Revista de Metalurgia
October-December 2021, 57 (4), e207
ISSN-L: 0034-8570
https://doi.org/10.3989/revmetalm.207

Tank bioleaching of a copper concentrate using the moderately thermophilic microorganisms *Sulfobacillus thermosulfidoxidans* KMM3 and *Sulfobacillus acidophilus* KMM26

Zahra Manafi, Mohammad Kargar*, Farshid Kafilzadeh

Department of Microbiology, Jahrom Branch, Islamic Azad University, Jahrom, Iran
(*Corresponding author: mkargar@jia.ac.ir)

Submitted: 1 March 2021; Accepted: 23 October 2021; Available On-line: 30 December 2021

ABSTRACT: Laboratory scale bioleaching of a copper concentrate was conducted using moderately thermophilic microorganisms to evaluate the technical capabilities as an alternative to the conventional smelting, and also to find the optimum conditions for copper extraction in terms of the pulp density, pH, and grain size of concentrate particles. For this purpose, a set of experiments was carried out in a 5-litre controllable bioreactor using two *Sulfobacillus* species. The results showed that more than 80% of Cu could be extracted from chalcopyrite concentrate within 12 days. The optimum conditions for Cu extraction were a pulp density of 5% (w/v), an initial pH 1 and a particle size (d80) of 45 µm. The results of this research will contribute to the design of an industrial tank bioleaching plant with an annual capacity of 50000 t cathodic copper by the Iranian Babak Copper Company (IBCCO).

KEYWORDS: Bioleaching; Bioreactor; Copper concentrate; Moderate thermophiles

Citation/Citar como: Manafi, Z.; Kargar, M.; Kafilzadeh, F. (2021). "Tank bioleaching of a copper concentrate using the moderately thermophilic microorganisms *Sulfobacillus thermosulfidoxidans* KMM3 and *Sulfobacillus acidophilus* KMM26". *Rev. Metal.* 57(4): e207. https://doi.org/10.3989/revmetalm.207

RESUMEN: *Biolixiviación en tanque de un concentrado de cobre utilizando los microorganismos moderadamente termofílicos Sulfobacillus thermosulfidoxidans KMM3 y Sulfobacillus acidophilus KMM26.* Se ha investigado, a escala de laboratorio, el proceso de biolixiviación de un concentrado de cobre utilizando microorganismos termófilos moderados como una alternativa al proceso de fundición convencional; asimismo se han evaluado las condiciones experimentales óptimas para la extracción de cobre en términos de densidad de pulpa, pH y tamaño de partículas del concentrado cuprífero. La experimentación se llevó a cabo en un biorreactor de 5 l de capacidad. Y utilizando dos especies de *Sulfobacillus*. Los resultados mostraron que, después de 12 días, más del 80% del cobre se puede extraer del concentrado de calcopirita. Las condiciones óptimas para la extracción del metal fueron: una densidad de pulpa del 5% (p/v), un pH inicial de 1 y un tamaño de partícula (d80) de 45 µm. Los resultados de esta investigación contribuirán al diseño, por parte de la Compañía Iranian Babak Copper (IBCCO), de una planta industrial de biolixiviación en tanques de una capacidad anual de 50.000 t de cátodo de cobre.

PALABRAS CLAVE: Biolixiviación; Biorreactor; Concentrado de cobre; Termófilos moderados

ORCID ID: Zahra Manafi (https://orcid.org/0000-0002-6217-0975); Mohammad Kargar (https://orcid.org/0000-0002-9531-3645); Farshid Kafilzadeh (https://orcid.org/0000-0003-2506-7140)

1. INTRODUCTION

Recent investigations indicated that low kinetics of sulfide minerals leaching and bioleaching is the main reason to limit industrial usage of bio-hydrometallurgy (Hedrich *et al.*, 2018; Close, 2021; Rasoulnia *et al.*, 2020). Different methods have been suggested to increase chalcopyrite dissolution include increasing pressure and temperature, advance grinding and mechanical activation, addition of chlorine and silver, usage H_2O_2, addition of ferric ions, roasting, and atmospheric leaching. All of these methods can increase the Cu recovery from copper sulfide minerals. Bioleaching of copper concentrate is an environmentally friendly alternative to conventional pyrometallurgical and chemical metallurgy processes (Rawlings *et al.,* 2003; Jones *et al.,* 2011; Wang *et al*., 2020; Tao *et al*., 2021). Besides its less complexity, the principal benefits of the bioleaching process would be its lower required control, labor operation costs, energy, and also its lower capital investment and reduced greenhouse gasses emission (Hedrich *et al.,* 2018). Most of secondary copper sulfides, such as chalcocite, digenite, bornite and covellite can be bioleached successfully by mesophilic microorganisms. However, the bioleaching of chalcopyrite which is a primary sulfide mineral is still a major challenge due to slow kinetics and low Cu recovery (Jones *et al.,* 2011; Wang *et al*., 2019; Close, 2021). Many researchers have investigated the possibility of using thermophilic microorganisms to improve the chalcopyrite bio-extraction rate instead of mesophilic microorganisms (Jones *et al.,* 2011; Hedrich *et al*., 2018). Using thermophiles to leach sulfide minerals not only greatly improves the reaction kinetics, but also avoids excessive chalcopyrite "passivation" which hinders the extent of bioleaching treatment (Jones *et al*., 2011). However, the use of the term "passivation" is not universally accepted, most likely because of only a superficial resemblance, if any, to the well-known passivation behavior of metals and the lack of a clearly identifiable surface layer (Crundwell, 2015; O'Connor and Eksteen, 2020). The majority of extreme thermophiles surviving above 60 °C are classified as archaea, which are lacking a typical cell wall and cannot survive at high pulp density due to strong stirring shear. Consequently, it is very difficult to apply extreme thermophiles in the biomining industry. On the other hand, moderately thermophilic bacteria such as *Sulfobacillus spp* can tolerate a higher pulp density than extremely thermophilic archaea, thus having an advantage in the application of chalcopyrite bioleaching (Jones *et al*., 2011). Various species of Sulfobacillus are widely used in industrial reactors of the bioleaching industry. All species of the genus Sulfobacillus are moderately thermophilic or thermotolerant acidophiles. *Sulfobacillus* are Gram-positive, acidophilic, rod-shaped, mobile bacteria and endospore forming. They could usually be found in low-pH environments, such as waste dumps/tailings at mine sites and acidic water streams. It has been reported that, in the presence of small amounts of yeast extract, the species of the genus Sulfobacillus can obtain energy by oxidizing, elemental sulfur, ferrous iron, and sulfide minerals. With sulfur- and iron oxidizing activity, microorganisms of this genus are important in the oxidative dissolution of sulfide minerals. Their best growth temperature is 50 to 58 °C, and their optimal pH is between 1.9 and -2.4. (Hedrich and Shippers, 2021; Zhang *et al.*, 2021).

