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Original Research

Catalytic Efficiency of A*cidithiobacillus ferrooxidan*s for Bioleaching Copper from Chalcocite Containing Sulfide Ore from Reko Diq Deposits

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Abstract

Bioleaching of low-grade secondary copper sulphide ores using different microbial strains is an ecologically safe technology for the recovery of metals in the mineral and mining industry. The purpose of the present study was to analyze the mineral contents of Reko Diq deposits and to assess the dissolution of copper from sulfide ore by an indigenously isolated strain of acidophilic iron- and sulfur-oxidizing bacterium (BSTFe-2) in shake flask experiments. X-ray diffraction (XRD) analysis of the ore sample suggested that it contained 0.81% Cu on dry matter basis and contained chalcocite (Cu_2S) and covellite (CuS) as main copper minerals. Pyrite (FeS_2) was also present as a sulfide mineral. The other minerals detected in the ore matrix were muscovite (a di-octahedral mica mineral), quartz, feldspar (anorthite) and calcite. Quartz (SiO_2) was the main silicate mineral present in the sample. Calcite ($CaCO_3$) was found as the main acid-consuming gangue mineral. We observed that about 80-90% of the total Cu content present in the ore matrix was solubilized during 30 days of the leaching process mediated by *Acidithiobacillus ferrooxidans* at 30ºC. Copper dissolution from ore was found to be directly related to the reaction pH (1.5-1.9). The leaching data obtained from the pulp densities (5, 10 and 20% wt/vol) at

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30ºC are comparable. The results of the present study concluded that the bioleaching can be a suitable alternative for conventional hydrominerological processing of low-grade copper ores.

Keywords: bioleaching, copper sulfide ore, chalcocite, covalite, acidophilic iron- and sulfur-oxidizing bacterium (*Acidithiobacillus ferrooxidans*)

Introduction

The impurities in valuable minerals in various geological settings have been a serious concern both in terms of value and environmental impact [1]. Globally, low-grade copper ores containing high oxides and carbonate gangue represents a major proportion of available resources of copper. The extraction of minerals from parent rock materials has been quite challenging owing to the complexity of refractory ores and high concentrations of associated impurities [2, 3]. These factors subsequently compromise overall operational efficiency of the conventional recovery processes. Under the notion of sustainability, environmental regulations and economic considerations, conventional metal recovery processes are less desired today in the mineral and mining industry [4]. The microbiological-driven processing of copper ores, technically bioleaching, under versatile geological settings has been considered an efficient technology on account of its economic and environmental benefits. The ferrous iron and sulfur oxidizing microorganisms are the main players for the bioleaching process. These microorganisms release the metals from the ores by a series of biochemical reactions in which sulfide minerals are oxidized to produce acidic species at the first stage, followed by acid consumption, ferric ion generation and ferric ion consumption respectively [5]. Different acidophilic iron- and sulfur-oxidizing bacteria have been previously known for their potential for bioleaching, such as *Acidithiobacillus ferrooxidans*, *Acidithiobacillus caldus and Acidithiobacillus thiooxidans* [6]. However, the bioprocess based on *Acidithiobacillus ferrooxidans* has been practiced in varying experimental settings such as stirred tank leaching and heap and dump leaching due to the ability of the bacterium to use various ions as an energy source [7-9]. *A. ferrooxidans* is a versatile chemolithotroph mostly isolated from acid mine drainage sites. Besides the potential role of *A. ferrooxidans* in bioleaching operations, this bacteria is known to contribute effectively in metals and sulfur recycling in the environment [10].

