World Academy of Science, Engineering and Technology
International Journal of Chemical and Molecular Engineering
Vol:18, No:2, 2024

Bioleaching for Efficient Copper Ore Recovery

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***Abstract*—**At the Aktogay deposit, the oxidized ore section has been developed since 2015; by now, the reserves of easily enriched ore are decreasing, and a large number of copper-poor, difficult-to-enrich ores has been accumulated in the dumps of the KAZ Minerals Aktogay deposit, which is unprofitable to mine using the traditional mining methods. Hence, another technology needs to be implemented, which will significantly expand the raw material base of copper production in Kazakhstan and ensure the efficient use of natural resources. Heap and dump bacterial recovery are the most acceptable technologies for processing low-grade secondary copper sulfide ores. Test objects were the copper ores of Aktogay deposit and chemolithotrophic bacteria *Leptospirillum ferrooxidans* (L.f.), *Acidithiobacillus caldus* (A.c.), *Sulfobacillus acidophilus* (S.a.), represent mixed cultures utilized in bacterial oxidation systems. They can stay active in the 20-40 °C temperature range. Biocatalytic acceleration was achieved as a result of bacteria oxidizing iron sulfides to form iron sulfate, which subsequently underwent chemical oxidation to become sulfate oxide. The following results have been achieved at the initial stage: the goal was to grow and maintain the life activity of bacterial cultures under laboratory conditions. These bacteria grew the best within the pH 1,2-1,8 range with light stirring and in an aerated environment. The optimal growth temperature was 30-33 ºC. The growth rate decreased by one-half for each 4-5 °C fall in temperature from 30 °C. At best, the number of bacteria doubled every 24 hours. Typically, the maximum concentration of cells that can be grown in ferrous solution is about 10^7/ml. A further step researched in this case was the adaptation of microorganisms to the environment of certain metals. This was followed by mass production of inoculum and maintenance for their further cultivation on a factory scale. This was done by adding sulfide concentrate, allowing the bacteria to convert the ferrous sulfate as indicated by the Eh (> 600 mV), then diluting to double the volume and adding concentrate to achieve the same metal level. This process was repeated until the desired metal level and volumes were achieved**.** The final stage of bacterial recovery was the transportation and irrigation of secondary sulfide copper ores of the oxidized ore section. In conclusion, the project was implemented at the Aktogay mine since the bioleaching process was prolonged. Besides, the method of bacterial recovery might compete well with existing non-biological methods of extraction of metals from ores.

***Keywords*—**Bacterial recovery, copper ore, bioleaching, bacterial inoculum.

I. Introduction

HEAP and in-situ leaching processes, which encompass bacterial leaching, hold significant importance in extracting metals directly from the source, such as abandoned mines at depth, off-balance ores, and low-grade deposits.

Heap bacterial leaching for commercial purposes was initially introduced in 1958 at the Bingham Canyon copper mine (Utah, USA) [1] to extract copper from substandard ores. Heap bioleaching is now extensively utilized for the recovery of copper from secondary copper ores containing the minerals chalcopyrite (Cu_2S) and covellite (CuS). Subsequently, from the 1980s onward, numerous copper heap bioleaching plants have been established in many countries across the globe. By the end of the last century, global copper production through bioleaching had accounted for 25% of the world's copper production [1].

In the USA, over 15% of copper and a substantial amount of uranium were extracted using bacterial leaching methods. Bioleaching copper from naturally occurring off-balance ores containing 0.1-0.3% of metal is 2-5 times more cost-effective than traditional pyro- and hydrometallurgical treatments [2], [3].

In Bulgaria [4], the method of heap bacterial leaching produces copper from dumps containing 0.1-0.15% copper at three times the cost of copper produced by the traditional method. Practical data shows that thione bacteria accelerate the dissolution of chalcopyrite by 12 times, arsenopyrite by 8 times, covellite, and bornite by 18 times. These microorganisms can oxidize almost all sulfides of heavy metals. They are strict autotrophs, able to exist on the mineral medium due to the energy released during the oxidation of reducing compounds of sulfur and iron [4].

Heap and in-situ leaching processes, including bacterial leaching, are of particular importance in the direct extraction of metals from ores in their natural locations (at depth in worked-out mines), off-balance ores, and low-grade deposits [5]. These technological methods require an extended period, ranging from 1 to 3 years, as they are conducted under natural uncontrolled conditions. These conditions encompass a wide range of temperatures, redox potential, and pH levels, along with various degrees of irrigation, aeration, and nutrient availability [6].

In Kazakhstan, Uzbekistan, Armenia, and Russia, bacterial leaching technologies are being actively developed, primarily for uranium, gold, copper, and nickel [1]. This was due to the abundant raw material base of these metals and the well-established structure of mining and processing enterprises that operated under traditional technologies. A bioleaching technology for the extraction of gold from refractory ores found in Akbakai [1], Vasilkovskoye [1], and Bestobe [1] deposits has been developed and is in use. This technology results in a 15-20% increase in the recovery of valuable metal compared to classical cyanide processing [1]. Additionally, another developed biotechnology for enriching the aged waste materials of the Pribalkhash [1] and Akbakai [1] enrichment plants enables the production of conditioned gold-containing concentrates with a noble metal content of 25-30 g/t, achieving a recovery rate of up to 70%. These concentrates could undergo further processing through cyanidation [10],

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[12].

This study aims to the possibility of applying bacterial-chemical leaching to extract copper from the sulfide-oxidized ores of the Aktogay deposit, to evaluate the feasibility of implementing this process on an industrial scale.

