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Evaluation of copper extraction potential of *Pseudomonas aeruginosa* from low-grade copper ore

Awais Iqbal[1*], Khansa Ahsan[2], Sumera Afzal[3], Zhang-Huan Shang[1], Wasim Sajjad[4], Muhammad Maqsood Ur Rehman[1], Khadija Usman[2], Mian Laiq Ur Rehman[5], Muhammad Usman[1]

[1]*School of Life Science, Lanzhou University, Lanzhou 730000, PR China*

[2]*Department of Microbiology, Faculty of Biological Sciences, Quaid-i-Azam University, Islamabad 45320, Pakistan*

[3]*Center of Biotechnology and Microbiology, Faculty of Life and Environmental Sciences, University of Peshawar, Peshawar, Pakistan*

[4]*Key Laboratory of Petroleum Resources, Gansu Province/Key Laboratory of Petroleum Resources Research, Institute of Geology and Geophysics, Chinese Academy of Sciences, Lanzhou 730000, PR China*

[5]*College of Environmental Science and Engineering, Nankai University, Tianjin 300350, PR China*

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Abstract

Bioleaching is an eco-friendly and low-cost technology compared to conventional methods of metals extraction from their respective low-grade ores. Owing to the substantial amount of metals existence in low grade ores, these are prospective resources of metals. This study was aimed to evaluate the copper dissolution potential of environmentally isolated *Pseudomonas aeruginosa*. Bioleaching was carried out in shake flask experiment. Dissolved copper was quantified by using Atomic Absorption Spectroscopy (AAS). Bacterial count and pH profiling of the bioleaching system was regularly evaluated. The bioleached residues after bioleaching were studied under scanning electron microscopy (SEM). Initially, abrupt increase in the bacterial count was reported and after 5 days of incubation, slight decline was observed. Slight decrease in pH was reported, which shows that this bacterial strain was capable to produce organic acids. The maximum copper dissolution carried out by *P. aeruginosa* was 36.1% at particle size of (53-63 µm) during 5 days of experiments. The SEM analysis showed significant porosity and corrosion on the ore surface compared to compact control. Given the importance, this study provided an essential optimized condition that could be helpful for upscaling the bioleaching procedure for copper extraction.

* **Corresponding Author:** Awais Iqbal ✉ awais.iqbal16@hotmail.com

Introduction

Minerals are imperative constituent of life as they have been employed in food processing, establishment of buildings, providing shelter/housing, vehicles, communications, pharmaceuticals, and various other leisure activities (Soetan *et al.,* 2010; Gholami *et al.,* 2011; R. Zhu and Buchwald, 2012; Mikesell, 2013; Horeh *et al.,* 2016). Since ancient civilization, use of copper for ornamental purposes and properties like non-corrosiveness, ductility, malleability, non-magnetism and thermal conductivity has made it a competent and conventional metal. In nature it occurs as native metal, copper oxide and copper sulphide. Copper ores exist in the form of pyrite or chalcopyrite ($CuFeS_2$), Chalcocite or copper glance (Cu_2S), Malachite green ($CuCO_3.Cu(OH)_2$), Azurite blue ($2CuCO_3.Cu(OH)_2$), Bornite or peacock ore ($3Cu_2S.Fe2S_3$) and Melaconite (CuO).