The bioleaching process is strongly associated with various intrinsic factors, including mineral composition of the parent rocks. On the other side, bacterial specie, the availability of nutrients for microbial growth, pH, aeration, temperature and impurities affect mineral solubilization rates in a given bioleaching process [11, 12]. Among all aforesaid parameters, properties of minerals are of paramount importance. Wu et al. [8] reported preferential leaching rates of different sulfide minerals treated with bacteria. These materials include chalcopyrite, covellite, enargite, chalcocite and bornite. Besides a vast disparity in composition of rocks, biohydrometallurgy proved to be a high throughput approach and economically viable option as compared to the pyrometallurgic processing, allowing for robust extraction from low-grade and relatively complex copper ores that otherwise would be considered waste [11, 13].

The Reko Diq mines in a desert area of Changi District, Balochistan, are in close proximity to the Pakistani-Afghan-Iranian border. According to geological survey, Reko Diq represents one of the largest copper reserves in the world, having estimated deposits of 5.9 billion tons of ore-grade 0.41% copper [14]. Based on different exploration projects carried out between 1933-1997, 48 pockets have been identified for the extraction of copper and gold from the Reko Diq mine complex [15]. The extraction of minerals from rock materials is a laborious process involving a number of technical steps. As mentioned, the recovery rate obtained by conventional processing methods from Reko Diq geological settings is only 0.41%, producing 5.3pc of grade copper [16]. Bioleaching could be an alternative and economically more attractive approach for the extraction of valuable minerals from this site. It is likely that the application of microbial-enhanced extraction of copper could transform Reko Diq mineral resources into more profitable reserves. Various efforts have been made to evaluate different process parameters. Nevertheless, the microbiology of heap bioreactors remained unclear due to the complexity of microbial systems and their working efficiency under experimental conditions. The present research typically focused on analyzing the mineral composition of Reko Diq ores and isolating acidophilic iron and sulfur-oxidizing bacterium for application in bioleaching of low-grade copper ore.

Material and Methods

Collection of Copper Ore Samples

A Reko Diq copper ore sample (50 Kg) was received from the Liaison Office, Baluchistan Copper Gold Project (BCGP), Islamabad, for the present studies. The sample was mixed thoroughly with repeated coning and quartering techniques and at last a representative copper ore sample of about 1 kg was prepared with the help of a laboratory sample divider. The representative ore sample was ground to fine powder to pass through a 200-mesh

particle size test sieve (<74 μm) by using a vibrating cup mill for mineralogical and chemical analyses and present bacterial leaching studies.

X-ray Diffraction Analysis of Reko Diq Ore Sample

X-ray diffraction (XRD) techniques were carried out for mineralogical analysis of the copper ore sample. XRD analysis of the top fill fine particles mount was done by using CuKα-radiation (1.54056 Å) and a vertical broad range goniometer equipped with diffracted ray monochromator. The black shale specimen was scanned from 3˚2Θ to 70˚2Θ in increments of 0.02˚ by 0.5-seconds step time. The matching of X-ray diffractogram was carried out in a JCPDS powder diffraction library in an automatic mode, and for conformation it was checked manually against the automated identification of phases.

Preparing Culture Media

For the isolation of required chemolithotroph from the acid water samples, a previously reported 9K basal salt media was used with the following composition of salts (g/L): $(NH_4)_2SO_4$ 3 g, KCl 0.1 g, K_2HPO_4 0.5 g, $MgSO_4.7H_2O$ 0.5 g, and $Ca(NO_3)_2$ 0.01 g. The pH of the medium was adjusted to 2.0 using 5 M H_2SO_4. After sterilization, the media was supplemented with 44.7 g/L sterilized ferrous iron (9K-Fe) and 19 g/L heat-sterilized sulfur to make 9K–S media. These culture media were used for enrichment and submerged fermentation [17].

The 9K Gelrite-Fe solid medium was used for the isolation and enumeration of acidophilic iron and sulfur oxidation. Briefly, 4.0 g of Gelrite was soaked in 300 mL distilled water for 20 minutes and then autoclaved and mixed with the previously mentioned 9K-Fe and 9K-S media.

