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Research Article

BIOMINING-A PROMISING ECOFRIENDLY TECHNOLOGY

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ABSTRACT

Biomining is nowadays an emerging technology of bioremediation as well as resource recovery. *Acidithiobacillus ferrooxidans* is a gram (-), obligate chemoautotrophic bacteria which is capable of oxidation of metal sulfides like FeS, ZnS etc, thus capable of recovering heavy metals from mining wastes as well as industrial sludge. This study has focused on Zn recovery from the sludge of electroplating industry. Experimental observation reveals that after 7, 14 and 21 days of incubation under shaking and static condition in media containing pure ZnS and ZnS recovered from sludge, maximum efficiency of the bacteria has been found under shaking condition after 21 days of incubation from pure ZnS (45.4%) in comparison to ZnS recovered from the electroplating industrial sludge (26.2%) which indicates that as *A ferrooxidans* is an aerobic bacteria thus maximum bioleaching efficacy depends on aeration, optimum condition, number of bacterial cells in the culture media and purity of metal sulfide.

INTRODUCTION

Biomining is a technique of extracting metals from ores and other solid materials typically using prokaryotes or fungi. The process has also proven to be effective at viably extracting minerals from low grade ore and tailings previously considered unusable. Due to safer and cleaner technology biomining has become one of the most promising totally natural biological processes for is efficacy. These refinement methods need little intervention to initiate, produce no toxic byproducts or emissions, and require no external fuel sources. As heavy metals could be recovered by biomining it reduces the toxicity generated from mine wastes thus biomining and bioleaching is considered as one of the most promising bioremediation technique nowadays. *Bioremediation* is a waste management technique that involves the use of organisms to neutralize pollutants from a contaminated site. According to the United States EPA, bioremediation is a "treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non toxic substances". These processes are especially attractive for low-grade ores and are used on an industrial scale mainly for sulfidic ores. The main aim of this technology is to utilize the biotransformation to decompose the mineral matrix and expose the entrapped valuable metals. biomining process may recover metals including; Fe, Ni, Zn, Co, Sn, Cd, Mo, Pb, An, As, Se etc from low-grade sulfide containing ores. It is cost-effective, use little energy, can function well at low concentration of metals, do not usually produce harmful emissions, reduce the pollution of metal-containing wastes. Generally, biomining comprises two different processes-namely, "bioleaching" and "biooxidation"[1]. Both processes are attractive alternatives to conventional smelting processes, which discharge large amounts of carbon dioxide, sulfur dioxide, and various toxic materials such as arsenic.

In the case of bioleaching, the target metals are solubilized as a result of mineral dissolution. In the case of biooxidation, the valuable metal remains in the solid phase, but becomes enriched. Thus, it can be used as a pretreatment process to degrade minerals sulfides to obtain concentrates. The integrating of bioleaching into mining strategies provides different advantages. The technology enables the extraction of metals from low-grade ores, polymetallic ores of difficult refractory concentrates where traditional methods fail. Further, microbes can help to remove arsenic or other toxic elements from concentrates in an environmentally-stable form[2.] Commercial bioleaching processes of sulphidic ores are well-established and at the same time are relatively easy and reliable with regard to maintenance and infrastructural costs. Bioleaching is therefore attractive, especially for smaller deposits and remote locations. Industrial processes that are currently applied focus on the bioleaching of sulfidic ores are reviewed in[3]. These approaches mainly rely on the use of