Pakistan is endowed with significant mineral resources (Shah *et al.,* 2010) and emerging as a potential area for exploration of mineral deposits. Therefore, efficient recovery of these mineral is of great significance for the socio-economic development of our country. In Pakistan, copper reservoirs are located at Shinkai, Boya, North Waziristan, Bulashgah, Gilgit, Saindak, Sasht-e-Kain, Chagai, ZiaratPir Sultan, Kabul Koh, Missi, Bandegan and RekoDiq. Increase in population reflects its demand thus affecting the availability of copper from natural ores. Pyro-metallurgy and hydro-metallurgy are among the earliest extraction methods for extraction of copper (Xin *et al.,* 2012; Hadi *et al.,* 2013). Although effective, release of toxic gases such as sulphur dioxide, the main cause of acid rain, has made these techniques controversial. Therefore, alternative method is required for the extraction of copper especially from low grades ore (Demergasso *et al.,* 2010; Panda *et al.,* 2012). The trend is now moving towards the use of biological methods which comprises the utilization of living cells (Demergasso *et al.,* 2010; Panda *et al.,* 2012). Bioleaching is considered the most preferable and economically efficient method as it consumes less energy and is eco-friendly (Panda *et al.,* 2015; Carolin *et al.,* 2017; Awasthi, Kumar, & Li 2017).

Copper extraction amidst other metals have been listed as commercial applications of bioleaching (Shabani *et al.,* 2013). Up-till now, many studies have suggested the use of microorganisms to extract metals from low-grade ores (Gholami *et al.,* 2011; N. Zhu *et al.,* 2011; Johnson, 2014; Horeh *et al.,* 2016). Microorganisms when provided with suitable conditions, can utilize enzymes to oxidize metals from their respective ores (Awasthi *et al.,* 2017). Much has been published on *Thiobacillus ferrooxidans*, *Lactosprillium, Thiobacillus thiooxidans* and *Sulpholobus spp.* for efficient extraction of metals (Shabani *et al.,* 2013; Awasthi *et al.,* 2017), however, they require stringent growth conditions. In contrast, *Pseudomonas aeruginosa,* being a pioneer specie for bioremediation and biodegradation studies (Abinaya Sindu and Gautam, 2017), can be easily handled and maintained at cheap resources. Therefore, the prime focus of this study was to utilize *Pseudomonas aeruginosa* for bioleaching of copper from Saindak ore, Pakistan.

Materials and methods

Ore Sample

Bulk ore sample was collected from Mana area, Barang, Bajaur agency, Khyber Pakhtunkhwa, Pakistan (Fig. 1). Initial pH of the sample was 7.5. The obtained sample was oven dried and ground to 200 mesh size by ASTM standard sieves series.

Bacterial strain and growth conditions

Pure culture of *Pseudomonas aeruginosa* was obtained from Environmental laboratory of COBAM (Centre of biotechnology and microbiology), Peshawar, Pakistan to evaluate the bioleaching efficiency of the selected strain due to resistive adaptation of bacterium. The strain was gradually optimized by increasing the copper concentration from 50-400µg/100mL and glucose from 2-6%. Growth conditions were kept constant at 35±2°C and cultures were stored at 4 °C, by harvesting bacterial cells in their logarithmic phase.

