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Research article

Identification of microorganisms at different times in a bioleaching process for the recovery of gold and silver from minerals in oxide form

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ABSTRACT

In this study, gold and silver were recovered through a bioleaching process conducted at room temperature over 11 days. Native bacteria and varying ratios of mineral pulp to culture medium (20/80, 37.5/62.5, and 50/50 %) from a mining operation in Zacatecas, Mexico, were evaluated. The mineral was crushed to a particle size of 0.125 inches or smaller, containing gold and silver concentrations of 0.609 g/ton and 138.89 g/ton, respectively. Four native microorganisms were identified using molecular biology techniques and a 16S rRNA gene fragment: *Acidovorax citrulli*, *Brevundimonas albigilva*, *Sphingomonas korenensis*, and *Methylobacterium organophilum*. The bioleaching system achieved metal extractions of 84.12 % and 63.93 % for gold and silver, respectively. Different microorganisms were identified at various processing times: *Sphingomonas korenensis* (days 1, 2, 5, 8, and 11), *Methylobacterium organophilum* (days 1 and 2), *Paenibacillus dongdonensis* (days 1 and 2), *Brevundimonas albigilva* (day 5), *Ureibacillus manganicus* (day 5), *Peribacillus simplex* (day 8), *Niallia circulans* (day 8), *Massilia atriviolacea* (day 11), and *Bacillus licheniformis* (day 11). The dominant bacterium throughout the process was *Sphingomonas korenensis*, which appeared at all stages of the experiment.

1. Introduction

The increasing global demand for gold and silver has driven the development and implementation of various extraction processes, such as cyanide leaching and flotation, to extract these metals from mineral matrices. However, these methods are often inefficient for extracting gold and silver from mineral oxides [1,2]. An innovative alternative for gold and silver recovery from different mineral matrices is bioleaching, a process that utilizes microorganisms in direct or indirect contact with metals in an acidic system. This system solubilizes metals from the solid matrix into the solution. The solubilization of metals in bioleaching depends on three key factors: the

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material being leached, the leaching medium, and the bacteria. These bacteria obtain energy by using the compounds in the system, specifically by extracting electrons from the metals of interest, which leads to the solubilization of the metals into the solution without the need for external energy input into the acid system [3,4]. Additionally, these bacteria can utilize the metals embedded in the solid matrix as final electron acceptors in their respiratory chain, causing metal polarization and subsequent solubilization.

Bioleaching processes involve various types of microorganisms, including autotrophic, mixotrophic, and chemolithotrophic bacteria, each suited for specific conditions and metal types. In the mining industry, bioleaching systems typically employ microbial consortiums—combinations of different bacteria working together—resulting in increased metal recovery productivity due to the consortium's ability to adapt to environmental changes.

Changes in the system can lead to shifts in the bacterial community, where one group of bacteria may increase in abundance while another may decrease. This phenomenon, known as bacterial dominance, is influenced by environmental factors such as system geometry, materials, oxygen availability, pH, temperature, and the adaptation phase of the bacteria [5–10]. Bacteria that are best adapted to changes in the system—due to their osmotic regulation and nutritional cycles—are more likely to dominate the process [11–13]. However, even bacteria present in small quantities can proliferate if the system undergoes environmental changes or if they occupy specific niches within the system [14]. Bacterial dominance and their metabolic processes can significantly impact the operational costs of any system. Therefore, identifying the microorganisms present in mining extraction systems—whether they are present in total or in dominant forms—underscores the need to microbiologically characterize these processes. This is especially important for identifying native bacteria, as well as the molecules involved in regulating functions such as gene expression, transmission, and storage of genetic information. However, many molecules, amino acids, and proteins found in microorganisms may be similar across species. In such cases, the most reliable method for identification is the analysis of the 16S ribosomal DNA substructure. This polyribonucleotide sequence, approximately 1500 nucleotides in length, contains hypervariable regions with unique amino acid fragments that differ between species. These fragments can be extracted, sequenced, and compared against databases or bioinformatics programs to identify similarities and establish phylogenetic relationships among species [15,16]. This research aims to analyze the native species present in a mineral and track their population changes over time during a silver and gold bioleaching experiment. The study used the optimal conditions for bioleaching gold and silver from oxide minerals as determined by Contreras-Mora et al. (2023) [17].

