American Journal of Nano Research and Application
2015; 3(1): 6-11
Published online February 27, 2015 (http://www.sciencepublishinggroup.com/j/nano)
doi: 10.11648/j.nano.20150301.12

The Studing of Silver Nanoparticle Effect on the Copper Bioleaching Output from Low Grade Sulfidic Ores

Jamshid Raheb[1, *], Sorur SHaroknyan[2], Fatemeh Nazari[1], Yasin Rakhshany[3]

[1]National Institute of Genetic Engineering and Biotechnology, Tehran, Iran
[2]Department of Environment, Science and Research Branch, Islamic Azad University, Tehran, Iran
[3]Department of Microbiology, Islamic Azad University North Tehran Branch, karaj, Iran

Email address:
jamshid@nigeb.ac.ir (J. Raheb)

To cite this article:
Jamshid Raheb, Sorur SHaroknyan, Fatemeh Nazari, Yasin Rakhshany. The Studing of Silver Nanoparticle Effect on the Copper Bioleaching Output from Low Grade Sulfidic Ores. *American Journal of Nano Research and Application.* Vol. 3, No. 1, 2015, pp. 6-11.
doi: 10.11648/j.nano.20150301.12

Abstract: The extraction of metals from ores causes various environmental pollutions. Since Iran is located on the so-called 'copper belt' and holds a significant share of the world's copper mines and resources, reduction of pollution from these mines can have an important effect on the overall reduction of pollution. Copper processing methods include pyrometallurgy and hydrometallurgy. Pyrometallurgy is mainly used in high grade mines whereas hydrometallurgy process is used in lower grade mines. In low grade copper sulfide mines, hydrometallurgy processes are used which use a lot of energy to covert mineral deposits into oxide forms which are then leached using sulfuric acid, or are extracted using bioleaching process. In acidic leaching, a lot of environmental pollution is created. Bioleaching process is an environmentally-friendly method which is mainly used in mines where the common physicochemical methods are not profitable. In this study, we have tried to increase the efficiency of bioleaching process by adding silver nanoparticle in order to increase the popularity of this method. For this purpose, initially the indigenous bacteria were separated from the ores and after adoption to silver, the bacteria were used in bioleaching tests. Three concentrations of silver component were used for the bioleaching tests. The results were compared to cases where no bacteria and no silver compounds were used, which showed significant increase in copper extraction efficiency. In the next step, the optimum concentration of silver was used in the percolation column. In this stage, four columns were set up for 'with bacteria and silver', 'with silver', 'with bacteria' and 'without bacteria and silver'. Results show that the column with bacteria and silver produced the highest efficiency of copper extraction.

Keywords: Silver Nanoparticle, Bioleaching, Copper, Ores

1. Introduction

The minerals present on the surface the earth's crust according to their importance, are being considerable by the man, and trying to find new mining areas and using more from these present mining areas is continuing, too. As simple as possible, we can say that without these mineral materials, our lives will not possible. According to increasing progress in using and consuming from these materials in different and various industries, Nowadays economics minerals are taken into account as one of the most important and essential foundations in every country[1].

The investing a researcher divided the history of mankind indifferent periods according to these minerals such as:

Rock age, Bronzeage and Iron age. Cheap minerals frequency is being created necessary field for establishing the industrial civilization and man societies general welfare is related to consume per capita these mineral productions[2].

Current living of mankind is dependent in to these new technology and industries and the mankind is not able to separate itself from the all sources which are providing all the living devices. The environment is a set of very huge and complicated different parameters and elements which have being created by the organisms gradual evolution of any living and the elements of making the earth surface, and then it has been influence on human activities and it is influenced by these activities. By doing mining operations, the surrounding environment has exposed to different changes and if the man often doesn't pay attention on controlling and monitoring these effects، may cause environment pollution[3].

The most and serious environment problem is related to having acidic mine sewages, which have caused harmful

effect on ground and underground waters. Most mines are sulfide minerals especially pyrite minerals.

