Bioleaching of Copper Concentrate by Indigenous Isolates of Iron and Sulfur-Oxidizing Bacteria from Acid Mine Drainage

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ABSTRACT: *Chalcopyrite is the most abundant copper mineral in the world, and its bioleaching suffers from low dissolution rates, which is often attributed to passivating layers. Hence, these passivating layers must be overcome to use bioleaching technology to its full potential to process chalcopyrite. Leaching must occur at a low Oxidation/Reduction Potential (ORP) to prevent these passivating layers from forming, but chemical redox control in bioleaching heaps is difficult and costly. As an alternative, selected weak iron-oxidizers could be employed that are incapable of scavenging exceedingly low concentrations of iron and, therefore, raise the ORP just above the onset of bioleaching but not high enough to allow for the occurrence of passivation. This study isolated four bacterial strains from acid mine drainage in one of Mongolia's most significant copper mining sites. Three of these strains were identified based on their partial sequence of the 16S rRNA gene. Also, we studied the electrochemical properties of the bioleaching process of sulfide ore by one of the isolates obtained from the acid mine drainage. Our results show that strains ER-1a and ER-1c are closely related to Candidate division OP10 bacterium P488 (AM749768), and ER-1d is closely related to Fimbriimonas ginsengisoli Gsoil 348 (GQ339893). Bioleaching of copper concentrate was monitored by the electrochemical method. During 18 days of oxidation, only three types of oxidations*

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were observed. The solubility of copper reached 615 mg/L and 53.37%, while 83.7% of ferrous ions were converted to iron (III). The CV-cyclic voltammetry oxidation current peak intensity gradually increased until day 15 and then decreased on day 18 during the bioleaching experiment.

KEYWORDS: *Electrochemistry; Copper concentrate; Iron-oxidizers; Bioleaching.*

INTRODUCTION

Bioleaching of copper sulfide ores by acidophilic chemolithoautotroph bacteria such as *Acidithiobacillus ferrooxidans* is a well-researched and fully-fledged process for copper recovery [1-3]. Mongolia is home to some of the largest copper mines in Asia and copper is mainly in the form of pyrite and chalcopyrite. Erdenet, one of the largest copper reserves in Mongolia, is mostly infilled with chalcopyrite in its veins [4,5]. Mongolian copper is processed mainly by classical ore processing methods. However, classical mining methods that employ chemical leaching leave a considerable amount of copper in the mine tailings, wasting an economically valuable resource. Besides, processed mineral ore waste accumulates in tailing ponds. Due to microbial activity, this waste results in soil and water acidity that may cause ecological danger to the region. Therefore, bioleaching, an environmentally friendly technique, is vastly employed in the processing of sulfide ores where ferrous and sulfur-oxidizing bacteria are involved in the dissolution and removal of ferrous and sulfur in the ore, making copper extraction feasible from low-grade ores. Bioleaching of iron and sulfur-containing copper ores such as pyrite and chalcopyrite are done by *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans*, *Thiobacillus thiooxidans*, *Sulfolobus* species, and others. These microorganisms derive energy from the oxidation of ferrous and sulfur ions and make biomass from carbon dioxide fixation. Bioleaching of chalcopyrite from mining sites in Inner Mongolia, located in the same Central Asian Orogenic Belt as Mongolian copper reserves, has been reported [6-8]. On the other hand, the industrial application of bioleaching for processing copper ores was introduced to Mongolian mines only in the last three decades. However, still more research is required to explore iron and sulfur-oxidizing microorganisms from local sources and study the mechanism involved in bioleaching in detail[9]. On the other hand, indigenous species that are adapted to the local environment are preferable for employing in microbial processing such as bioleaching.

Furthermore, the application of iron and sulfur-oxidizing microbial species isolated from acid mine drainage is not limited to metal extraction from sulfide ores by bioleaching. For instance, it is reported that *Acidithiobacillus ferrooxidans* can be used in the bioremediation of chromium (VI), which has been causing a big problem because of the release of untreated water from tanneries into the effluent of urban sewage treatment plants in Ulaanbaatar, Mongolia [10,11]. Also, *Acidithiobacillus ferroxidans and Acidithiobacillus thiooxidans* can be used in the desulfurization of fuels and industrial gases. Therefore, there is considerable interest in isolating indigenous strains of iron-sulfur oxidizing species and introducing them to mineral processing and bioremediation [12-14].

In addition, by combining biological oxidation with electrochemical oxidation technology with other traditional physical, biological, and chemical treatment methods (pre-treatment, post-treatment, or integrated-treatment), it is possible to the leaching of refractory ores and treatment of Persistent Organic Pollutants (POPs) from industrial wastewater [15-18]. The relative cost of combining biological oxidation with electrochemical oxidation technology is advantageous. Therefore, many researchers have studied biodegradable, inexpensive substitutes made from natural resources for treating industrial wastewater [19-22].