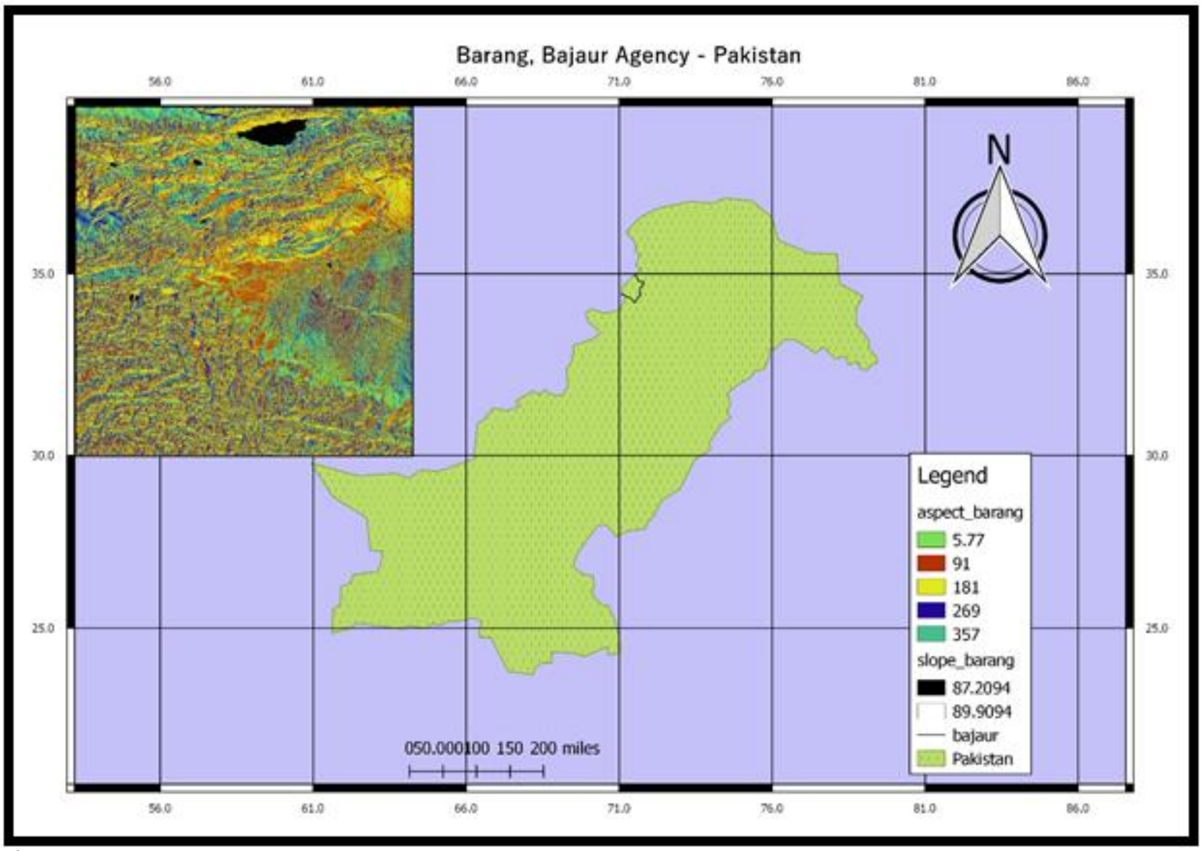

Fig. 1. Map of Barang, Bajaur agency, Pakistan.

Bioleaching experiment

Copper resistant and optimized bacterial cultures were inoculated (20:80 v/v) in 250 mL Erlenmeyer flask containing sterilized broth medium supplemented with 1 g/100 mL of ore sample (53-63 µm size) and incubated at shaking incubator with 80 rpm at 35±2°C for 5 days. Initial pH of the medium was adjusted to 7 by H_2SO_4. Samples were withdrawn every 24hrs for various analysis including CFU and metals dissolution analysis by using AAS.

Bacterial count

CFU was performed for 5 days by standard plate count method. Initially, samples were serially diluted and cultured on nutrient agar plates. Cultured plates were incubated at 35±2°C for 24hrs and CFU was counted for each day. This method allows the counting of living cells only eliminating the dead cells.

Copper analysis

AAS was used for analyzing copper dissolution within the bacterial samples. Before analysis, samples were centrifuged at 10,000×8 rpm for 15 minutes at 28°C along with control. Supernatant was diluted 10 folds with 10% HNO_3. Samples were stored aseptically at 4 °C.

Results and discussion

Biological extraction

Bacterial Count

Maximum CFU of 84/mL was calculated during 48hrs (Fig. 2). Initially, increase in the bacterial cells could possibly be due to the presence of glucose as sole source of energy and nutrient availability while it reduced in later stages due to less availability of nutrients and production of different organic acids.

In the previous verdicts, it has been observed that, in the presence of glucose, bacterial growth increases while production of gluconic acid, pyruvic acid, citric acid, oxalic acid, malic acid and succinic acid enhance the cell mechanism of bioleaching but reduced the pH and CFU (Shabani *et al.,* 2013; Karwowska *et al.,* 2014). Furthermore, *P. aeruginosa* exhibit different metabolic pathways for glucose intake and utilizing it as a carbon source (Meylan *et al.,* 2017).