2. Materials and methods

2.1. Mineral pulp

The samples of mineral pulp were taken from the principal vein of a mineral consortium located in Zacatecas, México (Fig. 1).

For the detachment of the mineral, samples were removed using a hammer and chisel, until having a particle size $\leq$6 in. Later, the samples were processed with a Mitchel jaw crusher type Blake (double effect) model 512 to obtain a particle size of $\leq$0.125 in. The characterization of the mineral pulp was conducted using EPA 3050B for sample analysis via inductively coupled plasma mass spectrometry (ICP-MS) [18]. For mineralogical analysis, 1 g of the sample, which was previously pulverized and dried at 80 °C for 3 h, was used. To this, 5 mL of 5 % HNO3 was added, and the sample was heated to 95 °C. Hydrogen peroxide (H_2O_2) was then introduced, followed by the addition of nitric acid for reflux at 95 °C. The resulting solution was analyzed using an ICP-OES instrument (PerkinElmer Optima 5300DV). The analysis revealed concentrations of 0.609 $\pm$ 0.2 g/ton of gold (Au) and 138.89 $\pm$ 0.58 g/ton of silver (Ag). Other metals detected in the sample included iron (Fe) at 4.935 $\pm$ 0.25 g/ton, zinc (Zn) at 0.493 $\pm$ 0.006 g/ton, lead (Pb) at 0.417 $\pm$ 0.06 g/ton, copper (Cu) at 0.027 $\pm$ 0.008 g/ton, bismuth (Bi) at 0.019 $\pm$ 0.0009 g/ton, antimony (Sb) at 0.006 $\pm$ 0.0005 g/ton, and cadmium (Cd) at 0.001 $\pm$ 0 g/ton. X-ray diffraction analysis was performed using a Rigaku Mini-Flex 300/600 model, operated at 40 kV and 15 mA, with a scanning speed of 0.9°/min and a 2θ range of 5–90° [19]. The analysis indicated that the majority of the mineral

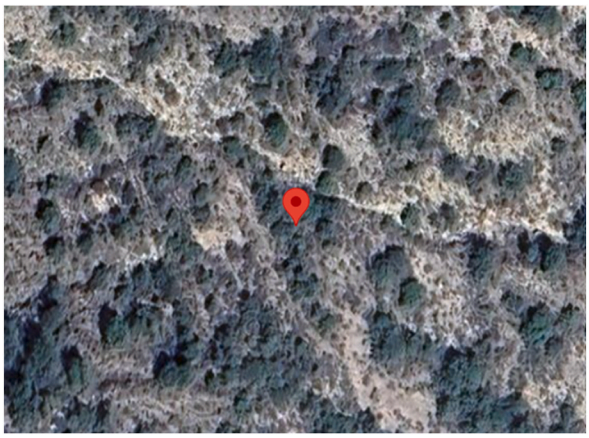

Fig. 1. Mine location (23°09′48.0″N 104°07′19.0″W).

components were orthoclase compounds ($KAlSi_3O_8$).

2.2. Culture medium

The culture medium proposed by Silverman et al. (1976) [20], was modified with the next compounds: 4 g/L of ammonium sulfate ($(NH_4)_2SO_4$); 0.1 g/L of potassium chloride (KCl); 0.5 g/L of potassium phosphate (K_2HPO_4); 0.5 g/L of magnesium sulfate heptahydrate ($MgSO_4 \bullet 7H_2O$), 0.14 g/L of calcium nitrate tetrahydrate ($Ca(NO_3)_2 \bullet 4H_2O$) and 1 % (vol/vol) of nitric acid (concentrated).