Having oxidation of these minerals cause to become condensed, especially pyrite and cause the formation of AMD.AMD is become specified by characteristics such as high Iron and Sulfate content and low pH [4]. Copper usually is found in the mineral form. The other minerals such as Azurite, Malachite and Bernite similar to other sulfites such as Chalcopyrite ($CuFeS_2$), Kovolin(CuS), Kalcozine (Cu_2S) or other oxides such as Copyrite (Cu_2o) are from the Copper sources [5]. The compounds such as Fleming solution which are used in chemistry and Copper sulfate which is used as toxin and is used as water filtration is another form of consuming Copper, today, too[6]. Nowadays, Bioleaching dumplings are very low cost techniques for Copper recovery from main mass which there are no other low cost techniques like these.

In addition to economical success Bioleaching dumplings, very little operations are needed for preparing these dumplings [7]. Chalcopyrite is the most important of the Copper source. About %70 of known sources are Chalcopyrite [8]. In the most mines in the world, masses of sulfite stone minerals are dissolved because of water penetration, and green solutions (containing Iron ion)and blue solutions (containing Copper ion) are sweat from those. Rodolf and Heilbroner discovered in 1922 that bacteria can extract metals from sulfide stones which have low purity and assay. By investigating and studying this phenomenon, the investigator were suspected to the performances of some bacterial which are being in these waters, and in next investigation, they proved that, these biological elements, when they have suitable environment for living, they receive their required food and energy from the Iron and Sulfur oxidation which are got from the sulfide minerals of these quarry.

In the next investigation, different kinds of these bacteria were explored, and each one of them in special physical and chemical situation (such as pH and temperature) could continue their living and performances[9]. The investigations show that adding other ions such as Bismuth, Plumb (Pb), Cobalt (Co), Magnesium (Mg), Zinc (Zn) and Nickel (Ni) don't have any influence on extracting Copper. Silver is a key element in Bioleaching and in extracting Copper from low purity and assay mines.

Hiroyoshi in his investigations find out that the suffering of biological leaching of chalcopyrite is related to its potential oxidation and reduction, and it is determined by Ferric iron [10]. Bioleaching of the metals which means extracting and exploiting the metals from the mines, is done by the neutral elements such as microorganisms.

During this procedure, an indissoluble metal (which is usually a metal such as Ni, Cu or Zn) is changed into a dissoluble from (which is metal sulfate) [11]. Present of the last extracted rich residue resources which are contained a lot of metals that are not justifiable economically[12]. The hyolro metallurgical procedures are included: leaching, extracting and exploiting solvent, Electrovining and Sendimentology are in recent decades, by worldwide several big mine companies, in order to extract low purity and assay resources which are not possible and economical by the piro mythological procedures, are being more attractive[13]. Bioleaching procedure for extracting the metals such as Copper, Nickel, Kobalt, Zinc and Gold and etc. using the low purity and assay sulfite resources according usual physicochemical methods and technologies are not profitable. Extracting metal from the mines is done by using natural procedures such as microorganisms. These microorganisms have the best growth in the pH(1.8-2) and temperature degree (20-45˚c) environment.

Bioleaching in contrast by usual physicochemical procedures, has the following advantages:

- All the microbiological process have very high coordination with the environment
- These procedures don't need too much energy and are profitable economically.
- They don't produce sulfur dioxide (sio_2) and other harmful gasses.
- In the case of quarry with low purity and assay, these procedures are usable. Most of these quarries are not recyclable or not profitable.
- Main bacteria of Bioleaching, have common physiological features. All of them chemo lithotrophic, and they can use from Iron or other nonorganic sulfur resources as electron donor. As the sub-production of sulfur oxidation is Sulfuric acid therefore, these organisms are acidophilus, and they grow at the pH 1/5 or 2. This acidophilus feature is correct about Bio minis organisms which they only oxidize the Iron. Although these bacteria can use of other receiver electrons which are non-oxygenic, But they commonly can grow better in aeration solutions [14].
- Microorganisms, in their structures, they need to a small amount of metals which their supplying resources of these metals are eco-environment. Most of bacteria, they deposit large amount of using metals into their inner cells, in order to use them when the amount of these metals are decreased in their environment, because they need these resources to supply their structural needs.

It is possible that the metal influence the phosphorylation oxidative and permeability of the cell membrane. We can see this condition in Vanadium and Mercury[15]. Bacterial active oxidation of Iron in soil and mines is done at the usual temperature, 10˚c. Extracting desirable of metal from mine sulfides by these bacteria, is happened at the temperature degrees between 25-45˚c.

