



Review Paper

Bioleaching of Heavy Metals by Sulfur Oxidizing Bacteria: A Review

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Available online at: www.isca.in, www.isca.me

Received 22th June 2015, revised 28th July 2015, accepted 1st September 2015

Abstract

Bioleaching, a novel biotechnological process, is used to eliminate heavy metals from contaminated sediments by acidification as well as solubilization of heavy metals. Metals from poor-quality ore and mineral compounds are removed by Bioleaching process, which is simple and low cost effective technology. Acidophilic sulfur oxidizing bacteria (*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*) transforms toxic metal sulphides to less toxic sulphates. Trace elements are processed by using sulfur oxidizing microorganisms (*Aspergillus niger*). Heap bioleaching of chalcocite ores is widely used as a relatively low cost process option, especially for marginal deposits. *Aspergillus niger* has good leaching efficiency in the extraction of Fe, Sn and Au. Now these days metal recovery technique is widely practiced for the recovery of copper, gold, iron, manganese and lead. The bacterial species generally used in bioremediation process are known as *Thiobacillus*. Decomposition and erosion by sulfur oxidizing microorganisms are the preliminary methods for the extraction of toxic metal ions from contaminated environment. Therefore, bioleaching has potential effect on metal retrieval and detoxification of waste products of industry, coal mine, sewage sludge and heavy metal contaminated soil.

Keywords: Bioleaching, direct bioleaching, indirect bioleaching, heavy metals, microorganisms, bacteria, fungi, *thiobacillus*, metal solubilisation.

Introduction

Heavy metal contamination in soil and water due to industrial, agricultural and domestic activities has created major environmental problem and dangerous for human health¹. For that reason, it is necessary to eliminate toxic heavy metals from soil and water. Unlike other environmental pollutants, removal of heavy metals from environment is difficult and these metals cannot be chemically or biologically degradable. Various physic-chemical treatment techniques have effectively applied for extraction of toxic heavy metals from soil and water, but they have some unavoidable drawbacks such as low capability and huge cost effect². Several biotechnological approaches such as bioremediation and phytoremediation have received a great attention in recent years for removal of heavy metals from environment, which is not only as scientific origination but also for the probable application in industry³. Sulfur oxidizing bacteria are responsible for the extraction of toxic metal ion to non toxic or less toxic metal ions. This process is going on for many years. They are the natural source of cleaning the environment⁴. One of the most successful Biotechnological approaches for removal of heavy metals is bioleaching.

Bioleaching is the process of dissolving (leaching) sulfide minerals and extracting metals easily by the increasing effect of the production of less toxic metals by unmediated discharging bacteria. The microorganisms intensively used in bioleaching process belong to the genus of *Thiobacillus*. Bioleaching process using acidophilic sulfur oxidizing bacteria

(*Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*) and neutrophilic microorganisms (*Aspergillus niger*) has been intensively investigated for successful removal of metals from sediment, municipal solid, and sludge⁶. Sulfur oxidizing bacteria oxidizes and produces acid for solubilization of heavy metals by obtaining power supplied from the utilization of elemental sulfur and thiosulfate in bioleaching process. The method for elimination of toxic metal contaminated soil and water by application of the sulfur bio cycle. Bio-oxidation reactions on the principle of direct or indirect oxidation often participate in the bioleaching process in parallel⁷.

Therefore, suitable conditions are needed in this solubilization process in the term of bioleaching for the expansion of pervasive sulfur oxidizing bacteria.

Microorganisms

***Thiobacillus*:** The highest important effective bacteria in bioleaching process originate to the group of *Thiobacillus*. They are colorless, rod shaped and strictly aerobic bacteria. They are chemolithoautotrophic bacteria meaning they utilize inorganic chemicals such as sulfur as energy source. These bacteria oxidize decreased sulfur compounds including sulfides, elemental sulfur and thiosulfate to sulfate⁸.

Thiobacillus ferrooxidans and *T. thiooxidans* are responsible for the bacterial leaching process, which is carried out in acidic environment where the pH value remain at 1.5-3 and other

metal compounds remain in solution. Various *thiobacilli* are capable to oxidize sulfur and sulfide to sulfate at higher pH in which condition other metal ions do not remain in solution. At lower pH acidophilic *Thiobacillus ferrooxidans* and *T. thiooxidans* can grow. The serviceable price range of bioleaching technique by *Thiobacillus ferrooxidans* is huge and they oxidize sulfur compound to sulfate gently compare to *T. thiooxidans*⁹.