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chemolithoautotrophic microorganisms such as *Acidithiobacillus spp., Ferrimicrobium* spp., and *Leptospirillum* spp. that are able to use ferrous iron and/or reduced inorganic sulfur sources as electron donor. In these reactions, sulfuric acid and ferric iron are produced as byproducts, thus contributing to mineral degradation[2]. The organisms are acidophilic and mostly grow within the pH range 1.5-2.0. In this study mainly the bioleaching process have been performed. The industrial waste water mainly sludge released from electroplating industries generally contains high concentration of heavy metals which are toxic pollutants if released but these heavy metals if recovered by bioleaching can be reused and through a clean technology. Traditional mining extractions involve many expensive steps such as roasting and smelting, which requires sufficient concentrations of elements in ores while low concentrations are not a problem for bacteria because they simply ignore the waste which surrounds the metals, attaining good percentage of extraction. Applying microbiological solubilisation processes, it is possible to recover metal values from industrial wastes which can serve as secondary raw materials. Now with the gaining importance this mechanism is being used to recover metals such as gold, copper, iron, uranium. A variety of mineral oxidizing bacteria readily found can easily oxidize iron and sulfur containing minerals. These include the iron- and sulfuroxidizing *Acidithiobacillus ferrooxidans*, the sulfur-oxidizing *Acidithiobacillus thiooxidans* and *Acidithiobacillus caldus* and the iron-oxidizing *Leptospirillum ferrooxidans* and *Leptospirillum ferriphilum* [4, 5, 6]. Several species of fungi can be used for biomining. Experiments have shown, that two fungal strains *Aspergillus niger* and *Penicillium simplicissimum* were able to mobilize Cu and Sn by 65% and Al, Ni, Pb and Zn by more than 95% [7].

Biomining Mechanism: Microorganisms involved in biomining actually gain energy by breaking down minerals into their constituent elements. The mineral dissolution reaction is not identical for all metal sulfides. It have been observed that the oxidation of different metal sulfides proceeds via different intermediates[8]. They proposed a *thiosulfate mechanism* for the oxidation of acid-insoluble metal sulfides such as pyrite (FeS_2) and molybdenite (MoS_2) and a *polysulfide mechanism* for acid-soluble metal sulfides such as sphalerite (ZnS), chalcopyrite ($CuFeS_2$), or galena (PbS). In the *thiosulfate mechanism*, solubilization is through ferric iron attack on the acid-insoluble metal sulfides, with thiosulfate being the main intermediate and sulfates the main end-product. Using pyrite as an example the reactions proposed[8] are:

$FeS_2 + 6Fe^{3+} + 3H_2O \rightarrow S_2O_3^{2-} + 7Fe^{2+} + 6H^+$ eqn (1)

$S_2O_3^{2-} + 8Fe^{3+} + 5\ H_2O \rightarrow 2SO_4^{2-} + 8Fe^{2+} + 10H^+$ eqn (2)

In the case of the *polysulfide mechanism*, solubilization of the acid-soluble metal sulfide is through a combined attack by ferric iron and protons, with elemental sulfur as the main intermediate. This elemental sulfur is relatively stable, but can be oxidized to sulfate by sulfur-oxidizing microbes (Eqns 3-5)[9]

***Effects of Temperature and pH*:** Physical conditions like temperature and pH are although the inherent property of micro-organism and so vary with micro organisms; still it is mainly decided by the dominant bacteria in the consortium like *T. ferrooxidans* and *Leptospirillum ferrooxidans*. The major reason for the dominance of '*Leptospirillum*' and *T. ferrooxidans* in industrial processes is almost certainly the Fe^{3+} / Fe^{2+} ratio i.e. redox potential[10]. However, there may be other reasons which contribute to the dominance. The optimum pH for the growth of *T. ferrooxidans* is within the range pH 1.8-2.5, whereas *L. ferrooxidans* is more acid resistant than *T. ferrooxidans* and will grow at a pH of 1-2[11]. With regard to temperature, *T. ferrooxidans* is more tolerant of low temperatures and less tolerant of high temperatures than is *L. ferrooxidans*. Some strains of *T. ferrooxidans* are capable to oxidize pyrite even at 10°C[12] but 30-35°C is considered to be optimal. an upper limit of around 45°C [12] with a lower limit of 20°C[13] biooxidation processes which are used to treat gold-bearing arsenopyrite ores or concentrates operate at 40 °C and pH 1-6[14]

$MS + Fe^{3+} + H^+ \rightarrow M^{2+} + 0{:}5H_2S_n + Fe^{2+}$ (n=2) eqn (3),
(Metal sulfide)

$0{:}5H_2S_n + Fe^{3+} \rightarrow 0{:}125S_8 + Fe^{2+} + H^+$ eqn (4)

$0{:}125S_8 + 1.5O_2 + H_2O \rightarrow SO_4^{2-} + 2H^+$ eqn (5).