Enrichment and Isolation of Iron- and Sulfate-Oxidizing Bacteria

The acidic bed-rock seepage water samples from different sites of black shale deposit of Diameer District, KPK were collected in pre-sterilized bottles. The isolation of *A. ferrooxidans* was carried out by taking 1-liter of bedrock seepage water filtrate. After filtration, the membrane filter was positioned in a conical flask containing 20-ml sterilized water of pH 2.5 and incubated in a shaking incubator at 30ºC for 1 hr to liberate the bacterial cells attached on a membrane filter. From this, 0.1-mL of the suspension was spread onto 9K Gelrite $FeSO_4$ solid medium incubated for 2 weeks to get bacterial growth. A single colony was packed into 50 mL 9K-Fe liquid medium and incubated till the medium color changed to brick red, indicating oxidation of ferrous iron. From this, 0.1 mL culture broth was re-spread onto Gelrite 9K-Fe solid medium to obtain the pure culture. The pure isolate was obtained after five to six sequential re-spreadings and named strain BSTFe-2.

Morphology and Physiology of the Strain BSTFe-2

The cells of bacterial strain having the ability of iron-oxidation were observed under a phase contrast microscope to examine cell size, shape and motility. The growth pattern of the bacteria was determined in 9K-Fe liquid medium under the effect of varying pH levels and temperatures. For evaluating optimum pH, 1 mL of the growing culture of the strain BSTFe-2 was inoculated into 100 mL flasks containing 9K-Fe broth and incubated at 30ºC in an orbital shaker and standard inoculums with approx. 10^9 cells per ml used in all experiments. Whereas for determining optimum growth temperature, aforesaid conditions were set at different temperatures (between 15-50ºC). The growth of bacteria (direct cell count) was monitored using a Petroff–Hausser chamber at different time intervals during 6-8 days of the incubation period. The pH of the fermentation broth during the growth of bacteria was monitored using a digital pH meter. Titration method as described in [18] using $K_2Cr_2O_7$ with a diphenylamine as the indicator was employed to determined iron concentration. The concentration of sulfate in 9K-S medium was calculated by barium sulfate turbidimetry [19].

Shake Flask Bioleaching Studies of Copper Ore

Shake flask leaching experimentation was done in a 250-ml Erlenmeyer flask (150 rev/ min) with copper ore in 9K mineral salt solution. The starting pH 2.5 of leach slurry was maintained with 5M H_2SO_4 or NaOH solution. The inoculated flasks were made with 5-ml inoculums containing about 10^9 cells/ml of locally isolated strain of acidophilic iron- and sulfur-oxidizing bacterium. In chemical (sterile) controls, thymol (0.08% wt/vol final concentration) dissolved in ethanol were included in each experiment. The effect of acid concentration, pulp density and leaching time on Cu solubilization from ore were studied in shake flask leaching experiments. Periodically, leach liquor samples were aseptically taken for monitoring pH, redox potential (Eh) and the analysis soluble copper in leach solutions. Copper and associated metals in ore and leach solution were analyzed by standard atomic absorption spectrometry (Varian Model SpectrAA 30/40AAS) as well as induced coupled plasma optical emission spectrometry (iCAP 6500 Thermo Fisher Scientific, USA).

Results and Discussion

Analysis of Reko Diq Copper Ore

In order to characterize the different proportion of each mineral phase in the copper ore sample, X-ray diffraction (XRD) analysis of top fill mixture mount was performed. The minerals identified were quartz,