2.3. Isolation of native microorganisms

The microorganisms were extracted from 100 g of homogenized mineral, which was placed in a 250 mL beaker and flooded with distilled water. The beaker was then immersed in a Baku ultrasonic bath (model BK-3550) for 15 s at 35 W. The liquid was homogenized and transferred into five 50 mL Corning tubes. The tubes were centrifuged at 2500 rpm for 2 min using a Solbat centrifuge (model C-40). After centrifugation, the supernatant was decanted, and 15 mL of distilled water was added to each tube. The tubes were then centrifuged again at 2500 rpm for 1 min, followed by sonication at 15 W for 15 s. The liquid from the tubes was transferred into new 50 mL Corning tubes and centrifuged at 3500 rpm for 15 min. After centrifugation, the supernatant was decanted, and the bacterial pellet was recovered in solid form. The pellet was then inoculated onto plates containing bacteriological agar and incubated in a FELISA incubator at 30 °C for 8 days. Following incubation, several bacterial colonies were obtained, each of which was transferred to a new agar plate and incubated for an additional 9 days at 30 °C.

2.4. DNA extraction

Each bacterium was recovered from the plates by adding 4 mL of distilled water and gently agitating with a glass rod. The suspension (containing the bacteria) was transferred into an Eppendorf tube, which was then centrifuged at 13,500 rpm for 1 min using a Thermo IEC Microlite centrifuge (Germany). The supernatant was decanted, and 1 mL of lysis buffer was added to the tube, followed by homogenization in a Vortex-Genie 2 (USA) at 13,500 rpm for 1 min. The liquid was decanted again, and 300 μL of lysis buffer and 100 μL of lysozyme were added, then mixed using a vortex. The tube was incubated at 37 °C for 5 min, after which 30 μL of 20 % SDS was added, and the mixture was vortexed and incubated at 37 °C for another 5 min. Next, 50 μL of phenol, 40 μL of chloroform, and 2 μL of isoamyl alcohol were added to the tube, which was then mixed with a vortex and centrifuged at 13,500 rpm for 5 min. This step was repeated in the same tube with the same quantities of reagents. After the second centrifugation, the upper liquid phase was transferred into a new Eppendorf tube, and 800 μL of 95 % ethanol and 40 μL of sodium acetate were added and gently mixed. The tube was then centrifuged at 13,500 rpm for 5 min. The supernatant was decanted, and 500 μL of absolute ethanol was added, followed by another 5-min centrifugation at 13,500 rpm. The supernatant was decanted, and the pellet was dried for 30 min at room temperature. Finally, 100 μL of nuclease-free water was added to the tube to resuspend the DNA.

2.5. Amplification of the fragment of 16S ribosomal DNA

The DNA amplification was performed using an Eppendorf tube with 5 μL of Gotaq buffer; 1 μL of dNTPs; 3 μL of $MgCl_2$; 1 μL of taq polymerase; 1 μL of R oligonucleotide (16STHR (CCGTCAATTCCTTTGAGT)) and 1 μL of D oligonucleotide (16STHD (GTGCCAGCMGCCGCGGTAA)). This reaction was an endpoint PCR performed on a Bio-Rad T100 equipment (USA), with the amplification process conducted under a temperature gradient ranging from 55 °C to 72 °C.

2.6. Preparation of bioleaching system

The experiment was conducted at room temperature over 11 days, with medium changes using a modified 9K medium (90 mL of 9K medium and 1 mL of nitric acid) on days 4 and 8 to maintain optimal growth conditions for the microbial cultures. The experiments were performed in plastic trays (dimensions: 15 cm × 23 cm x 15 cm), where the ratio of native bacteria to mineral pulp/culture medium was systematically varied to assess its effect. The evaluated ratios were 20/80, 37.5/62.5, and 50/50.

In addition to the bacterial-medium ratio, another key variable in this study was the particle size of the mineral samples. Particle sizes were categorized as $\leq$ ¼", $\leq$1/8″, and $\leq 3.543 \times 10^{-7}$", allowing for an analysis of how particle size might influence microbial growth and activity.

