

Bioleaching of Pyrite Ores Using Facultative Anaerobes



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ABSTRACT

The study of bioleaching using anaerobic bacteria and fungi at different physico chemical parameters was estimated using heterotrophic microorganisms. The incubation period, pH, temperature and ore concentration was performed to measure the ions released from the Mn, Cu, Pb & Fe ores during the process of growth of the organism from 24 hours to 144 hours. The results for the order of affinity showed as Pseudomonas aerogens: Cu>Fe>Mn>Pb, E.coli: Cu>Fe>Mn>Pb, Aspergillus niger: Fe>Mn>Cu>Pb and Trichoderma sp.: Mn>Cu>Pb>Fe. The incubation time was found for the bacteria more effective between 72- 96 hours and for the fungal organism as 120 hours. The pH was observed between 7 for bacteria and 5 for fungal organism as maximum for bioleaching.

Introduction

Worldwide reserves of high-grade ores are diminishing at an alarming rate due to the rapid increase in the demand for metals. However there exist large stockpiles of low and lean grade ores yet to be mined. The problem of recovery from metals using conventional techniques is very expensive due to high energy and capital inputs required (Kandemir 2004). Another major problem is impact on the environment due to high level of pollution from these technologies. Environmental standards continue to stiffen, particularly regarding toxic wastes, so costs for ensuring environmental protection will continue to rise. With increase in industrialization coupled with population growth, the demand of metals has increased and is likely to go up further in years to come. This has resulted in irreversible impacts on diminishing high-grade ores with simultaneous generation of solid wastes and effluents containing metals. It is thus important to tackle the problem for control of pollution and recovery of metal values in a cost-effective method. Bioleaching is adopted today as the valuable process for the recovery of harmful metals from soil and water. It is also a process considered to be less expensive novel practice and has proved to be very fruitful for the environment in the extraction of heavy metals. (Satrupa et al 2015).

The metals from earth crust can be recovered by two different methods (1) bioleaching by microorganism and (2) biosorption using microbial biomass. There are two ways **the bacteria which are naturally associated with the rocks can lead to bioleaching are viz., (i)** direct action of bacteria on metal ores oxidize them and solubilize to extract; and second method (ii) indirect action of bacteria produce enzymes that oxidizes ferrous ions to ferric ions. The ferric ions are final oxidizing agent bio-regenerate ferric sulphate. It enhances the bioleaching sys-

tem in absence of oxygen and with the help of bacteria chemical substances such as sulfuric acid and ferric iron which extract the metal (indirect action) (Cvetkovska et al 2011). Bioleaching has been a valuable metal recovery process due to less expensive and eco-friendly method. Microbial leaching of non-sulphide ores that contain no energy to support the growth of the microbes is a great challenge (Gregory J Olson, 2001, 1992).

The organism plays an important role in recovery of heavy metal from industrial waste are majorly of acidophilic group. The acidic pH (2.0-4.0) help the metals dissolve from solid phase to aqueous phase. *Thiobacillus ferrooxidans*, *Sulfolobus* sp., *T. thiooxidans* are commonly used organisms. Fungal organisms like *Penicillium* sp. *Aspergillus niger* also helps effectively in metal leaching process (Debraj,2010). The thiobacilli and chemolithoautotrophic species which uses carbon dioxide from the atmosphere to synthesize its cellular materials has been commonly used. *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, helps in rapid oxidation of elemental sulphur to sulphuric acids that reduces the pH of the medium between 1.5-3.0. (Bosecker, 1997). The industrial wastes, spent petroleum, catalysts, electronic scraps, sewage sludge, fly ash etc. majorly produced contains Ni, Co, Cu, Mo, Pb, Zn & Cr like metals. (Cui et al, 2008, Marafi et al, 2008, Kucha & Mayer, 1996).

Takayanagietal (1996), isolated thermophilic organism *Sulfolobus hakonensis* from a geo thermal area in Hakone in Japan. The isolate was observed an aerobic, facultative chemolithotrophic organism that grew on SO and reduced sulphur compounds optimally at pH 3.0. These are usually found associated with other sulphides or oxides other metal ores in either refined quantity or as a major component. For the successful rate of the recovery various parameters can be optimized like physico-chemical and

biological factors, nutrient, oxygen, carbon, temperature, pH, incubation period, solid-liquid ratio, detention of microorganisms to the metal ions and period of bioleaching (Satrupa *et al* 2015).

For our experimental study we have selected four different facultative anaerobic microorganism that could grow on reduced oxygen medium. The bacterial strains selected was *Escherichia coli* and *Pseudomonas aerogens*. Another two fungal strains *Aspergillus niger* and *Trichoderma reesei* was used for bioleaching.