Moreover, many studies have noted that the main habitat of sulfur-oxidizing bacteria can be isolated from Acid Mine Drainage (AMS) [23, 24]. It has been observed that research using bacterial consortia is important for the further expansion of bioleaching techniques in the recovery of metals [25]. However, some drawbacks, such as the sensitivity of bacterial cell walls to high pulp density and lesser tolerance of cells towards metals and bacterial

communities have restricted its upscale application. But some studies are being carried out to eliminate the shortcomings of the bioleaching process[26].

From the viewpoint of electrochemical measurement, there are many methods of determining metals in dissolution studies (electrochemical methods, atomic absorption spectrometry, inductively coupled atomic emission spectrometry, plasma excitation, X-ray fluorescence, optical sensing, etc.) The electrochemical analysis, is the most significant field analysis method due to its small equipment size, ease of installation, and capabilities of multi-element detection. Some research work on the use of Screen-Printed (SP) electrodes in electrochemical analysis has been publisheds and describes the voltammetric behavior of various Screen-Printed Carbon (SPC) electrodes. Electroanalytical methods offer low cost, short analysis time, and miniaturization and automation potential that allow the development of highly sensitive methodologies. Simple portable devices of electroanalytical methods allow fast screening and in-field / on-site monitoring. Screen-printed electrodes are a consolidated tool in this field, with a demonstration of applicability in the environmental analysis[27]. Many researchers have used SPC electrodes in their studies. Its advantage lies in the ability to study the oxidation of substances from a small amount of solution[28]. Cyclic voltammetry measurements were carried out by portable electroanalytical equipment. The three-electrode screen-printed platforms (SPCE DS114, working and auxiliary electrodes made of carbon, pseudo reference electrode made of silver) were placed in the bottom of a Bipotentiostat/Galvanostat μStat 400 (DropSens) Teflon conic cell, specially designed to perform analysis on up to 2 mL solutions[29]. In this study, we used electrochemical methods in combination with ICP-OES and spectrophotometric analysis methods to control the leaching of metals from copper concentrates into solutions by microorganisms.

This study aims to isolate iron and sulfur-oxidizing indigenious microorganisms, preferably *Acidithiobacillus* species, from acid mine drainage in Mongolian mining sites and investigate the bioleaching efficiency of the isolates with the future intention of industrial application for processing low-grade ore using the microorganisms.

Here we have isolated and identified strains of iron and sulfur-oxidizing bacteria from acid mine drainage from the heap leach solution. Also, the biodegradable activity of isolated bacterial strains of copper concentrates through the sulfuric acid leaching process was investigated by electrochemical methods.

EXPERIMENTAL SECTION

Culture media

Here we used three types of culture mediums for bacterial enrichment and isolation of pure culture. The same culture media were used for the growth of microorganisms during bioleaching. These are NCIMB medium no.18 (0.4 g/L $(NH_4)_2SO_4$, 0.4 g/L K_2HPO_4, 0.4 g/L $MgSO_4 \cdot 7H_2O$, 33.4 g/L $FeSO_4 \cdot 7H_2O$, pH=3.0) , 9K basal medium (3.0 g/L $(NH4)_2SO_4$, 0.1 g/L KCl, 0.5 g/L K_2HPO_4, 0.5 g/L $MgSO_4 \cdot 7H_2O$, 0.01 g/L $Ca(NO_3)_2$, 44.22 g/L $FeSO_4 \cdot 7H_2O$, pH=2.0) and 9K+C (9K basal medium with 2g/L glucose and 3 g/L peptone as organic carbon source, pH=2.0). For each medium, $FeSO_4 \cdot 7H_2O$ solution was prepared separately and filter-sterilized before adding to autoclaved (121^0C, 15 min) solution containing other salts, and pH was adjusted with H_2SO_4 (pH=3.0 for NCIMB medium no.18, pH=2.0 for 9K or 9K+C medium). Solid media was prepared by adding 2% agar to salt solution before autoclaving.