II. Experimental

A. Materials

Nutrients and Trace Elements: Ammonium sulphate GOST 3769-78 (pure qualification) was acquired from Labkhimprom LLP; Dipotassium hydrogen phosphate anhydrous CAS 7758-11-4 Chemondis; Magnesium sulfate heptahydrate GOST 4523-77 (pure qualification) was purchased from Labkhimprom LLP; Iron (II) sulphate heptahydrate GOST 4148-78 (pure qualification) was acquired from Krisanalit LLP; Potassium sulfate GOST 4145-74 (pure qualification) was purchased from Krisanalit LLP; Ammonium phosphate GOST 3771-74 (pure qualification) was purchased from Krisanalit LLP; Elemental Sulfur TU 6-09-2546-77 (pure qualification) is purchased from Krisanalit LLP.

B. Equipment and Supplies

The equipment utilized included the OMAX 40X-2500X microscope for microscopic examination. Helber Bacteria counting chambers were employed to measure microbial concentration, alongside the Marien Field cell counting chamber for the same purpose. Additionally, the measurement of water activity was carried out using the Novasina Labswift water activity meter. Micropipette Scientific was employed for precise measurements. For the growth of inoculum, Carboy 7 gallons Kegco 3PSD-7340J was utilized, alongside Tank 55 gallons Chem-Tainer TC2236CA and Tank 275 gallons Ronco Plastic 275HDPE. Heating elements included a Hot Plate UC152D from Cole Palmer, an Electric fermentation Heater 41987 from Northern Brewer, and a Temperature controller ITC-308 from Inkbird. Additionally, an SS PTHF-304 Heater and a Temperature thermocouple controller CN-TOT-A-175JF-240V were utilized for temperature control. Thermocouple probes ICSS-IM30G-300-PFA were employed for temperature monitoring. The Active AQUA PIMP4 AAPA15L Hydrofarm served as an air supply, while the Mixer System, Cole Palmer EW-50006-01, facilitated mixing of the culture. Lastly, the Dissolved Oxygen Meter Hach HQ1130 was used for measuring dissolved oxygen levels.

C. Growth and Maintenance of Bacterial Cultures

The bacterial inoculum consisted of three types of cultures cultured from microorganisms obtained from the Aktogay deposit: *Leptospirillum ferrooxidans* (L.f.), *Acidithiobacillus caldus* (A.c.), Sulfobacillus Acidophilus (S.a.) [7], [8].

Nutrients and Trace Elements

The typical nutrient solution used for isolating and cultivating mesophilic cultures was a nutrient medium (base). This solution contained nitrogen in the form of ammonium salt, phosphorus in the form of potassium salt, magnesium in the form of magnesium sulfate, and iron in the form of ferrous sulfate. The pH of the nutrient medium was adjusted to pH 1.3-3.5 range by adding 1 ml sulfuric acid solution.

The base nutrient solution contained compounds necessary to support the growth of cellular structures. The addition of ferrous (divalent) iron or elemental sulfur to the base solution provided the bacteria with an energy source. In some cases, ore or sulfide concentrates were added to support bacterial growth and metal adaptation. The energy source helped sustain the growth of the bacterial population. The addition of divalent iron or sulfur depends on the type of bacteria being grown. For iron-oxidizing bacteria, divalent iron was added, whereas for sulfur-oxidizing bacteria, elemental sulfur was added. In mixed or two-species cultures, both sulfur and divalent iron were added.

The improved nutrient base medium was prepared by adjusting the volume to one liter with DI water and incorporating Table I.

TABLE I
NUTRIENTS

Formula	Reagent name	Weight, g/l
	Laboratory nutrients (base)	
$(NH_4)_2SO_4$	Ammonium sulphate	5.000
K_2HPO_4	Dipotassium hydrogen phosphate	0.75
$MgSO_4$-$7H_20$	Magnesium sulphate heptahydrate	0.83
H2SO4	Sulfuric acid	9.81
H_2O	Deionized water	balance
	Laboratory nutrients	
$FeSO_4$-$7H_20$	Iron (II) sulphate heptahydrate	44.2
S	Elemental Sulfur	0.25
RAF	Rafinate	~200
	Plant nutrients	
K_2SO_4	Potassium sulfate	0.025
$NH_4H_2PO_4$	Ammonium phosphate	0.0503
(NH4)2SO4	Ammonium sulphate	5.000

Culture Maintenance

The bacteria were initially cultured in small volumes. The setup included a 2000 ml beaker, a heating plate equipped with an electric motor, a stirrer, and a small air pump to supply oxygen. As the culture expanded, successive dilutions were performed to sustain the necessary nutrient and metal concentrations. This process was referred to as "subculturing" or "reseeding." To facilitate continuous addition, nutrient salt solutions were prepared as outlined in Table I.

The challenge was to cultivate a substantial culture from a relatively small source and sustain its viability over an extended period for future use.

The following steps were performed to construct the setup:

- ✓ A mixture of 500 ml of nutrient salt base medium and 500 ml of fresh culture medium was prepared and placed in a 2000 ml beaker. Regular stirring was initiated, and the temperature was adjusted to the desired level (30 ± 5 °C).
- ✓ Aeration was employed to maintain a dissolved oxygen concentration of at least 4-6 ppm.
- ✓ The pH level was adjusted to 1.3–1.8 using sulfuric acid.
- ✓ For iron-oxidizing bacteria, 5 g/L of ferrous sulfate was added. For sulfur-oxidizing bacteria, 1-2 g/L of elemental

sulfur was added.

