

Biotechnology for Metal Extraction, Mineral Beneficiation and Environmental Control

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Abstract

With the rapid depletion of high grade ores and concerns about environmental degradation, the necessity for utilisation of lean grade mineral resources have become all the more urgent. With the advent of bioleaching since the early 1960's, possibilities of metal extraction in an environment-friendly fashion have emerged. As of now three metals namely copper, uranium and gold are commercially produced around the world using biooxidation in the presence of Acidithiobacillus ferrooxidans.

Bioleaching of base metal concentrates such as those containing copper, zinc and nickel has also been proved to be commercially viable during this decade. Bioreactor technology using thermophilic bacteria holds the key for the successful and efficient bioleaching of chalcopyrite, sphalerite and pentlandite concentrates.

Microorganisms find use in environmental control and mineral beneficiation as well. Microbially-induced mineral flotation and flocculation have been proved to very cost-effective and environment-friendly.

In this paper, biotechnology as applied to metal extraction, mineral beneficiation and environmental control is illustrated.

INTRODUCTION

The microorganism, *Acidithiobacillus ferrooxidans* which is known to be effective in the leaching of several minerals was first isolated in the laboratory in 1947 from the acid mine drainage of bituminous coal mines. Bioleaching processes are now becoming increasingly significant due to rapid depletion of high grade ore reserves, increasing energy costs and environmental concerns.

Large quantities of copper, uranium and gold ores are processed by microbial technology on an industrial scale. The recovery of several other metals are also possible by such methods.

In-situ, dump, heap and bioreactor leaching are the techniques practised in microbial leaching. In particular, heap leaching in the presence of bacteria is a very useful method to recover copper, uranium and gold from low grade ores and tailings. The contribution of bioleaching is estimated to be approximately 15, 13 and 25% of the total world production of copper, uranium and gold. For gold-containing refractory sulfide concentrates, bioreactor technology has been practised around the world (Natarajan 1998).

In this paper, the status of mineral biotechnology in metal extraction as well as future prospects are discussed. Involvement of microorganisms in the generation of Acid Mine Drainage and biotechnological methods of mine pollution control are also illustrated. Microbially-induced mineral flotation and flocculation is illustrated with examples.

Role of *Acidithiobacillus Ferrooxidans* in Leaching Processes

Acidithiobacillus ferrooxidans is the most widely studied and implicated bacterium in the leaching of sulphidic minerals.

Bioleaching is critically dependent on factors such as bacterial concentration, activity and its growth, rate of biooxidation reactions, and iron oxidation ability of bacteria. The activity of *Acidithiobacillus ferrooxidans* and other oxidizing bacteria is often measured indirectly in terms of oxidation rate and monitoring of Eh and pH, while the estimation of protein, nitrogen and carbon content of the cells give a quantitative idea of bacterial activity. Growth of *Acidithiobacillus ferrooxidans* in a copper containing ore suspension has been enumerated by counting the number of colony forming units in the solution and as attached to the ore particles (Rossi 1990).

Development of adapted cells play a vital role in the efficient dissolution of minerals at a faster rate. Bacterial adaptation before bioleaching significantly enhances dissolution rates of sulphides, reduce the lag phase and decrease the solution pH. High metal tolerance can be achieved by the gradual adaptation of bacteria before their use in bioleaching. Bacterial growth is affected by the presence of increased concentrations of heavy metals such as Cu, Zn, Ni and Fe in solution. Adapted strains have been found to be more effective for metal extraction. Thus, an adapted strain of *Acidithiobacillus ferrooxidans* exhibited high copper and nickel extraction from a Cu and Ni concentrate. An enhanced sphalerite dissolution has been observed by using a pyrite adapted *Acidithiobacillus ferrooxidans* strain.

The association of bacteria with several sulphide minerals has also been observed and there is ample evidence regarding the attachment of *Acidithiobacillus ferrooxidans* to minerals. The nature of attachment to mineral surfaces may be physical or chemical. Studies carried out on adhesion of the bacteria to mineral surfaces show that dislodgement of bacteria from mineral surfaces is difficult. Although selective bacterial attachment has been noticed with respect to a low grade waste rock, the attachment was not uniform.

Current Commercial Bioleaching Practices

Major current commercial applications of bioleaching are extraction of copper and uranium from low grade ores and waste ore burden and enhancement of gold recovery from sulphidic, refractory ores and concentrates.

COMMERCIAL COPPER BIOLEACHING

Three methods commercially employed around the world for extraction of copper from sulphide and mixed oxide-sulphide ores are

- In situ leaching or solution mining
- Dump leaching
- Heap leaching

All the above methods have been practiced over the last four decades with advantages such as low cost, suitability for different mines and lean grade ores and environmental acceptability (Brierley 1978, 1982 and Acevedo 1993).

In Situ Leaching

Microbial in situ leaching (or solution mining) can be considered as a unique method of metal extraction from run of mine ores and nonprofitable underground ore bodies. Underground mined stopes can be leached in this manner. Leach solutions are injected into rock cavities and the pregnant solution recovered by pumping back. Selective blasting of rock is practised to gain access to richer regions of mineralization.