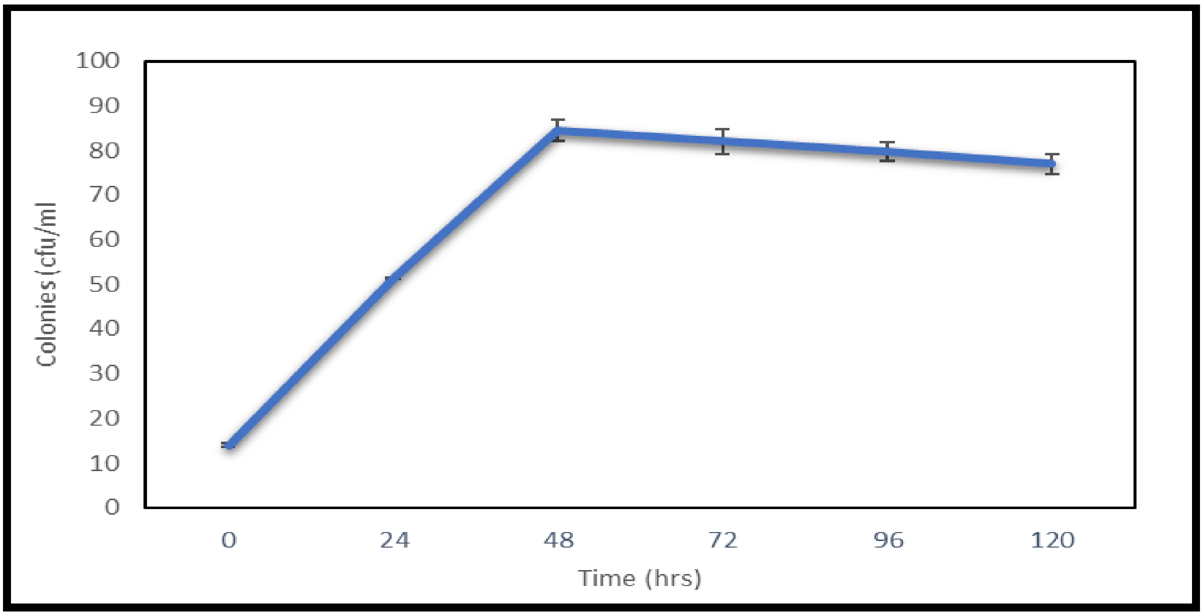

Fig. 2. Colony forming unit (CFU/ml) calculated during 120hrs of experiment.

In contrast, nutrient depletion poses drastic effects on overall bacterial count (Shabani *et al.,* 2013).

pH Profile

The pH profile of the medium is shown in the (Fig. 3). It was reduced to 5.2 during bioleaching experiment. Decrease in the pH could possibly be due to the formation of citric acid by microbial oxidation of carbon sources. Results were in correspondence with the findings of (Chen and Nielsen, 2016), which states that, by using heterotrophic bacteria, pH was declined by 2-4 units during a leaching experiment.