This explains why strictly sulfur-oxidizing bacteria, such as *A. thiooxidans* or *A. caldus*, are able of solution and to leach some metal sulfides but not others. The ferrous iron produced during metal dissolution and biomining resulting metal-enriched solutions are recovered might also be reoxidized by iron-oxidizing organisms to ferric iron.

$2Fe^{2+} + 0{:}5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$ eqn(6)

The role of the microorganisms in the solubilization of metal sulfides is, therefore, to provide sulfuric acid is piled up. (Eqn 5) for a proton attack and to keep the iron in the oxidized ferric state (Eqn 6) for an oxidative attack on the mineral.[9]

$Cu_2S + 2Fe_2(SO_4)_3 \rightarrow 2CuSO_4 + 4FeSO_4 + S$ eqn 7

Chalcocite
$CuS + Fe_2(SO_4)_3 \rightarrow CuSO_4 + 2FeSO_4 + S$ eqn 8

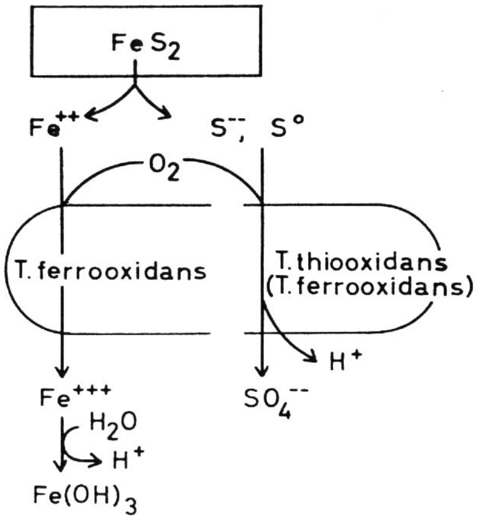
Covellite
Eqn 7 and 8 shows the microbial solubilization process of copper[15]

In case biooxidation of Galena (PbS)-
$PbS + H_2SO_4 + 1/2O_2 \rightarrow PbSO_4 + H_2O + S^0$
and in presence of iron the oxidation is expressed as:
$PbS + Fe_2(SO_4)_3 \rightarrow PbSO_4 + 2FeSO_4 + S^0$

Gold Extraction- During biodischarge of gold in the first stage bacteria catalyses the breakdown of mineral arsenopyrite (FeAsS) by oxidizing the sulfur and metal (arsenic ions) to higher oxidation states and reducing dioxygen by H_2 and Fe^{3+}. This allows the soluble products to dissolve. This biochemical process produce energy for the bacteria. In the second stage, bacteria then oxidize Fe^{2+} to Fe^{3+} and reduce O_2

$FeAsS_{(s)} \rightarrow Fe^{2+}_{(aq)} + As^{3+}_{(aq)} + S^{6+}_{(aq)}$ eqn 9
Arsenopyrite

Acidithiobacillus bacteria: *Acidithiobacillus* are acidophilic, gram negative obligate chemoautotrophs that use elementary sulfur, tetrathionate and ferrous iron as electron donors. They assimilate carbon from carbon dioxide The genus comprises motile, rod-shaped cells that can be isolated from low pH environments including low pH microenvironments on otherwise neutral mineral grains. *Acidithiobacillus ferrooxidans* is commonly found in acid mine drainage and mine tailings. Nitrogen fixation also is an important ecological function carried out by some species in this genus.



Ref. Diagram: Bacterial leaching of ores and other minerals : Feb,1986; R. Naveke Institut fur Mikrobiologie, Technische Universitat Braunschweig, Fed. Rep. Germany.