Materials & Method

The thioglycolic acid acts as an oxygen absorbing agent resulting in anaerobic conditions for sodium thioglycolate medium. The major composition used (g/L): L-Cysteine 0.5g, Sodium Chloride 2.5g, Yeast extract 5g, Dextrose 5.5g, Peptone 15g, Thioglycolic Acid 1ml. The experiment was carried out for different concentrations of ore (%): 0.5, 1.0, 2.0, 5.10, 15 & 20 in 100ml broth. Different incubation period, optimum pH and temperature was performed to evaluate maximum ion recovery during the process of bioleaching of Fe, Cu, Pb & Mn ores.

Collection of Ores:

Four types of pyrite ores was collected for the experiment from, the Ajjanahalli mines in Tumkur district, Karnataka, India.

Microorganisms: *Pseudomonas aerogens*, *Escherichia coli*, *Aspergillus niger* and *Trichoderma reesei* were selected for the experiment.

Effect of various ore concentration:

The 100ml thioglycolate broth was taken in 250ml conical flasks for the different concentrations of ore viz., 0.5%, 1%, 2%, 5%, 10%, 15% & 20%. Bacteria was incubated at 37°C for 24-144 hours and fungi at room temperature. On completion of incubation periods bacterial cell was centrifuged at 7000 rpm for 15 minutes at 4°C. The supernatant was collected for further estimation and biomass was discarded. For the fungal organism the cell was homogenized. The cell suspension was centrifuged at 7000 rpm for 15 minutes at 4°C. The supernatant was collected for further estimation.

Effect of Incubation period on final ore recovery:

The bacteria and fungi was incubated with 10% ore concentration for 24, 48, 72, 96, 120 & 144 hours.

Effect of pH on final ore recovery:

The experiment was set up for optimizing different pH ranging from 5, 6, 7 & 8 with 10% ore concentration for bioleaching using all the microorganisms including bacteria and fungi.

Effect of temperature on final ore recovery:

Four different temperature was used to optimize the maximum recovery from 10% of ore concentration by the microorganism viz., 25°, 30°, 37° & 40 ° C.

ANALYSIS OF ORES: The Standard solution (100mg/ml) was prepared by dissolving 5g of standard salt (FeSO₄, PbSO₄, CuSO₄, MnSO₄) in 50ml distilled water (BIS, 2004).

CHEMICAL ANALYSIS OF ORES:

Iron Ion: The iron ions when treated with 10% potassium thiocyanate a blood red coloured ferrocyanate was formed. Whose Optical density was measured at 680 nm. 1ml of the prepared aliquots in different test tubes. Added 0.5ml potassium thiocyanate and shaken well till coloration was uniform and OD was measured.

Copper Ion: The copper ions already have a faint blue color. The addition of ammonium hydroxide (0.1N) deepens the faint blue colour enabling measuring its OD at 600 nm. In different

test tubes 1ml of the prepared aliquots was taken. Added 5ml of ammonium hydroxide. Incubated in dark for 10 min. Measured OD at 600 nm.

Lead Ion: The lead ion form a yellow compound of lead iodide when treated with potassium iodide. Since the solubility of lead iodide is low, the solution must be chilled to avoid precipitation. Take 1ml of the prepared aliquots in different test tubes. Add 1ml potassium iodide. Shaken well and incubated in ice water for 5 mins and OD of yellow color formed at 640 nm was measured.

Manganese Ion: Manganese ions when oxidized in acidic conditions yield a brown colored compound of manganese oxide. Whose optical density can be measured at 680 nm. Take 1ml of the prepared aliquots in different test tubes. Add 0.1ml HCl and shaken well. Added 1ml of 10% K₂Cr₂O₇ and shaken till uniform brown coloration appear. Measured OD at 680 nm.