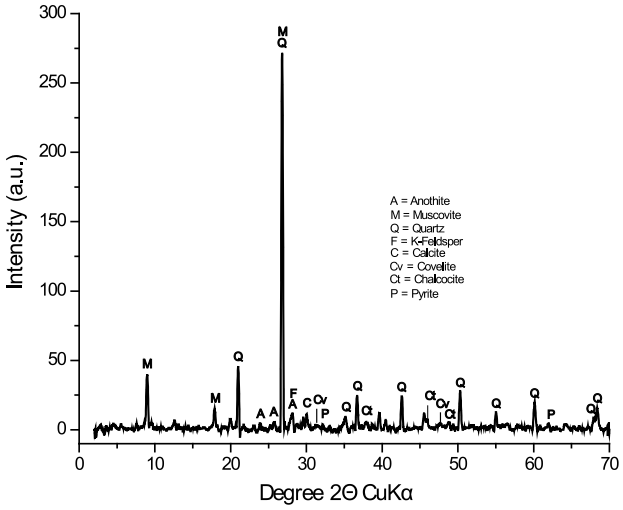

Fig. 1. XRD diffractogram of Reko Diq copper ore sample with symbol designation: A = anorthite, C = calcite, Cv = covellite, Ct = chalcocite, F = K-feldspar, M = Muscovite, P = pyrite, Q = quartz.

muscovite (a dioctahedral mica-mineral), anorthite (Ca-feldspar), microcline (K-feldspar), calcite, pyrite, covellite and chalcocite. XRD diffractogram of the copper ore sample is shown in Fig. 1.

Quartz (SiO_2) was the major mineral existing in the copper ore sample. Quartz, muscovite, microcline, and anorthite were the main silicate-phases present in the ore sample. Covellite (CuS) and chalcocite (Cu_2S) were the main copper minerals identified in the ore matrix. Both these Cu-sulfide minerals are known as secondary copper sulfide minerals in nature. Pyrite (FeS_2) was also identified as an iron sulfide mineral in the ore-matrix by the XRD analysis. Calcite ($CaCO_3$) was present as a main acid-consuming gangue mineral. X-ray diffraction provide a comprehensive analysis of the minerals and have been employed extensively for compositional analysis of different copper ores [20, 21].

The chemical analysis of the copper ore sample is presented in Table 1.

The ore sample contained 1.02% CuO (0.81% Cu) on a dry matter basis. The ore sample also contained 58.72% SiO_2, 17.71% Al_2O_3 and 6.72% Fe_2O_3 as major constituents present in the ore matrix. In addition, alkali and alkaline earth metals like Na, K, Ca, Ba and Mg with some toxic metals (Cr and Sn) were also present in the ore sample. Manganese (Mn) and phosphorus (P) contents of the ore were found to be 0.041% $MnO_2$and 1.798% P_2O_5. The sulfur (S) content of 3.195% analyzed in the ore sample was the main constituent of covellite (CuS), chalcocite (Cu_2S) and pyrite (FeS_2) present in the ore sample. The disparity in the chemical composition of various ore samples is typically associated with the geographic location of the reservoir and pattern of rock formation [22, 23].

Table 1. Chemical analysis of Reko Diq copper ore sample.

Constituents	Results (%)
SiO_2	58.70
Al_2O_3	17.71
Fe_2O_3	6.72
CaO	1.08
CuO	1.015 (0.81% Cu)
MgO	0.913
P_2O_5	1.798
MnO_2	0.041
Cr_2O_3	0.021
Na_2O	0.295
K_2O	1.419
BaO	0.025
SnO_2	0.054
S	3.195
TiO_2	0.370
V_2O_5	0.012
ZnO	0.011
Loss on ignition at 1000°C	5.41

Isolation of the Bacterial Strain

The isolation of iron and sulfate-oxidizing bacterial strain was carried out from acid water samples. After enrichment in 9K-Fe liquid medium, bacterial colonies were obtained on plates containing Gelrite 9K-Fe solid media. Reddish-brown colonies of iron-oxidizing bacteria appeared on the agar plates after one-week incubation at 30ºC (Fig. 2). Each single colony was picked and cultivated in liquid iron medium (9KFe^{2+})