The acclimatization of bacteria in the new medium occurred over a period of 5-7 days. pH, redox reaction (Eh), temperature, and the presence of Fe^{2+} and Fe^{3+} were monitored daily. Bacterial reseeding was scheduled after the bacterial Eh level was restored to at least 610 mV for iron-oxidizing bacteria and for sulfur-oxidizing bacteria when the pH dropped to 1.0-1.2. The turbidity of the solution could also be considered a sign of bacterial activity; typically, cultures grown on elemental sulfur exhibit a milky color, indicating a high growth of the cell population. When the bacterial concentration reached 10^7 cells per milliliter or more, the cultures reached maximum density in 2-3 days under ideal conditions. A decrease in divalent iron indicated the activity of iron-oxidizing bacteria.

Once the above criteria were achieved, on day 3, the bacterial culture underwent a 2-fold dilution. The dilution was accomplished by adding base nutrients, including iron sulfate and elemental sulfur at every dilution. Appropriate measurements were conducted, and the dynamics of bacterial growth in the 2000 ml reactor were monitored twice a day. The results are presented in Fig. 1.

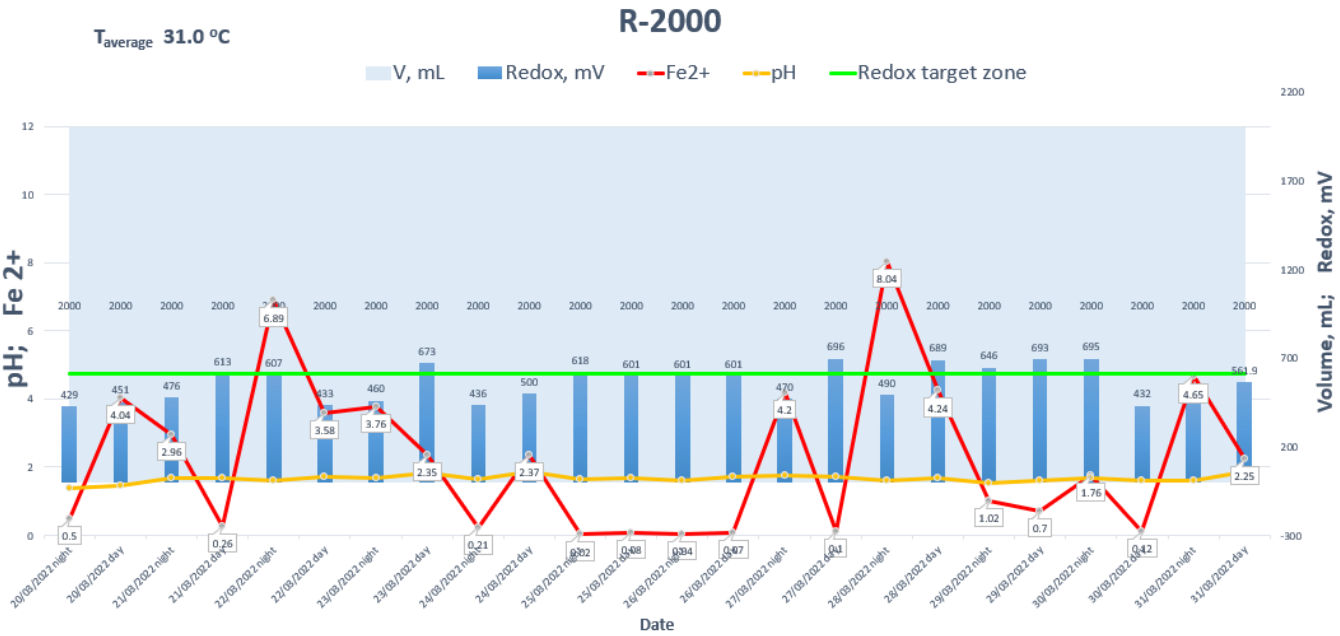

Fig. 1 Diagram of bacterial growth rates

Large Scale Inoculum Development

The laboratory inoculum was cultivated in a volume of up to 2 liters. When the culture was ready for further cultivation, half of its volume was extracted and transferred to another 20-liter reactor.

The volume extracted from the small reactor was replenished with nutrients, with iron and sulfur added as per requirement. The volume transferred to the larger reactor was adjusted to the original volume using nutrients like iron and sulfur added as needed. For instance, from an initial 2-liter seed, 1 liter was extracted and transferred to a 20-liter reactor. The remaining 1 liter of bacterial solution was adjusted to the original 2-liter volume using a nutrient solution. The 1 liter transferred to another reactor was diluted with an equal amount of the appropriate nutrient solution (1 liter).

To cultivate bacterial cultures that were resilient or adapted to elevated metal concentrations, additional metals, aside from iron, needed to be introduced. In a laboratory setting, raffinate was incorporated, not exceeding 20% of the overall inoculum volume. From the obtained healthy material, 1 liter was separated and transferred to a 20-liter reactor. The air supply, agitation, and temperature were maintained at the same parameters as the 2-liter reactors. In this diluted nutrient volume, raffinate was added to make up 20% of the total added nutrient volume. Alternatively, sulfide concentrate was gradually introduced until the copper concentration in the solution reached 1-2 g/L. The criteria for reseeding remained consistent with those for the 2-liter reactors. This process was reiterated until the desired metal values were attained. The approximate metal values after extended adaptation were as follows: Fe 30 g/l, Cu 20 g/l, As 20 g/l, Zn 100 g/l, Ni 10 g/l. The solids content needed constant monitoring and should not surpass 10% of the solution mass. Elevated concentration of solid particles could detrimentally impact cell structure integrity and the oxygen mass transfer. The growth dynamics of the cultures were observed twice a day, and the results were depicted in Fig. 2.