Each experimental condition was performed in triplicate to ensure statistical reliability and reproducibility. To monitor the progress of the experiment, pH and oxide-reduction potential (ORP) were measured every 24 h directly from the samples using a multiparametric Hanna model HI 991003 (Romania). These parameters were tracked to evaluate chemical changes occurring throughout the experiment. For microbial recovery, samples were collected at regular intervals on days 1, 2, 5, 8, and 11, and analyzed to assess growth dynamics and changes in the microbial population over time.

2.7. Epifluorescence of bacteria present in the mineral of the system

A sample of mineral from the system with the highest recovery of gold and silver was collected before the process, as well as on days

1, 2, 5, 8, and 11. The sample was submerged in 1 % formaldehyde for 10 s and then dried at room temperature. It was subsequently stained with acridine orange (prepared by dissolving 20 mg of acridine orange in 100 mL of sodium acetate buffer (1 mol) and 90 mL of 1M HCl) for 2 min. After staining, the sample was washed with distilled water. Next, the sample was decolorized using a 50/50 alcohol-acetone solution for 10 s, washed again with distilled water, and dried at room temperature. Once dry, the sample was treated with fluorescein (prepared by dissolving 0.002 g of fluorescein sodium salt in 52 mL of acetic acid (0.2 mol) and 48 mL of sodium acetate (0.2 mol)) for 2 min. After this, the sample was washed with distilled water and dried at room temperature. The sample was then examined under a Leica MZ16 FA microscope (Germany) with an emission wavelength of 490 nm and a blue filter. The fluorescence images were analyzed using Leica AF Lite software. Each sample was analyzed in triplicate. The images were processed using Fiji software, which involved using the "Measure" command, along with the "Area" and "Mean Gray Value" (IntDen), as well as the "Raw Integrated Density" (RawIntDen) options.