T. thiooxidans isolated by Waksman and Joffe in 1922 is famous for the oxidation of elemental sulfur¹⁰. The most significant role of bioleaching process is carried out by *Thiobacillus ferrooxidans*¹¹. *Thiobacillus ferrooxidans* differs from other *thiobacilli* as they use ferric iron as alternative electron acceptor.

One more acidophilic and facultatively chemolithoautotrophic *Thiobacilli* is *Thiobacillus cuprinus*. This bacterium oxidizes metal sulfides but do not oxidize ferrous ion. This was isolated from copper mine and they preferentially mobilize copper from chalcopyrite in aerobic condition¹².

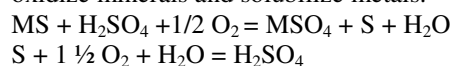
Thermophilic bacteria: *Thermophilic* sulfur bacteria are the part of sulfur oxidizing bacteria and they arise on pirate and chalcopyrite at the temperature start from 50°C. Extremely thermophilic bacteria growing above the temperature of 60°C was first identified by Brierley and co-workers¹³. *Acidianus brierleyi* is facultatively chemolithoautotrophic and aerobic bacteria grow on the presence of elemental sulfur, metal sulfur and ferrous ion and they are extremely acidophilic *Archaeon*. They formally come under the genus *Sulfolobus*. Under anaerobic condition elemental sulphur is reduced to H₂S as an electron acceptor. The group comes under *Sulfolobus* are facultatively chemolithoautotrophic, aerobic bacteria and mature on metal sulfur and elemental sulfur. Ferrous ion, elemental sulfur and sulfide minerals are oxidized and used as energy source by *Sulfobacillus thermosulfidooxidans*, which is a endospore forming facultative chemoautotrophic sulfur bacteria. Yeast extract is the main source for the growth of these bacteria.

Heterotrophic bacteria: Organic compounds are the source of the maturation of heterotrophic bacteria. Moreover, sometimes power supply is needed for metal leaching. Highly oxidized metal compounds does enzymatic reduction for metal solubilization in manganese leaching process^{14,15}. Manganese leaching is affected by generation of organic substrates such as citric acid, lactic acid, gluconic acid and oxalic acid. And also it is affected by some selective compounds generated by the organic compounds. These components disintegrate toxic metals by the formation of soluble macro-element complexes, chelates and complete utilization of toxic compounds. There is no such report of the benefit of the metal leaching of heterotrophic microorganisms¹⁷. The group of *Thiobacillus* species has huge and important effect of bioremediation of toxic environment. Some members of the genus *Arpergillus* and *Penicillium* have

the maximum effect on metal bioleaching. A heterotrophic microorganism *Bacillus circulans*, was used for the removal of heavy metals such as Cu, Ni, Pb and Co. This bioleaching process is direct process mediated by membrane bound reductase enzyme. Reduction of Manganese and Iron by bacteria leads to release of valuable metals from the host lattice thereby dissolving them chemically^{17,18}. Indigenous micro flora was used for recovery of Nickel. In this case, most effective organic compounds such as oxalic acid and citric acid are responsible in removal of the metal ions from Nickel ore as differentiated to other organic metabolites. A negligible amount of nickel could be solubilised by organic metabolites of microbial growth¹⁹. Heterotrophic bacteria produce amino acids (mainly alanine) during leaching process.

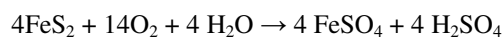
Bioleaching Mechanisms

Direct Bioleaching: In direct bioleaching, bacteria directly oxidize minerals and solubilize metals.



Physical contacts are present between bacterial cells and mineral sulphide surfaces in direct bioleaching mechanism. Oxidation of sulfate undergoes several chemical processes²⁰.

In this mechanism, Iron sulfate is the final oxidation product generated through some reaction steps.

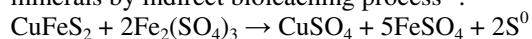


In direct bioleaching process, minerals undergo direct enzymatic attack by the microorganisms. The patents by Basson et al. WO 01/18266 and Steemson et al. WO 94/28184 have already been described the recently developed techniques for direct bioleaching of zinc concentrates as an alternatives to conventional roast-leaching. Low-grade zinc concentrates can be treated by these direct bioleaching process which does not generate any environmentally hazardous emissions or residues. Iron precipitate to purify the leach solution in the pregnant leach stream (PLS) followed by the recovery of zinc by solvent extraction and electro winning. Disadvantage of direct bioleaching is sulfur is completely converted to sulfuric acid in the bioleach, resulting in increased oxygen and neutralizing agent consumptions.