To establish a continuously operating seed production system, it was essential to cultivate an initial seed crop with a volume adequate to fill ¼ of the tank volume according to the TK300 system, which amounts to 43 m^3(Fig. 3).

Large volumes of inoculum were generated through sequential dilution in several smaller vats. The sequence of vats used served as the primary source for inoculating

systems, including 20-liter, 200-liter, and 1000-liter reactors.

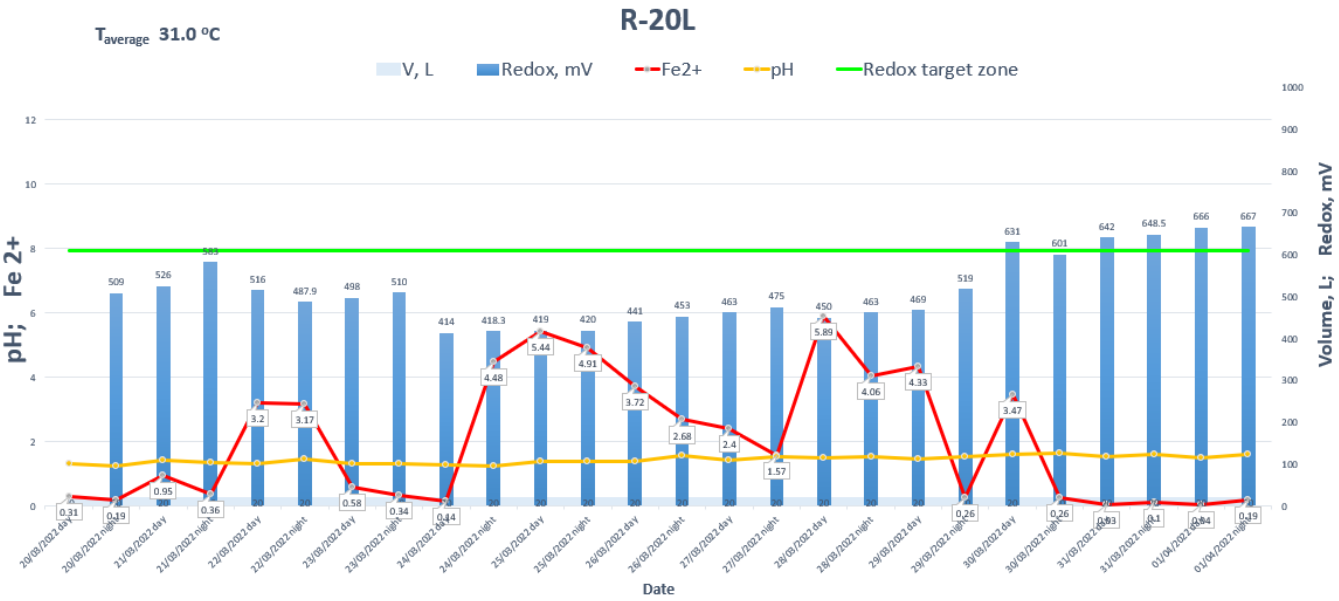

Fig. 2 Diagram of bacterial growth rates

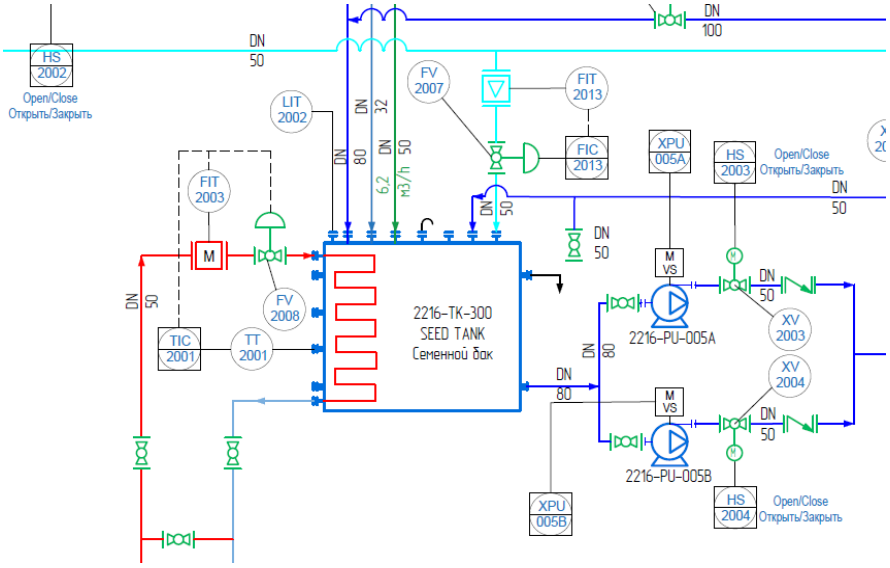

Fig. 3 Layout of the tank

After filling the 20-liter reactor, half of its volume was transferred to the 200-liter reactor and diluted with nutrients according to the instructions provided earlier. This process was then replicated with the 1000-liter reactor. The cycle of cultivation, transfer, and dilution was repeated until the desired seed volume was achieved.

The process of cultivation, transfer, and breeding continues until the required seed volume is attained [9]. Theoretically, more than 12 dilution cycles were needed to achieve the necessary volumes. A portion of the dilutions is illustrated in Table II.