The application of in situ leaching depends on the geological and topographical features of the ore body, as well as the hydrological and rock-mechanical properties of the host rock. Feasibility of in situ leaching need be established in the first place with respect to rock permeability and accessibility.

Dump Leaching

Open cast mining operations over a period of time generate huge quantities of waste ore which analyses on an average 0.1 – 0.4% Cu. Such waste ore dumps remain at the mine site and cannot be economically processed for extraction of copper by any conventional methods. Bioleaching offers an economical alternative to process such waste dumps. Most of the dumps are generated without any scientific planning and contain different sizes of rock materials – boulders to smaller fractions. Experiences with dump operations have provided a wealth of information on dump geometry conducive for efficient leaching. Dumps positioned parallel to each other and greater in length than width and height are called ‘finger dumps’ which have dimensions of the order of 800 m x 35 m x 200 m. Permeability of the fragmented rock mass constituting the dump is a critical parameter. Low permeability is not conducive for efficient leaching. Blasting can disintegrate the rock and generate fines, too much of which also affect percolation of leaching solution.

Dump leaching essentially involves percolation of an acid lixiviant through the mineralised particles across the mass. Microorganisms present indigenously are allowed to proliferate by providing the necessary growth conditions. The mineral sulphides are biologically solubilized in the process and the effluent collected at the bottom of the dump is enriched with the metal content. The leach solutions are then subject to recovery processes such as cementation with iron scrap to recover the dissolved metal.

Heap Leaching

As different from dumps, in heap leaching appropriate heaps of low grade ores or waste materials could be prepared on impervious bottoms with control exercised with regard to amount of the ore materials, particle size, height and irrigation techniques. The heap leaching operation otherwise is similar to dump leaching. The following aspects need be considered while preparing heaps.

- a) Topography, geological and mineralogical features
- b) Geography, environmental and ecological aspects of the area
- c) Operational procedures including particle size distribution, dimensions and irrigation methodology.

Heaps are generally laid out in the immediate vicinity of the mines. Ground impermeability is important. Nonporous pads must be created on which heaps are rested. There are several ways of preparation of such impermeable bottoms such as use of concrete, asphalt, pitch, coal-far or synthetic polymeric sheets. Slopes need be maintained to allow collection of percolated leach solution. Particle size distribution and porosity of the fragmented ore body in the heap should be such as to enable penetration of leach solution and oxygen as well as bacterial activity. In many commercial heaps, run-of-mine ores containing about 4% - 100 mm and 25% - 20 mm particles are used. Excessive fines would exhibit a pronounced tendency for slime formation, impeding percolation of the leach solution. In many cases, the as-mined ore is subjected to one or two stages of crushing to derive particles of about –5 mm size. In case of excessive fines, prior agglomeration may become necessary. This is especially true in the case of fine tailings. The height of the heap need be chosen to attain adequate oxygen diffusion through natural draft. Heap sizes of 6.0 m height, 137 m length and 107 m width containing more than 50,000 tonnes of ore are common.

BIOLEACHING OF URANIUM

Along with copper, uranium ore leaching is an example of successful commercial application of biohydrometallurgy. Uranium, a nuclear metal occurs essentially as uraninite (UO₂) or pitchblende

(U_3O_8) in nature and the ores are of very lean grade (less than 0.1%). Tetravalent uranium is acid insoluble and only its hexavalent form is readily soluble in sulphuric acid solutions. Hence its becomes essential to oxidise the naturally occurring uranium to the hexavalent state before resorting to leaching. Acidic ferric sulphate (Fe^{+3}) is used as an oxidant in commercial leaching operations.

The major role of bacteria such as *Acidithiobacillus ferrooxidans* in uranium hydrometallurgy is essentially an indirect one, namely, the dissolution brought about by bacterial metabolite, namely, acidic ferric sulphate. Uranium ores occur usually with pyrite. In the presence of iron oxidizing bacteria such as *Acidithiobacillus ferrooxidans* the pyrite gets converted to ferric sulphate and sulphuric acid which act as lixivants.

In situ bioleaching is also applicable to recover uranium from ore bodies for which conventional stoping methods are unprofitable. The Stanrock mine in Ontario, Canada and the Pitch mine in Colorado, USA carried out several bioleaching tests and recovered sizable uranium. An in situ bioleaching process using a virgin ore body was taken up at the Agnew lake mine in Canada in 1975. Bore holes were drilled by explosives and rock samples disintegrated from stopes. The operations produced more than 100 tons of U_3O_8 from the surface heaps and underground stopes. In situ uranium mining has become very attractive. Many ore bodies consist of sandstone layers with uranium minerals sufficiently permeable. The special geometry of the ore body at Denison mines in Ontario, Canada is found to be ideal for bioleaching. The presence of sufficient concentrations of pyrite-pyrrhotite in the ore body makes it conducive for the growth and activity of *Acidithiobacillus ferrooxidans*.