Furthermore, the weak iron oxidation ability of *Sulfobacillus thermosulfidooxidans* strain improves chalcopyrite bioleaching through maintaining a favorable redox potential, and chalcopyrite leaching by this strain would not be inhibited in the presence of 200 mM NaCl (Zhang *et al.*, 2021).

Bioleaching in stirred tank reactor (STR) can provide a more homogenous reacting mass and allow close control of the main process variables, compared with the heap and column leaching operation (Acevedo and Gentina, 1989; Rawlings, 2008).

This article investigates the most important factors affecting the bioleaching efficiency such as pulp density, grinding method, particle size fraction, and acidity. The effect of grinding method on the recovery was also studied using four different milling conditions. Results of current research would be considered to develop an industrial plant for bio-treatment of the chalcopyrite concentrate with capacity of 50000 t/annual cathodic copper.

2. MATERIALS AND METHODS

2.1. Microorganisms

Following the method introduced by Ñancucheo and colleagues (Ñancucheo *et al.*, 2016), two strains of *Sulfobacillus thermosulfidooxidans* and *Sulfobacillus acidophilus* were isolated and identified from the samples received from Miduk Copper Mine in Kerman- Iran. A basal medium was prepared and inoculated by the samples containing the microbial community and while kept on orbital shaker at 150 rpm, inoculated for 7 days at 30 °C. Using a common laboratory practice (Johnson and Hallberg, 2007), pure cultures of the detected strains were then isolated from the solid medium (Johnson, 1995).

The number of bacteria in the solution was counted daily under an optical microscope (Zeiss, Axioskop 40) using a blood cell counting chambers (Eftekhari *et al.*, 2020a; Eftekhari *et al.,* 2020b). The initial inoculation containing 7×10^7 cells/mL was added to the reactor in 10% (v/v). The bacterial counts reached from about 6×10^6 to about 8×10^8 cells/mL, from early days to the end of the experiment (day 7).

To extract genomic DNA, pure cultures were cultivated in broth medium and then, 5 ml of the bacterial cultures were centrifuged (15 min at 13000 rpm) and washed twice with Tris buffer (pH 8). According to modified protocol (Webster *et al.,* 2003), employed for DNA extraction. The fragment of 16SrDNA was amplified by PCR (polymerase chain reaction) using designed primers and then PCR was carried out (Webster *et al.*, 2003).

PCR products were sequenced after purification from the gel by Bioner South Korea. The desired sequences received the registration tracking code by blasting and registering on the NCBI site. Sequences derived from 16S rRNA gene were registered in the name of the researchers, in the World Gene Bank. The phylogenetic trees were constructed using neighbor-joining algorithm implemented in Molecular Evolutionary Genetics Analysis software (MEGA 6.0).

2.2. Copper concentrate

A copper concentrate from Miduk Copper mine was used in all experiments. The concentrate is produced by the Iranian Babak Copper Company (IBCCO) using a flotation method. Table 1 shows data of the chemical and mineralogical analyses of the concentrate. All elemental analyses were performed by atomic adsorption spectroscopy (by AAS, A gilent 200, Asturalia) and mineralogy was determined with X-ray diffraction (Philips X'pert-MPD system, CuKa, K ~ 0.154 nm, 40 kV, 40 mA).

2.3. Bioleaching tests

Three effective parameters including pulp density, pH and particles size (d_{80}) were comprehensively studied (Table 2). A 5-litre glass reactor (Sina-Shisheh, Iran) (Fig. 1) with the stirrer speed of 500 rpm was used for all bioleaching experiments (Anderson *et al.,* 2002).

The contents of the 9K culture medium with some additive and modifications summarized in Table 3 (Anderson *et al.,* 2002).

To start the experiments, 4500 ml of 9K medium was added to the reactor and inoculated by 450 ml bacterial suspension containing 10^7 cells·mL^{-1}. The reactor was set at 50 °C (by coupling it with a water

Table 1. Results of elemental atomic absorption analysis (using weight percentage of each element) and mineralogical results (using mineral imaging)

Chemical analysis (%)					Mineralogical analysis (%)				
Cu	**Fe**	**S**	**Zn**	**Pb**	**Chalcopyrite ($CuFeS_2$)**	**Pyrite (FeS_2)**	**Covelite (CuS)**	**Chalcocite (Cu_2S)**	**Quartz (SiO_2)**
31.3	24.59	28.79	0.96	0.26	44	25	13	3	6

Table 2. The conditions of bioleaching treatments for chalcopyrite concentrate

Test N°	Pulp density (%)	pH	particle size (d_{80}) (µm)
1	10	1.5	10
2	5	1.5	10
3	10	1	10
4	10	1.5	45
5	5	1.5	45
6	10	1	45
7	5	1	45
8	5	1	10

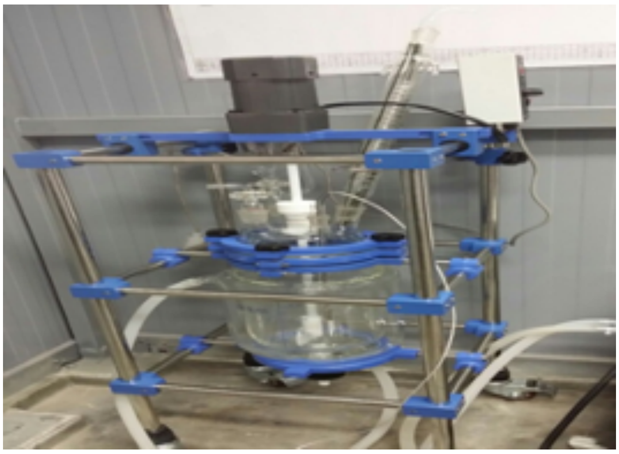

Figure 1. Bioreactor used for the bioleaching tests.