Bacterial enrichment and strain isolation

A sample of pregnant solution from acid mine drainage of heap leach solution was obtained to isolate and identify iron and sulfur-oxidizing bacteria. Bacterial enrichment was carried out in NCIMB medium no.18, 9K, and 9K+C. For bacterial enrichment, the initial culture was made by adding 10 mL of the pregnant solution into a 250 mL Erlenmeyer flask containing 100 mL of culture medium and incubating in a temperature-controlled water bath shaker at 30^0C and 110 rpm for three days. Twice, the culture was enriched by transferring 10 mL to a 100 mL fresh culture medium after every three days of incubation at 30^0C at 110 rpm. After nine days of total incubation for bacterial enrichment, 400 μL of culture was withdrawn (200 μL for the case of 9K with organic carbon source) from each media for enrichment culture and spread on respective agar medium (2% agar, pH=3.0 for NCIMB medium no.18, pH=2.0 for 9K or 9K+C medium) and incubated at 30^0C for seven days. After seven days of incubation, distinctive single colonies grown on the agar media were picked and streaked on fresh agar medium for single colony isolation and incubated at 20^0C for seven days.

Table 1: Elemental composition of the copper concentrate.

Element	Cu	S	Fe	Au	Ag	As	Al	Cd	Pb	Mo
Content [%]	23.07	34.07	30.08	0.003	0.005	0.039	1.43	<0.001	0.033	0.31

Strain characterization and identification

Morphological properties: Colony morphology was examined by stereomicroscope after pure colony isolation. Cell morphology was examined microscopically. Using ocular and stage micrometers, cell size was measured. Cells were stained by Gram stain to determine as either Gram-positive or Gram-negative.

DNA isolation, PCR, sequencing, and sequence alignment: Bacterial DNA was isolated from each strain by standard methods after growing bacteria in a liquid medium for 2-7 days [30][31]. All amplicons were produced by PCR using polymerase (polymerase enzyme name, company) and amplification parameters recommended by the manufacturer. PCR primers 27F-AGA GTT TGA TCM TGG CTC AG, 1492R-TAC GGY TAC CTT GTT ACG ACT T were used to amplify 1465 bp long 16S rRNA gene sequence [32-34]. The PCR amplification was carried out as follows: 50 ng of genomic DNA, 2.0 mM $MgCl_2$, 0.5 mM dNTPs, 0.25 μM each of sense and antisense primers, 2.5 μL of 10X reaction buffer, and 1 U of Taq DNA polymerase (Takara, Dalian, China) in a total volume of 25 μL. The PCR program consisted of one cycle of DNA denaturation for 3 min at 95^0C. Then 30 cycles were performed using the following parameters: 45 s at 94^0C to denature, 45 s at 55^0C to anneal, and 120 s at 72^0C to extend, followed by a final extension of 10 min at 72^0C. Finally, the PCR product was purified and submitted for sequencing. The nucleotide sequences of 16S rRNA for the bacterial isolates were searched for homology by BlastN at the NCBI server(http://www.ncbi.nlm.nih.gov/BLAST/Blast) and submitted to Genbank. Bacterial identities were confirmed based on their phenotypic characteristics and 16S rDNA sequences.

Composition of copper concentrate

The sample of copper concentrate used in the bioleaching experiment originated from Mongolia. The mineral sample was crushed and sieved, with diameters of the particles less than 75 μm, which was applied to the bioleaching experiment. X-ray diffraction (XRD X'PERT PRO, PANalytical, Netherlands) indicated that ore comprised 70.62% chalcopyrite, 14.2% pyrite, 1.32% bornite, 11.8% quartz, and 2.06% others. The elemental composition was analyzed using ICP-OES (iCAP 6300, Thermo Scientific) and ICP-MS (XSeries 2, Thermo Scientific) after the dissolution of the mineral in acid digestion. Besides minor elements, it contained 23.07% copper and 30.08% iron (Table 1). The sulfur content was 34.07% analyzed by gravimetry (combustion).

Analysis of main physicochemical properties in solution

The physicochemical parameters were measured every three days. The pH value was measured by a digital pH meter (PHS-3BW, Biobase Biodustry, Shandong China). The oxidation-redox potential (ORP) was monitored by a platinum electrode (213–01, Biobase Biodustry, Shandong China) using an Ag/AgCl reference electrode (218, Biobase Biodustry, Shandong China). The concentrations of copper, total iron, and ferrous iron were measured by ICP-OES and 1,10-phenanthroline spectrophotometric method, respectively [35-37]. Ferric iron concentration was calculated by subtracting ferrous iron concentration from total iron concentration.

pH and redox potential measurements and determination of ferrous iron: pH and redox potential were measured in a fresh medium before the addition of pregnant leach solution and before every culture transfer using a pH meter. Ferrous iron content in the culture medium was determined by the same method used in the bioleaching experiment described in the next section.