BIOPROCESSING OF REFRACTORY GOLD ORES AND CONCENTRATES

Biotechnology could be effectively applied to recover gold from the following unconventional and waste resources:

- a) Tailing dumps accumulated at the mine sites over a period of several years amounting to several millions of tonnes, which in few locations contain as high as 1 gram / tonne of gold
- b) Lean grade sulfide ores containing less than 2-3 gram/tonne of gold, which cannot be economically processed through the current practice.
- c) Refractory ores-sulphidic or carbonaceous, where finely disseminated gold particles are locked up, making them unsuitable for direct cyanidation

Gold occurs in nature essentially in its native state and can be considered to exist in three different types of ores, namely, free milling, base metal and refractory. Free-milling ores are those from which the precious metal can be efficiently liberated from the host rock through appropriate size reduction. Most of the current gold-processing plants around the world utilize such deposits to recover the metal through the century-old cyanidation process. Base metal ores containing copper, lead, zinc and iron as their sulphides often contain small quantities of gold which is recovered as a by-product metal. The third type, namely, refractory ores, is becoming increasingly important as a potential primary source of gold. Among the refractory gold ore occurrences, two types are relevant, namely, sulphidic and carbonaceous. In the former case, the precious metal is finely disseminated within sulphide minerals such as pyrite and arsenopyrite and as such the encapsulated gold particles cannot be satisfactorily liberated even after fine grinding making direct cyanidation very inefficient for metal recovery. In the case of refractory gold bearing carbonaceous ores also, gold particles remain locked-up, making recovery by gravity or cyanidation methods unacceptably low. Another problem with carbonaceous materials is 'preg-robbing' which means even if liberated gold particles are cyanided, they get absorbed onto carbon matter making recovery extremely difficult.

The use of mixed strains containing *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* and *Leptospirillum ferrooxidans* could prove to be more effective in sulphide mineral oxidation due to

synergistic mechanisms in operation. The use of thermophiles such as *Sulfolobus* which have optimum activity at about 60-80°C would ensure enhanced leaching kinetics.

It should be understood that bioprocessing of sulphidic gold-bearing minerals is essentially a pretreatment process aimed at liberation of locked-up gold particles. The acid bioleached residues need be alkali treated before subsequent cyanidation. In the absence of prior biotreatment, even finer grinding of such refractory ores would result in very poor gold recoveries, as little as 6% and at any rate never exceeds 30-40% even under ideal processing conditions. The improvement in precious metal recovery conferred by prior biooxidation would be significant, often exceeding 95%.

There exists a direct relationship between the degree of sulphide mineral oxidation and percent gold recovery. Complete oxidation of sulphides is not necessary to achieve significant enhancement of gold recovery. On the basis of the sulphide entity, high gold recoveries can be obtained with as low as 50% oxidation of the total sulphides. The entire biotreatment process is agitation leaching, carried out in stirred bioreactors or Pachuca type reactors. The extent of mineral oxidation depends on the type of sulphide involved, whether pyrite or arsenopyrite.

Though gold bearing sulphides can be directly bioleached, it is often preferable to treat a flotation concentrate of the ore since it enables easy handling of a reduced tonnage of enriched material. The following variables need be closely controlled to achieve optimum efficiency.

Choice of appropriate preadapted bacterial culture

Particle size – fine size favours faster oxidation

Pulp density – 20-30% optimum

Oxygen and carbon dioxide availability

Agitation and homogeneous mixing of pulp

Temperature 30°C to 45°C (use of moderately thermophilic strains)

pH 1 to 2

Residence period 2 – 7 days

Eh 700-900 mV

Availability of nutrients

Removal of toxic and interfering oxidation products

Large-scale applications of biotechnology for refractory gold ores and concentrates have been coming up during the last five to ten years. The most prominent commercial application is the 10 to 12 tonnes per day plant treating flotation concentrates at the Fairview mine in South Africa started in 1986. Since then, many commercial operations have been reported. Ashton Mining's Harbour Lights operation in Western Australia is one of the largest. Asarco, Australia has commissioned at Wiluna, W.Australia a concentrate bioleaching plant. Ashanti gold mines in Ghana initiated bioleaching for enhanced gold recovery. The General Mining, Metals and Minerals group of S.Africa operates the Sao Bento mine in Brazil.

