FEASIBILITY STUDY ON THE MICROBIAL SEPARATION OF IRON FROM SLIME

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Life science

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CERTIFICATE

This is to certify that the thesis entitled "**Feasibility Study on the Microbial Separation of Iron from Slime**" submitted to National Institute of Technology; Rourkela for the partial fulfilment of the Master degree in Life science is a faithful record of bonafide and original research work carried out by N.Rohini under my supervisions and guidance.

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DECLARATION

I hereby declare that the thesis entitled **"Feasibility study on the microbial separation of iron from slime"**, submitted to the Department of Life Science, National Institute of Technology, Rourkela for the partial fulfilment of the Master Degree in Life Science is a faithful record of bonafide and original research work carried out by me under the guidance and supervision of Dr. (Miss.) Bismita Nayak, Assistant Professor, Department of Life Science , National Institute of Technology, Rourkela. No part of this thesis has been submitted by any other research persons or any students.

Date: N.ROHINI

Place:

Acknowledgement

If words are considerable as symbols of approval and taken as acknowledgement then let the words play a heralding role in expressing my gratitude.

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ABSTRACT

Bio-mineral processing is the generic term that describes the processing of metal containing ores, concentrator tailings, newly mined run-of-the-mine (ROM) material, and intermediate to high-grade ores using (micro-) biological technology. The slime generated by the Tata Iron and Steel Company is becoming a major problem for the Company. Since, it contains a high quantity of Iron (around 56%), it can be recycled for the generation of Steel. Bioleaching comes to the rescue of such a problem. As it contains a high percentage of alumina and silica as its component, it can be treated as a non- sulphide system. Heterotrophic organisms can be used to leach out the alumina and silica. We have thus tried to see the feasibility of Bacillus to leach the slime and increase the Iron content in it. The conditions like pH, time and inoculum size have been optimized. The results showed that, there was a maximum recovery of iron (around 79%) in the slime and the optimum conditions at which this was obtained were at pH of 7, a time of 5 days and inoculum size of 20%.

Chapter 1

INTRODUCTION

INTRODUCTION

Iron ore is a major raw material for any steel-making company and Tata Steel has its own captive mines, at Noamundi in Jharkhand and at Joda in Orissa. The impurities in the raw iron ore, namely alumina and silica (also called gang minerals), are separated at the mine site through a process known as beneficiation. The usable iron minerals are transported to the steel plant and the rejects, or slime — fine in size and slurry in form — are stored at the mine site in deep ponds. The slime cannot be dumped just anywhere because it will contaminate the land and the water. So Tata Steel, and every other steelmaker in India, has to find land within its mining area to bury the waste. This storage situation has worsened in recent times, taking up more and more land due to the huge increase in steel production, especially so over the past two decades. This slime contains some amount of iron that can be used in steelmaking, and till now there has been no technology to extract it from the slurry. Slime contains very fine size particles of below 25 microns and the content of gangue is also high as compared to Run of Mine (ROM) ore. This by-product of iron ore beneficiation has high alumina (Al_2O_3) content and lower iron content which makes it unsuitable for using it directly as blast furnace feed. Therefore, beneficiation of slime to enrich the iron (above 62%) and reduce alumina content to the desired level (below 1.5% alumina) has to be achieved. The gangue materials in such slimes comprise of variable amounts of alumina and silica in different discrete or combined forms and some traces of metallic values too. These alumino-silicates at times entrap the iron values as well but are prone to weathering by microbes in due course of time, causing iron values to entail into the ground water system.

The use of heterotrophic bacteria is of great importance if biologically assisted leaching is to be extended to non sulphide systems. They form/ secrete various kinds of complex forming organic species/ compounds (chelators) which react with alumina/ silicates or silicates to solubilise them. Bioleaching or bioremediation of the slime using these heterotrophic organisms may be more environmentally acceptable than the use of many chemical leaching agents.

The present batch investigations have been undertaken to study the feasibility of the separation of alumina and silica from the iron ore slime using *Bacillus* species, which is a very common bacteria found everywhere. Attempts have been made to optimize process parameters like pH, inoculum size to leach out maximum quantity of slime using suspended batch culture of the bacteria.

