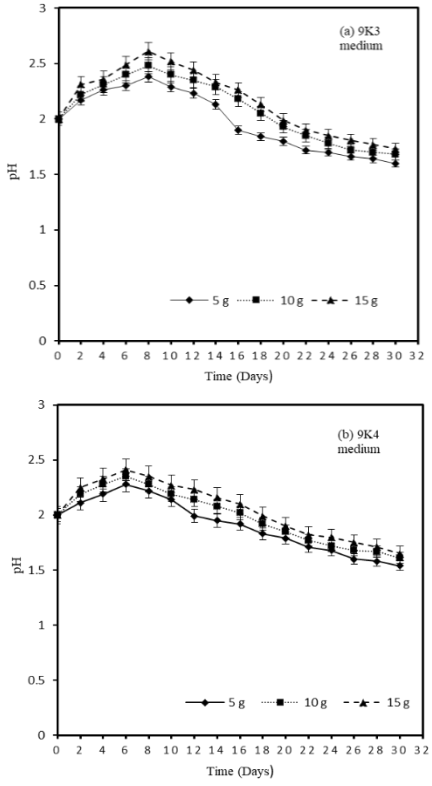

Fig. 5. pH variation as a function of time at different pulp densities: 5% wt/vol; 10% wt/vol; 15% wt/vol in the (a) 9K3 medium and (b) 9K4 medium

3.4.2. Effect of changes in oxidation-reduction potential (ORP)

The oxidation-reduction potential is a measure of the tendency of a solution for electron transfer. High dissolution rates are achieved at low potentials in the range of 450-650 mV. During the oxidation of ferrous ions by the bacteria, the ORP of the system gradually increases. In the presence of thermoacidophilic bacteria, due to the reduction of oxygen solubility at high temperatures, ORP reaches a maximum of 600 mV [39]. Fig. 6 shows the changes in oxidation-reduction potential during 30 days of copper bioleaching at different pulp densities in the 9K3 and 9K4 media. As shown in Fig. 6, the decrease in potential in the early days of the bioleaching process reinforces the hypothesis of a direct correlation between the negative effect of alkalinity of mineral tailing on the growth and oxidative activity of bacteria and the use of ferric ions for the oxidation of sulfide minerals [40]. According to Fig. 6, in the 9k3 medium from the 8^{th} day, and the 9k4 from the 6^{th} day to the final day of the process, the oxidation-reduction potentials began to increase. The potential increase in the medium with 5% wt./vol pulp density was more than the one with 15% wt./vol, which is a sign of more oxidative activity of the bacteria in the former. Also, according to the results, the amount of potential increase in 9k4 (up to 585 mV) was higher than the 9k3 medium (up to 541 mV) containing 5% wt./vol of pulp density, which could be due to the optimal conditions of this medium for the bacterial cell growth and activity. In addition, the increasing trend of the potential is reduced in the last few days, which can be due to the increased oxidation of ferrous to ferric ions by the bacteria [40] as well as the deposition of ferric ions as jarosite on the surface of mineral tailing. These could be the reason for the reduced reaction between the mineral tailing and the leaching solution, resulting in no electron transfer [13].

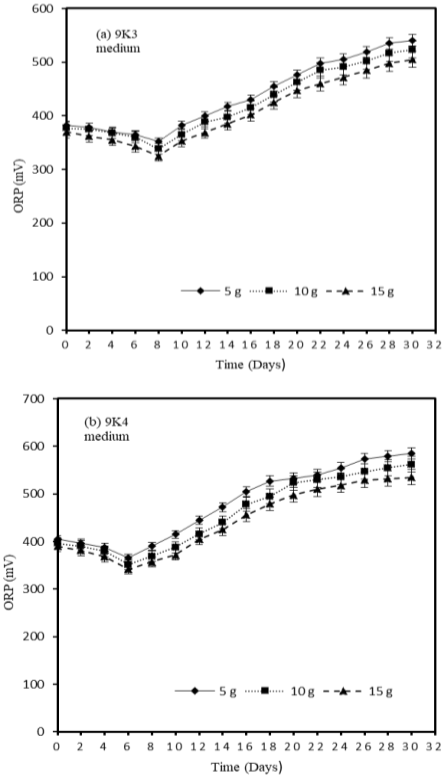

Fig. 6. Oxidation-reduction potential (ORP) variation as a function of time at different pulp densities: 5% wt./vol; 10% wt./vol; 15% wt./vol in the (a) 9K3 medium and (b) 9K4 medium.