Result & Discussions

The batch process for the bioleaching of metal ores was performed for heterotrophic bacteria and fungi. To recover maximum amount of ions during bioleaching different ore concentrations under optimum conditions of pH, temperature and incubation period was recorded. The ore concentration ranging from 0.5% to 20% was measured for every 24 hours of incubation including bacteria and fungi. The fungi was found to be more effective in comparison to bacteria as the mycelial structure of fungi promoted better bioleaching. At 15% of initial ore concentration addition in the medium was found to be higher recovery in all the microorganisms. The ore concentration was measured in terms of ions released gram equivalent weight of the metal. The bioleaching by bacteria was found to be higher for copper as in *E.coli* and *P. aerogens*. *Aspergillus niger* released Fe>Mn>Cu>Pb with 15% initial ore concentration (Table 1). According to the trend the nature and nutrient requirement of the organism shows the intensity of bioleaching of ores. *Trichoderma reesei* data represents the maximum efficiency in comparison to other fungi and bacteria. Increase in alkaline leaching can be enhanced in presence of organic nitrogen (protein, amino acids, purines, pyrimidines, etc.) as a result of deaminations yielding in the aqueous solution ammonia that converts into ammonium hydroxide by the catalytic action of heterotrophs. Autotrophic organism have high requirement for compounds such as NADPH to reduce their carbon source (CO₂) to produce sugars, nucleotide and amino acids and other molecules from which new cell mass is synthesized whereas heterotrophs group do not demand high for NADPH as their carbon source is more reduced than CO₂ & H atoms from their source of nutrition (Brauser, 2012). Some other metals like copper, cobalt, and zinc, forms compound (NASA, 1992), by ammonium hydroxide. The incubation period was optimized from 24 hours to 144 hours where 96 hours represents more ore release for bacteria and 120 hours for fungal organisms. This may be due to the utilization of the organic matter to produce other acidic products with a higher metabolic rate. The fungal organism with the increased incubation period produced higher biomass hence the rate of utilisation was prolonged and due to the acidic nature of the medium the ore concentration was found to be higher in *A. niger* and *T. reesei*. (Agatzini, 2003) (Table 2).

Table 1: Bioleaching by organisms at various concentrations of ore.

Microorganism	Ore Con (%)	Amount of Ions released (g eq)			
		Fe	Cu	Mn	Pb
<i>E. coli</i>	0.5	0.05	0.12	0.08	0.03
	1	0.15	0.18	0.1	0.08
	2	0.3	0.5	0.4	0.25
	5	0.6	0.8	0.7	0.4
	10	0.7	1.1	1.0	0.5
	15	1.1	1.4	0.91	0.63
	20	0.85	0.96	0.7	0.42

Microorganism	Ore Con (%)	Amount of Ions released (g eq)			
		Fe	Cu	Mn	Pb
<i>Pseudomonas Aerogens</i>	0.5	0.1	0.13	0.05	0.08
	1	0.22	0.16	0.15	0.2
	2	0.4	0.7	0.5	0.6
	5	0.8	0.9	0.8	0.7
	10	0.9	1.0	0.9	0.8
	15	1.13	1.15	1.06	0.89
<i>Aspergillus sp.</i>	0.5	0.01	0.02	0.1	0.07
	1	0.03	0.03	0.15	0.1
	2	0.05	0.1	0.2	0.14
	5	0.85	0.85	0.95	0.8
	10	1.2	0.95	1.3	1.0
	15	1.56	1.1	1.26	0.92
<i>Trichoderma sp.</i>	0.5	0.02	0.04	0.02	0.1
	1	0.05	0.06	0.07	0.16
	2	0.1	0.06	0.15	0.2
	5	0.65	0.95	0.75	0.75
	10	1.0	1.2	1.2	1.2
	15	1.12	1.28	1.32	1.27
20	0.98	0.94	0.87	0.79	

The non-enzymatic reduction of pyrolusite [Mn (IV)] oxides to +2 oxidation state by *Pcitrinum* from the top soil isolated from Joda east Manganese mine area, India was observed due to the metabolites such as oxalic acid and citric acid. They reported 64.6% of maximum solubilisation of Mn with a particle size of -45um ore at the pulp density (w/v), sucrose concentration 10% (w/v) and inoculum size (10% v/v) for an incubation period of 30 days. (Acharya, 2004). Under similar acidic conditions Mukherjee 2003, added reducing agents like HCl, H₂SO₄ etc. to enhance the oxidation activity and the recovery was also increase to 25% Co, 25% Ni, 40% Cu, 10% Mn and 40% Fe at pH 2.0 during the bioleaching of ocean nodules by *Bacillus* sp. (Agatzini, 2003).

Many other scientists have reported pH as a major influencing parameter for bioleaching by any organism. Kibin *et al* 2014 found at low pH acidity increases and this leads to decrease in the oxidising activity of bacteria showing an inhibitory activity. Our experimental result also shows as the increase in pH is adjusted the activity drops but in case of fungi it increases.

Figure 1 a, b, c, d shows another important physical parameter was temperature. At 25°C and 30°C the efficiency was maximum in fungi as due to low pH effect and to be the optimum the heat generation in the medium increases the rate of solubilisation. But the bacteria shows slow and low release of ions at lower pH but at 30-37°C it has produced more ions into the medium.