Chapter 2

REVIEW OF LITERATURE

REVIEW OF LITERATURE

The term **Bioleaching** refers to the bacterial conversion of an insoluble metal (usually a metal sulfide, e.g., CuS, NiS, ZnS) into a soluble form (usually the metal sulfate e.g., CuSO4, NiSO4, ZnSO4). When bioleaching takes place, the metal is extracted into water (Kelly *et al*., 1979; Torma 1977). The first bacterium discovered for bioleaching was able to oxidize these minerals and was identified to be *Acidithiobacillus ferrooxidans* (*At. ferrooxidans*, previously *Thiobacillus ferrooxidans*), a gram-negative, acidophilic, chemolithoautotrophic, non-spore forming rod. Nutritionally, *At. ferrooxidans* isolates are considered as obligate autotrophs. *At. ferrooxidans* is able to utilize either ferrous iron or a wide variety of reduced inorganic sulfur species as an electron donor compounds. It is also able to grow using ferric iron as an electron acceptor, provided by an electron donor, such as reduced inorganic sulfur compound is present in the surrounding. Energy is derived from the oxidation of reduced iron and sulfur compounds, including ferrous ion, sulfide, elemental sulfur and thiosulfate, with final oxidation products being ferric ion and sulfate (Rawlings, 2002; Leduc and Ferroni, 1994).

Other microorganisms considered important in commercial mineral biooxidation processes are: *Acidithiobacillus thiooxidans*, *Acidithiobacillus caldus*, *Leptospirillum ferrooxidans, and Acidiphilium acidophilum* (Rawlings, 2002). Bioleaching has emerged as a simpler, safer and less expensive process than other alternatives for most limestone, granitic, or other host rocks that have secondary replacement of pyritic minerals containing metal values. In recent years, biooxidation has shown itself to require less capital, reduced operating cost, and less skilled operating and maintenance personnel than the traditional pressure oxidation or roasting techniques (Lynn, 1997). This technology has been used for treating specific mineral ores, mainly copper and gold bearing ores (Acevedo, 2002; Shuey, 1998; Songrong, 2002). Moreover, bacterial leaching in acid medium has been successfully applied in: uranium metallurgy (Mathur *et al*., 2000); silver, gold and lead recovery (Frias *et al*., 2002); zinc (Harvey et al 2002); and new processes have been developed for cobalt recovery (Wiertz *et al*., 1999; D'Huges 1997). A complex sulfide ore is an association of galena (PbS), sphaelerite (ZnS) and chalcopyrite (CuFeS2), disseminated in a pyritic matrix. Besides of lead, zinc and copper as valuable metals, such deposits may contain significant quantities of

silver, gold, arsenic, antimony, bismuth and mercury. Numerous economically important deposits of these ores exist in the world (Gomez *et al.,* 1999). Complex ores are often characterized by particularly fine intergrowth of the mineral values. Due to these specific mineralogical characteristics, it is necessary to finely grind and concentrate the ore prior to the solubilization of the valuable metals. To obtain separate concentrates by selective flotation involves high unit-cost, poor quality of the concentrates and relatively low overall recoveries (Ortega and Bonella 1983).

The possible uses of microorganisms in the processing of minerals and in the remediation of mineral industry waste streams are numerous. To date microorganisms have been used industrially to assist the leaching of sulfide ores and in the bio-oxidation of refractory sulfide precious metal ores. Additionally, there are current efforts to use biosorbents to clean up heavy metal waste streams. Many other, sometimes novel, uses are possible. Also of importance in the field of minerals bio-processing are developments in identifying and producing new strains of microorganisms, both through natural adaptation procedures and by genetic engineering. Simple identification of entirely new and novel species of organisms to perform various mineral processing tasks should also become important, as will also the overall characterization of these organisms (including detailed surface characterization). Not to be overlooked is the importance of modeling and industrially controlling the bioprocesses developed. The different fields of bioprocess that has played major role in various ares are.

1. Bioleaching of sulfide ores and bio-oxidation of sulfide precious metal ores.
2. Bioleaching of non-sulfides.
3. Bioremediation of mineral industry aqueous wastes.
4. Microorganisms in mineral flocculation and flotation.
5. Modeling of mineral bioprocesses.