This process stopped completely, because of bacterial decreasing in proteins and enzymes in the temperature degree 55˚c and in the temperature degree above 55˚c, only the chemical oxidation of the stone mine is happened.

Thiobacillusferroxidans, is done the metal extracting operation in the temperature degree between 10-40˚c.

Because of protoplasmic special compound of organism, the least temperature for metals bacterial extraction in every region, is related to the special condition of that region [6].

2. Materials and Methods

2.1. Isolation of Bacteria

First of all, the surface stones are collected from the mine and for bacterial separate ,is sent in genetic researcher lab. In order to create a natural environment for Bioleaching bacteria the stone samples collected from the mine are put in big plastic dishes and they are sprayed by 1/5 normal sulfuric acid every 4 hours a day.

These samples are kept in similar green house at the temperature degree 30˚c. Extracting chromosomal DNA is done by the DNA FAST Kit which is made by National Institute of Genetic Engineering and Biotechnology.

In order to detect those separated bacteria, the gens of the 16 RNA region are duplicated by the PCR set. That PCR production, after doing the electrophoresis, It is send for doing sequence test, and during the sequencing tests, present nucleotide sequences in the duplicated DNA, are determined or sequenced. Those obtained sequences are compared by the existing sequences which are in the genetic data base by the Blast program, and then by using these data Blast, the bacteria are detected by point of molecule.

2.2. Adaptation of Bacteria

Having more than certain amount of heavy metals are poisonous for bacteria, and they prevent the growth of bacteria. Then we must pay attention to the amount of them, and we must adapted the bacteria to silver in order to it haven't any problem for the growth of bacteria and Bioleaching tests.

In order to adapt the bacteria, first of all we use the 0.005g in 100cc culture solution and after have been completed the growth of bacteria, The bacteria sub cultured and 0.01g silver was added to culture and in every time, bacteria sub culturing was added about 0.005g silver to the previous culture. At the end the amount of 0.03g of silver has been detected. In order to prepare 9k bacteria culture ,we added 500 ml distilled water in an 1000 ml flask and then the following substances are added, respectively too.

K_2HPO_4(0.34 g), $Ca(NO_3)_2$ (0.02 g), KCL(0.2 g), $MgSO_4.7H_2O$(0.43 g), $(NH_4)_2SO_4$(1.5 g), $FeSO_4.7H_2O$ (25 g).Then after adding these substances, flask is received to 1000 ml.

2.3. The Bacteria Growth Test

The growth of bacteria is examined by adding the silver. Thus growths of all added substances in adaptation are measured separately. This work is done by measurement every day and then the bacteria growth diagram is drawn which is included bacteria plus seven different amounts of silver nitrate.

2.4. Iron Oxidation Tests

In order to determine the Iron oxidation, at the first standard solutions of 10 Fenantronin ($1H_2O$), Acetatsodium and Feroamonium sulfate ($6H_2O$) is prepared. Then by the help of Feroamoniumsulphate, the standard diagram of Iron is drawn. 50 μ of the sample which is contained bacteria and 5cc of Fenantroine and 4cc Acetat sodium are poured into falkon 50 and after 20 minutes, its OD is read in the wave length of 509 nanometer by the help of spectrophotometer.

2.5. The First Bioleaching Tests

In order to study the release Cu and Fe by the separated bacteria, we were used 12 flask with the volume 100cc. Three amounts of 0.01, 0.02, 0.03silver nanoparticles, is selected for doing these tests. Every one of these tests were contained 4 flask and they contained 0.01, 0.02, 0.03silver nanoparticles and 10 g of Nochonestone mines and 5cc *Thiobacillus ferroxidans* bacteria in the 95cc cultural solution which was homogeneous completely. Every one of three tests by having different amounts of silver which are prepared in 4 similar dishes, are removed in the first and second and third week. Three remained flasks of three amounts which were in the twenty eighth day or fourth week, are removed, too. The sediments are separated from the solutions by filter paper after the complete washing of the sediments, then sampling of the remained solution is done and these samples are sent to Atomic Energy Organization for analyzing ICP (Atomic spectrometer). The evidence tests which are included twelve above tests with similar amounts silver, are done without adding any more bacteria. Other evidence tests which are included tests of without silver and by adding bacteria and the tests that any without bacteria and silver are done, too.