Bacterial preparation for bioleaching: Preculture inoculum (10 % v/v) was added to a 250 mL flask containing 45 mL of 9K medium at an initial pH of 2.8. The flask was incubated in a rotary shaker at 30^0C and 200 rpm. The cells were harvested after they had reached the exponential phase (2-7 days), and the culture was filtered through filter paper (Whatman No. 1) to eliminate iron precipitation and other solid particles. Next, the culture was centrifuged at 5000 rpm for 10 minutes to separate the cells and washed three times with the same medium (without ferrous ions) used for growth. The cells were then diluted to a known concentration with the medium and used for subsequent experiments. Cell number was determined by the Neubauer chamber and confirmed by spectrometry.

Experimental procedure for bioleaching: Bioleaching experiments were carried out with newly isolated strains using a copper concentrate on a rotary

Table 2: Single colony characteristics on NCIMB, 9K, and 9K+C

Solid medium	Colony shape, margin, surface	Colony Size	Colony color
9K+C	round, uniform, elevated	2-3 мм	Orange
	round, wavy, wrinkled	2-3 мм	Pale orange
9K	Growth along the streak changing media color to yellow		yellow
NCIMB medium no.18	round, uniform, elevated	1-2 мм	Pale orange

shaker at 110 rpm for 30 days with 5.0% (w/v) pulp density. Each strain with an initial cell density of 1.0×10^5 cells/mL and an abiotic control were set for bioleaching. At every three days of incubation, aliquots were taken to determine copper, total iron, and ferrous iron concentration. Also, at 3-day intervals, aliquots were taken from the bioleaching solution to monitor pH, redox potential, and electrochemical measurements.

Electrochemical analysis: Bioleaching of copper concentrate was carried out in 3 different media (NCIMB, 9K, 9K + C) and strain ER-1c. Electrochemical studies gave the best results with cultures in a 9K nutrient medium. Electrochemical behaviors of bioleaching processes were studied by recording voltammetric curves using cyclic voltammetry with three-electrode integrated screen-printed carbon electrode SPCE DS114 (DropSens, Spain). Here we used a modified carbon electrode as the working electrode, Ag/AgCl electrode as the reference electrode, and a carbon electrode as the counter electrode. The SPC electrode is suitable for handling droplets of micro volumes from solution and ideal for decentralized analysis and developing specific biosensors. The SPC electrode was rinsed with 0.6 M H_2SO_4 and 0.1 M NaOH solution, washed with distilled water and ethanol several times, and dried. Electrochemical measurements were carried out on Dropsens μStat 400 potentiostat/galvanostat and controlled by DropView software on the computer. Cyclic voltammetric (CV) experiments were performed in the potential range from -1 to 1 V, with a scan rate of 20 mV/s, and a sterile 9K medium as an electrolyte (pH=2.0, adjusted by H_2SO_4). All experiments were carried out at room temperature (22 ± 2^0C). All the bacterial cultures were sub-cultured into basal salts medium supplemented with 5g of copper concentrate in 100ml solution. During leaching, drops of samples were withdrawn at three-day intervals from the leaching system and applied on the SPC electrode to cover the three-electrode area for monitoring oxidation reaction by CV.

RESULTS AND DISCUSSION

Bacterial enrichment and strain isolation

Iron and sulfur-oxidizing bacterial isolates were obtained by culturing a pregnant leach solution from a copper mining heap. After nine days of culturing for bacterial enrichment, the medium color turned from colorless to orange with a red tint for NCIMB medium no.18 and 9K. In contrast, the 9K+C culture medium color changed to beige. Measuring ferrous iron concentration in each enrichment culture showed a decrease in ferrous iron level after nine days of incubation, indicating the possible oxidation of ferrous iron to ferric iron with the help of enriched bacteria. 400 µl (200 µl in the case of 9K+C) culture was spread onto a solid medium and incubated at 28^0C for seven days. After seven days of incubation, several colonies appeared on the solid media, and the characteristics of the colonies were recorded (Table 2).

Identification of bacterial isolates

Three bacterial isolates obtained from acid mine drainage of open-pit tailing were identified based on their 16S rRNA gene sequence. Using the genomic DNA extracted from each bacterial isolate as template DNA, about 1500 bp long fragments were amplified using a pair of universal PCR primers 27F/1492R. An intense single band is visible on 1% agarose gel stained with ethidium bromide Fig. 1. The sequencing has been done from the forward and reverses directions for the PCR product using the BDT V3.1 cycle sequencing kit on the ABI 3730XL genetic analyzer. Assembled sequences of ER-1a, ER-1c, and ER-1d were submitted to the NCBI gene bank with Genbank accession numbers MZ7689528, ON532894, and MZ723878, respectively. The percentage of 16S rRNA gene sequence match was analyzed for all closely related data using BLAST search. The result shows that ER-1a and ER-1c strains are closely related to Candidate division OP10 bacterium P488 (AM749768), with percent similarities of 78.21% and 93.39%, respectively. ER-1d is closely related to *Fimbriimonas ginsengisoli* Gsoil 348 (GQ339893)