BIOLEACHING OF SULPHIDE ORES

Hydrometallurgical extraction leaching of copper from ore and the precipitation of copper from the resultant solution by treatment with metallic iron cementation is an ancient

technology. The Chinese practiced a form of this technology as far back as 100–200 BCE (Before Christ Era) and probably even earlier. The discovery in ancient times of the principles underlying this technology without knowledge of modern chemistry seems remarkable when we consider that the appearance of a blue solution of copper sulfate resulting from the aqueous leaching of copper ore, or of the crystals of blue vitriol ($CuSO_4.5H_2O$) formed when the water of the blue vitriol be derived from a solution of blue vitriol must have come from the chance discovery that metallic iron in contact with such a solution resulted in the precipitation of copper. Copper was important to the ancients as a metal and as an ingredient of bronze, a copper–tin alloy. Historical records indicate that copper ore leaching and cementation were also known in Europe and Asia Minor (Rossi, 1990). The technology was probably known to these civilizations much earlier. Whether this knowledge came from China, was carried to China, or was discovered independently is not known. As we now realize, leaching was probably the only way the ancients had to extract copper from sulfidic ores because smelting in very ancient times, run in open hearths, was effective only with copper oxides and carbonates. Not until the introduction of crucibles could smelting be successfully applied to sulfidic copper ores. The practice of copper leaching and cementation was refined through the centuries and has continued to the present day. The Moors during their conquest of Spain appear to have instituted heap leaching at the Rio Tinto Mines. Other records show that more than 2 million tons of copper have been leached from the copper deposits of the Falun Mine in central Sweden since 1687 (Hallberg and Rickard, 1973).

Despite the long-standing practice of leaching of sulfidic copper ores, the involvement of certain kinds of bacteria in this process was not discovered until the middle of the twentieth century. The reason for this very belated discovery was that the existence of bacteria in general was not known until the middle of the seventeenth century. It was Anton van Leeuwenhoek who in 1676 first described what has been interpreted to have been bacteria in a peppercorn infusion, which he examined with his ingeniously fashioned simple microscope. He thought he was observing little animals because the creatures moved under their own power. Little did he and those to whom he revealed his discovery suspect that other tiny creature, very like the ones he saw with his microscope, are able to extract metals from ore. Leeuwenhoek made no attempt to determine how these creatures arose. Other naturalists over the next century and a half mostly thought that they arose by spontaneous generation. Not

until Louis Pasteur and John Tyndall was this notion thoroughly disproven. The work of Pasteur and others showed furthermore that bacteria and other microbes were the cause of specific chemical changes in their environment and were not the product of chemical change, as Justus von Liebig and his followers thought. Although by 1875, Ferdinand Cohn had abandoned the idea that bacteria were little animals e.g., infusoria and classified them with plants (Brock, 1961; Thimann, 1963) it was not until the 1960s that they were recognized to be a special group of organisms distinct from plants and animals. The introduction to bacteriology after World War II of transmission electron microscopy and ancillary techniques, such as cell sectioning and inorganic staining, revealed that bacteria had a unique cell organization. As a result, bacteria were now classified as prokaryotes. The unraveling of the genetic code inscribed in DNA and its analysis led Carl Woese to conclude in 1977 that the prokaryotic bacteria should be divided into two distinct phylogenetic groups, the eubacteria now bacteria and the archaebacteria now archeota. (Woese and Fox, 1977). Both of these groups include members of special importance to bioleaching.

Colmer, Temple and Hinkle reported in 1950-1951 that acid coal mine drainage was the result of bacterial oxidation of pyrite inclusions in bituminous coal seams exposed to air and moisture, initial reports of bacterial involvement in copper leaching appeared L.C. Bryner, J.V. Beck, and their students at Brigham Young University in Provo, Utah found the same bacteria, *Thiobacillus ferrooxidans* and *T. thiooxidans*, in copper mine drainage from Kennecott's open-pit mine in Bingham Canyon, Utah that had previously been discovered in acid coal mine drainage. They showed in laboratory experiments that *T. ferrooxidans* was capable of leaching various copper sulfide minerals as well as molybdenite (Bryner *et al.*, 1954). However, molybdenite was only leached in the presence of pyrite. This was because the oxidation of pyrite generated ferric iron, which precipitated molybdate, which is poisonous to *T. ferrooxidans*. Demonstration of bioleaching of some other metal sulfides like ZnS, NiS, and PbS soon followed. The chief process in bioleaching of sulfidic ores is the mobilization of metal constituents. This is accomplished through microbially promoted oxidation of the metal sulfides. Silverman and Ehrlich distinguished between two modes of bacterial attack, indirect and direct. In the indirect mode, $Fe^{3+}$was seen as the oxidant whereas in the direct mode it was O_2. In the indirect mode, the chief function of *T. ferrooxidans*, which