To ensure the sustainable operation of the bio-oxidation system, a substantial number of seed must be cultivated. Hence, the inoculum needs to be continuously introduced into the process. Within the stirred reactor, the bacterial population was consistently replenished through in-system growth. Typically, sulfide bio-oxidation involves a series of bioreactors arranged sequentially. The bacteria's residence time in the reactor is adequate to cultivate them in significant numbers to compensate for the bacteria loss in the effluent. The standard residence time is 4 days. The nutrient composition utilized aligns with Table I.

The traditional method of bacterial inoculation of ore on heap leach pads relies on existing bacteria in the system [11], particularly wild-type microorganisms present in the environment. However, this method poses a challenge as it

may take years to cultivate a substantial and viable culture in an irrigation solution due to the adherence of most microbes to ore particles. To address this, a technological approach has been developed based on a separate inoculation system that produces bacteria specifically for the heap leaching process [13]. These bacteria were then delivered to the ore via an irrigation system.

TABLE II
DILUTION CYCLE

Cycle	Volumes	Reactor and Transfer Volumes (L)				
		2 Liter	20 Liter	200 Liter	1000 Liter	TK-200
1	Initial Volume	2.0	0.0			
11	Volume	2.0	20.0	200.0	1000.0	0.0
	Volume Removed	-1.00	-10.0	-100.0	-500.0	0.0
	Volume Added	0.00	1.00	10.00	100.00	500.00
	Nutrients Added	1.00	9.00	90.00	400.00	500.00
	New Volume	2.00	20.00	200.00	1000.00	1000.00
12	Volume	2.0	20.0	200.0	1000.0	1000.0
	Volume Removed	-1.00	-10.0	-100.0	-500.0	0.0
	Volume Added	0.00	1.00	10.00	100.00	500.00
	Nutrients Added	1.00	9.00	90.00	400.00	1500.00
	New Volume	2.00	20.00	200.00	1000.00	3000.00
15	New Volume					43000.00

This system offered the advantage of providing a large viable population of bacteria, ensuring effective bioleaching. However, due to a notable disadvantage, the need to establish an initial culture was detected to allow the system to operate in a self-sustaining mode. The approach involves a system comprising a vat and a tank. Initially, the vat, with a residence time of 12 to 24 hours, is used to mix nutrients and heat to the temperature necessary to stimulate bacterial growth. The process then progressed to the tank, where the residence time was extended to 3 to 4 days, ensuring further seed development. Both the vat and tank were equipped with forced aeration to facilitate optimal conditions for bacterial growth. The nutrient medium was derived from the process drain, and the seed dosage was determined based on production requirements. The necessary seed quantity could exceed 10 m^3/hour.

To establish a continuous seed production system, it was essential to create an initial seed crop with a volume adequate to fill half of the first vat in the system. Once this condition is fulfilled, the growth of the seed can commence within the existing system.

D. Measuring Microbial Concentration

When culturing bacteria, cell counts or bacterial concentrations were measured (Fig. 4), typically to an order of magnitude. Microbes facilitate enzymatic catalysis, accelerating chemical reactions; thus, higher bacterial concentrations lead to faster leaching. Upon reaching a critical level of bacterial concentration, other limiting factors, like the mineral particle surface area, start influencing the leaching rate. Determining the precise number and concentration of bacteria in biological leaching was challenging due to the majority of bacteria adhering to ore particles. The recorded results of bacteria counts were consistent at least 10^7 cells per milliliter.

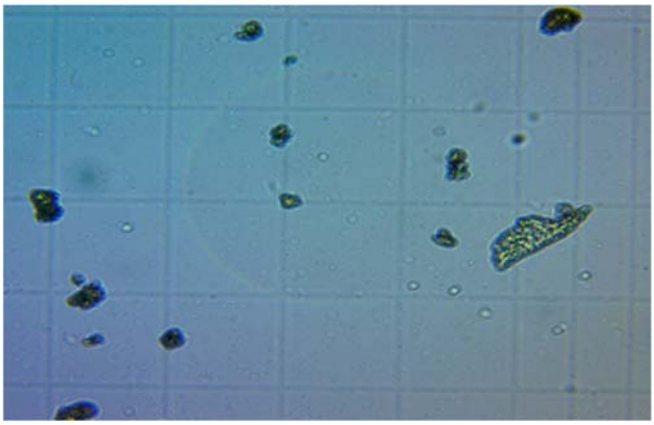

Fig. 4 Counting bacterial cells

The sample was thoroughly mixed to ensure an even distribution of bacteria. A known volume of the liquid was placed on a counting slide, and the bacteria were counted by examining the slide under a microscope. If the microbial population was too diluted (less than 10^6 cells per milliliter), the sample's concentration should be increased by centrifuging and re-dispersing the bacteria in a smaller volume. Conversely, if the concentration was too high for accurate reading, the sample should have been appropriately diluted. A Hoxley Helber chamber was used for counting, which has a depth of 20 µm with marks defining 50 µm by 50 µm squares. The chamber, when filled to the top, had a volume of 5×10^{-8} ml. Thus, the total number of bacteria counted in this square was multiplied by 2×10^{-7} ml to determine the number of cells in one milliliter. Typical *A. ferrooxidans* bacterial concentrations ranging from 10^8 to 10^9 cells/mL corresponded to approximately 5-50 bacteria per 50 square µm.

To count the cells within the square, the microscope was focused first on the cells attached to the bottom surface of the chamber and then on those attached to the top surface. The known distance between the top and bottom surfaces, nominally 20 µm, allowed for many small cells (ranging from 0.5 to 1.0 µm) to fit within this gap. While cells typically adhere to the glass surfaces, it was also important to estimate the number of cells suspended in the solution between the top and bottom surfaces.

