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An Overview: Application of Microorganisms in Bio-Mining of Metals

Shazia Sana*, Deepesh Neelam, Varsha Gupta, Devki and Ravi Rahi
Department of Microbiology, JECRC University, Sitapura, Jaipur-303905, Rajasthan, India.

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***Corresponding Author Email:** sana.sazi92@gmail.com

Abstract
Biomining of copper from ores was started during ancient era without even knowing the role of microorganisms in the process. The ever-increasing demand of metals, declination of natural resources, huge reserves of low-grade metallic ores and generation of massive amount of metallic wastes from mining and beneficiation process has led to the evolution and commercial scale adoption of biomining. The ability of microorganisms to bioamine metals depends on redox reactions, organic or inorganic acid formation and the release of complexing agents. Redox reaction is the key step of biomining process which is based either on direct or indirect bioleaching. The main difference between the two mechanisms is the direct contact between the microorganisms and the reduced minerals. Apart from the economic benefits, biomining also reduces the problem of acid mine drainage (AMD). Biomining is successfully utilized in metal recovery from low grade ores, mine tailings, mine wastes, municipal solid waste dump sites, legacy wastes, incinerator ash, E-wastes and, shales and tars etc. In this way, biomining inhibits the release of heavy metals from various types of wastes to the environment and release of toxic gases from the municipal solid waste dump sites and legacy wastes.

Keywords
Archaebacteria; bioleaching; chemolithoautotrophic bacteria

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INTRODUCTION
The history of mining is as ancient as the history of civilization. Many important cultural eras are identified by specific metals and its derivatives and in other words discovery of metals has shaped the evolution of civilization. [1-3.] Together with agriculture, mining was the basic industry of early human civilization. Mining has a pivotal role in energy/ power industry, for infrastructure development, manufacturing industry and with fertilizer industry. Besides these benefits mining has been detrimental to environment, and human health.[1-3]. The environmental impacts of mining ranges from air pollution, ground water contamination, surface water pollution, siltation of water bodies and agriculture lands deforestation, loss of wildlife, land degradation to acid mine drainage etc.

Due to rapid economic and infrastructure development metallurgy industry has witnessed increased demand of metals in post industrialization era. The situation gets further complexes by decrease in grades and quality of ores over the decades.[1-3]. Obtaining same output from the lower grades of ores is energy-, water-, capital- and labor-intensive process and simultaneously it generates larger quantity of waste[2] which is economically not viable by traditional mining methods.

As accessible primary metal ores become lower grade, it is economically more viable to recycle the mine wastes.[4] This also reduces the problem of AMD.[5] On the other hand, potential metals present in low grade ores, mine waste dumps and mine tailings can be recovered using the native microorganisms by process called biomining. It is particularly useful in the large-scale application for economical metal recovery.[6] Unlike the conventional mining, this is less energy intensive as well as eco-friendly approach which is devoid of discharge of any hazardous substances whether it is solid, liquid or gas into the environment.[7] Biomining in a broad term is the extraction and recovery of metals from their ore and waste materials through the application of living organisms.[8] It includes both bioleaching and bio-oxidation processes.[9]

In bioleaching, microorganisms convert solid metal values into their water-soluble state, whereas, in bio-oxidation minerals get oxidized by microbial activity while the metal values still remain in the solid phase in a more concentrated form.[10] Bio-oxidation is generally used as a preconditioning process. Microbes mediated oxidation of host minerals (which comprises the metal of concern) is used for concentrating precious metals in the ore (for example gold) and other such metals existing in the ore that cannot be directly recovered easily.[11] Microbial solubilization of metals involves both chemical and biological process: chemical because the metal is extracted by the action of ferric ion and/or acid, and biological because microorganisms are responsible for producing these leaching agents.[11] A variety of lithotrophic and organotrophic microorganisms are known to mediate these processes.[11] Microorganisms have been actively involved since the early beginning of life during both mineral formation and decomposition process in earth's crust.[12] This inherent ability of mineral decomposition of microorganisms was being used in the past in leaching of copper without even knowing that this was a microorganism mediated process[12] and was a pure empirical process then.[13] Johnson reported that globally bio-mining accounts for production of ~15% of copper and 5% of total gold production while, the contribution of other metals is even smaller.[14]