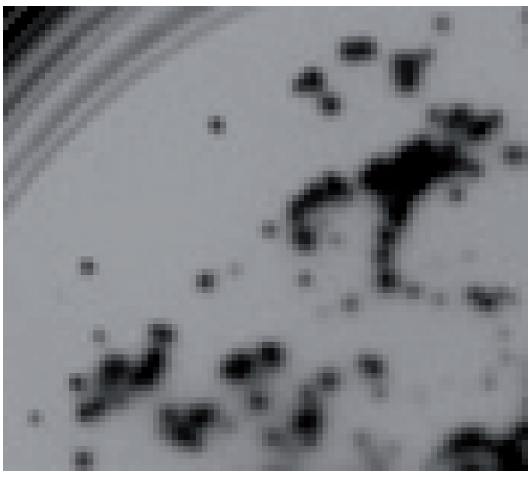

Fig. 2. Deep brown colonies of enriched culture of strain BSTF-2 on solid Gelrite K9-$FeSO_4$ medium.

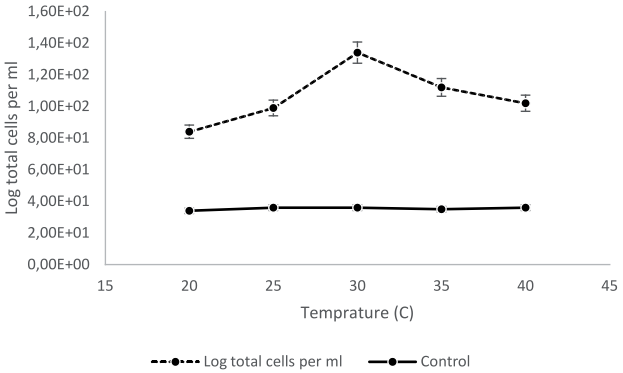

Fig. 3. Optimization of temperature for strain BSTF-2.

of pH 2.0. Under continuous shaking conditions, the cultures were grown for 5 days at 30ºC and the medium turned reddish-brown after 4 weeks of incubation, indicating the biochemical transformation of Fe^{2+} to Fe^{3+}. The harvested cells of iron-oxidizing bacteria were inoculated onto solid Gelrite-$FeSO_4$ plates. In modified Gelrite 9K-Fe- solid medium, gellan gum was used instead of agar for solidification and supporting growth of bacterial strains. It has been reported frequently in the literature that the chemoautotrophs show considerable sensitivity to organic compounds, including polysaccharide in agar. Moreover, galactose found in agar has an inhibitory effect on the growth of *A. ferrooxidans* [24]. The addition of gelrite instead of agar was quite promising owing to its ability to form clear and rigid gels in the presence of cations, compatibility with various nutrients and thermal stability [25, 26]. Colonies of enriched isolated strain of acidophilic iron-oxidizing bacterium on Gelrite-$FeSO_4$ plates were minute (0.2-0.7 mm), circular, and entire. The phase-contrast microscopic observations of isolated strains of acidophilic iron- and sulfur-oxidizer resembling *Acidithiobacillus ferrooxidans* (designated as BSTF-2) was motile, single rod-shaped and Gram-negative bacterium (Fig. 2).

The isolated strain (BSTF-2) oxidized Fe^{2+} to Fe^{3+}, pyrite, sulfur and reduced sulfur-compounds like sodium thiosulfate ($Na_2S_2O_3$) and potassium tetrathionate

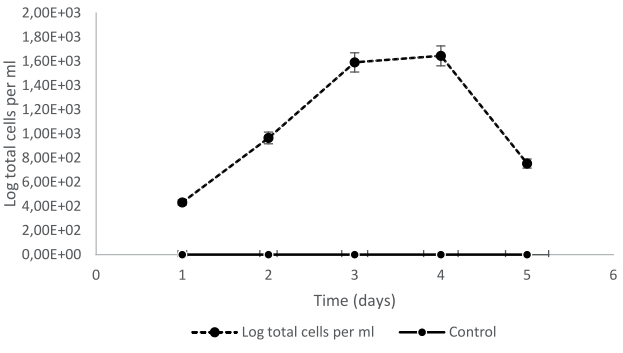

Fig. 4. Optimization of incubation time for strain BSTF-2.