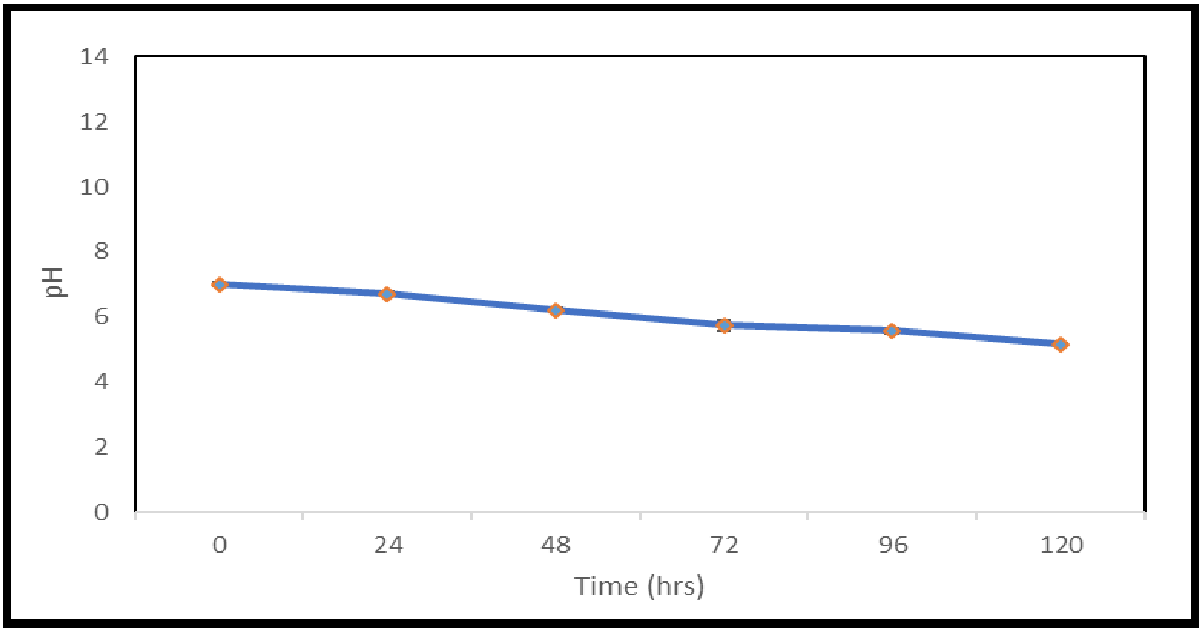

Fig. 3. Variation in the pH of the medium during 120hrs of experiment.

Bioleaching

AAS was used for determination of copper bioleaching by *P. aeruginosa*. The selected bacterial strain was capable to extract 36.1% of copper during 120hrs (Fig. 4). Bioleaching of copper and other metals is greatly influenced by the production of organic acids. *P. aeruginosa* being heterotrophic bacteria has the potential to tolerate and adapt itself towards high concentration of heavy metals such as Copper, Zinc, Nickel (Shabani *et al.,* 2015). Hence, 53% of copper bioleaching was reported by *P. aeruginosa* for its heterotrophicity and ability to produce organic acids (Shabani *et al.,* 2013). In a recent study, (Hassanien *et al.,* 2014) reported the bioleaching of rare earth metals using *P. aeruginosa*. The study revealed the secretion of organic acids such as citric acid, oxalic acid along with the production of 2-ketogluconic acid.