DEVELOPMENTS IN BIOLEACHING OF METAL CONCENTRATES

A number of companies have developed biooxidation processes for use in metal extraction. Some of these have found commercial application while others are still in the experimental or pilot plant stages (Brierley 2001, Anon 2002 and Miller et al., 1999)

Newmont Mining	BIOPRO™ Process – heap leaching of refractory gold ores
Gold Fields, Ltd	BIOX™ Process – agitated tank oxidation of refractory gold ores
BHP Billiton, Ltd.	BioCOP™ Process – agitated tank oxidation and leaching of copper sulfides BioNIC™ Process – agitated tank oxidation and leaching of nickel sulfides BioZINC™ Process – agitated tank oxidation and leaching of zinc sulfides
BacTech Enviromet	BacTech/Mintek Process – agitated tank oxidation and leaching of copper sulfides
GeoBiotics, Inc.	GEOCOAT™ Process – heap leaching sulfide mineral concentrates

BacTech Enviromet Corp., in conjunction with Mintek, has developed proprietary technologies for the high temperature leaching of copper concentrates. Working in conjunction with Industrias Penoles SA de CV, one of Mexico's largest and most diverse mining companies, they operated a 2.2-metric tons per day (mt/d) stirred-tank copper-concentrate bioleach demonstration plant in Monterrey, Mexico during 2001. A decision will be made later regarding construction of a plant capable of producing 25,000 metric tons of cathode copper per year.

BHP Billiton and Codelco, in a joint venture, Alliance Copper Ltd., are constructing a demonstration plant at Chuquicamata, in northern Chile, that will produce 20,000 tons of cathodes a year starting in 2003 using Billiton's patented BioCOP™ process. One purpose of the project is to process "dirty concentrates," *i.e.*, those containing high levels of arsenic (enargite, Cu_3AsS_4) that are not desirable to be processed by smelting for environmental reasons.

MICROBE-MINERAL INTERACTIONS RELEVANT TO MICROBIALLY-INDUCED MINERAL BENEFICIATION

Microbe-mineral interactions result in several significant consequences of relevance in mineral beneficiation. The end results of such biogenic reactions manifest as mineral formation and conversion, surface modification, dissolution of mineral constituents, as well as biometal accumulation. Selective leaching, flotation and flocculation are some of the processes involved in microbially-induced mineral beneficiation.

The mechanisms through which the bacteria oxidize the sulfide minerals are complex and not fully understood. The interaction between the cell and the surface has been shown to be dependent on a number of physical and bio-chemical parameters. The bacteria are postulated to adhere to the mineral surface directly and utilize cell surface-associated or extracellular biopolymers to catalyze chemical reactions on the surface of the mineral.

Several type of autotrophic and heterotrophic bacteria, fungi, yeasts and algae are implicated in mineral beneficiation processes. However, the role of only a few type of bacteria, namely, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans*, Sulphate reducing bacteria (*Desulfovibrio*) and *Paenibacillus polymyxa* have been studied in detail.

Use of *Acidithiobacillus Ferrooxidans* in Sulfide Mineral Beneficiation

Acidithiobacillus ferrooxidans is the most widely implicated autotroph in bioleaching and biomineral beneficiation. It is a Gram-negative, non-spore forming, rod shaped bacterium, mobile by means of a single polar flagellum and occurring singly or occasionally in pairs. These chemolithotrophs obtain their energy by the oxidation of ferrous iron, sulphur and reduced valence sulphur compounds.

Relative hydrophobicity of the cells of *Acidithiobacillus ferrooxidans* has been studied through hydrophobic interaction chromatography and phase partitioning after growing them in a liquid 9K medium and on solid mineral substrates such as sulphur, pyrite and chalcopyrite (Devasia et al., 1993). Bacterial cells of the bacteria grown on mineral substrates were observed to be increasingly

transferred to an organic phase, hexadecane, unlike those grown in a liquid medium. Mineral-grown cells of *Acidithiobacillus ferrooxidans* thus exhibit higher hydrophobicity. Common immunoreactivity was observed between the various mineral-grown cells, while liquid-grown cells did not exhibit such a behaviour, indicating that a secretion is released on mineral-grown cells alone for aiding surface adhesion. FTIR analyses of the cell surfaces grown on sulphide mineral substrates readily indicated the presence of NH₃, NH₂, NH, CONH, CO, CH₃, CH₂, CH and COOH functional groups. Such a proteinaceous secretion synthesized by *Acidithiobacillus ferrooxidans* was completely absent if grown in a liquid nutrient medium, suggesting the unique environmentally necessitated alteration of bacterial cell composition. The bacteria prepare the mineral particle surfaces for adhesion through the secretion of surface proteins and firmly attach thereafter.

Acidithiobacillus ferrooxidans have been shown to bind selectively to pyrite in coal and this property has been used to condition coal prior to flotation achieving significant pyrite suppression. The depression of pyrite was due to the specific and selective attachment of the organism on the surface of pyrite (Ohmura 1993). This selective adhesion behaviour of the bacterium towards the ferrous sulphide mineral was successfully utilized for the removal of pyrite from both synthetic and natural coals during flotation.