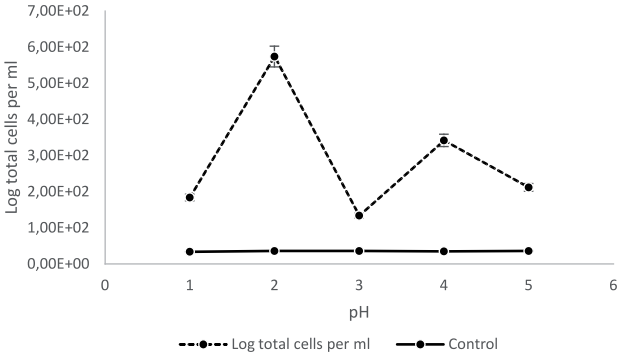

Fig. 5. pH Optimization for strain BSTF-2.

($K_2S_4O_6$). Similar results have been previously reported by Zhang et al. [9, 27].

Optimum Growth Conditions for Strain BSTF-2

Although the strain BSTF-2 isolated from the acid water was found to be mesophilic in nature, it showed the ability to survive between 20 to 40ºC with an optimum growth temperature of 30ºC (Fig. 3).

Maximum growth was observed between the 3rd and 4th days of incubation (Fig. 4).

The optimum pH for the growth of strain BSTF-2 was found to be pH 2 (Fig. 5). After this pH a sharp decline in the growth of *A. ferrooxidans* was observed. However, interestingly, at pH 4, a mild increase in the bacterial growth was observed.

It is possible that between pH 2 and 4, the decrease in the growth (pH 3) was due to some intermediate compounds generated because of mineral decomposition.

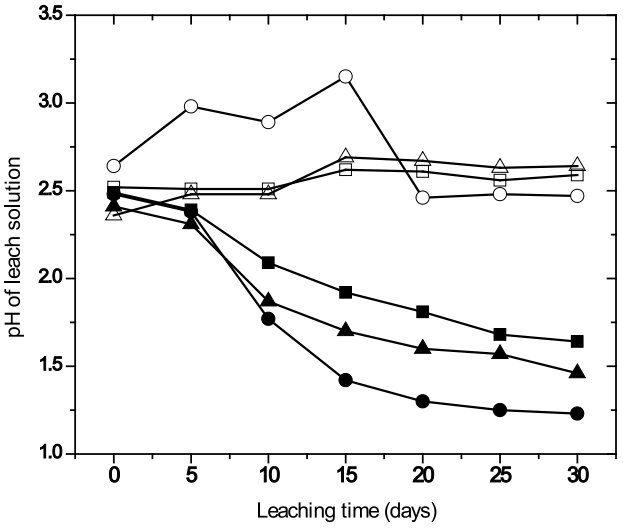

Fig. 6. a) pH and b) redox potential profiles of leach solutions obtained from various pulp densities of copper ore with isolated strain of acidophilic iron- and sulfur-oxidizing bacteria; symbol designations: □= control 5% (w/v), ■ = inoculated 5% (w/v), △= control 10% (w/v), ▲= inoculated 10% (w/v), ○ = control 20% (w/v) and ● = inoculated 20% (w/v).

At further increase in pH, these intermediate compounds could have been converted into less toxic species and favored the growth of *A. ferrooxidans* [28]. We previously reported that the optimum temperature for the growth of *A. ferrooxidans* was 30ºC and at pH 2 the bacterium showed maximum growth and biochemical activities [11].

Bioleaching of Copper Ore

Shake flasks leaching experiments were carried out to investigate the possibility of copper bioleaching from Reko Diq ore using an isolated strain of acidophilic iron-oxidizing bacterium (BSTFe-2). In the leaching process, 9K mineral salts medium of initial pH 2.5 containing copper ore (1%wt/ vol. ore pulp density) was investigated at 30˚C under shaking conditions (Fig. 6).