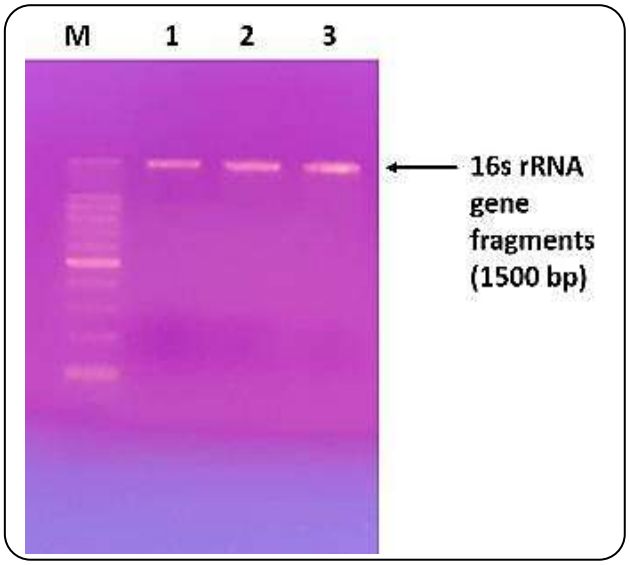

***Fig. 1: An image of 1% agarose gel indicating 16S rRNA gene amplicon* (1: strain ER-1a, 2: strain ER-1c, 3: strain ER-1d)**

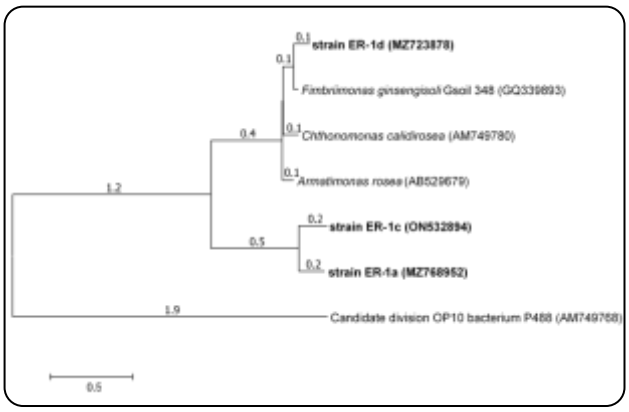

Fig. 2: Phylogenetic tree based on 16S rRNA gene sequences of close homologs of strain ER-1a, ER-1c, and ER-1d. Neighbor-joining cladogram constructed in MEGA6 [27]; alignment of the sequences was done with CLUSTALW [28], and the scale bar represents 0.5% sequence divergence

with percent similarity of 78.78%. Candidate division OP10 bacterium P488 and *Fimbriimonas ginsengisoli* Gsoil 348 are species belonging to the phylum *Armatimonadetes*. A phylogenetic tree was constructed using the four cultivated representatives of the phylum Armatimonadates and our isolates ER-1a, ER-1c, and ER-1d (Fig. 2). Armatimonadetes is a moderately abundant and phylogenetically diverse bacterial phylum previously known as candidate phylum OP10. Environmental bacterial isolates from candidate phylum OP10 are reported to be present in methylmercury contaminated mine tailings, biological heap leaching, and iron-dominated flocculent mats in deep-sea hydrothermal environments [38-40]. However, their exact role in a biological heap leaching or metabolic process in their living environments, such as mine tailings or hydrothermal environments, is unknown. Studies on their involvement in iron and sulfur oxidation and their role in the metal leaching process are scarce. Thus, further analysis must be done to clarify the physiological and biochemical characteristics of the acid mine drainage isolates obtained in this study.

Variation of leaching procedure

The main bioleaching parameters using strain ER-1c in a 9K medium containing copper concentrate are shown in Fig. 3. The final concentration of extracted copper was 615 mg/L and 53.37%.

The highest copper extraction rate was obtained from the 28th to the 58th day, and in this stage, the cell density kept the maximum value (5.3 × 108 cells/mL). The ferric iron concentration continuously increased to 1.66 g/L before the 70th day. It has been reported that ferric ions are the primary oxidants during the bioleaching of chalcopyrite, and the high ferric iron concentration would benefit copper extraction. However, the ferrous iron concentration is always kept at a low value. These may be due to two reasons: once ferrous iron was leached out from chalcopyrite, it would be oxidized quickly by the bioleaching microorganism.