Table 3. Salts used in 9k culture medium, and additives of yeast extract, ferrous ions, and elemental sulfur

(Solution A)	$g \cdot l^{-1}$	(Solution B)	$g \cdot l^{-1}$	(Solution C)	W/V in water
$(NH_4)_2SO_4$	3	$FeSO_4.7H_2O$	42	Yeast extract	1
KCl	0.10	S	8		
K_2HPO_4	0.50				
$MgSO_4.7H_2O$	0.50				
$Ca(NO_3)_2$	0.014				
Distilled water	1000 ml	1000 ml		Distilled water	20 ml
pH	2 - 2.4				

Table 4. The mills properties used for size reduction

Mill type	Ball mill with ceramic balls	Ball mill with steel balls	Ceramic mill	Planetary mill
Dimension	30 cm	20 cm × 25 cm	15 × 20 cm	-
Volume	1 l*	7850 cm^2	3500 cm^2	0.5 l
Grinding media	3 mm	13.5 kg 40 - 15 mm	2.68 kg 10 - 25 mm	Steel ball. 3 cm
Grinding time**	840	840	150	10

l*: liter, Time**: min

bath) which was aerated by 360 mL·min^{-1}. The temperature was controlled by a digital sensor to avoid sharp fluctuations. Redox potential, pH, and copper concentration were daily analyzed. After calculating the recoveries, a chemical control test without any bacteria was also carried out to compare its results with the test with highest Cu recovery.

2.4. Milling and grinding

To study the effect of grinding methods on the bioleaching efficiency, four different mills were used to decrease the particle size to smaller than 10 µm. Table 4 shows the mills properties. Scanning electron microscopy (SEM) studies were also carried out to investigate the particle shapes after grinding by each milling condition.

2.5. Aggregation and sedimentation

To study the agglomeration of the particles, a series of sedimentation tests have been operated at different pH values from 0 to 12 (totally 13 tests). For this purpose, the copper concentrate was mixed with the reagent at different pH values. Sulfuric acid and lime were used to set the pH. The pulp was transferred to 50 ml scaled cylinders and remained for 120 minutes. To complete the sedimentation studies, the point of zero charge was measured for the Cu concentrate sample (Particle Metrix, Germany).

3. RESULTS AND DISCUSSION

3.1. Identification of bacteria

The two isolated bacterial strains were identified and named as *Sulfobacillus thermosulfidooxidans KMM3 (MW175495)* and *Sulfobacillus acidophilus KMM26 (MW175507)*. Their phylogeny is shown in Fig. 2. After identification, the bacteria were stained and examined microscopically (Fig. 3). The two bacteria showed a similar morphology.

3.2. Optimization of bioleaching process

In total eight bioleaching tests along with one abiotic control test were conducted; the results are presented in Table 5. Generally, the test with a pulp density 5 %, pH 1 and a particle size (d_{80}) of 45 µm showed the highest copper recovery.

By decreasing the particle size, the specific surface area increases, thereby improving mass transfer and consequently enhanced bioleaching efficiency. It was expected that a greater surface area exposed to bacterial attack at smaller particle sizes (e.g., -10 µm) would result in metal dissolution increases. However, it was observed that decreasing particle size under 30 µm did not improve bioleaching rates, and resulted in lower recoveries. It was observed that at the particle sizes less than 30 µm, the solid particles were aggregated and thus, the bacteria could be pos-

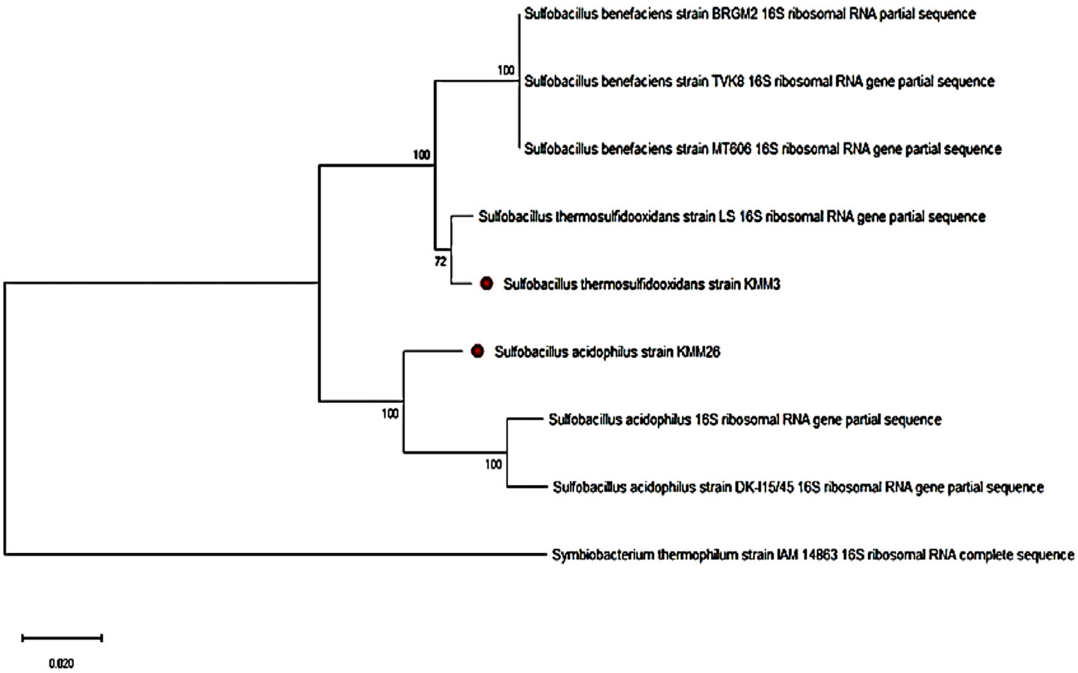

Figure 2. Phylogenetic tree of Sulfobacillus strains using Neighbor-joining method with keeping bootstrap coefficient value of 100.