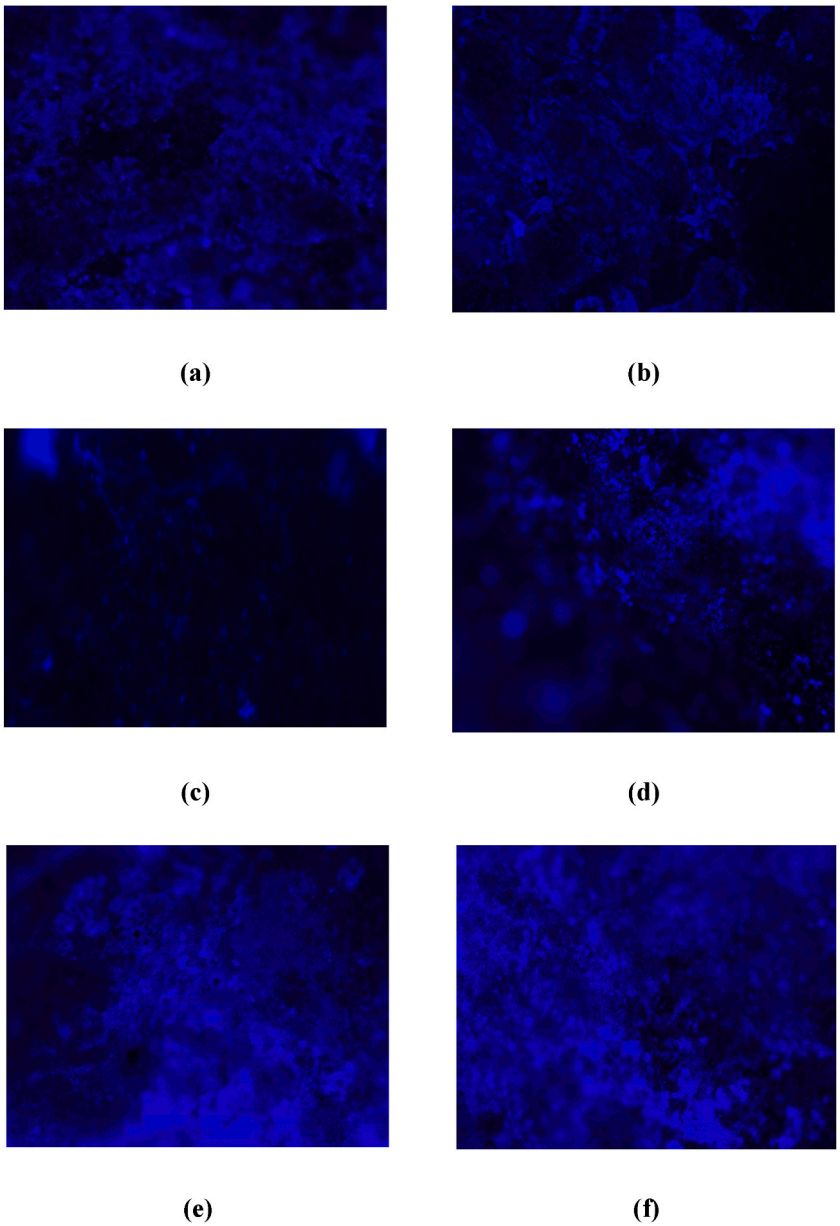

Fig. 2. Epifluorescence images of acridine orange-stained mineral samples: a) unprocessed mineral, b) mineral after 24 h of processing (day 1), c) day 2 of processing, d) day 5 of processing, e) day 8 of processing, and f) day 11 of processing.

3. Results

3.1. Identification of native bacteria

The bacteria found in higher proportions in the minerals without bioleaching were *Sphingomonas koreensis* (95 % similarity), *Methylobacterium organophilum* (92 % similarity), *Acidovorax citrulli* (82 % similarity), and *Brevundimonas albigilva* (97 % similarity).

3.2. Bacteria identification present in the bioleaching process

The system with 50 % pulp (vol/vol) and native bacteria exhibited the best conditions for gold and silver recovery, achieving 84.13 % and 63.93 %, respectively. At zero hours, the raw, unprocessed mineral contained the following bacteria: *Acidovorax citrulli* (82 % similarity), *Brevundimonas albigilva* (97 % similarity), *Sphingomonas koreensis* (95 % similarity), and *Methylobacterium organophilum* (97 % similarity) (Fig. 2a: IntDen = 112.04, RawIntDen = 5,422,940). After 24 h (day 1), the system exhibited an oxide-reduction potential of 565.33 ± 5.3 mV and a pH of 1 ± 0.041. The fluorescence of the samples decreased (Fig. 2b: IntDen = 88.267, RawIntDen = 4,272,101), and the bacterial concentration was $9.55 \times 10^6 \pm 4.06 \times 10^6$ cells/mL. The identified bacteria were *Sphingomonas koreensis* (95 % similarity), *Methylobacterium organophilum* (89 % similarity), and *Paenibacillus dongdonensis* (92 % similarity). After 2 days, the system showed an oxide-reduction potential of 613.3 ± 4.3 mV and a pH of 1 ± 0.001. The bacterial concentration decreased to $3.82 \times 10^6 \pm 1.16 \times 10^6$ cells/mL (Fig. 2c: IntDen = 45.18, RawIntDen = 2,186,902). The bacterial population changed, with the identified species being *Sphingomonas koreensis* (95 % similarity), *Methylobacterium organophilum* (89 % similarity), and *Paenibacillus dongdonensis* (92 % similarity) (Table 1). On day five, the system's changing conditions led to further alterations in the bacterial population, which included *Sphingomonas koreensis* (95 % similarity), *Brevundimonas albigilva* (90 % similarity), and *Ureibacillus manganicus* (92 % similarity). The bacterial mass decreased to $1.13 \times 10^7 \pm 1.32 \times 10^6$ cells/mL (Fig. 2d: IntDen = 104.07, RawIntDen = 5,037,352). The pH remained stable at 1 ± 0.004, while the oxide-reduction potential increased to 645 ± 3.7 mV. On the eighth day, the system showed a slight decrease in oxide-reduction potential (631.3 ± 3 mV) and maintained a constant pH of 1 ± 0.04, with a bacterial mass of $1.76 \times 10^7 \pm 2.72 \times 10^6$ cells/mL (Fig. 2e: IntDen = 7,696,550, RawIntDen = 7,696,550). The identified bacteria at this stage were *Sphingomonas koreensis* (95 % similarity), *Peribacillus simplex* (91 % similarity), and *Niallia circulans* (88 % similarity). At the end of the experiment, the system had an oxide-reduction potential of 649.66 ± 3.6 mV and a pH of 1 ± 0.004. The bacterial mass had decreased to $1.29 \times 10^7 \pm 1.16 \times 10^6$ cells/mL (Fig. 2f: IntDen = 841,806, RawIntDen = 8,418,064). The identified bacteria included *Massilia atriviolacea* (93 % similarity), *Bacillus licheniformis* (87 % similarity), and *Sphingomonas koreensis* (95 % similarity).