was the only organism capable of promoting leaching that was recognized at the time, was to regenerate ferric ion from ferrous ion in the bulk phase,

$$2Fe^{2+} + 0.5O_2 + 2H^+ \rightarrow 2Fe^{3+} + H_2O$$

The ferrous ion resulted from the chemical oxidation of the metal sulfide in the ore by ferric ion,

$$2Fe^{3+} + MS \rightarrow 2Fe^{2+} + M^{2+} + S^0$$

MS represents a metal sulfide, and M^{2+} the divalent metal ion formed in the oxidation of MS. In addition to oxidizing Fe^{2+}, *T. ferrooxidans* and or *T. thiooxidans,* which is also detected in bioleach processes, were visualized as oxidizing the S^0, formed in the chemical oxidation H_2SO_4.

$$S^0 + 1.5O_2 + H_2O \rightarrow H_2SO_4$$

In the direct mode of bacterial attack of metal sulfide, Silverman and Ehrlich postulated that the bacteria attack a metal sulfide by attaching to its surface and oxidizing it enzymatically by conveying electrons to O from the reduced moiety of the 2 mineral, usually the sulfide, but in the case of Cu_2S also from the cuprous copper,

$$MS + 0.5O_2 + 2H+ \rightarrow M^{2+} + H_2O$$

Clear evidence of the ability of *T. ferrooxidans* to attach readily to the surface of metal sulfides was developed subsequently. A more detailed version of Silverman and Ehrlich's model of direct attack also emerged later. In this modified model, electron transfer from sulfide-S, or from cuprous copper in the case of Cu_2S, involves FeIII bound in the cell envelope and exopolymer (Ingledew et al, 1980; Sand et al, 1997; Gerhke et al, 1995). This bound Fe acts as an electron shuttle between the electron donor and the electron transport system of the cell, which conveys a major portion of the electrons to O and the rest to CO_2. Thus, the Fe III bound in the cell envelope and exopolymer is thought to undergo reversible reduction and oxidation in this electron transfer. The sites on a metal sulfide particle for

bacterial attachment and attack seem to be finite. Thus, once maximum attachment has been achieved, further multiplication of attached cells, if it occurs, should result in the displacement into the bulk phase of one of the two daughter cells of each dividing bacterium, which may then participate in indirect attack by oxidizing Fe^{2+} to Fe^{3+} in the bulk phase. If the two models of Silverman and Ehrlich describe the process of bio-oxidation of metal sulfides correctly, the iron requirement for an optimal rate of metal sulfide oxidation by the direct mode of attack should be significantly smaller than for the indirect mode. Differences in reaction kinetics between exclusively direct and indirect modes of attack can also be expected.

Sand and collaborators have recently suggested that because FeIII oxidizes metal sulfide in both the direct and indirect mechanisms, there is no difference between the two mechanisms (Sand *et al.,* 1995). Their model emphasizes a similarity in the chemistry of attack of the sulfide moiety by iron in the two modes and makes no distinction between ferric iron in the bulk phase and ferric iron bound in the cell envelope. Although initial studies of bioleaching suggested that *T. ferrooxidans* was the only active organism in bioleaching of metal sulfides, subsequent studies showed that other, phylogenetically unrelated organisms could also be active. These include not only autotrophs but also heterotrophs (Johnson, 1995), and not only mesophiles but also thermophiles, all of them acidophilic and all of them Fe II oxidizers (Johnson, 1995). Indeed, recent findings have shown that in many cases *Leptospirillum ferrooxidans*, which cannot oxidize reduced forms of sulfur, seems to dominate the metal-sulfide oxidizing microbial flora (Sand *et al.*, 1992). Because the scientific staff at Kennecott Copper and others in the early practice of bioleaching stressed that the interior of some leach heaps could reach temperatures above the upper limit tolerated by mesophiles due to the exothermic nature of metal sulfide oxidation, they suggested that bioleaching activity is probably confined to the top of leach heaps. This led to successful searches for thermophilic, acidophilic iron-oxidizers that could act within heaps (Brierley, 1978).