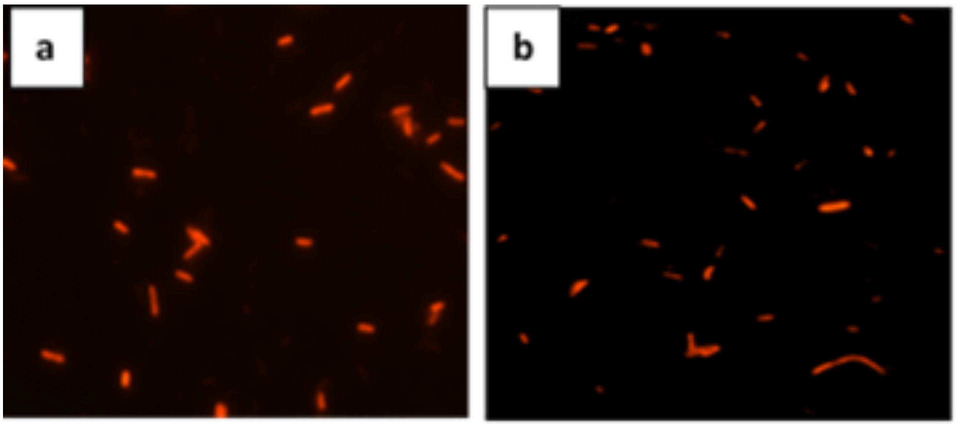

Figure 3. Stained thermoacidophilic bacteria: (a) *Sulfobacillus thermosulfidooxidans*, and (b) *Sulfobacillus acidophilus*.

sibly stocked in between the aggregates. Therefore, although viable cell counts were not significantly changed, their access to the minerals was limited and the overall recovery was reduced. This might be attributed to possible cell damage and deactivation and consequently, the reduced recoveries recorded by finer particles.

Figure 4 shows the SEM images for Cu concentrates before and after grinding. The EDS studies indicated that large particles after grinding contained higher amounts of iron and sulfur than fine particles, which contained more copper and less iron and sulfur (data not shown). This obviously shows that chalcopyrite is enriched in smaller particles compared to pyrite and other minerals. The samples also contained low concentrations of Al and

Table 5. The copper extraction for different tests

Test No.	Copper recovery (%)
1	33
2	57
3	30
4	41
5	76
6	50
7	82
8	81
Control test	25

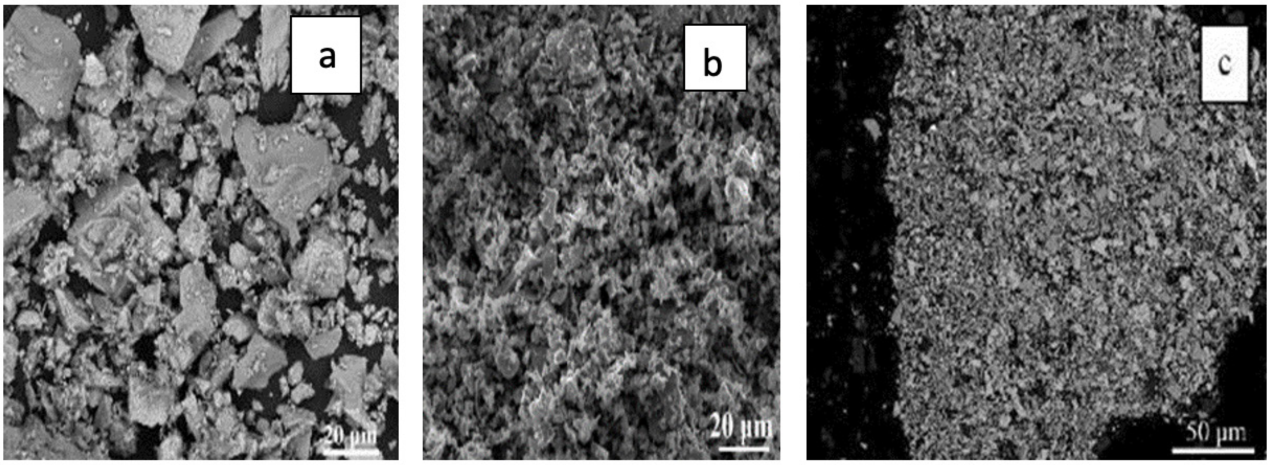

Figure 4. The SEM images, (a) before ultra-fine grinding, (b) after grinding with ceramic balls, (c) with steel balls.

Si, which indicated that the concentrate contained lower amounts of silicates as gangue.

Smaller particle size distribution (Fig. 4b) using grinding by ceramic media can also be one of the reasons for low recovery as finer particles become more agglomerated under the bioleaching conditions.

Figure 5 shows pH and oxidation-reduction potential (ORP) changes in different bioleaching tests. Among the selected parameters, acidity (pH) had lowest effect on the dissolution rate. Via the production of S^0, chalcopyrite dissolution could be considered as an acid consuming reaction (Smart *et al.*, 2000), in contrast to an expected complete oxidation of the sulfur in the chalcopyrite to sulfuric acid according to Eq. (1) (Tanne and Schippers, 2019):

$$4CuFeS_2 + 10H_2O + 17O_2 \rightarrow 4Cu^{2+} + 4Fe(OH)_3 + 8SO_4^{2-} + 8H^+ \quad (1)$$
$$2Fe^{2+} + 2H^+ + H2O2\ (aq) \rightarrow 2Fe^{3+} + 2H_2O \quad (2)$$
$$CuFeS_2 + 4Fe^{3+} \rightarrow 5Fe^{2+} + Cu^{2+} + 2S^0 \quad (3)$$

This finding indicates incomplete sulfur oxidation due to inactive bacteria towards the end of the experiments and probably accumulation of elemental sulfur occurring during abiotic chalcopyrite oxidation according to Eq. (2) (Tanne and Schippers, 2019).

Eftekhari and Kargar reported that increasing the pH causes iron precipitation in form of iron hydroxide or jarosite which can cover the minerals surface and prevent reagent access to surface (Eftekhari and Kargar, 2018; Eftekhari *et al.,* 2020b; Eftekhari *et al.,* 2020c).

The microscopic images (by Leica DMLP optical microscope) of the concentrate before and after grinding, and after bioleaching showed pyrite, chalcopyrite and covellite as the main minerals with various sizes down to d_{80}10 μm (Fig. 6). The microscopic observation of un-milled concentrate (a), milled concentrate (b) and milled concentrate after bioleaching process (c) are presented accordingly.