The pH value and ORP results are shown in Fig.3 (c–d). pH firstly increased before 12 days and then decreased quickly to 2.20 on the final day. The pH increase at the beginning of bioleaching was mainly due to proton consumption in the bioleaching solution. In contrast, the decrease in pH value originated from the oxidation of elemental sulfur to sulfuric acid. An increase in ORP was observed with an increase in Fe^{3+} concentration and a reduction in pH value. During the bioleaching, ORP augmented from 235 mV to 386 mV and then kept stable. It is reported that the variation of ORP is related to the Fe^{3+}, Fe^{2+}, and H^+ concentrations in the solution [41]. Therefore, the increase in ORP is mainly due to the rise in the $[Fe^{3+}]/[Fe^{2+}]$ ratio and decrease in pH value.

Electrochemical behavior of bioleaching at SPEs

The electrochemical behavior of a copper concentrate bioleaching using strain ER-1c in 9K medium was studied by cyclic voltammetry for 18 days at three-day intervals, and the resulting cyclic voltammograms were analyzed.

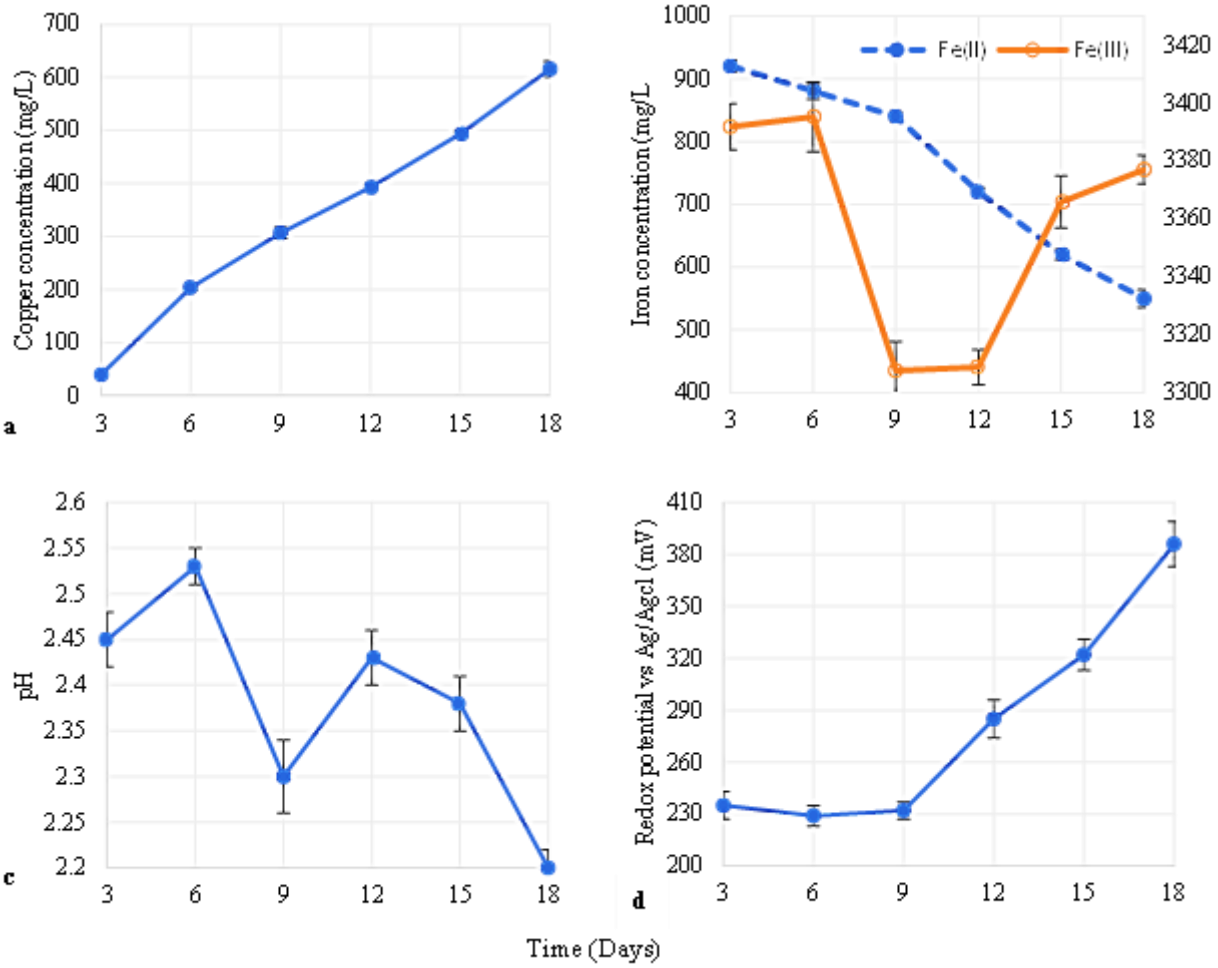

Fig. 3: Variation of physicochemical parameters in leaching solution the bioleaching process. (a) Copper concentration; (b) Iron concentration; (c) pH; (d) Redox potential