The area of each small square was 1/400 mm², and the depth of the liquid with a properly lapped cover glass is 0.02 mm. The volume of liquid contained in one square was calculated as follows:

$$\text{Volume} = 1/400 \times 0.02\ \text{mm}^3 \quad (1)$$

$$\text{Volume} = 0.0025 \times 0.02\ \text{mm}^3 \quad (2)$$

$$\text{Volume} = 5\times10^{-5}\ \text{mm}^3 \text{ or } 5 \times 10^{-8}\ \text{cm}^3 \quad (3)$$

The calculation of the number of cells per milliliter was determined by:

Number of cells per milliliter = Number of microbes (of small squares × 5 × 10^{-8} cm^3)

III. Results and Discussion

The circuit diagram of the bioleaching apparatus for the site of the oxidized ore is illustrated in Fig. 5. When the TK-300 tank is filled, a portion of the bioculture was transferred to the TK-200 tank, which has a volume of 437 m^3. Subsequently, the material proceeded to the inoculant pond and is then dispatched to the heap leaching site.

The planned nutrient consumption was 73 tons per year. Currently, the consumption was 23 tons, attributed to the slow growth of bacterial cultures. According to the initial estimation, the inoculum supply should have been 31 m^3/hour, but the experimental results showed only 10 m^3/hour was supplied. To address the sluggish crop growth, several experimental studies were conducted to assess the efficacy of bacteria on ore materials.

Due to the slow growth of bacterial cultures, several experimental studies were conducted to evaluate the effectiveness of bacteria on ore materials.

Power Supply for PD-200

- Initial data: PD200 (2 liter): + $(NH_4)_2SO_4$ - 0.372 g, K_2SO_4 - 0.056 g, $NH_4H_2PO_4$ - 0.1 г, $FeSO_4 \times 7H_20$ – 2 g, air supply.
- Redox – 480 mV, pH – 1.47, temp. – 27 °C, O_2 – 5 ppm, Fe^{3+} - 1.63 g/l, Fe^{2+} - 0.65 g/l, Fe_{tot} – 2.28 g/l.

On day 4, the Redox was 618 mV.

The adaptation time during feeding (redox growth) was 4 days (Fig. 6).

R-2L from PD200			Measurements						
Date	Shift	Duration	Volume	pH		Redox (mV)		Temperature	DO
		days	Liter	Initial	Final	Initial	Final	°C	ppm
30-Aug-22	Day	1	2						
30-Aug-22	Night	1	2	1.55	1.54	461	463	21	8.74
31-Aug-22	Day	2	2	1.54	1.55	464	464	21	8.65
31-Aug-22	Night	2	2	1.50	1.45	473	503	21	8.67
01-Sep-22	Day	3	2	1.37	1.35	482	492	21	8.36
01-Sep-22	Night	3	2	1.48	1.52	470	487	21	8.12
02-Sep-22	Day	4	2	1.37	1.34	510	557	21	8.62
02-Sep-22	Night	4	2	1.51	1.52	602	618	21	8.33

Fig. 5 Measurement values when feeding the PD-200 pond

Comparison of Copper Extraction Using Raffinate Solution and Inoculant on Oxidized Copper Ore Material

- Initial ore data: Cu_{tot} – 0.31%, phase analysis: Cu_{oxide} – 0.28%, $Cu_{sec.sulph.}$ – 0.017%, $Cu_{prim.sulph.}$ – 0.065%.
- Initial Raff data: Cu_{oxide} – 0.09 g/l, H_2SO_4 – 19.7 g/l, redox – 400 mV, pH – 0.89, temp. – 25 °C, O_2 – 7.06 ppm, Fe^{3+} - 1.20 g/l, Fe^{2+} - 3.26 g/l, Fe_{tot} – 4.43 g/l.
- Initial inoculate data R-20L.4: Cu_{tot} – 0.09 g/l, H_2SO_4 – 6.55 g/l, redox – 645 mV, pH – 1.81, temp. – 29 °C, O_2 – 7.41 ppm, Fe^{3+} - 2.50 g/l, Fe^{2+} - 0.95 g/l, Fe_{tot} – 3.49 g/l.

The purpose of the experimental test was to determine the point at which bacteria become effective upon contacting with oxidized ore material (ground to a size fraction of 0.075 mm). To achieve this, an initial phase analysis of the oxidized ore's minerals (referred to as initial ore data) was conducted, yielding information on phases such as copper in oxidized minerals, copper in secondary sulfides, and copper in primary sulfides shown in (Fig. 6). The expectation is that bacteria would primarily interact with the copper phases in secondary and primary sulfides. This hypothesis could be confirmed by conducting a subsequent phase analysis of the ore being tested after an unspecified duration. To provide a basis for comparison, the same leaching process was conducted using a raffinate solution. Throughout the test, analysis of the total copper in the solution was performed to quantify the concentration of free copper ions in the leached solution.

1) 20 g of the ground sample was placed in a flask, and 100 ml of raffinate (initial raffinate data) was added.
2) 20 g of the ground sample was placed in a flask, and 100 ml of inoculant was added (initial inoculant data).