Table 5.2: Effect of incubation time on Bioleaching of ores

Microorganism	Incubation time (hr)	Amount of Ions released (g eq)			
		Fe	Cu	Mn	Pb
<i>E. coli</i>	24	0.1	0.6	0.4	0.3
	48	0.6	0.8	0.7	0.4
	72	0.65	0.9	0.9	0.7
	96	0.68	0.95	1.0	0.75
	120	0.79	1.14	0.97	0.81
	144	0.64	0.94	0.84	0.65
<i>Pseudomonas Aerogens</i>	24	0.2	0.8	0.5	0.2
	48	0.4	0.9	0.8	0.7
	72	0.7	1.0	1.0	1.0
	96	0.7	1.0	1.1	1.1
	120	0.69	1.14	0.87	0.94
	144	0.61	0.98	0.72	0.63
<i>Aspergillus sp.</i>	24	0.04	0.3	0.15	0.1
	48	0.8	0.85	0.95	0.8
	72	1.2	1.2	1.8	1.6
	96	1.6	1.3	2.2	2.2
	120	2.01	1.41	2.38	2.27
	144	1.86	1.17	2.12	1.91

Microorganism	Incubation time (hr)	Amount of Ions released (g eq)			
		Fe	Cu	Mn	Pb
<i>Trichoderma sp.</i>	24	0.01	0.1	0.2	0.05
	48	0.5	0.95	0.75	0.75
	72	1.0	1.15	1.6	1.8
	96	1.5	1.6	2.3	2.5
	120	1.89	1.95	2.62	2.63
	144	1.47	1.62	2.27	2.19

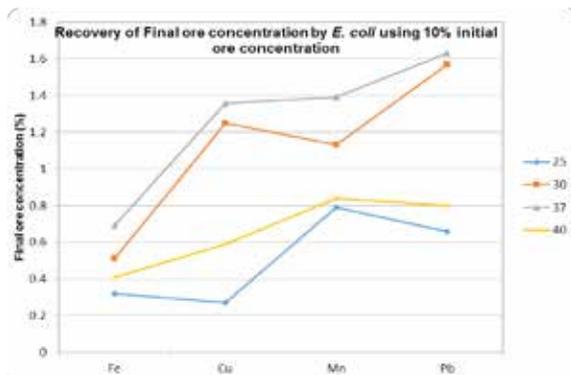


Fig. 1 a: Recovery of ore by *E.coli* at pH 7.0 and 10% ore concentration after 72 hrs of incubation.

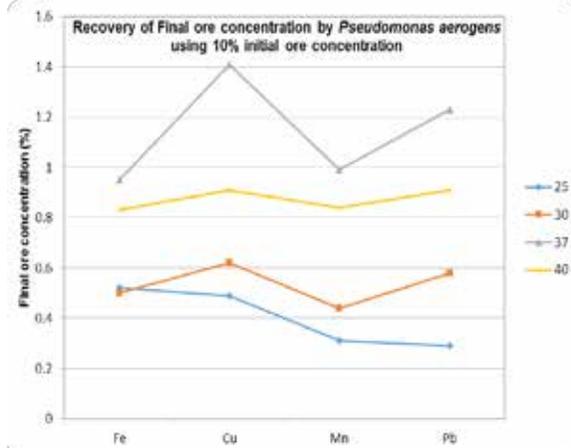


Fig. 1 b Recovery of ore by *P.aerogens* at pH 7.0 and 10% ore concentration after 72 hrs of incubation.

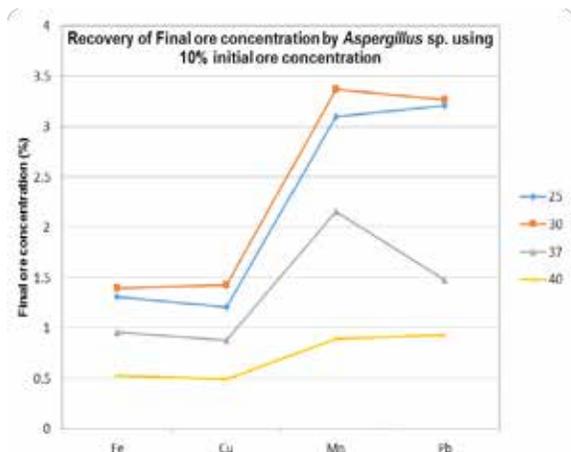


Fig. 1 c: Recovery of ore by *Aniger* at pH 5.0 and 10% ore concentration after 120 hrs of incubation.