***Bioleaching**:* The bioleaching process may be of two types direct bacterial leaching and indirect bacterial leaching. In case of direct leaching there should be a physical contact between bacterial cell and mineral sulfide and the oxidation of metal sulfide takes place via several reactions. The reactions are as follows:

$$4FeS_2 + 14O_2 + 4H_2O \xrightarrow{\text{bacteria}} 4H_2SO_4 + 4FeSO_4$$

$$2H_2SO_4 + 4FeSO_4 + O_2 \xrightarrow{\text{bacteria}} 2Fe_2(SO_4)_3 + 2H_2O$$

Investigations[16] have shown that the following non iron metal sulfides can be oxidized by *Acidithiobacillus ferrooxidans* in direct leaching method, like- Covellite (CuS), Chalcocite (Cu_2S), Sphalerite (ZnS), Galena (PbS), Molybdenite (MoS_2), Stibnite (Sb_2S_2) etc.

In indirect bioleaching process the bacteria generate a lixiviant which chemically oxidizes the sulfide mineral and the bacteria donot need to be in contact with mineral. The reaction is as follows.

$$\underset{\text{Metal sulfide}}{MeS} + \underset{\text{lixiviant}}{Fe_2(SO_4)_3} \rightarrow MeSO_4 + 2FeSO_4 + S^0$$

For both direct and indirect bioleaching process the metal sulfides should occur in an acid environment below pH5.0
In case of zinc sulfides the reaction is

$$ZnS + 1/2O_2 + H_2SO_4 \xrightarrow{\textit{Thiobacillus ferrooxidans}} ZnSO_4 + H_2O + S$$ [17]

$$ZnS + 4Fe_2(SO_4)_3 + 4H_2O \xrightarrow{\textit{A ferrooxidans}} ZnSO_4 + 8FeSO_4 + 4H_2SO_4$$

Wastes generated from electroplating industries are heavily polluted with heavy metals, acids, cyanide and other toxic chemicals. Mainly these industries release waste water, acids and hydroxide sludge which can also be considered as low grade metal resource. In this experiment Direct bioleaching process is studied.

Experimental

***Sampling Site**:* Low grade deposits like sludge or industrial waste may be sources of many minerals which can be recovered through biomining. The sampling site was an electroplating industry located at Gouribari lane, Kolkata-700004, where mainly zinc plating is performed on iron rods and other metal equipments. Thin films (0.3 to 0.7 mm thickness) of zinc coating on iron, steel and other metal equipments provides protection against atmospheric or indoor corrosion and it is cost effective than nickel or chromium coating. Bright zinc (bleached chromate conversion) which is commonly used also provides a decorative finish. Approximately 200 litres/day of waste water is generated from the industry which is treated by using caustic soda 10kg/day.
In this experiment the Direct bioleaching process has been studied. All the chemical parameters of waste water were analysed following the standard techniques[18] and the characterization of sludge was done by AAS method and Cyanide by Aldrige method.

The results of chemical analysis both treated and untreated waste water of New Tara electroplating Industry is described in Table 1 and the chemical character of the sludge is given in Table 2.

METAL RECOVERY METHODOLOGY

Study in our laboratory revealed that the sludge precipitation completes when the pH rises to pH 3. The untreated waste water is highly acidic (pH=0.25) and 10 ml of waste water generates 0.591gm sludge (dry weight) which is rich in heavy metals. 58.08 gm of ZnS can be recovered from 100gms of slugde although not in pure form. As the concentration of zinc and iron was maximum in the sludge the recovery of these two metals were cost effective. This experiment was concentrated on zinc recovery. The procedure is described below-

Digestion of sludge sample: For the estimation of heavy metal concentration in sludge sample it was first digested, then the heavy metal concentration was estimated by AAS method. 0.5gm of dried sludge was dissolved in 10ml of concentrated nitric acid. The solution was heated in 80^{o}C for 5 minutes. When the sludge sample was totally dissolved in acid the solution was cooled and volume was made upto 50ml by adding distilled water.

To isolate Zn from the sludge the following steps were followed.

Step1- Zn was first separated as Na_2ZnO_2 from other heavy metals in the sludge and the concentration of Zn was estimated by AAS in mg/l.