2.6. Tests of Percolation Column

After doing shaking flask tests, these tests are done in the higher level volume and in the semi-test condition and in the percolation column. In these columns is prepared the suitable environment condition for the growth of bacteria and amount of 0.03 silver is added to the columns of containing silver. The column tests were containing four columns with 7cm the diameter. The weight of the stones by the diameter of 400-500 micron particles were 2400 g. Too, amount of 3700cc distilled water is added to the system bins and the pH of solution is lowered by 3cc of acid and spraying is done by the speed of 5 lh/m^2 in every column. The pH is measured every day and if it goes higher than 2, then it is lower by adding twice Sulfuric acid. For preparing the first column, amount of 25cc bacteria in 475cc distilled water is dissolved, and this solution is added to the column from the above of it. The bacteria are stayed in the same position for 5 days in order to adhere or join on the stone. Then 3700cc distilled water and 58.25cc silver solution %1 is added to the column. The second column is contained the same amount of bacteria without adding any silver and the third column is contained silver without adding any bacteria. In order to compare the results, in the forth column, we don't add any of the bacteria and silver because of we want to measure the influence of every factors.

3. Results and Discussion

After separating, the bacteria are observed by the fluorescent microscope and then they are observed rod-shaped bacteria (Fig 1).

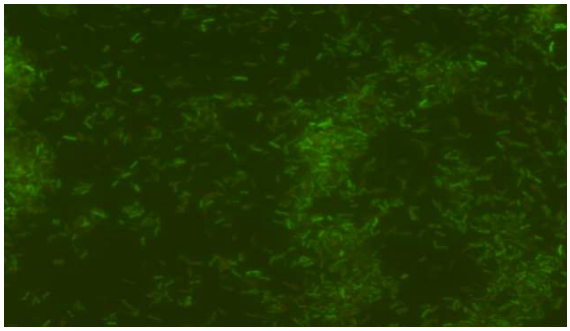
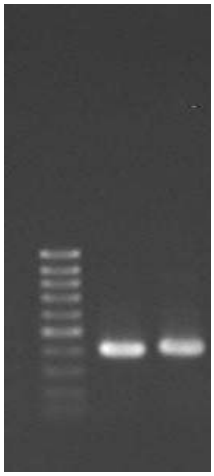


Fig. 1. *thebacterias are observed by the fluorescent micros cope.*

The result of electrophoresis of the PCR products, on the agarose gel %1 has shown the length of 500bp bound. The compared result of this sequence with the other available data in the world wide bank has shown that this bacteria is *Thiobacillus ferroxidans* (Fig 2).



500 bp

Fig. 2. *The result of electrophoresis of the PCR products, on the agarose gel %1.*

3.1. The Tests of Bacteria Growth

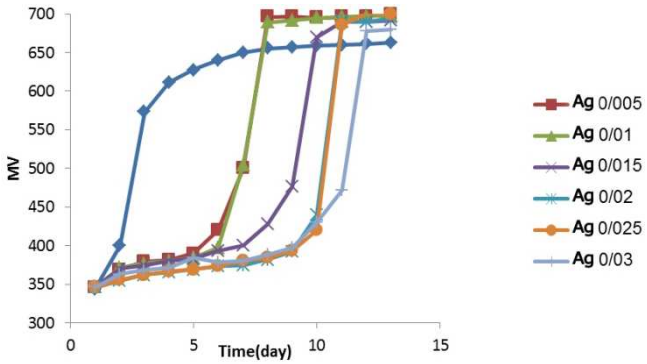


Fig. 3. *The tests of bacteria growth.*

In the growth tests had shown that whatever the more amount of silver was added, the time of starting the growth of bacteria was more delayed (Fig. 3).

3.2. Iron Oxidation Tests

Iron oxidation tests had shown that in compare with the standard bacteria DSM 583, it had more quickly growth. The result had been shown in diagram, too (Fig. 4).