HISTORY OF BIO-MINING

Leaching technique of copper extraction was acknowledged by Georgius Agricola (1494–1555) a German physician and mineralogist in his work *de re metallica*.[15] Rossi reported in his book (Biohydrometallurgy) that bioleaching of copper from ores seems to have started at least 100-200 BC in China and it was also likely to be utilized in Europe around the second century.[16] Roman writer Gaius Plinius Secundus (23-79 AD) in his report described the process of copper recovery through slow passage of water through a mine in winter and subsequent evaporation of the leachate in summer.[17] On commercial scale copper extraction through bio-leaching was done in Rio Tinto, Spain in 1752.[16] For this the heaps containing low grade copper ore were stockpiled for 1-3 years to decompose naturally and were leached by acidic ferric ion solution (Fe^{3+}) for copper recovery without knowing the role of bacteria in the process. The discovery of formation of acid mine drainage (AMD) by bacterial action (*T. ferrooxidans*)[18] and the description of involvement of bacteria in copper leaching[19] started a revolution in bio-mining. *T. ferrooxidans* was later renamed *A. ferrooxidans*.[20] The chemoautotrophic acidophilic iron oxidizing bacteria was recognized to have the potential to draw the energy indirectly from the sulfide minerals and to maintain iron in an oxidizing state for the oxidation of sulfide copper ore to solubilize copper in an extreme acidic environment.[6,14] The modern era of biomining started with leaching of copper run-of-mine waste rocks (Dump leaching) in USA during mid-1960s.[14] In-situ bioleaching of uranium from extract residual uranium was also carried out in Canada during 1970s.[21]

The early commercial bioleaching applications which were limited to dump bioleaching of sub-marginal copper bearing rocks are of immense economic importance.[6] The surge in biomining began in the late 20th century after the development of modern approaches (heap leaching and tank leaching) of bioleaching/ bio-oxidation and with the application of bioleaching for other transition metals and polymetallic ores.[14] Biooxidation of refractory sulfidic gold ores is another successful and extensive commercial application of biomining.[22]

MICROBIOLOGY OF BIOMINING

In terms of the mode of nutrition generally chemolithoautotrophic bacteria and archaea, heterotrophic bacteria and heterotrophic fungi are used for biomining process. These organisms are often referred as mesophilic because they carry out their activity at a temperature in the order of 40 °C or less.

Chemolithoautotrophs used in biomining are acidophilic microorganisms, which are having the ability to fix CO_2 by oxidizing the ferrous iron or reduced sulfur and produces ferric iron or H_2SO_4.[12] Solubilization of metal sulphides by ferric iron or H_2SO_4 decreases the pH of surrounding and as a result further enhance the solubilization of metals,[23] because low pH (1.5-3.0) is best for microbial leaching due to the fact that at low pH most of the metals stay in solution.[24] Chemolithoautotrophic acidophilic *Acidithiobacillus ferrooxidans,* *Leptospirillum ferrooxidans and Acidithiobacillus thiooxidans* mediate in the process of bioleaching.[25] Among these *Acidithiobacillus ferrooxidans* is the most extensively explored and commercially used bacteria for biomining.[26] *Acidithiobacillus thiooxidans* being a facultative aerobe also thrives in anaerobic conditions by using reduced compounds of sulphur and ferric iron as an alternate acceptor of electron.[27] Application of *Acidithiobacillus thiooxidans* and *Acidithiobacillus ferrooxidans* has been well established by different studies for the leaching of filter dust,[28, 29] filter press residues,[28] sediment,[30] spent catalyst,[31, 9] spent nickel-cadmium battery,[32] electronic scrap,[33, 34] coal fly ash,[35] and municipal solid waste fly ash.[36] Recovery of metals (Al, Co, Cu, Zn, Mo, U, V) and radionuclides from their respective ores by chemolithoautotrophs is gaining more attention.[37-38] Thiobacillus and other autotrophs are also capable in bioleaching of shales, schist, and ores originating from fossil fuels.[37, 39]