The fluorescence emitted by the bacteria growing on day 5 (Fig. 2d), achieved the highest concentration on day 8 (Fig. 2e) and had a minor decrease in their population, as well as in the fluorescence of Fig. 2f (day 11 of processing).

4. Discussion

4.1. Oxide-reduction potential and pH

The pH potential throughout the experiment remained low, with values approaching 1. This is due to the mineral composition, as most of the minerals are orthoclase compounds ($KAlSi_3O_8$), which are minimally reactive with the nitric acid used in the experiment. Additionally, these compounds are less basic compared to those typically used in metal recovery via cyanide leaching. This results in a chemical leaching system for gold and silver [21,22], which is further supported by bacterial activity. The bacteria utilize the metals to aid in protein stability in hostile environments. Moreover, bacteria in low-pH media can survive due to their reduced membrane permeability, as well as the rigidity of the membrane and changes in the membrane pores that help protect against external stressors. Extracellular polymeric substances (EPS) also contribute to bacterial survival in these conditions [23].

Table 1
Bacteria identification in bioleaching system.

Time of experimentation (Days)	pH of the Bioleaching system	Bacteria identified
1	1 ± 0.041	*Methylobacterium organophilum* *Paenibacillus dongdonensis* *Sphingomonas korenensis*
2	1 ± 0.001	*Methylobacterium organophilum* *Paenibacillus dongdonensis* *Sphingomonas korenensis*
5	1 ± 0.004	*Brevundimonas albigilva* *Sphingomonas korenensis* *Ureibacillus manganicus*
8	1 ± 0.04	*Niallia circulans* *Peribacillus simplex* *Sphingomonas korenensis*
11	1 ± 0.004	*Bacillus licheniformis* *Massilia atriviolacea* *Sphingomonas korenensis*

The oxide-reduction potential (ORP) over the course of the experiment remained above 550 mV, which facilitates the leaching of metals, particularly gold and silver. High millivolt readings indicate significant metal leaching, often associated with a high concentration of oxidizing elements [24–26]. The silica mineral material plays a crucial role in maintaining redox levels, as it interacts with the metals in the system. This behavior contrasts with materials that have been processed or have different compounds added to the matrix [27]. The redox balance in the system was maintained by the bacteria, as they can utilize electrons from metals like gold and silver for respiration through the membrane. This process involves the assembly of porin–cytochrome complexes and membrane proteins, which generate organic acids that indirectly solubilize metals [28,29].

4.2. Identification of native bacteria

The changes in bacterial populations occurred after 24 h, with an increase in the abundance of certain bacteria, such as *Sphingomonas korenensis*, while less abundant bacteria from other species were also affected. This shift in population could play a key role in bacterial survival in the newly introduced leaching environment [30]. The new medium contained fewer nutrients, prompting the bacterial community to alter gene expression (e.g., lasI, lasR, rhlI, and rhlR) to regulate proteins involved in electron transfer and cellular respiration. Additionally, enzymes like acylase were expressed to disrupt quorum sensing (QS) and regulate bacterial population growth in response to the new bioleaching medium and the particle size of the mineral [31–34]. These changes affected part of the initial community, as some bacteria began to use the mineral as a substrate, utilizing metals in their metabolism. This led to the proliferation of bacteria such as *Sphingomonas korenensis* and the inactivation of others, such as *Methylobacterium organophilum*. This inactivation could change depending on the increase or decrease of certain components in the system, a trend observed early in the experiment with the population of *Brevundimonas albigilva* (a native bacterium), which reappeared on the fifth day of the experiment [35–37].