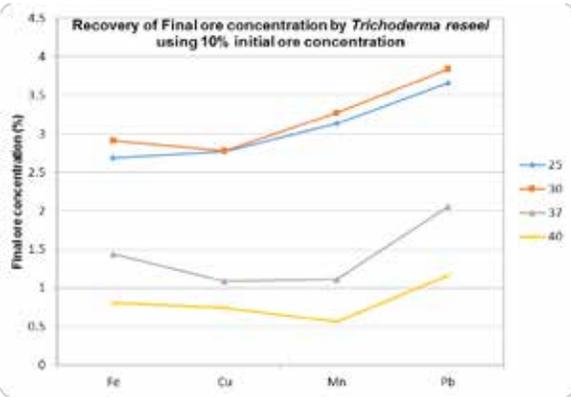


Fig. 1 d: Recovery of ore by *T. reesei* at pH 5.0 and 10% ore concentration after 120 hrs of incubation.

Table 3 represents that bacteria shows higher amount of recovery at pH 7 considered to be the optimum condition and for the fungi pH 5 shows an increasing trend. In 2009, Anjum *et al.* recovered metals from low grade black shale ore using *Penicillium notatum* supplementing various organic wastes as substrates. The results showed 49.29% Cu, 53.51% Co, containing glucose and molasses, & 9.11% Zn, in acidified mango peel medium.

Recent report shows the effect of pH (2-9), H₂O₂ (0.5-6g/L), Fe concentration (0.5-4g/L) and leaching time as (5-60 mins) by Fenton method for Zn, Cd, Pd & Cu from waste water sludge, the yield in the form of recovery was measured as 92%, 100%, 100% & 80%. The leaching was completed in 15 mins. (Azhdarpoor 2015.) Figure 2 indicates the gradual increase in the ore concentration increases the yield in all the strains but after 5% till 20% the activity changes from organism to organism. In spite of all the optimum conditions the complete recovery was observed after 96 hours. The yield obtained at 2% ore concentration for copper by *E.coli* was 25% & 35 % by *P. aerogens* but eventually, 5% was maximum for Mn by *T. reesei* and *A.niger* 10% was the ore concentration supplemented to yield 34% & 32% respectively. The nutrient utilization by the organism is depleted due to the inoculum level maintained constant with the increase in ore concentration due to lesser biomass the yield was decrease with increase in the ore concentration.

Table 3 : Effect of pH on release of ions by *E.coli*, *P. aerogens*, *A niger* & *T. reesei*

Micro organism	Amount of ions released (g eq)				
	pH	Fe	Cu	Mn	Pb
Escherichia coli	5	0.42	0.76	0.73	0.31
	6	0.51	0.89	0.77	0.38
	7	0.83	1.17	1.09	0.76
	8	0.32	0.39	0.43	0.82
Pseudomonas aerogens	5	0.46	0.51	0.4	0.36
	6	0.84	0.9	0.89	0.36
	7	1.16	1.24	1.19	0.65
	8	0.91	0.94	0.87	0.96
Aspergillus niger	5	1.55	1.62	1.25	1.2
	6	1.46	1.13	1.36	0.79
	7	1.08	1.05	1.06	0.94
	8	0.83	0.42	0.96	0.73
Trichoderma reseei	5	1.14	1.22	1.17	1.32
	6	1.03	1.36	1.51	1.15
	7	0.59	0.42	0.39	0.48
	8	0.8	0.95	0.79	0.86

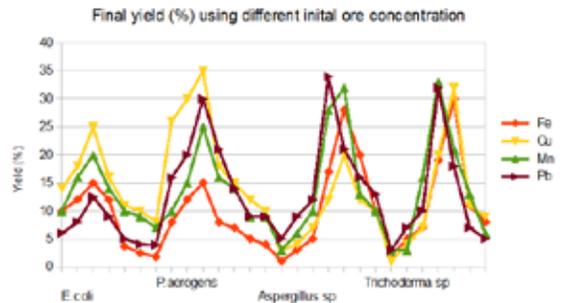


Fig. 2: Estimation of yield by various organisms at 0.5, 1.0, 2.0,5.0,10,15 & 20 (%) Ore concentration.

Conclusion

The present study can be interpreted as the organisms shows recovery under anaerobic conditions. The yield can be improved by genetic engineering so as the organism can withstand the acidic conditions and help in release out of the low grade ores associated with the major ores. The cell wall of the fungal organism if modified will enhance the extraction and recovery process to a great extent which could support for the control of environmental pollution. Biosorption can be cost effective choice for the recovery as the biomass produced can be reutilized by a minimum requirement and can be used as packed bed for remediation of the ore from the compounds.

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