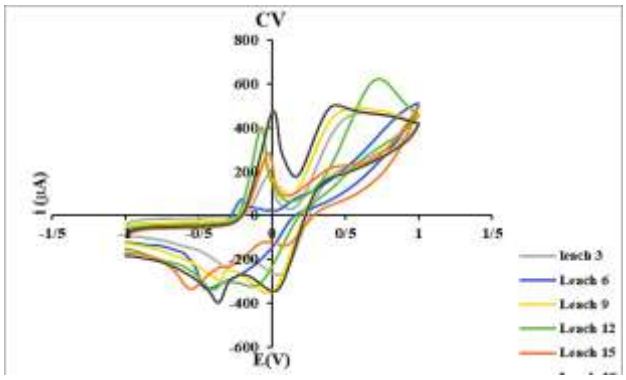

Fig. 4: Cyclic voltammograms of copper concentration in 9K medium bioleaching from 3 to 18 days. Sweep rate=20mv/s; Reference electrode: Ag/AgCl electrode

As can be seen, well-defined oxidation peaks of cyclic voltammetry were observed, which improved over the days (Fig. 4). In the cyclic voltammogram, A1, A2, and A3 peaks were considered important in the bioleaching of the copper concentrate. Thus, we further illustrated the change in current at these peaks in Fig. 5 and the change in oxidation potential in Fig. 6. Oxidation behavior can be observed by the current peak in the cyclic voltammogram [42-44]. As seen in Fig. 5 , the current peak intensity gradually increased until day 15 and then decreased on day 18 during the bioleaching experiment, which shows that oxidation is saturated from day 15. Oxidation potential of A1, A2 and A3 peaks range between -0,072 V and 0,044 V (vs.Ag/AgCl), 0,29-0,43V (vs.Ag/AgCl), 0,62-0,98 V (vs.Ag/AgCl); respectively (Fig. 6).

Three distinct anodic peaks were observed in the anodic scan from -1 V, including peak A1, peak A2, and peak A3. In addition, two different cathodic peaks C1 and C2,

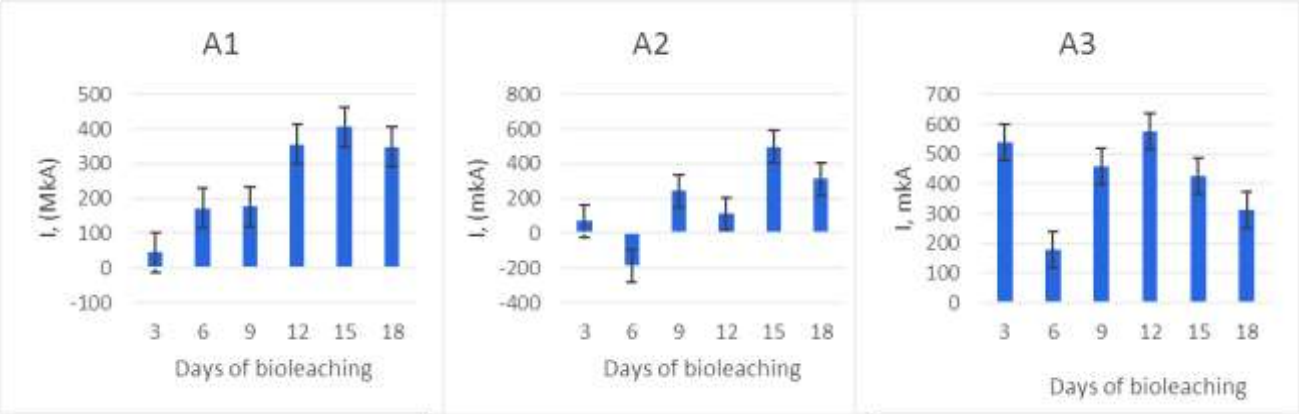

Fig. 5: CV oxidation currents in (A1), (A2), and (A3) peaks of copper concentrate during 3-18 days of bioleaching using SPC electrode