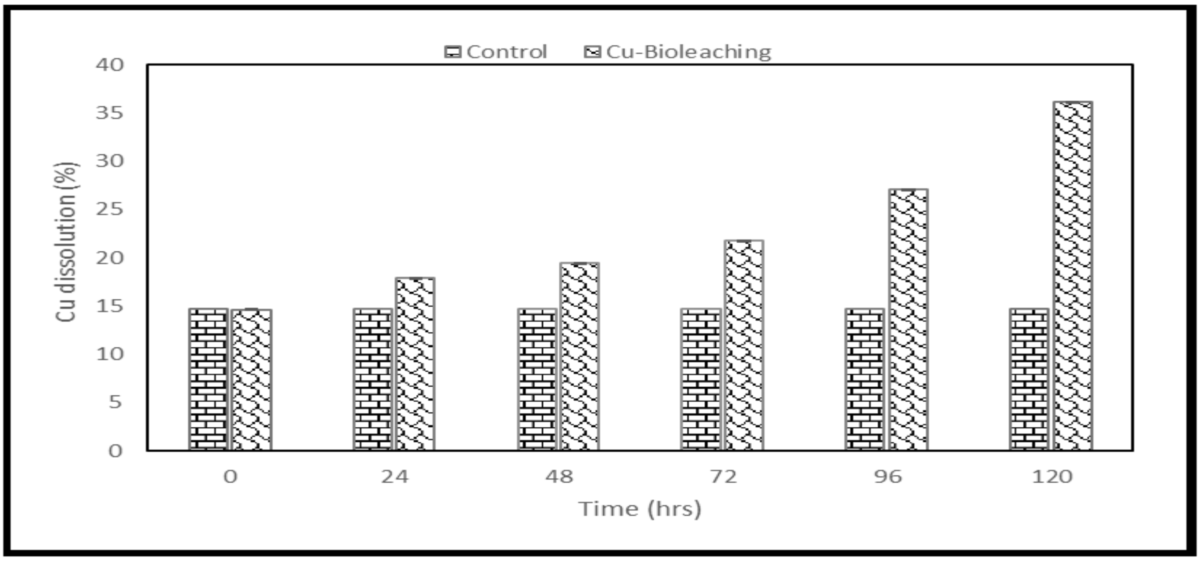

Fig. 4. Percentage of Cu bioleaching by *Pseudomonas aeruginosa*.

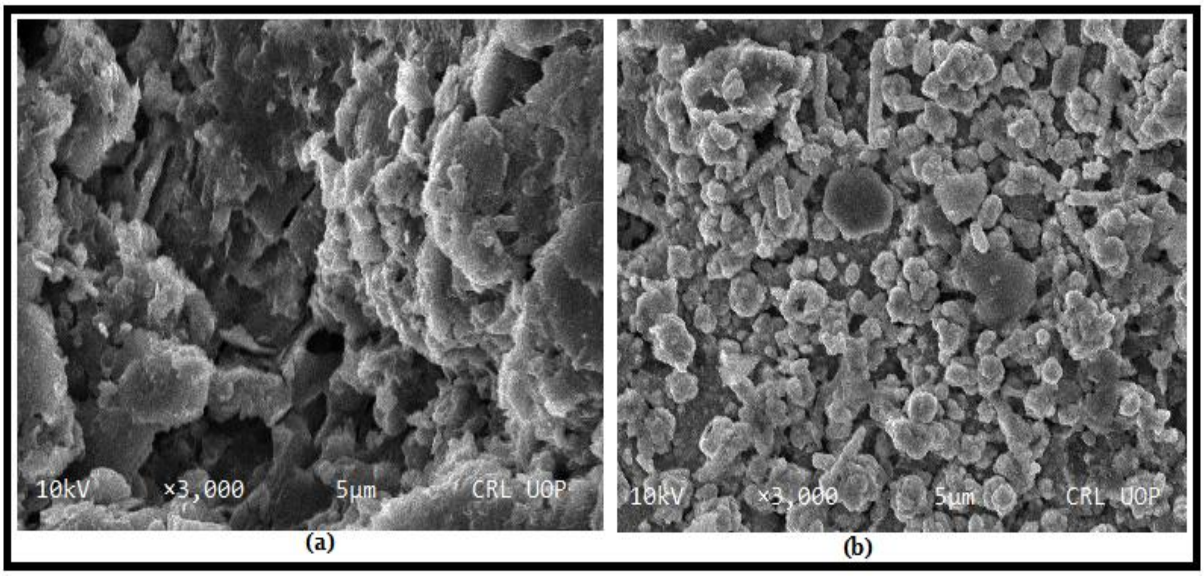

Fig. 5. SEM images of ore samples: (a) Before leaching and (b) after leaching.

Scanning Electron Microscopy (SEM) analysis

The SEM analysis revealed the surface morphology of the mineral. As shown in the (Fig. 5), some parts of the mineral were dissolved by the reagents produced through bacterial activity, hence increasing porosity of the mineral.

Conclusion

Our study revealed that *Pseudomonas aeruginosa* has the potential to bioleach copper from a sulphide ore. The ability to bioleach copper is attributed to the production of organic acids which solubilizes metal constituents of the ore. The selected strain was found to bioleach 36.1% of copper at a particle size of (53-

63µm) with an optimum temperature of 35±2°C during 5 days of shake flask experiment.

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