Procedure: 0.5gm dry sludge sample was fused with 2gmNaOH in a nickel crucible. After cooling the melt it was acidified with slow addition of 15ml conc. HCl. Then 5ml 10(v) H_2O_2 was added and warmed gently to maximum temperature of 60^{0}C. diluted to 250ml. 50ml solution was taken from it and 2gm NH_4Cl was added. NH_4OH was added until smell ammonia. The solution was filtered and the residue was rejected. 10ml NaOH 1(N) was added with 5ml 10(v) H_2O_2 into the filtrate. The solution was filtered and the filtrate is Na_2ZnO_2. The conc. of Zn in filtrate was measured by atomic absorption spectrophotometer .

Step2- the filtrate was acidified by acetic acid and H_2S was passed, white precipitate appeared i.e, Zn precipitated as ZnS

58.08 gms of ZnS was recovered from 100gms of sludge sample although not in pure form. The bioleaching of zinc using *A ferrooxidans* was performed in the in vitro condition using both pure ZnS and ZnS recovered from sludge under both static and shaking condition

Step3- Bioremediation processs

The obtained ZnS by chemical method was not in pure form. In laboratory *Acidithiobacillus ferrooxidans* [strain NCIB 111, the clonal isolate of NCIB 8455, collected from Bose Institute , Kolkata] was used for the bioleaching of ZnS to $ZnSO_4$. This experiment would indicate the efficiency of *A ferrooxidans* to convert ZnS to $ZnSO_4$ and it would also indicate the purity of chemically obtained ZnS.

Procedure of adaptation of A ferrooxidans strain NCIB 111 in laboratory condition

The bacterial strain was first grown in 100ml $N9K^+$ medium (containing $FeSO_4$) for 5 days and 10% of this culture was reinoculated into fresh medium containing elemental sulfur powder. This was repeated twice. After another 10 days of incubation the culture suspension was centrifuged in 12000rpm for 20 minutes. Then the cell suspension was washed with 0.01 (N) H_2SO_4 and recentrifuged in same rpm for 20 minutes. 10% of the obtained cell suspension was added in six experimental set ups containing $ZnS\text{-}9K^+$ media, among which 3 set ups containing pure ZnS and 3 set ups containing ZnS recovered from the sluge of electroplating industry. Similar six set ups were prepared. One of the six set up was kept in gyratory shaker and another was kept in incubator in static condition. The temperature was maintained at 28^0C and pH at 1.3 to 1.5.

Table 3 Iron containing 9K media preparation for *A ferrooxidans*[19]

Solution A(9K)		Solution B (containing Fe)	
$(NH_4)_2SO_4$	**3.0 gm**	**FeSO4, 7H2O**	**44.2 gm**
KCl	0.1gm	Distilled water	300ml(vol. make up)
K_2HPO_4	0.5gm		
$MgSO_4$, $7H_2O$	0.5gm		
$Ca(NO_3)_2$	0.1gm		
Distilled water	700ml (vol. make up)		
10 (N) H_2SO_4	1.0ml		

Sol A was autoclaved at 15 psi for 15 mins and Sol B was sterilized through milipore filter. The final pH was made between 1.3 to 1.5. The supernatant of Sol B was decanted into Sol A. the yellow ppt is to be discarded. This modified 9K medium is used for adaptation of the strain at pH 1.3.

9K medium containing sulfur

1gm/100ml elemental sulfur powder in place of $FeSO_4$, $7H_2O$ added in sol B other compositions all same.

9K medium containing ZnS

0.5 gm/50ml ZnS (either pure or recovered from the sludge) was added in place of elemental sulfur powder in the sol B. other compositions all same.