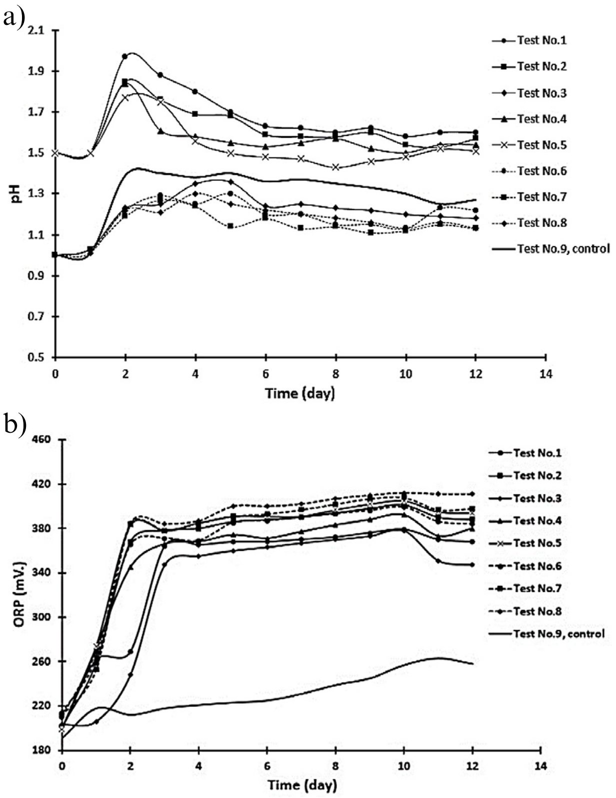

Figure 5. Variations of pH and ORP in the eight bioleaching treatments and one control test: (a) variations of pH, and (b) variations of ORP.

Microscopic examinations clearly show agglomeration of fine particles during the leaching process (Fig. 6). As this figure shows, very fine grinding results in the production of very fine particles (Fig. 6b) with much more surface area available for bioleaching. However, in an oxidizing leaching environment, their agglomeration prevents to achieve high copper recoveries because of not leached mineral particles (Fig. 6c).

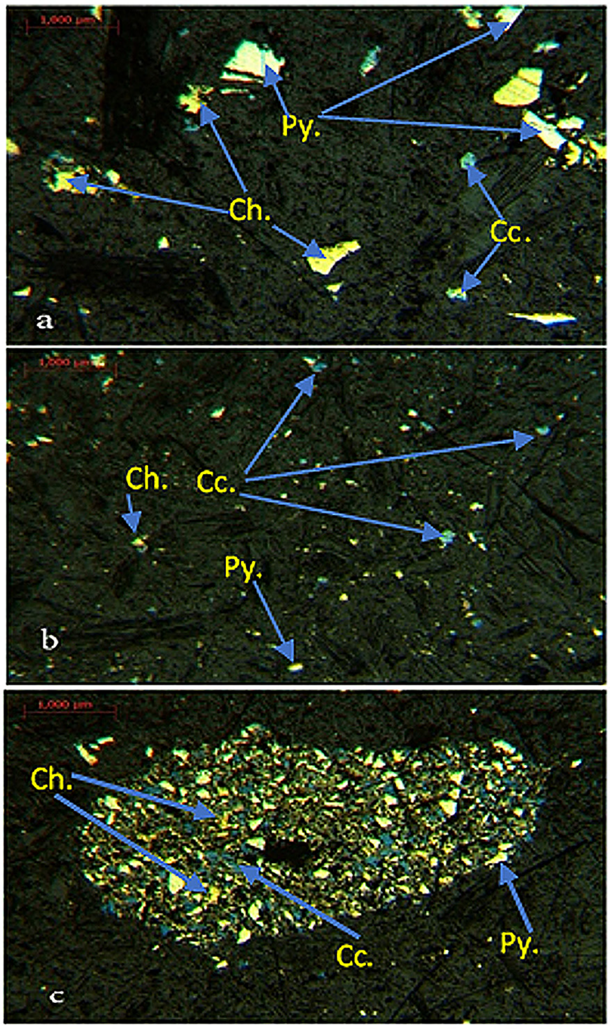

Figure 6. The microscopic observation of (a) un-milled concentrate, (b) milled concentrates, and (c) milled concentrate after bioleaching process.

Pulp density was the most important factor. The rate of copper extraction decreases with increasing the pulp density, probably due to shear stresses resulted from higher percentage of solids, and consequently increases the toxicity of metal ions (Wang *et al.,* 2014). It has shown that grinding the concentrate could increase the copper recovery to more than 10 % (Manafi, 2021). At the same pulp density, when the particle size gets smaller, the easier and more effective access to the surface of the mineral and metal content increases the reaction rate. On the other hand, a significant decrease in particle size increased the apparent viscosity of the pulp, and reduced the oxygen transmission rate which could be harmful for the bacterial activity (Derjaguin and Landau*,* 1993; Anderson, *et al.,* 2002).

3.3. Effect of grinding on the bioleaching efficiency

In tank bioleaching tests, non-agglomerated particles are critical to achieve maximum contact surface. When the particles are close enough, the van der Waals attraction can interact between particles causing agglomeration of particles. Further, electrostatic equilibrium (DLVO theory) and steric and electrostatic force cause agglomeration. Agglomerated particles could significantly decrease the metal extraction efficiency. It is evident that type of fragmentation and milling in this series of tests was quite effective. Sphere particles have the lowest surface to volume ratio. If the grinding is such that instead becoming orb, particles can be created in angular shape, the surface energy of the angular particles increases. Therefore, choosing the right method for grinding has great importance on the extraction recovery (Marshall, 1949; Biggs and Healy, 1994).

Therefore, in order to create spherical particles with minimal surface changes due to regrinding, different grinding intermediates (grinding media) were used to regrind the concentrate. Although the surface area of the particles obtained using ceramic grinding media was more uniform and also relatively larger, the higher recoveries were obtained using steel metal media. One of the possibilities is perhaps the drastic structural changes of the concentrate particles when using steel balls, which could be considered as a kind of mechanical activation. In this case, the defected crystalline concentrate particles dissolve more rapidly in the leaching environment due to these drastic structural changes. Another purpose of using ceramic pellets was to prevent the possible formation of reactive oxygen species that are toxic to microorganisms. However, better results obtained from steel pellets show that, firstly, the probability of the formation of active radicals at this level of crushing (d_{80} 8 µm) is very low. Secondly, the results of solid leaching studies (SEM and mineralogical photomicrographs) reinforced the conclusion that the main reason for not extracting 100 % of copper is the agglomeration of very fine particles during the leaching process. Therefore, it seems that although softening is quite useful in increasing recovery, arrangements must be made so that the mineral particles have maximum dispersion during the bioleaching period. This, of course, needs to be examined more closely to determine what happens to the particle surface during the leaching period. Accordingly, it may be possible to overcome this problem by surfactants or by precisely controlling the pH of the environment or by adjusting other operating parameters.