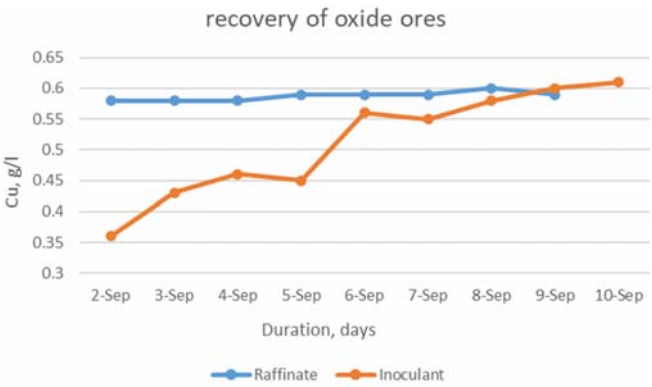

Fig. 6 Copper ion growth indicators in raffinate solution versus inoculant on oxidized copper ore material

Based on the data presented in Fig. 6, it can be noted that there was no increase in concentration observed in the flask with the refined product, and it remained at the same level. Slow growth was observed in the flask with the inoculant. This difference could potentially be attributed to variations in sulfuric acid concentration, potentially resulting in a slower leaching process in the test sample with the inoculant. Alternatively, this difference might be linked to the predominant presence of copper ores in oxidized mineral form. The phase analysis of the test samples is illustrated in Fig. 7.

In samples of oxidized ores with refined copper minerals, extraction was uniform across all phases. In samples of oxidized ores with the inoculant, the percentage of extraction of oxidized copper minerals was lower, but compared to the minerals of primary ores it was higher for 2% (Fig. 7).

Duration	Cu oxide	Cu sec.sulph.	Cu prim.sulph.	∑ Cu
6 days	%			
Heap leach pad	0.284	0.022	0.065	0.362
Heap leach pad 20g + Raff orig 100 cm^3	0.042	0.003	0.009	0.054
Heap leach pad 20g + inoculant 100 cm^3	0.105	0.003	0.008	0.116
% recovery with Raffinate	85%	86%	86%	86%
% recovery with Iniculant	63%	86%	88%	79%

Fig. 7 Phase analysis indicators and extraction percentage on oxidized copper ore material

Comparison of copper extraction using a raffinate solution and an inoculant on transitional material: The material is worn, sieved to a fraction of 0.075 mm from copper ore:

- Initial ore data: Cu_{tot} – 0.354%, phase analysis: Cu_{oxide} – 0.156%, $Cu_{sec.sulph.}$ – 0.133%, $Cu_{prim.sulph.}$ – 0.068%.
- Initial Raff data: Cu_{oxide} – 0.09 g/l, H_2SO_4 – 19.7 g/l, redox – 400 mV, pH – 0.89, temp. – 25 °C, O_2 – 7.06 ppm, Fe^{3+} - 1.20 g/l, Fe^{2+} - 3.26 g/l, Fe_{tot} – 4.43 g/l.
- Initial inoculate data R-20L.4: Cu_{tot} – 0.074 g/l, H_2SO_4 – 8.0 g/l, redox – 601 mV, pH – 1.75, temp. – 30.6 °C, O_2 – 7.41 ppm, Fe^{3+} - 2.25 g/l, Fe^{2+} - 0,82 g/l, Fe_{tot} – 3.07 g/l.

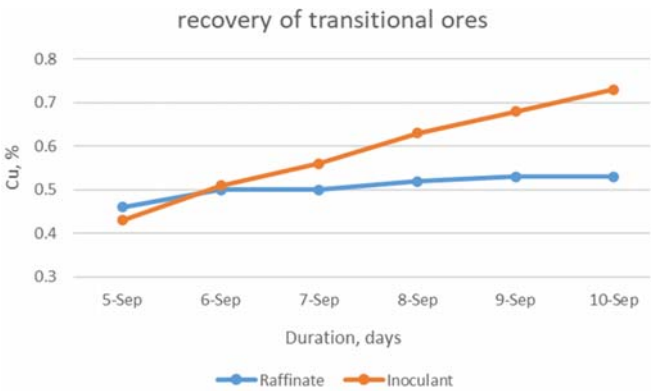

Fig. 8 Copper ion growth indicators in raffinate solution versus inoculant on tranzitional copper ore material (0.075 mm)

A slight increase in copper concentration was observed in the refined flask, whereas a noticeable increase in copper concentration was noted in the flask with the inoculant (Fig. 8).

The copper content in the solution with the inoculant was 0.2 g/l higher compared to the raffinate.

Duration	Cu oxide	Cu sec.sulph.	Cu prim.sulph.	∑ Cu
6 days	%			
Heap leach pad	0.156	0.133	0.068	0.356
Heap leach pad 20g + Raff orig 100 cm^3	0.014	0.038	0.087	0.139
Heap leach pad 20g + inoculant 100 cm^3	0.021	0.041	0.079	0.141
% recovery with Raffinate	91%	71%	0%	61%
% recovery with Iniculant	86%	69%	0%	60%

Fig. 9 Phase analysis indicators and extraction percentage on tranzitional copper ore material (0.075 mm)

Phase analysis of the test samples (Fig. 9) revealed no significant difference in tests with the inoculant compared to tests with the refined solution. It was estimated that this occurred due to the reason that bacteria need more time to work effectively.

Comparison of copper recovery by raffinate solution and inoculant on transitional material: The material is crushed to a 2 mm fraction of copper ore.

- Initial ore data: Cu_{tot} – 0.354%, phase analysis: Cu_{oxide} – 0.156%, $Cu_{sec.sulph.}$ – 0.133%, $Cu_{prim.sulph.}$ – 0,068%.
- Initial Raff data: Cu_{oxide} – 0.09 g/l, H_2SO_4 – 19.7 g/l, redox – 400 mV, pH – 0.89, temp. – 25 °C, O_2 – 7.06 ppm, Fe^{3+} - 1.20 g/l, Fe^{2+} - 3.26 g/l, Fe_{tot} – 4.43 g/l.
- Initial inoculate data R-20L.4: Cu_{tot} – 0.074 g/l, H_2SO_4 – 8.0 g/l, redox – 601 mV, pH – 1.75, temp. – 30.6 °C, O_2 – 7.41 ppm, Fe^{3+} - 2.25 g/l, Fe^{2+} - 0,82 g/l, Fe_{tot} – 3.07 g/l.