Yelloji Rao et al. (Yelloji Rao et al., 1992) investigated the utility of *Acidithiobacillus* sp. for selective removal of galena from sphalerite. The depression of galena after interaction with the bacteria has been explained. The utility of this bacterium for selective pyrite separation from associated ferrous sulfides has also been assessed. Chandraprabha et al. (Chandraprabha et al., 2004a,b) were able to achieve effective separation of pyrite from both chalcopyrite and arsenopyrite at acidic and neutral pH by biomodulation using *Acidithiobacillus ferrooxidans*. *Acidithiobacillus ferrooxidans* thus appears to play a dual role, promoting flotation under certain conditions while enhancing depression of minerals under some other processing conditions. Promotion of flotability of sulphide minerals in the presence of *Acidithiobacillus ferrooxidans* could be understood in the light of elemental sulphur formation on mineral surfaces through biooxidation. Bacterial interaction for prolonged periods of time leads to reoxidation of the sulphur to sulphonyl compounds and ultimately to sulphate. Gradual build-up of such oxidized layers on mineral surfaces would impede flotation.

The availability of initial biomass as well as the duration of conditioning of the mineral with the bacteria were observed to be important variables influencing the flotability of both sphalerite and galena. With increase in cell concentrations and duration of biopretreatment, flotability of both sphalerite and galena was found to be deleteriously affected. For sphalerite, 94% recovery obtained after treatment with 10⁸ cells/ml decreased to about 53% when the cell concentration was increased by ten fold. On the other hand, flotability of galena was observed to be more seriously affected by increasing the initial biomass. Thus, it would thus be possible to preferentially float sphalerite from a mixed zinc-lead sulphide ore through bacterial pretreatment under carefully controlled conditions.

Separation of pyrite from other ferrous sulfides such as chalcopyrite and arsenopyrite is not an easy task due to the similar flotation behaviour these minerals have with xanthate collectors. Separation by biomodulation using bacterial cells or their metabolic products would not only result in effective separation of these minerals, but also overcome the limitations of the conventional flotation processes with respect to environmental concern. *Acidithiobacillus ferrooxidans* was successfully utilized for the selective removal of pyrite from a mixture of pyrite and chalcopyrite (Chandraprabha et al., 2004a,b). When the individual minerals were conditioned with bacterial cells prior to flotation using Potassium isopropyl xanthate (PIPX) as collector, pyrite was depressed while chalcopyrite exhibited significant flotability. Table 1 portrays the results obtained for the selective separation of pyrite from chalcopyrite.

Table 1: Differential Flotation of Pyrite-Chalcopyrite Mixture After Conditioning with Collector (5×10^{-4} M PIPX) and Cells (4×10^8 Cells/MI)

Flotation pH	Weight % in floated fraction	
	Chalcopyrite	Pyrite
4.5	84	24
6.5	85	22
8.5	82	15

Selective separation of arsenopyrite and pyrite by microbial flotation and flocculation at acidic and neutral pH using *Acidithiobacillus ferrooxidans* cells was successfully performed (Chandraprabha et al., 2004a,b). Adhesion of the bacterium on the surface of arsenopyrite was very slow compared to that on pyrite resulting in a difference in surface modification of the minerals subsequent to interaction with cells. Adhesion studies also revealed that the cells exhibited higher affinity towards pyrite when compared to arsenopyrite. Subsequent to interaction with cells, the isoelectric point of the minerals shifted to higher pH values and the electrophoretic mobility became more positive. But the shift with respect to arsenopyrite was lower when compared to that of pyrite. Subsequent to interaction with minerals, the bacterial cell surface also exhibited similar changes and the changes were more significant for pyrite interacted cells. The cells were able to effectively depress pyrite flotation in presence of collectors like potassium isopropyl xanthate and potassium amyl xanthate. On the other hand the flotability of arsenopyrite after conditioning with the cells was not significantly affected. Selective separation could also be achieved by flocculation of biomodulated samples. When flocculated in presence of cells, pyrite was almost completely flocculated while arsenopyrite remained dispersed. Table 2 portrays the results obtained for the selective separation of arsenopyrite from pyrite.

Table 2: Selective Flocculation of Pyrite-Arsenopyrite System (1:1 Wt%) using *Acidithiobacillus Ferrooxidans* Cells

Experimental conditions	Pyrite		Arsenopyrite	
	% settled	% dispersed	% settled	% dispersed
Absence of cells (pH 4.5)	19.3	79.8	24.4	73.9
Presence of cells (pH 4.5)	96.3	3.2	25.9	71.7

Use of *Acidithiobacillus Thiooxidans* in Lead-Zinc Flotation and Flocculation

Surface chemical changes induced on sulfide minerals such as galena and sphalerite by an acidophilic chemolithotroph such as *Acidithiobacillus thiooxidans* has been studied. After interaction with *A. thiooxidans*, the isoelectric points of both galena and sphalerite were seen to be shifted towards higher pH values presumably due to specific adsorption of bacterial cells. Prior bacterial cell adsorption studies have indicated that larger number of cells are adsorbed on galena surfaces than on sphalerite (Santhiya et al., 2000, 2001a, b, 2002).

To understand the implications of such surface chemical changes brought about by bacterial interactions, single mineral flotation of both galena and sphalerite was carried out before and after interaction with *T.thiooxidans*. Flotation recovery of galena was observed to be significantly decreased (from 100% in the absence of bacterial interaction to about 10% after biotreatment), while that for sphalerite was promoted.