3.4. Effect of milling method

As discussed in section 2.4, bioleaching efficiency can be influenced by the milling method.

The particle size distributions for Cu concentrate before and after ultra-fine grinding are demonstrated in Fig. 7. The d_{80} for sample before grinding was 47.6 µm. 50 % and 10 % of particles were finer than

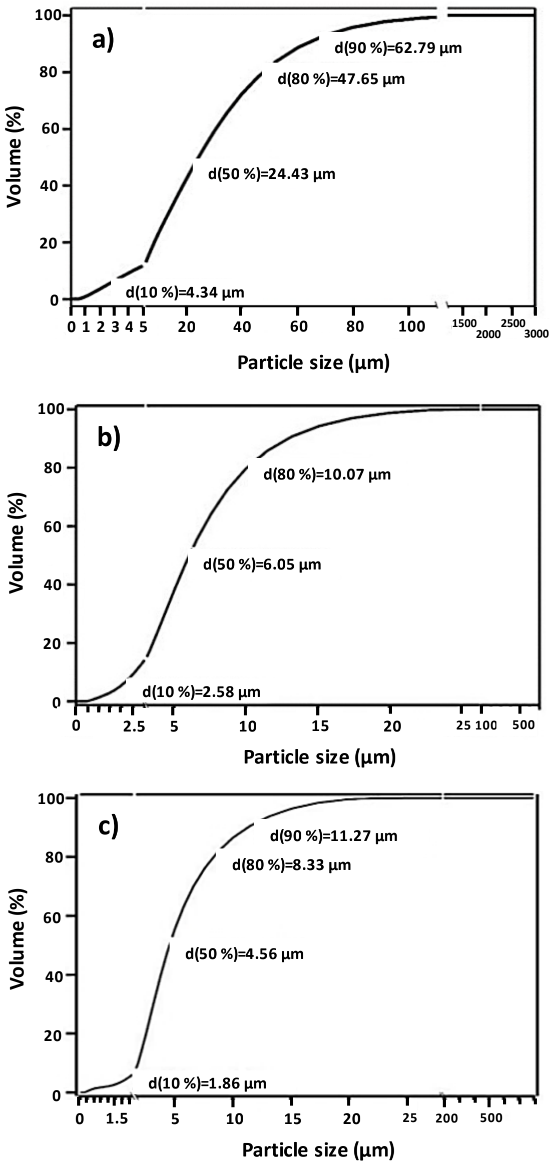

FIGURE 7. Distribution of particles sizes of Cu: (a) concentrate, (b) after 840 min grinding in ball mill with ceramic balls, and (c) with steel balls.

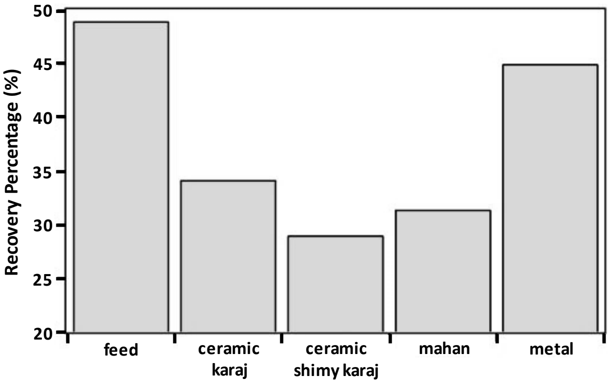

FIGURE 8. Effect of milling process on Cu recovery during bioleaching process.

24.5 and 4.5 µm, respectively. The d_{80} for ball mill with ceramic balls after 840 min grinding reach to ~10 µm and with steel balls reach to ~8 µm.

Figure 8 shows the copper recoveries for unmilled concentrate and concentrate ground with different mills. According to Fig. 8, with 49 % the highest Cu recovery was achieved for original Cu concentrate without any grinding process.

3.5. Effect of particles size

The Cu recoveries for copper concentrate at different d_{80}s are depicted in Fig. 9. All samples were milled with a ball mill using ceramic or steel balls as grinding media. The copper recovery dropped by decreasing the particle sizes to d_{80} 10 µm, even using high-energy planetary mill with highly mechanical activation effect. By decreasing the particle size, the agglomeration increases and this phenomenon cause decreasing Cu recovery. While using concentrate with d_{80} 20, 25 or 30 µm had a negligible effect on Cu recovery. Therefore, it is suggested to avoid over-grinding for bioleaching process. In the other words, decrease the particles size cause increase the energy consumption without any significant improvement in bioleaching efficiency.

The agglomeration phenomenon is detectable from the solid residuals achieved from different experiments. In fact, by reducing size to d_{80} 10 µm the agglomeration increased. Numbers of small particles were observable around each large particle. The obtained results showed that the recovery increased when steel balls used which concluded that the grinding method effect on some physiochemical properties of particles such as roundness (Fig. 9). The particle roundness was calculated by using following equation (Gilgannon *et al.,* 2017):

$$Roundness = \frac{4\pi(pore\ area)}{(pore\ perimeter)^2} \times 100$$

Based on above equation and SEM images (Fig. 4), particles which were ground by steel balls had roundness ~44 while particles milled by ceramic

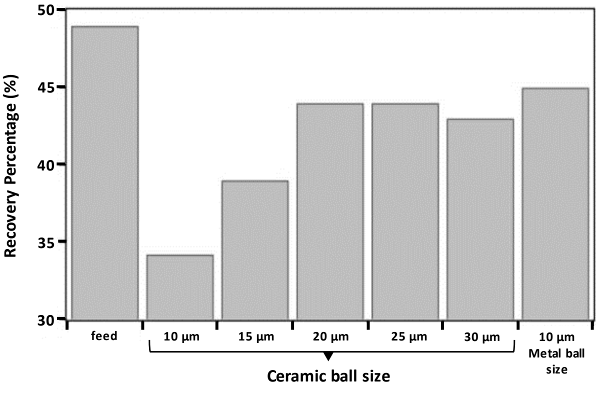

FIGURE 9. Recovery of Cu concentrates at different particle size (d80).

balls had roundness ~34. This means that grinding with steel balls increased the free space and this improved the bioleaching efficiency. Optical photomicrographs of the minerals indicated that there was not any chalcocite in bioleaching residuals, which shows complete decomposition of the chalcopyrite, but the chalcopyrite content also decreased from 24.97 % to 13.01 %. On the other hand, the covellite content increased from 8.79 to 30.6 %.