3.4.3. Effect of medium composition

The bacteria, which were used in the metal extraction, require an energy source; such as C, N, P, S, Mg, etc. [41]. According to Table 3, the highest bacterial cell concentration was for the 9K3 and 9K4 media. Fig. 7 and 8 show the copper extraction in the 9K3 and 9K4 media, respectively. The results showed that the copper extraction in the 9K4 medium was more than that in the 9K3 medium. Increasing the bacterial growth in the 9K4 medium decreases the pH and increases the copper bioleaching rate compared to the 9K3 medium.

3.4.4. Effect of the pulp density

By increasing the pulp density, the transportation of oxygen and carbon dioxide decreases. Furthermore, the acidity decreases which has a negative effect on the growth and activity of the bacteria and the solubilization rate [42, 43]. Fig. 7 and 8 show the percentage of the copper extraction as a function of time for the two different media of 9K3 and 9K4 with different pulp densities. According to Fig. 7, in the 9K3 medium with a pulp density of 5% wt/vol, the highest copper extraction was 76% on the 30th day. Also, increasing the pulp density of the tailing to 15% wt/vol decreased the percentage of copper extraction to 44% in the 9K3 medium. Fig. 8 demonstrates that in the 9K4 medium with a pulp density of 5% wt/vol, the highest copper extraction was 83% on the 30th day. Once more, by increasing the pulp density to 15% wt/vol, the percentage of copper extraction decreased to 52%. According to previous studies, increasing the pulp density decreases the oxygen concentration and consequently, decreases the bacterial growth, and the amount of metal extraction, but increases the destruction of the bacterial cells [44, 45].

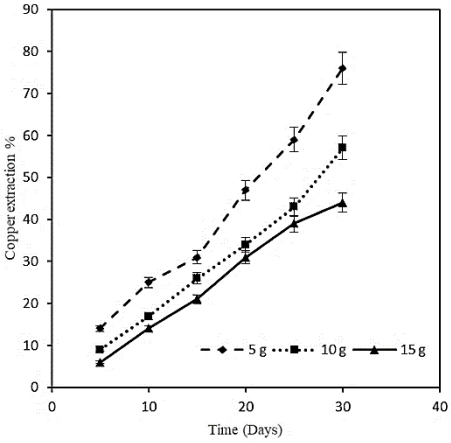

Fig. 7. Copper extraction as a function of time at different pulp densities: 5% wt/vol; 10% wt/vol; 15% wt/vol in the 9K3 medium

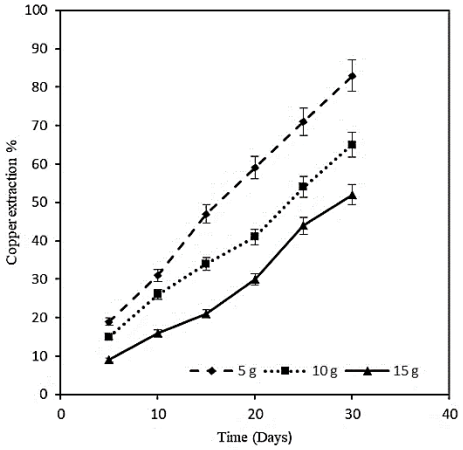

Fig. 8. Copper extraction as a function of time at different pulp densities: 5% wt/vol; 10% wt/vol; 15% wt/vol in the 9K4 medium.

4. Conclusion

- The isolated indigenous thermoacidophilic bacteria (*Strain SCM1*) from Sarcheshmeh low-grade copper ore belonged to *Delftia acidovorans* with 95.73% of identity.
- The optimal growth condition was reported in the 9K4 medium containing sulfur (10 g/L), glucose (10 g/L), yeast extract (2 g/L), and at the pH of 2.00 at 50°C and 150 rpm.
- Due to the alkaline characteristics of the pulp with a higher density, the pH increased and the ORP decreased in the early days of the process. Eventually, on the final days, due to the increased bacterial oxidative activity, bacterial cell concentration, sulfur oxidation, and acid production, the pH decreased and the ORP increased.
- The copper extraction in the media with a lower pulp density was higher due to the increased bacterial cell concentration and the activity of the sulfur-oxidizing bacteria, as well as the positive effect of the alkaline characteristics of the pulp with lower densities on maintaining the optimum pH (2).
- The isolated strain (*Strain SCM1*) that was previously unidentified in the Sarcheshmeh copper mine is practical for the extraction of copper from the low-grade tailing.

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