Heterotrophic microbes thrive on organic carbon produced by autotrophs for survival and excrete organic acids as metabolites.[12] These metabolites (Lactic acid, gluconic acid, citric acid and oxalic acid) make the pH of medium in the range of 4-6 and provide suitable medium for metal solubilization, whereas ferric iron is precipitated.[12] Heterotrophs also improve the biomining ability of autotrophs by oxidizing acid soluble metal sulfide. Bioleaching of non-sulfidic rare earth elements can also be carried out by some chemoorganoheterotrophic microorganisms without maintaining low pH and adding sulfur and/or iron to the system.[40] Heterotrophs can also consume the inhibitory substances excreted by autotrophs during oxidation and support the autotrophs by providing energy substrate which is generated during reduction of ferric iron into ferrous iron.[41] The external factors such as nitrogen, sulphur, phosphorus concentration and presence of other trace elements control the growth of heterotrophic bacteria by terminating the metabolism at low concentration of nitrogen and phosphorus compounds.[42]

Bacillus (*Bacillus licheniformis* and *Bacillus polymyxa)*[43, 44] and *Pseudomonas* (*Pseudomonas putida*) are the most used heterotrophic bacteria for metal solubilization.[45, 25] AMD and black shale are the prominent sources of these heterotrophic bacteria from where they have been isolated and characterized.[46-48] As far as fungi is concerned, the genera *Aspergillus* and *Penicillium* are most widely used fungi in biomining.[24] *Aspergillus* and Penicillium have been extensively used to leach and detoxify metals from silicates,[49] low grade laterite ores,[50] gold mine tailings,[51] electro-filter dust from heavy oil combustion,[29] coal fly ash.[52]

Role of archaebacteria of genera *Sulfolobus, Acidianus, Metallosphaera, and Sulfurisphaera* in bioming has also been well recognized which are extremely thermophilic sulfur and iron oxidizers.[53, 54] Mesophilic and acidophilic iron oxidizing archaebacteria of species *Ferroplasma acidiphilum* and *Ferroplasma acidarmanus* are also known which are capable in metal leaching.[55]

MECHANISM AND TECHNIQUES OF BIOMINING

The microbial (bacteria and fungi) ability of metal leaching and mobilizing from solid substances involves three principles. These are (i) redox (oxidation and reduction) reactions, (ii) acid formation (organic or inorganic acids), and (iii) the secretion of complexing agents.[56]

In terms of the contact between microorganisms and the minerals to be leached, the facilitation by redox reaction is based either on direct or indirect bioleaching. In direct bioleaching electrons are transferred directly from the reduced minerals (Metal sulfide) to bacterial cells. In this case close contact between bacterial cell and reduced mineral is needed. The adherence of cells to metal sulfide takes few minutes to hours[57] with the help of electrochemical process.[6] Microbial growth and metabolism can be negatively affected by dissolved metals, which in turns limits productivity.[58] Ferric sulfate is produced by oxidation of pyrite in this mechanism.[59] Non-iron metal sulfide can also be oxidized by direct bioleaching.[60]

The precursor of indirect leaching a lixiviant [Ferric iron (Fe^{3+})] formed by the microbes assisted oxidation of ferrous iron (Fe^{2+}) present in the minerals. Ferric iron being an oxidizing agent oxidizes metal sulfides and is itself reduced to ferrous iron which, in turn, get further oxidized to ferric iron by microbial activity.[56] Indirect bioleaching method is most appropriate for lower sulfur and sulfide minerals as sources for H_2SO_4 production by autotrophs.[23]

In addition to the laboratory leaching techniques (Percolator leaching, submerged leaching and column leaching), leaching techniques used in industrial scale leaching are dump leaching, heap leaching, tank leaching and underground leaching. Brief of the industrial leaching techniques is described in Table 1.