From the results obtained it could be concluded that consequent to interaction with the cells for 2 h, galena is almost totally depressed while sphalerite is completely floated. The significant differences in the adsorbabilities of the cells onto galena and sphalerite coupled with the nature of the interaction products, be it the respective sulphates or hydroxides, are the contributing factors for the diametrically

opposite flotation behaviour observed with respect to galena and sphalerite after interaction with the cells. It becomes of interest to logically extend the findings of these tests carried out on individual sulphide minerals to a synthetic mixture of the two minerals. The flotation test results obtained after interaction with the cells in the absence of collector/activator clearly demonstrated that while over 96% galena could be depressed, nearly 94% sphalerite is floated. In these tests, the minerals are interacted separately with the cells at pH 2.2-2.5 and mixed only at the time of flotation. However, when the minerals are interacted together with the cells, about 91% recovery each of galena and sphalerite was obtained in the tailing and concentrate fractions respectively.

The addition of the cells to a mixture of galena and sphalerite at pH 9-9.5 demonstrated that nearly 95% of sphalerite was dispersed, while 95% of galena was selectively flocculated.

It may be recalled that $ZnSO_4$ formed from sphalerite due to bacterial interaction is soluble unlike the case of lead sulphate. The changes in the surface morphology of galena and sphalerite after bacterial interaction could be clearly distinguished attesting to the higher adsorption of the cells onto galena as well as formation of $PbSO_4$.

Selective Flotation and Flocculation of Iron Ore Minerals in the Presence of *Paenibacillus Polymyxa*

Bacteria such as *Paenibacillus polymyxa* and their metabolic products, mainly, exopolysaccharides and bioproteins interact effectively with oxide minerals and bring about significant surface chemical changes (Namita et al., 1999b, 2001, Natarajan and Namita 1997a,b, 1998a,b, 1999a). Detailed electrokinetic studies have shown that interaction of the above bacteria with quartz, hematite, calcite, kaolinite and corundum bring about significant shifts in isoelectric points.

Such significant shifts in the isoelectric points of various minerals are indicative of chemical interaction. Bacterial cells adsorb on the mineral surfaces to different degrees of coverage and along with metabolite products form a biofilm. The adsorption tendency of the bacterial cells follow the sequence:

Kaolinite > Calcite ≥ Corundum ≥ Hematite > Quartz

Such an adsorption was also found to be almost independent of pH between 2 to 10 indicating non-electrostatic forces being responsible for bacterial mineral adhesion.

Quartz surfaces were rendered more hydrophobic after bacterial interaction while hematite and corundum became more hydrophilic. Similar tests indicated that kaolinite behaved similar to quartz while calcite surfaces were rendered more hydrophilic after bacterial interaction. Flotability of quartz and kaolinite was promoted by bacterial interaction. On the otherhand, bacterial interaction did not promote the flotability of corundum, hematite and calcite.

Eventhough quartz and kaolinite can be floated after bacterial interaction even in the absence of any flotation reagents, addition of trace amounts of appropriate collector reagents further promote their flotation. Efficient silica removal from iron oxides, alumina and limestone can be achieved through flotation through bacterial pretreatment. Flotation tests with 1:1 mixtures of hematite-quartz, alumina-quartz and calcite-quartz after biotreatment for 5 minutes in the presence of bacterial cells indicated that more than 95% of silica could be removed.

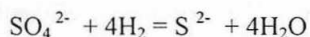
While the settling rates of hematite, calcite and corundum particles were increased after interaction with either bacterial cells or the metabolite, those quartz and kaolinite were seen to be significantly decreased after similar treatment. Flocculation of calcite, hematite and corundum and dispersion quartz and kaolinite is facilitated by bacterial interaction. Various polysaccharides such as starches and dextrans are used as depressants for iron oxides in iron ore flotation. Selective flocculation of iron oxide with dispersion of silica can be achieved by addition of the above polysaccharides.

Biopolymers such as those containing exopolysaccharides can interlink the mineral particles through polymer bridging and flocculate the mineral fines selectively. Increased affinity of bacterial cells and excreted polysaccharides towards hematite and corundum bring about their selective flocculation facilitating their rapid settling in an aqueous medium.

Results of selective flocculation tests carried out with mineral mixtures substantiate the above conclusion. It is possible to separate efficiently silica and silicates from alumina, calcite and iron oxide through bioflocculation. However, it is not possible to separate alumina from hematite efficiently using ordinary bacterial cells since bacterial interaction brings about similar surface-chemical changes on both of them. Tests with hematite – corundum mixtures have shown that both the minerals settled down faster in an aqueous medium after bacterial interaction without any selectivity. However, it has been observed that though the use of corundum-adapted strains of *P. polymyxa*, efficient separation of alumina from iron oxides can be achieved. At pH 7.0 more than 99% separation could be obtained between hematite and corundum. Unlike the case with unadapted cells, corundum particles could be effectively settled (flocculated) with the selective flocculation of only corundum using the corundum-adapted strain (hematite dispersed).