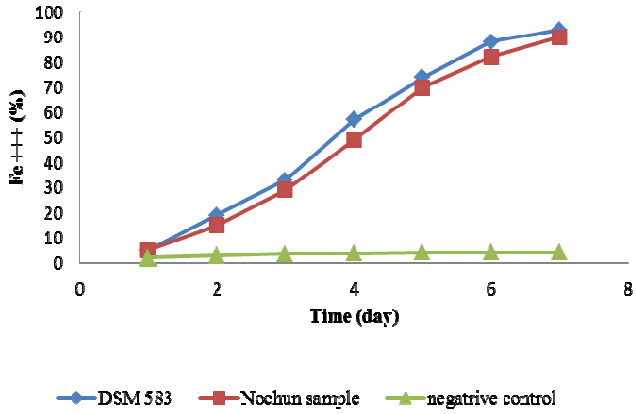


Fig. 4. *Iron oxidation tests.*

3.3. The Early Bioleaching Tests

The following diagram is shown the tests in three amount of silver without any bacteria. As it has been shown in the diagram, by adding more the amount of silver, the efficiency of releasing copper is increasing more, too (Fig. 5).

As it has been shown in the diagram, in the Bioleaching tests, by adding silver, the efficiency is increasing more, considerably. The first diagram which is related to the Bioleaching test without adding silver (Fig. 6) and the next diagram is related to the adding three amounts of silver (Fig. 7).

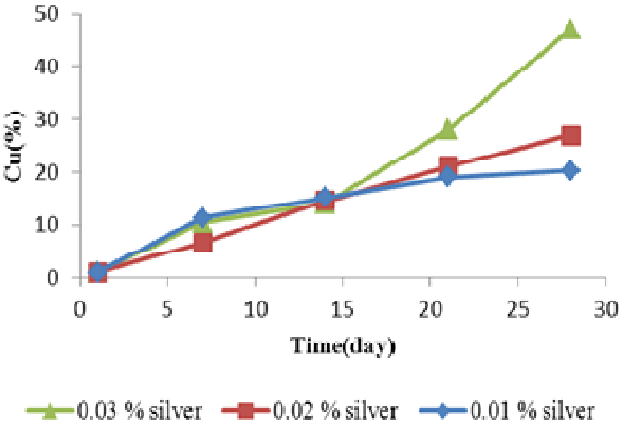


Fig. 5. *The early Bioleaching tests.*

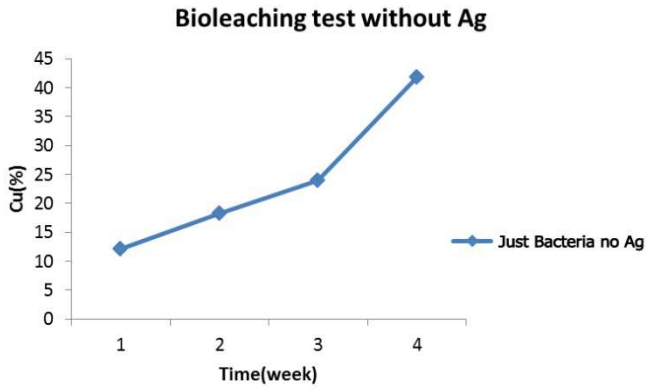


Fig. 6. *The early Bioleaching tests.*

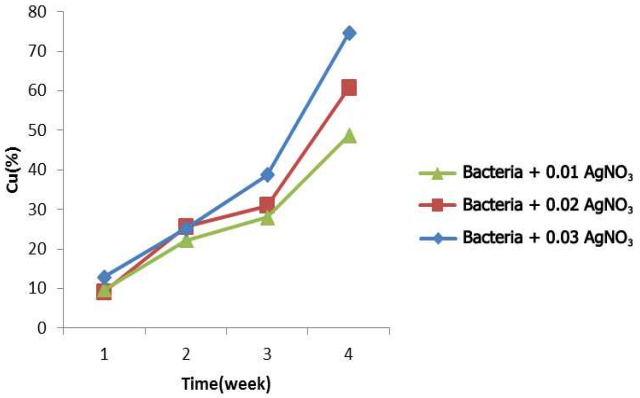


Fig. 7. *The early Bioleaching tests.*

3.4. The Column Results

In every column the previous result, are repeated and by adding the silver, releasing the Copper is increased, too (Fig. 8).