Table 1: Industrial scale bioleaching techniques

S. No.	Technique	Features	References
1.	Dump leaching	• Most ancient technique. • Height of dumps are in the range of 25-400 meter. • Dump is sprinkled/ flooded from the top. • Water, acidified water, or acidic ferric sulfate solution are used as lixiviant.	12
2.	Heap leaching	• The procedure of heap leaching is like that of dump leaching. • This technique is generally used for fine grained ores. • In the heaps also there is provision of oxygen supply to provide sufficient oxygen to the deeper portions.	24
3.	Tank leaching	• This technique is expansive, but the rate of metal extraction is higher. • Tank leaching is established as the most effective approach for bioleaching of ore concentrates and > 80% of the total zinc was leached from zinc sulfide. • Tank leaching is successfully used for extraction of gold from refractory gold ores.	24
4.	Underground leaching	• This is an in-situ method of leaching. • Underground leaching technique is generally done in abandoned mines and in ore deposits which are too low graded that cannot be extracted by conventional methods.	24

APPLICATION OF BIO-MINING

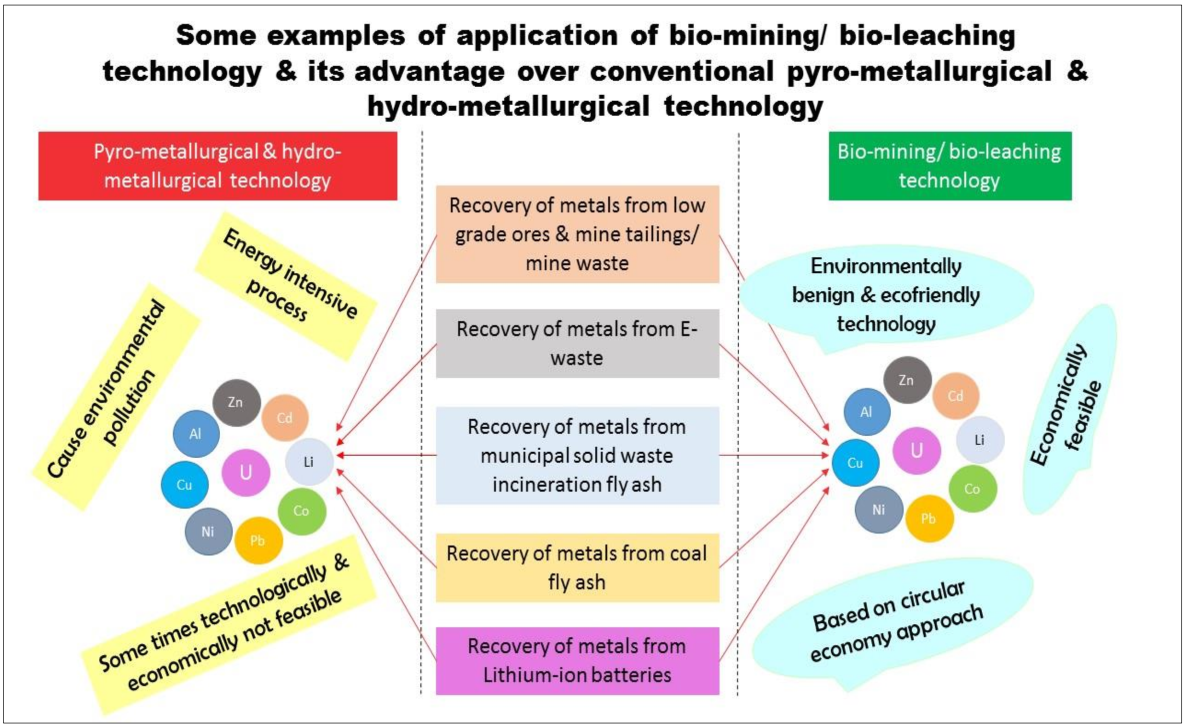
The applications of biomining as a benign and environmentally friendly technology is depicted in Table 2. Some examples of application of bio-mining/ bio-leaching technology & its advantage over conventional pyro-metallurgical and hydro-metallurgical technology is depicted in Figure 1.