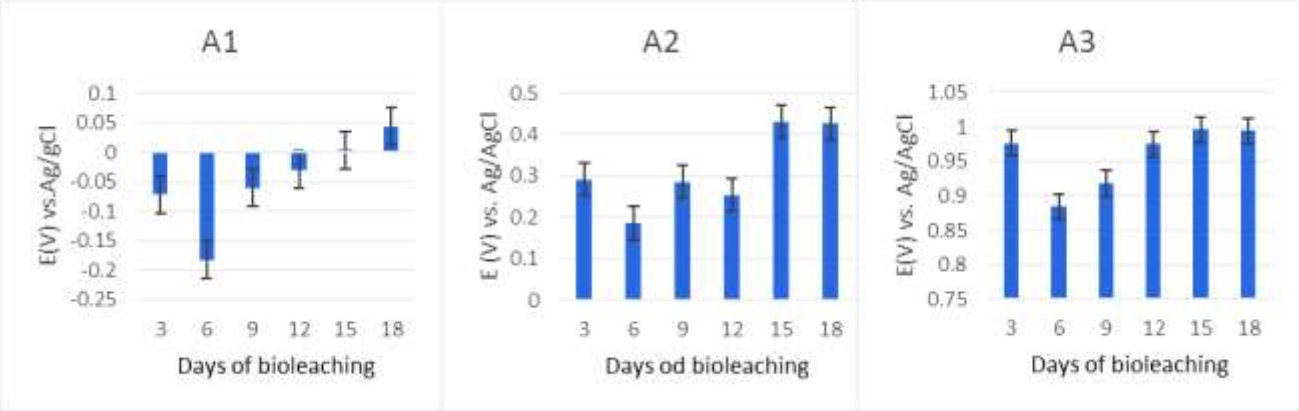

Fig. 6: CV oxidation potential vs. SHE (A1), (A2), and (A3) peaks of copper concentrate during 3-18 days of bioleaching using an SPC electrode

were observed. Possible reactions during the bio-oxidation of sulfide ore, occurring at the A1 peak at a potential from -0.07 to 0.044 V (vs. Ag /AgCl), the formation of chalcocite and covellite by oxidation of chalcopyrite and the oxidation of elemental copper to chalcocite occurs by the following reaction Equations (1-4). Based on the previous chemical reactions, peak A2, 0.3 to 0.5V (vs. Ag /AgCl), was considered as the oxidation of non-stoichiometric chalcocite and was related to the production of Cu_2S as shown in Equation (5-8). The peak (A3), when at potential values more than 0.6 V (vs. Ag/AgCl), represents the region of selective dissolution of elements from the crystal lattice of chalcopyrite to covellite, and covellite could be destroyed according to Equations (9-12), which multiple authors have reported [3,45-49].

$FeS_2+2H^++2e^-\rightarrow FeS+H_2S$ (1)

$2Cu+H_2S\rightarrow Cu_2S+2H^++2e^-$ (2)

$H_2S\rightarrow S^0+2H^++2e^-$ (3)

$CuFeS_2\rightarrow CuS+S^0+Fe^{2+}+2e^-$ (4)

$Cu_2S\rightarrow Cu_{1.92}S+0.08Cu^{2+}+0.16e^-$ (5)

$Cu_{1.92}S\rightarrow Cu_{1.6}S+0.32Cu^{2+}+0.64e^-$ (6)

$Cu_{1.6}S\rightarrow CuS+0.6Cu^{2+}+1.2e^-$ (7)

$CuFeS+3Cu^{2+}+3Fe^{2+}\rightarrow 2Cu_2S+4Fe^{3+}$ (8)

$6CuFeS_2\rightarrow 0.75Cu_2S+0.25Cu^{2+}+Fe^{2+}+1.25S^0+2.5e^-$ (9)

$CuFeS_2\rightarrow 2S^0+Cu^{2+}+Fe^{3+}+5e^-$ (10)

$CuFeS_2+8H_2O\rightarrow Cu^{2+}+SO_4^{2-}+16H^++17e^-$ (11)

$CuS\rightarrow S^0+Cu^{2+}+2e^-$ (12)

CONCLUSIONS

In this study we obtained three isolates from acid mine drainage and molecular identification by16S rRNA gene sequence homology search shows that strains ER-1a, ER-1c are closely related to Candidate division OP10 bacterium P488 (AM749768) and ER-1d is closely related to *Fimbriimonas ginsengisoli* Gsoil 348 (GQ339893). Bioleaching of copper concentrate by strain ER-1c was monitored by the electrochemical method. During 18 days of oxidation, only three types of oxidations were observed. The solubility of copper reached 615 mg/L, while 83.7%

of ferrous ions were converted to iron (III). The current peak intensity gradually increased until day 15 and then decreased at day 18 during the bioleaching experiment. The productivity of these cells per unit of time is 1.425 mg/L per hour.

The electrochemical process of biodegradation of sulfide ores by strain ER-1c in copper concentrate is determined according to the following scheme: $CuFeS_2 \rightarrow Cu_xS + S^0 \rightarrow Cu^{2+} + Fe^{3+} + SO_4^{2-}$.

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