METHODOLOGY

The bacterial culture was initially adapted with sulfur containing media then used for the bioleaching test. For each static and shaking condition 6 set ups were prepared each containing 50 ml of $ZnS\text{-}9K^+$ media, among which 3 set up contains pure ZnS and rest 3 containing ZnS recovered from sludge. All the set ups were autoclaved at 15 lb pressure for 15 minutes. 10ml of NCIB111 cell suspension was added to each of the above set ups. 6 set up were kept in incubator and rest 6 in gyratory shaker. At regular intervals of 7days one set up containing pure ZnS and one set up containing sludge recovered ZnS were withdrawn to estimate the utilization of ZnS. The % of conversion of $ZnSO_4$ from ZnS was calculated by gravimetric method. The obtained results are given in Table 4A and 4B.

RESULT AND DISCUSSION

The analytical results of chemical parameters of both treated and untreated waste water of the New Tara electroplating industry is given in Table 1.

Table 1 physic-chemical parameters of treated and untreated waste water in comparison to waste water discharge standards of electroplating industry

Parameters	Conc. in untreated waste water	Conc. in treated waste water (in lab)	Discharge standards
pH	0.25	7.0	6.00 to 9.00
Temperature	28^oC	27^oC	Shouldnot exceed 5^oC above the ambient temp of the receiving body
Suspended solids	260.46mg/l	60.2mg/l	100mg/l
cyanides	16.4mg/l	0.11mg/l	0.2mg/l
Total residual chlorine	10.65mg/l	1mg/l	1mg/l
Nickel	7mg/l	0.2mg/l	3mg/l
Zinc	210mg/l	0.5mg/l	5mg/l
Chromium	5.007mg/l	0.04mg/l	2mg/l
Copper	68.923mg/l	0.2mg/l	3mg/l
Lead	4.8mg/l	0.02mg/l	0.1mg/l
Iron	137.5mg/l	5mg/l	3mg/l

Table 1 shows that the untreated waste water is highly acidic, having high concentration of cyanide, residual chlorine and heavy metals like Zn, Ni, Cu, Cr, Pb and Fe. In treated waste water only the Fe concentration is elevated. The results of chemical analysis of the sludge of the electroplating industry is given in Table 2

Table 2 characterization of sludge of New Tara Electroplating Industry, Kolkata

Parameters	Concentration (mg/l)
Zinc	259.2
Lead	5.2
Copper	124.4
Nickel	10.9
Chromium	13.29
Iron	155.9
Cyanide	16.8

Table 2 shows that the concentration of zinc is maximum in the sludge (259.2mg/l). thus recovery experiment of this metal would be viable. Table 4A and 4B shows the obtained results of bioleaching potential of the bacteria.

Table 4A and 4B Conversion % of $ZnSO_4$ from ZnS using *Acidithiobacillus ferrooxidans*

4A: Under Shaking condition

Culture Type	Nature of Media	Days of incubation	conversion % as $ZnSO_4$
NCIB 111 clonal isolate of NCIB 8455 adapted	Pure ZnS containing media	7	15.6
		14	31.6
		21	45.4
	Media containing ZnS recovered from sludge in laboratory	7	10
		14	20.4
		21	26.2

4B: Under Static condition

Culture Type	Nature of Media	Days of incubation	conversion % as $ZnSO_4$
NCIB 111 clonal isolate of NCIB 8455 adapted	Pure ZnS containing media	7	13
		14	31.4
		21	40.2
	Media containing ZnS recovered from sludge in laboratory	7	7.8
		14	14.4
		21	20.6

Table 4A and B shows that the % of conversion from sludge recovered ZnS is almost 50.5% in comparison to conversion from pure ZnS which indicates its impurity. Under optimized condition (pH 1.5; temperature 28^{o}C) and incubation under shaking condition after 21 days of incubation the bacterial culture showed maximum efficiency 45.4% from pure ZnS and 26.2% from ZnS recovered from sludge which indicates that *Acidithiobacillus ferrooxidans* is an aerobic bacteria thus best grown under shaking condition and the efficacy of bacterial cells depends on increase in number of bacterial cells in the culture media which increases with time period and optimum condition.

CONCLUSION

It can also be concluded that this gram negative bacteria can also be used for the treatment of waste water of electroplating industry instead of chemical method but it will be a very slow process and need proper infrastructure.

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