Use of Sulfate Reducing Bacteria (SRB)

Unlike *Acidithiobacillus ferrooxidans*, Sulphate reducing bacteria (SRB) of the type *Desulfovibrio* are anaerobic heterotrophs occurring as curved rods and monoflagellated. They reduce sulphate to sulphide at an optimum pH in the range of 6-7 with the involvement of hydrogenase.



H₂S and other metal sulphides are generated during the growth of these organisms. From a flotation view point, these bacteria could serve as biological sulphidising agents. Many oxidized ores could be effectively sulphidised in the presence of SRB (Solozhenkin 1985 and Somasundaran et al., 2000). For example, flotation of cerussite was improved by 20-25% after pretreatment with SRB. Besides, they were also found to be effective desorbents of xanthate coatings from mineral surfaces. This observation is of significance in differential flotation processes wherein desorption of the collector coating from a mineral surface before addition of a new type of collector is often resorted to. Selective separation of lead and zinc from sulphidic ores after pretreatment with SRB has been attempted. Lead recovery in concentrate after bacterial treatment was about 95%, with the sphalerite recovery being about 4.5% under these conditions. Yet another reported application has been in the selective separation of chalcopyrite and molybdenite after biotreatment with SRB. Treatment with SRB was found to depress sphalerite and chalcopyrite, but not galena and molybdenite.

The ability of SRB to generate larger volume of H₂S and to convert metals and their salts to sulphides could be effectively used in a number of other mineral engineering applications. Through appropriate process control, the bacterial reaction can be so manipulated as to generate either a sulphide product (promotion flotation) or H₂S gas (depressant). These organisms can convert iron oxides to ferrous sulphide, malachite to covellite, silver carbonate/chloride to argentite and smithsonite to sphalerite. Dissolved metal ions from acidic solutions and effluents can be effectively precipitated as their sulphides by SRB, facilitating their flotation separation.

Microbial Beneficiation of Clays and Industrial Minerals

Silicon compounds in the form of clays stimulate certain microbial metabolism. Clays tend to modify the microbial habitat physicochemically. The role of biological factors in the silicon cycle is of great significance. Many autotrophic and heterotrophic bacteria are capable of breaking the siloxane bond, Si-O-Si. At high pH values, the biological destruction of quartz increases sharply. The solubilization may involve the cleavage of Si-O-Si or Si-O-Al bonds or removal of cations from the lattice of silicate, leading to disintegration of the silicate lattice structure. For example, *Pseudomonas* produces

2-keto gluconic acid from glucose dissolving silicates of calcium, zinc, magnesium and minerals such as apophyllite, wollastonite and olivine. Fungi such as *Penicillium notatum* and *Aspergillus niger* degrade spodumene. *Sarcina ureae* releases silicon readily from plagioclase and quartz.

Biologically mediated silica removal from magnesite ores has been reported using *Bacillus circulans*, *Bacillus mucilaginosus* and *Bacillus licheniformis*. Successful desilicisation of bauxite, iron ores, manganese and phosphate ores could be achieved through the choice of appropriate microorganisms and controlling the process parameters. The removal of silica from bauxite using silicate bacteria *Bacillus circulans* was achieved.

Bacterial removal of iron from kaolins and clays is of industrial significance in the production of commercial grade raw materials. Oxalic and citric acids produced by *Aspergillus niger* could effectively remove iron from clays. The metabolites were found to have higher iron removal rates if the process could be carried out at 90°C. The iron content of different kaolins could be brought down to 0.4-0.7% from an initial values as high as 1.5%. Such bioremoval could be effectively carried out in bioreactors at pulp densities in the range of 20-40% within a period of a few hours.

Removal of calcium and iron impurities from low-grade bauxite using *Paenibacillus polymyxa* for abrasive and refractory applications has been reported (Vasan 1998, 2001 and Phalguni et al., 1996). *Paenibacillus polymyxa* was shown to selectively remove calcium and iron impurities from low-grade bauxite (< 50% Al), for abrasive and refractory applications. An industrial scale formulation of Bromfield medium (called ISF-2) based on cane sugar and tap/mine water, was developed to successfully culture *Paenibacillus polymyxa* under aseptic conditions. The bioleached residue was found to be enriched in its alumina content with insignificant amounts of iron and calcium as impurities.

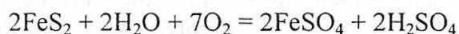
Removal of phosphorous and silicon from manganese ores is yet another example of biomineral beneficiation. *Hypomicrobium* sp. and *Aspergillus niger* are efficient phosphorous removers. Rock phosphates can be solubilized by strains of *Rhizobium* and *Bradyrhizobium*. Also, *Bacillus mucilaginosus* aids in phosphorous removal along with silica.

BIOTECHNOLOGY OF MINE ENVIRONMENTAL POLLUTION AND CONTROL

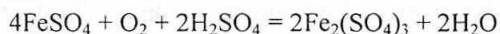
Microorganisms inhabiting ore bodies and water systems can also play a significant role in causing environmental pollution, such as acid water generation. At the same time, appropriate usage of various microorganisms can also bring about environmental protection.