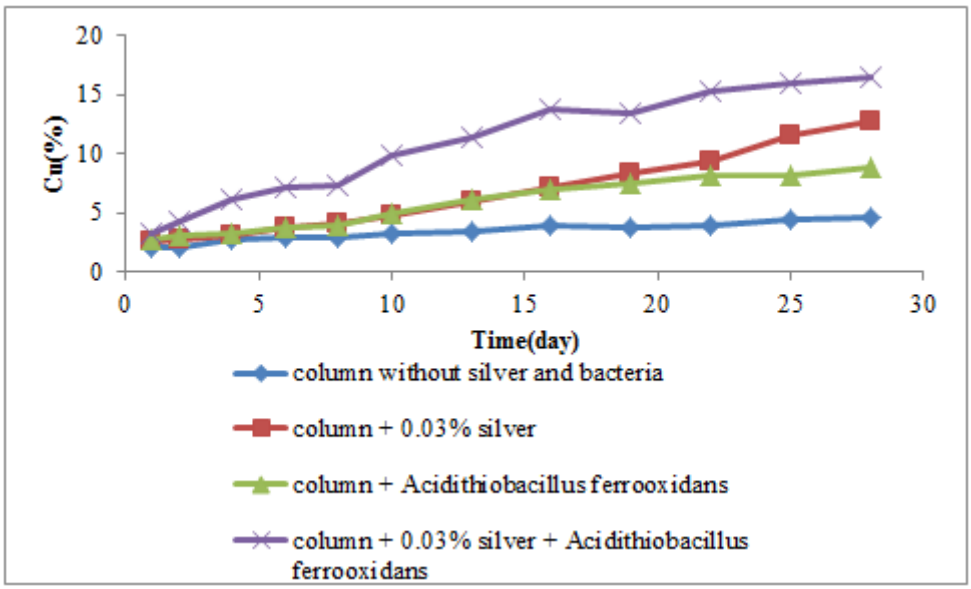


Fig. 8. *The column results.*

4. Discussion

Noadays, the procedure of ecoleaching (Bioleaching) has a special position in extracting metal technologies from mines, and many investigations in this field are doing now. Bioleaching (ecoleaching) is a suitable and economical technology for taking care of special mineral stones, according to the laboratory scale and semi-industry which is doing now, the noticeable amount of Bioleaching units in comparison with the developing countries (such as Iran) are establishing and working now. The reason of this subject is related to the simple operations and early cost investment for operating these units. In accordance with the fact that Iran has noticeable resources of valuable metals such as Copper and gold, the investigation in this field is very important. Having finding new microbial species by separating from mineral stones and studying the efficient parameters in the Bioleaching procedure, these stones will cause to progress this technology in the country. Therefore, first of all in this investigation, collecting the samples and species of *Thiobacillus* were separated suitably from Nochon mines, and after detecting those molecular factors, the effective factors on leaching procedure were evaluated, and finally the leaching in percolation system were evaluated, too. Comparing the exploiting methods of metals, especially Copper extraction, which has more mine in our country, has shown that the Bioleaching method has little damages on eco-environment, because this method doesn't need to consume more energy and those harmful gasses such as sulfure dioxide which are produced by the other methods, are not produced. The necessity which causes the detection of domestic mine bacteria was this, that the activity of every bacteria in its local region is more than non-local bacterias. The present of *Thiobacillus ferroxidans*and controlling of this bacteria in Bioleaching procedure, cause to produce Acid

higher than usual, and it has good influence on this economical procedure. This method is used for those stones which the exploiting of Copper from them is not profitable by previous methods because of low purity and assay, this method has coordination with the eco-environment and it is cost-effective. But this method needs too much time. Extraction of Copper from those composed Hypes is lasted several months, and for this reason this need is felt that some preparation activities must be considered to increase the efficiency of this method. One of these ways which were used in this project, was adding the silver compounds. The results showed that the released Copper in the presence of silver is increased, and by the noticeable achievements which were obtained in the laboratory site, the column tests for doing Bioleaching at a high and semi-industrial level were performed. The results were repeated in columns, too and they showed that adding silver cause to increase efficiency of the Bioleaching and then cause to increase the releasing the Copper.