3.6. Aggregation of the fined particles

The behaviour of the concentrate sedimentation at different pH values was visually observed in the bakers. The lowest stability occurred at tests with pH values of 3, 5, and 11, and the highest stability was at pH 0, 7 and 12. Interestingly, a little change in pH caused a significant change of sedimentation. For instance, although pulp had a high stability at pH 7, it had a low stability at pH 6 or 8. The point of zero charge (ZPC) is one of the most important factors that control the sedimentation. Figure 10 depicts the ZPC for the concentrate in comparison with the pure samples of chalcopyrite, chalcocite and covellite. By increasing the pH from 0 to 5 the zeta potential dropped from 47 to 5 mV. In addition, increasing the pH up to 7 -12 cause a ZPC decrease to lower than -40 mV. Generally, zeta potential of the concentrate has a decreasing-increasing-decreasing trend. A comparison between sedimentation tests and zeta potential results indicated that the highest stabilities happened at the highest and lowest zeta potential ranges (pH 0, 7 and 12). It should be noted that setting pH at 0 or 12 is better because at pH 7 few changes could result in significant changes in stability, which became important factor when regrinding the concentrate under 25 µm size distributions.

Thus, in general it can be concluded that at lower pH values the particles are more stable. However, changes in pH in the environment due to acid consumption can change this brittle stability. Of course, more important than these are the very large surface changes ("passivation" or by-product layers) in the mineral particles in the concentrate during bioleaching, which can completely invalidate these estimates.

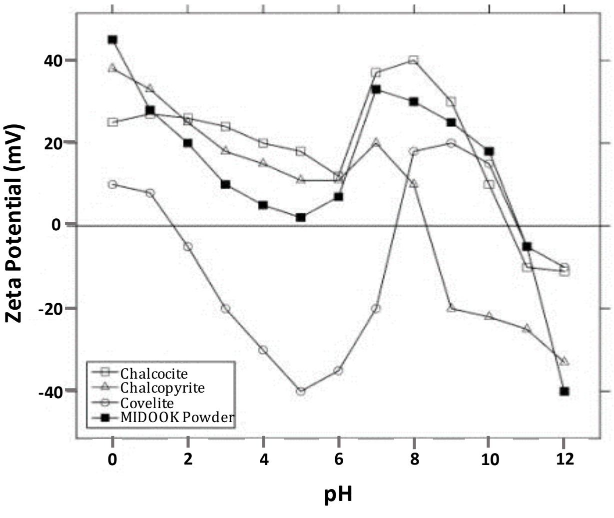

Figure 10. The zeta potential of Miduk concentrate, pure chalcopyrite, chalcocite and covellite samples.

4. CONCLUSION

- According to bioleaching tests, pulp density had a significant effect on copper recovery.
- The highest copper extraction was achieved from a test with pulp density of 5%, pH 1 and particle size of smaller than 45 µm.
- Concentrate grinding might have a dual effect on copper recovery in that it may increase agglomeration of the fine mineral particles and thereby negatively affecting the bioleaching process, and in contrast it may be also positive effect in the process through enhancing available reaction surface.
- Grinding media and milling methods (use of ball mills and high energy planetary mills) have a significant impact on copper extraction. The best method was to grind by means of a typical steel ball mill.

ACKNOWLEDGMENT

We acknowledge the financial support and facilities provided by the Iranian Babak Copper Company (IBCCO). The authors also appreciate the efforts and assistance of Dr. Nasim Eftekhari.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Acevedo, F., Gentina, J.C. (1989). Process Engineering Aspects of the Bioleaching of Copper Ores. *Bioprocess Eng.* 4, 223-229. https://doi.org/10.1007/BF00369176.

Anderson, C., Giralico, M., Post, T., Robinson, T., Tinkler, O. (2002). *Selection and Sizing of Biooxidation Equipment and Circuits. In: Mineral processing plant design, practice and control.* Mular, A.L., Halbe, D.N., Barret, D.J. (eds.), Society of Mining Engineers, Littleton.

Biggs, S., Healy, T.W. (1994). Electrosteric stabilization of colloidal zirconia with low-molecular-weight polyacrylic-acid. An atomic-force microscopy study. *J. Chem. Soc., Faraday Trans.* 90 (22), 3415-3421. https://doi.org/10.1039/FT9949003415.

Close, T. (2021). Kinetic analysis of leaching reactions in multi-component mineral systems. Ph. D. Thesis, Massachusetts Institute of Technology, Department of Chemical Engineering, pp. 167- 177. https://hdl.handle.net/1721.1/130666.

Crundwell, F.K. (2015). The semiconductor mechanism of dissolution and the pseudo-passivation of chalcopyrite. *Can. Metall. Q.* 54 (3), 279–288. https://doi.org/10.1179/1879139515Y.0000000007.

Derjaguin, B., Landau, L. (1993). Theory of the stability of strongly charged lyophobic sols and of the adhesion of strongly charged particles in solutions of electrolytes. *Prog. Surf. Sci.* 43 (1-4), 30-59. https://doi.org/10.1016/0079-6816(93)90013-L.

Eftekhari, N., Kargar, M. (2018). Assessment of optimal iron concentration in the precipitation of jarosite and the activity of *Acidithiobacillus ferrooxidans*. Modares. *Journal of Biotechnology* 9 (4), 525-529.

Eftekhari, N., Kargar, M., Rokhbakhsh Zamin, F., Rastakhiz, N., Manafi, Z. (2020a). Bioremoval of iron ions from copper raffinate solution using biosynthetic jarosite seed promoted by *Acidithiobacillus ferrooxidans*. *Rev. Metal.* 56 (4), e182. https://doi.org/10.3989/revmetalm.182.

Eftekhari, N., Kargar, M., Rokhbakhsh Zamin, F., Rastakhiz, N., Manafi, Z. (2020b). The catalytic activity of biological seeds and *Acidithiobacillus ferrooxidans* on the process of ammonium jarosite. *Journal of Microbial World* 12 (4), 355-363.