Acid Mine Drainage

Acidic waters originating from bacterial sulphidic mineral oxidation, especially from metalliferous and coal mines constitute a major environmental pollution problem. Both active and abandoned mines remain a source of this problem, termed, Acid Mine Drainage (AMD) leading to contamination of ground water tables, rivers, streams and even sea coasts. In nature, both iron and sulphur oxidising *Acidithiobacillus* group of bacteria are associated with mineral sulphides such as arsenopyrite, pyrite, chalcopyrite, sphalerite, galena, molybdenite, millerite, orpiment and antimonite, all of which serve as energy sources for the microbes. The abundance of iron and sulphur in natural sulphide mineralization makes it easier for the *Acidithiobacillus* group of bacteria to colonise on them. Biooxidation of pyrite and sulphur leads to the formation of sulphuric acid containing Fe⁺⁺⁺ which subsequently dissolve various toxic metal ions through its solvent action. Biogenic reactions involving sulphide mineral dissolution of relevance to acid mine drainage include



(pyrite)



Depending on the pH of the environment, precipitation of iron oxyhydroxides would occur. The role of synergism or symbiotic interactions among different *Acidithiobacillus* group of bacteria in generating acidic waters need to be understood. Under conditions existing in mines, ore stock piles and tailing dams, several types of microorganisms (autotrophic, heterotrophic, aerobic and anaerobic) could be active. Microbial activity due to aerobic, autotrophic iron bacteria in the pH range 6-7 could create anaerobic conditions due to oxygen consumption. Sulphate reducers can then proliferate producing H₂S and metal sulphides from acidic sulphate solutions. *Thiobacillus thioeparus*, another autotrophic aerobic active in the pH range 6 to 8 could further oxidise H₂S, S and sulphides to sulphate, producing acidity, lowering the pH of the water. Once the pH is brought down to 4-5, *Acidithiobacillus thiooxidans* can take over the sulphide oxidation process, lowering the pH further to 3. *Acidithiobacillus ferrooxidans* could then actively oxidise Fe⁺⁺ and sulphide compounds increasing the water acidity to a pH as low as 1-2.

The type of drainage depends on the type of the mine, mineralogy of the ore body and nature of geological surroundings. The intensity of acidification also depends on amount of sulphides, crystallinity, size and chemical stability of the minerals, nature and population of the bacteria, water level and the presence or absence of calcium and other basic gangue constituents in the ore.

Mine water microbiology relevant to acid mine drainage is similar to that involved in bioleaching and sulphide biomineralization. Ecological studies of coal and metalliferous mines have shown the presence of a wide range of microorganisms. The acid-tolerant autotrophs of mine waters include *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. A filamentous organism growing in iron-encrusted filaments similar to *Metallogenium* has also been isolated from several mine waters. Alkaline mine drainage contains different kinds of *Acidithiobacilli* that oxidise reduced sulphur compounds. *Thiobacillus thioeparus* and *Acidithiobacillus intermedius* are supported in alkaline environments.

Control of Acid Mine Drainage

Mining industries face the challenge of preventing acid drainage generation in a cost-effective manner. The best way to achieve this is to limit oxidation of sulfides or neutralise acid generation. Multi-layer soil covers have been found to be effective. Water covers could be economical. Due to low oxygen diffusion rate in water with respect to air, oxidation of reactive wastes can be minimised. Waters covers can significantly reduce acid generation. To further improve the effectiveness of water covers, use of biological materials and growth of aquatic plants in situ have been recommended. The benefit of an organic layer at the tailings and water interface is many-fold since oxygen will be consumed by bacterial activity in the organic material and upward metal fluxes from the tailings to the water cover will be prevented (Kuyucak 2000).

Most common passive-treatment systems are anoxic limestone drains, constructed anaerobic and aerobic wetlands and biosorption. Influent flow rate, contaminant concentrations and pH are extremely important to system performance.

Anoxic limestone drains treat acid drainage by adding alkalinity to water. The system consists of a bed of crushed limestone below the ground surface and covered with a fabric filter and clay to promote anoxic conditions.

Sulfate-reducers such as *Desulfovibrio spp.* can reduce sulfate to sulfide. The hydrogen sulfide generated forms insoluble metal complexes and lead to metal removal from effluents. SRB are known to be natural soil bacteria and can be found in sewage and manure. Industrial wastes such as molasses, sewage sludge, brewery waste and wood chips serve as cheap nutrients to these bacteria. SRB can be cost-effective for treating acid drainage and can be used in wetland system. SRB-based passive processes can treat seepage, small streams and water accumulated pits at mine sites.

Constructed wetlands are composite systems where various physico-chemical and biological process occur. Wetlands have ability to remove trace metals and improve alkalinity. In natural and constructed wetlands, general removal mechanisms include metal precipitation, settling and filtration, plant uptake, microbial sulfate reduction and metal sulfide removal. Wetlands can be aerobic, anaerobic or even a combined one.

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