References

[1] Najafi M. the series of articles from the first seminar about mine and related sciences, Azad university press of Tabas 1385.

[2] Rahimi N. procedures in using coal in power plants, the series of articles about strategic of the energy1376; vol. 1.

[3] Shokofeh N. Protecting of eco-environment in mines, eco-environment protection organization publicate1382.

[4] Orei and Tarigi. Benefitting of Iran coal mines Amir Kabir investigation and scientific publication1378; year 11, No. 41.

[5] Neale J. Bioleaching technology in minerals processin. Mintek, Biotechnology Division 2006; p. 110-115.

[6] Davis B, Carol S Nicolle J and Paul R .Ferrous iron oxidation and leaching of copper ore with halotolerant bacteria in ore columns. Hydrometallurgy 2008; 94(1–4): p. 144-147.

[7] Lundgren D G and Silver M. Ore leaching by bacteria. Annu. Rev.Microbial 1980; 34: p. 263-283.

[8] Munoz DB and Dreisinger WC. Silver- catalyzed bioleaching of low-grade copper ores, Part III Column reactors, Hydrometallurgy 2007; 88 P:35-57

[9] Dee Jay F, Debby F, Karen SM and Daphne LS. Evaluation of a Fluorescent Lectin-Based Staining Technique for Some Acidophilic Mining Bacteria. Applied and enviromental microbiology 2000; 5(66): p. 2208-2210.

[10] Barrie D and Jonhnson N O. Effect of temperature *on* the bioleaching of chalcopyrite concentrates containing different concentration of silver. Hydrometallurgy ; 2008 Vol 94 p:42-47.

[11] Foucher S, Battaglia-Brunet F, Hugues P, Clarens M, Godon JJ and Morin D. Evolution of the bacterial population during the batch bioleaching of a cobaltiferous pyrite in a suspended-solids bubble column and comparison with a mechanically agitated reactor. Hydrometallurgy 2003; 71(1–2): p. 5-12.

[12] Rawlings DE. Heavy metal mining using microbes. Annu Rev Microbiol 2002; 56: p. 65-91.

[13] Chen Z and Zhong L 2002. Growth Kinetics of Thiobacilli Strain HSS and Its Application in Bioleaching Phosphate Ore. Industrial & Engineering Chemistry Research 2002; 41(5): p. 1329-1334.

[14] Ac¸ıkel a, Ü,.Ers¸ana M. and Sag˘ Ac Y. Optimization of critical medium components using response surface methodology for lipase production by Rhizopus delemar. food and bioproducts processing. Microbiol 2010; 8 8: p. 31–39.

[15] Rohwerder T and Gehrke T. Bioleaching review part A: progress in bioleaching: fundamentals and mechanisms of bacterial metal sulfide oxidation. Appl Microbiol Biotechnol 2003; 63(3): p. 239-48.

[16] Gardner M.N, Deane S M. and Rawlings D E. Isolation of a New Broad-Host-Range IncQ-Like Plasmid, pTC-F14, from the Acidophilic Bacterium Acidithiobacillus caldus and Analysis of the Plasmid Replicon. Journal of Bacteriology. 2001; 183(11): p. 3303-3309.

[17] Kelly D P and Wood A P. Reclassification of some species of Thiobacillus to the newly designed genera Acidithiobacillus gen gov .,Halothiobacillus gen. gov. and and Thermithiobacillus gen. gov. International journal of systemic and evolutionary microbiology 2000; 50: p. 511-516.

[18] Watling H R . The bioleaching of sulphide minerals with emphasis on copper sulphides - A review, Hydrometallurgy 2006; 84(1–2): p. 81-108.

[19] Watling HR. The bioleaching of sulphide minerals with emphasis on copper sulphides - A review, Hydrometallurgy 2006; 84(1–2): p. 81-108.

[20] Xia Jin-lan, Peng An-an He, huan Yang yu, LIU, Xue-duan QIU and Guan-zhou.. A new strain Acidithiobacillus albertensis BY-05 for bioleaching of metal sulfides ores. Trans.Nonferrous Met.Soc.China 2007; 17: p. 168-175.