Table 2: Application of microorganisms in metal recovery

S.No	Application of bio-mining	Metals leached	Microorganisms applied	Reference
1.	Low grade uranium ore leaching at Uranium Corporation of India Limited, Jaduguda, India	U	*Acidithiobacillus ferrooxidans*	61
2.	Metal recovery from E-waste	Al, Cu, Pb, Ni, Sn, and Zn	*Thiobacillus thiooxidans, Thiobacillus ferrooxidans, Aspergillus niger* and *Penicillium simplicissimu*	33
3.	Metal recovery from printed circuit boards (E-waste)	Cu	*Leptospirillum ferriphilum* and *Sulfobacillus thermosulfidooxidans*	62
4.	Metal recovery from printed circuit boards (E-waste)	Al, Ca, Cu, Cd, Fe, Ni, Zn, Ag and Pb	*Streptomyces albidoflavus*	63
5.	Metal recovery from cellular phone printed circuit boards and computer goldfinger motherboards (E-waste)	Au, Cu, and Ni	*Aspergillus niger*	64

6.	Metal recovery from municipal solid waste incineration fly ash and bottom ash	Cu, Zn and Pb	Acidothiobacillus thiooxidans *and* Acidothiobacillus ferrooxidans	65
7.	Metal recovery from municipal solid waste incineration fly ash	Cd, Zn, Cu, Pb, Mn, Al, Cr, Fe, Ni	*Aspergillus niger*	66
8.	Metal recovery from coal fly ash	Cr, Pb, Cu, Zn	*Nostoc muscorum, Anabaena variabilis, Tolypothrix tenuis* and *Aulosira fertilissimia*	67
9.	Metal recovery from coal fly ash	Fe, Al, Zn, V, Ba, Mn, Pb, Ce, Y, La, Nd and Sc	*Candida bombicola, Phanerochaete chrysosporium* and *Cryptococcus curvatus*	68
10.	Metal recovery from spent Lithium-Ion Batteries	Li and Co	*Acidithiobacillus ferrooxidans and Acidithiobacillus thiooxidans*	69
11.	Metal recovery from spent Lithium-Ion Batteries	Cu, Li, Mn, Al, Co and Ni	*Aspergillus niger*	70

Figure 1: Some examples of application of bio-mining/ bio-leaching technology & its advantage over conventional pyro-metallurgical and hydro-metallurgical technology



CONCLUSION

Large number of Chemolithoautotrophic bacteria, heterotrophic bacteria, heterotropic fungi and archaebacteria are known to mediate the process of biomining. Among the chemoautotrophic bacteria *Acidithiobacillus ferrooxidans, Leptospirillum ferrooxidans and Acidithiobacillus thiooxidans* are most exploited bacteria for biomining. *Bacillus licheniformis, Bacillus polymyxa*, *Pseudomonas putida* are the most used heterotrophic bacteria and *Aspergillus niger*, *Penicillium simplicissimum* are the most used fungi for metal solubilization. Thermophilic archaebacteria of genera *Sulfolobus, Acidianus, Metallosphaera, Sulfurisphaera* and mesophilic and acidophilic archaebacteria of genera *Ferroplasma* are also known to mediate metal leaching.

At present microbial mediated leaching is the best available technology to extract metals from low grade ores, mine waste and tailings, dumps of municipal solid waste and e-waste even when the metals are present in very less concentration without harming the environment and ecology. Considering the benign nature of the technology further research is still needed for extensive application of this technology for the leaching of non-sulfide ores.

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CONFLICT OF INTEREST

Conflict of interest declared none.

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