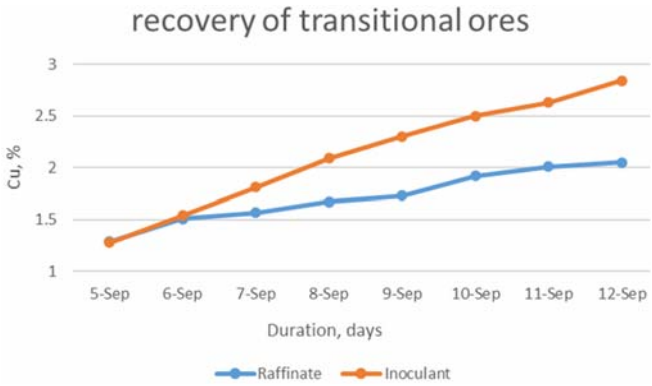

Fig. 10 Copper ion growth indicators in raffinate solution versus inoculant on tranzitional copper ore material (2 mm)

The copper content in the solution with the inoculant was higher by 0.79 g/l compared to raffinate (Fig. 10). Phase analysis of the test samples is depicted in Fig. 11.

Duration	Cu oxide	Cu sec.sulph.	Cu prim.sulph.	∑ Cu
6 days	%			
Heap leach pad	0.156	0.133	0.060	0.356
Heap leach pad 20g + Raff orig 100 cm^3	0.060	0.080	0.060	0.200
Heap leach pad 20g + inoculant 100 cm^3	0.050	0.069	0.057	0.176
% recovery with Raffinate	61%	40%	0%	44%
% recovery with Iniculant	68%	48%	0%	51%

Fig. 11 Phase analysis indicators and extraction percentage on tranzitional copper ore material (2 mm)

The phase analysis indicated that extraction with the inoculant was higher than with the refined solution by 6.8%. Furthermore, copper extraction from oxidized minerals with the inoculant was 6.42% higher than with the raffinate.

These results confirmed the superior extraction capability of the inoculant concerning transition ores and secondary sulfide forms of copper.

IV. Conclusion

Despite the positive and encouraging test results, obtaining a mass culture proves to be challenging. The expected copper extraction of 10% from sulfide ore has not been confirmed, and there have been no observable changes in the chemistry of technological solutions, such as oxidation-reduction potential (ORP), concentration of divalent iron, temperature, and copper concentration in the productive solution.

Observations from toxicity tests on the process solution for bacteria indicated a prolonged adaptation period, suggesting the difficulty in converting iron in a concentrated solution without additional water, even when the acidity falls within the suitable range for certain type of bacteria.

In the field of bacterial leaching of copper, extensive research and testing are ongoing, and the practical implementation of the technology was at the developmental and implementation stage. According to sources for Kazakhstan and for countries with a significant share of global mineral production and substantial mining potential, addressing the issue of industrial waste and low-grade ores utilization is of utmost importance. An important factor to consider is that the cost of commercial products derived from industrial waste was notably lower, ranging from 5 to 15 times less expensive than products obtained from traditional ore deposits. Studies underscored the critical need for the exploration and utilization of Kazakhstan's technogenic deposits.

Additionally, as per the literature data, experiments have been conducted involving sulfuric acid bioleaching on finely ground tailings and low-grade ores with particle sizes ranging from +0.15 mm to - 0.074 mm in experimental volumes. Mineral analysis had revealed that copper minerals are thinly dispersed within quartz and other minerals. The grain sizes of these copper minerals were smaller than 20 microns. This fine dispersion of copper grains explained the presence of a high copper content that remained unrecovered during the processing at the concentrator. To achieve full mineralization and recover copper inclusions, it was evident that mineral destruction was required. It has been suggested that optimal copper recovery from the tailings will necessitate leaching with pre-milling in the subsequent phases of the study.

During our work on bacterial adaptation, we encountered challenges related to their mass cultivation and the percolation leaching process. Furthermore, the ultrafine grinding required for mineral destruction has proven to be a costly procedure.

In summary, the comprehensive exploration of bioleaching and its immense potential as an eco-friendly, efficient method for extracting metals from ores was explored in this research. The texts covered various aspects, including the process parameters optimization, nutrient solutions selection, optimizing bacterial cultures conditions, and their effects on copper extraction.

Performed experiments provided valuable insights into optimizing bio-leaching processes. Key factors such as bacterial concentrations, nutrient composition, pH levels, and residence times were analyzed to research their impact on copper extraction efficiency. The results consistently emphasized the viability and promise of bioleaching as a sustainable approach in the mining industry.

However, challenges in scaling up the process and achieving mass bacterial cultures were evident. Addressing these hurdles was crucial in realizing the full potential of bioleaching. Additionally, the research underscored the importance of precise aeration and nutrient management for optimal bacterial growth and enhanced extraction rates.

Last but not least, the texts served as a valuable resource for researchers, practitioners, and stakeholders in the mining and metallurgy sector, while shedding light on the advancements and challenges in bioleaching. Further research and practical applications are essential to fully integrate and capitalize on this innovative and eco-conscious approach to metal extraction.

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