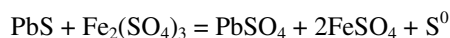
Indirect Bioleaching

In indirect bioleaching, the bacteria oxidizes ferrous ion in the form of ferric ion. Ferric iron is the final oxidizing agent. Direct and indirect mechanisms may occur simultaneously along with other physicochemical reactions in real bioleaching systems. A high amount of sulfide minerals is dissolved. Ferric sulfate is most important source of indirect bioleaching, because it proceeds in the absence oxygen and viable bacteria. Several

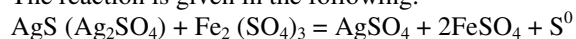
harmful mineral compounds are oxidized to less toxic sulfide minerals by indirect bioleaching process²¹.



Indirect bioleaching of zinc, silver and copper is mediated by bio-regeneration of ferric sulphate solution. After leaching of ferric sulphate and zinc are dissolved by electro winning. Treatment of leaching residue is maintained by sulphur oxidizing bacteria²². In indirect bioleaching Lead Sulphide is transformed to Lead Sulphate. The reaction is given in the following:



Silver has the similar pathway. Silver convert into Silver Jarosite ($\text{AgFe}_3(\text{SO}_4)_2(\text{OH})_6$). High temperature, low acidity and high ferric ion concentration are the three major components for the production of harmless Silver Jarosite²³. The reaction is given in the following:



Bioleaching of Heavy Metals: Generally, the study of bacterial leaching process of manganese from manganese dioxide ores is done by heterotrophic bacteria, which directly reduce the manganese dioxide to the soluble Mn^{2+} form and various research efforts on manganese bioleaching have been constructed on these heterotrophic bacteria. Glucose or other organic compounds are used as an energy source. pyrolusite is biodegraded by marine bacteria, and, probably, soil and fresh water species (e.g. *Bacillus Micrococcus*, *Pseudomonas*, *Achromobacter* and *Enterobacter*) through the process of enzymatic reduction, using a variety of carbohydrate nutrients (glucose, molasses) under aerobic and micro aerobic growth conditions. Certain fungi species, are known to act on Mn^{4+} (e.g. *Aspergillus niger*) form a mixture of extracellular reducing organic compounds (e.g. citric, formic and oxalic acids) during fermentative metabolism of sugars. The main drawback of manganese bioleaching is, complete manganese solubilisation can be achieved by heterotrophic microorganisms, but the use of nutrients such as glucose, and other refined sugars is cost effective²⁴.

FeS_2 and FeS are widely used to find out if metal sulphides are oxidized under anaerobic conditions at circum neutral pH. In chemical experiments, MnO_2 oxidizes FeS_2 and FeS . With MnO_2 as oxidant, elemental sulphur and sulphate are only elements of FeS oxidation, whereas FeS_2 is oxidized to a variety of sulphur compounds, mainly sulphate plus intermediates such as thiosulphate, trithionate, tetrathionate and pentathionate. MnO_2 oxidize thiosulphate to tetrathionate where other intermediates are oxidized to sulphate. The reaction products indicates that the thiosulphate mechanism is the main source of the oxidation of FeS_2 and polysulfide mechanism is the source of oxidation of FeS under anaerobic conditions which previously had been found for aerobic metal sulphide oxidation²⁵.

Heap bioleaching of chalcocite ores is one of the widely used areas of bioleaching as a relatively low cost process option, especially for marginal deposits. However, the process of copper extraction is rated slower than expected. Recent studies of chalcocite heap bioleaching have shown that the process is done by two-stage mechanism. The first step is done by supplying acid to the reaction sites and the second mechanism, which is comparably slower than the first one, is controlled by mineral oxidation kinetics. The results obtained from the column operated with a bacterial consortium grown under laboratory conditions are compared to those of a column run with a native culture and high-TDS (total dissolved solids) raffinate solution from the mine site. The results clearly indicate that among these two-stage mechanisms, the overall rate of copper extraction in the high-TDS column is significantly retarded²⁶.