Eftekhari, N., Kargar, M., Rokhbakhsh Zamin, F., Rastakhiz, N., Manafi, Z. (2020c). A review on various aspects of jarosite and its utilization potentials. *Ann. Chim. – Sci. Mat.* 44 (1), 43-52. https://doi.org/10.18280/acsm.440106.

Gilgannon, J., Fusseis, F., Menegon, L., Regenauer-Lieb, K., Buckman, J. (2017). Hierarchical creep cavity formation in an ultramylonite and implications for phase mixing. *Solid Earth.* 8 (6), 1193-1209. https://doi.org/10.5194/se-8-1193-2017.

Hedrich, S, Joulian, C., Torsten Graupner, Schippers, A., Guézennec, A.G. (2018). Enhanced chalcopyrite dissolution in stirred tank reactors by temperature increase during bioleaching. *Hydrometallurgy* 179, 125-131. https://doi.org/10.1016/j.hydromet.2018.05.018.

Hedrich, S., Schippers, A. (2021). Distribution of Acidophilic Microorganisms in Natural and Man-made Acidic Environments. *Curr. Issues Mol. Biol.* 40, 25-48. https://doi.org/10.21775/cimb.040.025.

Johnson, D.B. (1995). Selective solid media for isolating and enumerating acidophilic bacteria. *J. Microbiol. Methods* 23 (2), 205–218. https://doi.org/10.1016/0167-7012(95)00015-d.

Johnson, D.B., Hallberg, K.B. (2007). *Techniques for detecting and identifying acidophilic mineral-oxidizing microorganisms*. Rawlings, D., Johnson D. (Eds) Biomining, Springer, Berlin, Heidelberg: Springer Berlin Heidelberg. pp. 237–261. https://doi.org/10.1007/978-3-540-34911-2_12.

Jones, G.C., Corin, K.C., Hille, R.P.V., Harrison, S,T.L. (2011). The generation of toxic reactive oxygen species (ROS) from mechanically activated sulphide concentrates and its effect on thermophilic bioleaching. *Miner. Eng.* 24 (11), 1198-1208. https://doi.org/10.1016/j.mineng.2011.05.016.

Manafi, Z. (2021). Evaluation and improve the performance of moderate thermophiles in increasing of copper extraction from chalcopyrite concentrate. PhD thesis.

Marshall, C.E. (1949). *Theory of the stability of lyophobic colloids. The interaction of sol particles having an electric double layer*. Elsevier, pp. 413-414. https://doi.org/10.1002/pol.1949.120040321.

Ñancucheo, I., Rowe, O.F., Hedrich, S., Johnson, D.B. (2016). Solid and liquid media for isolating and cultivating acidophilic and acid-tolerant sulfate-reducing bacteria. *FEMS Microbiol. Lett.* 363 (10), fnw083. https://doi.org/10.1093/femsle/fnw083.

O'Connor, G.M., Eksteen, J.J. (2020). A critical review of the passivation and semiconductor mechanisms of chalcopyrite leaching. *Miner. Eng.* 154, 106401. https://doi.org/10.1016/j.mineng.2020.106401.

Rasoulnia, P., Barthen, R., Lakaniemi, A.M. (2020). A critical review of bioleaching of rare earth elements: The mechanisms and effect of process parameters. *Crit. Rev. Environ. Sci. Technol.* 51 (4), 378-427. https://doi.org/10.1080/10643389.2020.1727718.

Rawlings, D.E., Dew, D., du Plessis, C. (2003). Biomineralization of metal containing ores and concentrates. *Trends Biotechnol.* 21 (1), 38-44. https://doi.org/10.1016/s0167-7799(02)00004-5.

Rawlings, D.E. (2008). High level arsenic resistance in bacteria present in biooxidation tanks used to treat gold-bearing arsenopyrite concentrates: A review. *T. Nonferr. Metal. Soc. China* 18 (6), 1311-1318. https://doi.org/10.1016/S1003-6326(09)60003-0.

Smart, R.S.C., Jasieniak, M., Prince, K.E., Skinner, W.M. (2000). SIMS studies of oxidation mechanisms and polysulfide formation in reacted sulfide surfaces. *Miner. Eng.* 13 (8-9), 857–870. https://doi.org/10.1016/s0892-6875(00)00074-1.

Tanne, C.K., Schippers, A. (2019). Electrochemical investigation of chalcopyrite (bio)leaching residues. *Hydrometallurgy* 187, 8-17. https://doi.org/10.1016/j.hydromet.2019.04.022.

Tao, J., Liu, X., Luo, X., Teng, T., Jiang, C., Drewniak, L,, Yang, Z., Yin, H. (2021). An integrated insight into bioleaching performance of chalcopyrite mediated by microbial factors: Functional types and biodiversity. *Bioresour Technol.* 319, 124219. https://doi.org/10.1016/j.biortech.2020.124219.

Wang, Y., Zeng, W., Qiu, G., Chen, X., Zhou, H. (2014). A moderately thermophilic mixed microbial culture for bioleaching of chalcopyrite concentrate at high pulp density. *Appl. Environ. Microbiol.* 80 (2), 741–750. https://doi.org/10.1128/aem.02907-13.

Wang, L., Yin, Sh; Wu, A., Chen, W. (2019). Synergetic bioleaching of copper sulfides using mixed microorganisms and its community structure succession. *J. Clean. Prod.* 245, 118689. https://doi.org/10.1016/j.jclepro.2019.118689.

Wang, X., Ma, L., Wu, J., Xiao, Y., Tao, J., Liu, X. (2020). Effective bioleaching of low-grade copper ores: Insights from microbial cross experiments. *Bioresour. Technol.* 308, 123273. https://doi.org/10.1016/j.biortech.2020.123273.

Webster, G., Newberry, C.J., Fry, J.C., Weightman, A.J. (2003). Assessment of bacterial community structure in the deep sub-seafloor biosphere by 16S rDNA-based techniques: a cautionary tale. *J. Microbiol. Methods* 55 (1), 155–164. https://doi.org/10.1016/s0167-7012(03)00140-4.

Zhang, R., Hedrich, S., Jin, D., Breuker, A., Schippers, A. (2021). *Sulfobacillus harzensis sp.* nov., an acidophilic bacterium inhabiting mine tailing from a polymetallic mine. *Int. J. Syst. Evol. Microbiol.* 71 (7), 004871. https://doi.org/10.1099/ijsem.0.004871.