During the leaching process, a drastic change in the initial pH and redox potential (Eh) of leach solutions was observed. According to some previous reports [29, 30] the drop in pH could be due to the production of sulfuric acid in the medium as a result of bacterial oxidation of pyrite. After 30 days of leaching, about 95% of Cu was solubilized from the ore using *Acidithiobacillus ferrooxidans* strain BSTFe-2, which is comparatively higher than those reported earlier [31, 32]. Previously, 50 to 90% leaching efficiencies have been reported using iron- and sulfur-oxidizing bacteria. The differences in results are largely attributed to the quality and composition of the refectory ores [1, 33, 34]. Nonetheless, it is generally believed that the acidic environment (i.e., low pH 1.2 to 1.5 and high redox potential of 651 mV to 705 mV) facilitates copper dissolution from the chalcocite mineral [35]. The oxidative leaching process is sensitive to redox potential, and higher copper dissolution rate has been achieved at redox potential of 450-650 mV [36]. The present study is in line with aforesaid results that the bioleaching of copper is a function of acidic pH and redox potential of the medium.

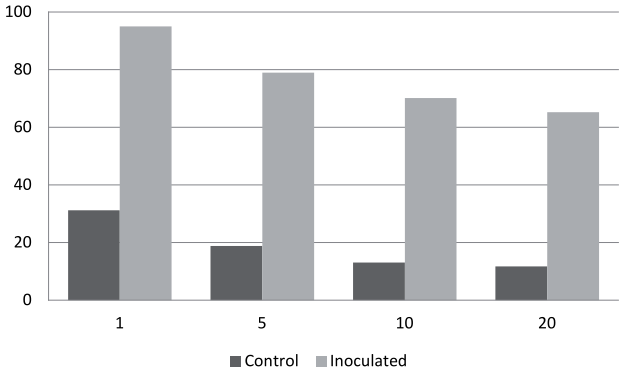

Fig. 7. Copper solubilized from ore at various pulp densities by isolated strain of acidophilic iron- and sulfur-oxidizing bacteria during 30 days of leaching time.

Effect of Ore Pulp Density on Copper Bioleaching from Ore

To investigate the role of pulp density (solid-to-liquid ratio) on the bioleaching process, shake flasks leaching experiments were performed with 5, 10 and 20% pulp densities (wt/vol). The results indicated that there was a marked change in the medium pH from 2.5 to 1.1, indicating biochemical activity of inoculated bacteria (Fig. 7).

Possibly, this drop in pH in medium was due to the biological generation of H_2SO_4 by the bacterial oxidation of pyrite; it was also observed that there was a drop in pH of the media by increasing pulp density (solid to liquid ratio)

At pulp densities of 5, 10 and 20% (w/v), the pH of the leach suspensions was 1.64, pH 1.46 and pH 1.23, respectively, after 30 days of incubation (Fig. 8). It could be suggested that sulfuric acid was biologically generated at a high concentration in the media as the solid to liquid increased.

This could be a logical reason for improved contents of pyrite in the media leading to increased pulp density during the bioleaching process. The interpretation of the data suggested that with an increase in ore pulp density, there was a decrease in Cu solubilization from copper ore. It has been reported that for gaining higher yield of copper from the ores, pulp density must be optimized [2, 37].

Conclusions

The main objective of the present study was to "eco-efficient" exploitation of the Reko Diq copper ore deposit for the extraction of copper. A special emphasis focused on the bioleaching of copper ore with isolated strain of acidophilic iron- and sulfur-oxidizing bacteria on dissolution of Cu during shake flasks studies. Bioleaching tests have shown that the copper ore was amenable to the leaching process regarding pyrite oxidation. The results of this study are very encouraging and can be useful for scientists, process engineers, geologists, and technical managers.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

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