The activation and inactivation of bacterial populations were influenced by changes in substrates. Some substrates acted as toxic agents for certain bacteria, effectively "inhibiting" them at specific times during the experiment [38]. For example, metals in the system induced stress in some bacteria, leading to a reduction in their population or inactivation, but this stress also enhanced the resistance and tolerance of the surviving bacteria to metals [39,40]. These bacteria promoted the adsorption and bioaccumulation of metals [41,42]. The bacterial community also responded to the nitric acid in the system, as silver ions released during the silver oxide leaching process have antimicrobial effects. These silver ions form complexes with the bacteria, disrupting their membranes and interacting with enzymes and DNA, causing damage to their functions. In response, the more adaptable bacteria survived due to increased membrane rigidity and changes in membrane pore structure through proteins like Porin (Omp40), as well as reduced membrane permeability and enzymes that help expel acidic charges [43–45].

4.3. Identification of native bacteria

The mineral harbored four types of bacteria: *Brevundimonas albigilva*, *Sphingomonas korenensis*, *Methylobacterium organophilum*, and *Acidovorax citrulli*. These bacteria are typically found in environments such as water, soil, leaf surfaces, nodules, grains, and air [46,47]. They can coexist synergistically, generating metabolites that benefit other microorganisms in the system, potentially promoting the growth of various species [48,49]. These bacteria are also capable of surviving the environmental conditions of the mining site, as they can adapt to both aerobic and anaerobic systems (Fig. 3). Additionally, some of them are resistant to the harsh conditions associated with hydrological stress, variations in water activity, and fluctuations in temperature across different seasons [50–53]. Furthermore, certain bacteria exhibit resistance to various salts [54–56] and toxic compounds commonly found in the mining industry [57]. A key factor for bacterial growth in minerals is the presence of metals. While some metals may inhibit specific bacterial species, these bacteria are able to thrive in the presence of metals such as Fe, Zn, Cu, and Ag. This resilience is attributed to their production of

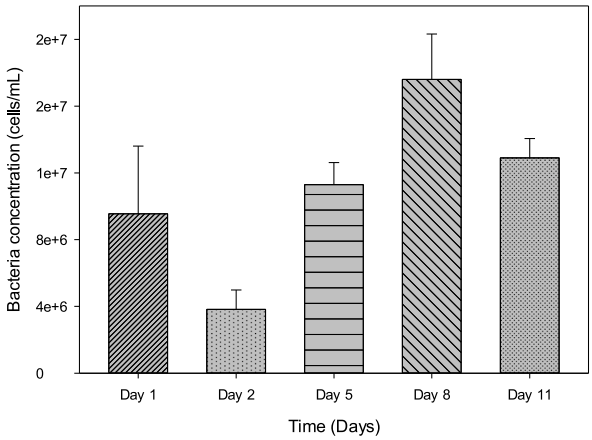

Fig. 3. Bacteria concentration (cells/mL) in bioleaching experiments.

extracellular polymeric substances, which protect them from environmental stress, and siderophores, which facilitate the capture of metals that can be transported into the cells for metabolic use [58–63].

The system changed throughout the experimentation, which impacted the bacterial population (Table 1) due to variations in the metabolites within the system. *Sphingomonas korenensis* and *Methylobacterium organophilum* exhibited the most suitable genes for nutrient cycling and showed the best adaptation to environmental changes and osmotic regulation. *Sphingomonas korenensis* belongs to the family *Sphingomonadaceae* in the order *Sphingomonadales* and is typically found in freshwater, marine habitats, and soils [64]. These bacteria can thrive in the environment of a bioleaching system, as shown in Fig. 3, thanks to their membrane and ABC transporters, mechanosensitive channels, and membrane proteins that protect the cell against osmotic stress. Additionally, they can bioaccumulate nutrients from the system and utilize compounds such as metals in their metabolism, a process mediated by intracellular proteins [40–65]. They also secrete extracellular polymeric substances (EPS), which further contribute to their survival. These mechanisms of metal adsorption and accumulation by the bacteria can lead to the separation of valuable metals from the mineral matrix. These metals are then released into the liquid system due to the bacteria's planktonic or sessile behavior [66,67].