Acidithiobacillus ferrooxidans and *Acidithiobacillus thiooxidans* are capable of oxidizing the sulphide to sulphate by releasing the metals in which respective sulphates become the soluble chemicals. Scientists have already been studied the use of *Acidithiobacillus ferrooxidans* monoculture and mixed culture of mesophilic bacteria. Application of mixed cultures is more efficient for the oxidation of the main sulphide minerals-arsenopyrite, pyrite and pyrrhotite. Elemental sulphur releases by the oxidation of sulphide concentrates. Bacterial oxidation of pyrrhotitepyrite-arsenopyrite concentrates derives maximum amount of elemental sulphur. The process of elemental sulphur bio oxidation is not terminated with the complete oxidation of pyrrhotite, arsenopyrite and some pyrite. Elemental sulphur has negative effect on the process of gold recovery. The first commercially available was constructed for the recovery of gold in Russia at the Olimpiada gold deposit. Although the microorganisms are several minerals amenable to bioleaching or bio oxidation on a commercial scale, only two metals, gold and copper, are currently recovered successfully using this technology²⁷.

In the last twenty years, industrial-scale bioleaching has known a comparably rapid growth with the start-up of about ten plants using bio heap leach, seven plants treating sulphide concentrates in agitated tanks and countless pilot-scale operations. A very critical aspect is the control of the performances, which is showed in real case situation and how to evaluate the efficiency of the process on real time. Gas analysis is applied for the performance to control and integrate the process of system²⁸.

In many cases, washing tests were performed using organic acids produced from the culture of *Aspergillus niger* and *Penicillium simplicissimum* in order to measure extraction potential and to indentify the mechanisms of solubilisation. Two types of mining residues were studied by the authors: one is Zn and Pb mine in New Brunswick, Canada and the second is a Ni mine in New Caledonia, where the concentrations of metals like Cu, Fe, Mn, Ni, Pb and Zn were high. Various factors controlled efficiency of the extraction at the end of the result. However, in

many cases, the slow kinetic of the bio oxidation processes has limited its commercial application. Different parameters such as biological, physicochemical, electrochemical and mineralogical factors attribute this slowness.

The catalytic effect silver, Ag (I), during the chalcopyrite leaching has been reported and the kinetics of chalcopyrite dissolution for the use of catalytic agents has been proposed. The dissolution rate is improved as a film of Ag₂S forms on the surface of chalcopyrite particles. The semi-conducting characteristics of sulphide minerals shows the electrochemical interactions (galvanic pairs formation), which is originated among different sulphide minerals in a same bioleaching system and this system could improve the selective dissolution of the most active minerals. Apart from all these, the system is complicated either to explain or predict their effect on the bioleaching rate in a sulphide mineral mixture. Mesophilic microorganisms are used in the presence as well as in the absence of Ag (I) to compare the results of bioleaching of the concentrate C2 doped with pyrite and chalcocite²⁹.

The main drawback of the copper bioleaching process is low kinetic reaction with high residence time. This high residence time do not permit this process for go further. Scientific research is going on the increasing rate of bioleaching process. Scientists are commercialising new techniques. Present research is focusing on the isolation and adaptation of new microorganisms, which are able to detoxify harmful metal ions. The use of catalysts has given positive result in this case. Various attempts have been made in order to evaluate the use of thermophilic bacteria and more recently, hyperthermophilic archaea, which grow at the temperature of 60°C or more than that. Some examples of these archaea are *Sulfolobus metallicus*, *Sulfolobus sulfataricus*, *Sulfolobus acidocaldarius*, *Acidianus infernus* and *Acidianus brierleyi*³⁰.

Apart from all these, bioleaching is affected by a number of parameters. Various physico-chemical and biological factors are present. The factors are i. nutrient, ii. oxygen and carbon dioxide supply iii. temperature of the leaching element iv. pH v. pre-culture period vi. liquid-solid ratio vii. physico-chemical states of the solid residue, viii. detention of microorganisms to metal ions and ix. bioleaching period. Maximum methods of bioleaching can be achieved when these parameters have been optimized. The success rate of bioleaching through microorganisms is dependent on these physico-chemical and biological parameters.

Conclusion

Bioleaching is the novel practice for extraction of harmful heavy metals from soil and water. Bioleaching is considered today not only the valuable process for recovery of metal contaminated soil but also a less expensive process. This is very fruitful process for the environment. Further research should be carried out for the development of these techniques. Destruction

of industrial waste causes environmental pollution. The waste products could be recycled through the process of heterotrophic leaching. Many less toxic compounds are generated which is not harmful for the environment. Rate of bioleaching with the help of the microorganisms is increasing by time. Many microorganisms are resistance to toxic heavy metals. Genetic improvement of microorganisms such as mutation, selection like genetic engineering process could be fruitful other than conventional processes like screening and adaptation. Solid-bed bioleaching is more efficient for the remediation of heavy metal polluted sediments, which is reported by Shen-Yi Chen, et al.

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