Methylobacterium species belong to the family *Methylobacteriaceae* and are commonly found in water, air, soils, biological soil crusts, and extreme environments. These bacteria can survive in the presence of heavy metals like lead, mercury, copper, nickel, and cadmium by producing siderophores that solubilize certain metals [68]. They also produce extracellular polymeric substances that protect their cells in bioleaching systems [69,70]. Furthermore, *Methylobacterium* can biodegrade toxic compounds in some systems [71] and produce nutrients that other microorganisms can use to survive synergistically [72]. These bacteria can associate with heavy metals at various stages of their growth, even during resting phases, through their cell wall, proteins, and extracellular polymers. This interaction results in the biosorption and bioaccumulation of metals, enabling the separation of metals from the mineral matrix [73].

Other bacteria present throughout the experiment include *Bacillus licheniformis*, *Brevundimonas albigilva*, *Massilia atriviolacea*, *Niallia circulans*, *Ureibacillus manganicus*, *Paenibacillus dongdonensis*, and *Peribacillus simplex* (Table 1). These bacteria are capable of surviving in environments with low mineral concentrations, and their presence persisted throughout the various stages of the experiment [74–76]. Their survival is attributed to their ability to generate spores [77,78] and produce extracellular polymeric substances, which protect the cells from external stressors [79]. A key factor in their resistance is the use of the 9K modified medium and the presence of heavy metals in the system, whether in solid or solubilized form [80–82], which the bacteria utilize in their metabolism to produce metabolites that benefit the bacterial community (Fig. 3). These metabolites can be either beneficial or harmful [83,84].

However, certain factors influenced the presence of these bacteria, particularly the pH of the system. The acidic experimental conditions (pH = 1 in all experiments) negatively impacted the growth of most bacteria, particularly alkaliphilic species. This is because the metabolic processes and metal leaching of most bacteria are typically optimized in alkaline environments [85–87], and room temperature further hindered bacterial metabolism. As a result, the bacterial community diminished over time (Table 1), as most of the bacteria were mesophilic and adapted to moderate temperatures [88–90].

5. Conclusion

This study successfully demonstrated the recovery of gold and silver through bioleaching using native microorganisms from a mining site in Zacatecas, Mexico. The process achieved an impressive extraction of 84.12 % gold and 63.93 % silver at room temperature over 11 days. The identification of native bacteria, including *Sphingomonas korenensis*, *Methylobacterium organophilum*, *Brevundimonas albigilva*, and others, revealed their dynamic presence and role at different stages of the bioleaching process. Notably, *Sphingomonas korenensis* was the dominant bacterium, appearing throughout the entire experimental timeline. The study highlights the potential of utilizing native microbial communities for efficient metal recovery, offering valuable insights for sustainable mining practices.

Data will be made available on request. For requesting data, please write to the corresponding author.

CRediT authorship contribution statement

Cuauhtémoc Contreras Mora: Writing – original draft, Resources, Methodology, Investigation, Formal analysis. **Juan Antonio Rojas Contreras:** Validation, Supervision, Methodology, Data curation, Conceptualization. **Mayra Cristina Rosales Villarreal:** Writing – original draft, Software. **José Luis Urban Martínez:** Validation, Methodology. **Efren Delgado:** Funding acquisition. **Hiram Medrano Roldan:** Supervision, Project administration, Conceptualization. **Felipe Samuel Hernández Rodarte:** Resources. **Damián Reyes Jáquez:** Writing – review & editing, Supervision, Project administration.

Declarations

On behalf of all authors of the manuscript entitled: "Identification of microorganisms at different times in a bioleaching process for the recovery of gold and silver from minerals in oxide form", as the corresponding author, I certify that they have NO affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

All authors would like to state the following.

1 The manuscript does not contain experiments using animals.
2 The manuscript does not contain human studies.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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