Further study of the microbes in pregnant solution from bioleaching operations showed that the acidophilic iron oxidizers were accompanied by many other kinds of organisms, including heterotrophic bacteria, fungi, and protozoa. Indeed, heap-, dump-, and

in-situ-leaching by native microbial flora in the field are probably the result of a consortium of acidophilic microorganisms including autotrophic and heterotrophic bacteria, fungi, and even protozoa. The autotrophic bacteria are generally believed to be the chief promoters of the actual metal leaching process, whereas an important role of the heterotrophs can be assumed to be to limit the concentration of organics that might otherwise inhibit the autotrophs (Turtle *et al*, 1976 and Arkesteyn *et al.*, 1980). Some of the heterotrophs can also promote formation of floc, as in the case of *L. ferrooxidans* (Sand *et al.*, 1993). Protozoans, in addition to aiding in the removal of dissolved organics, may control the size of the microbial population by preying on it. Both autotrophs and heterotrophs contribute to the weathering of the host rock gangue. This exposes ore mineral that is encapsulated in the gangue (Zimmerley *et al.*, 1958). Weathering of gangue is a microbial activity in bioleaching that has received very little consideration to date. In the weathering of aluminosilicates of gangue, sufficient Al may be mobilized to make its separation desirable (Zimmerley *et al.*, 1958). The weathering action is due in part to the sulfuric acid generated by the autotrophs in attacking pyrite and chalcopyrite minerals, which displaces alkali metals Na, K. and alkaline earths Ca and Mg. and causes rupture of Si-O and Al-O bonds in aluminosilicates. It also causes the dissolution of $CaCO_3$. Weathering may also be promoted by some of the less acidophilic heterotrophs that generate organic acids and or ligands. These may sequester Ca and Mg from the crystal lattice of aluminosilicates as well as cause rupture of Si-O and Al-O bonds. Even quartz may be attacked. Such weathering activity has been demonstrated by Huang and Keller (Huang and Keller, 1972), Bennett et al., Welch and Ullman, and Ullman et al. Four distinct approaches have been taken in the commercial exploitation of the ability of bacteria to mobilize metals in ores. These are heap-, dump-, in-situ-, and reactor-leaching. Zimmerley *et al.* (Zimmerley *et al.*, 1958) were issued the first patent on heap bioleaching on 24 October 1958. They assigned it to Kennecott Copper. This patent described a cyclic process of heap leaching of copper-, zinc-, copper-molybdenum-, chromite-, and titanium-ores. The last three ores were meant to be upgraded beneficiated by the process, i.e., the ore was enriched in metal value instead of the metal value being extracted. Such upgrading of the ores named in the patent seems never to have been commercially applied by Kennecott. Cu recovery from pregnant solution described in Kennecott's patent was by cementation with scrap iron.

Heap bioleaching has undergone various improvements over the years. Changes in the design of heaps to prevent slumping, and optimization of aeration have been a major factor in this improvement. Metal recovery from pregnant solution by cementation has been largely superceded by solvent extraction and electrolysis. Much effort has been expended to design a commercially viable process for bioleaching of ore concentrate in reactors. Progress has been gradual, with the chief stumbling block having been slow leaching rates. But breakthroughs are being achieved, making ore-concentrate bioleaching commercially feasible in certain instances. Advances in reactor design and, in at least one instance, the use of a moderately thermophilic acidophile as an agent of leaching (Miller, 1997) have been at the heart of this breakthrough. A rationale for turning to moderate thermophiles is a more limited cooling requirement for reactors. Ore concentrate treatment with hyperthermophiles by reactor leaching has been tried because of observations that leaching rates with such strains were higher than with mesophiles at ambient temperatures. However, more recent studies have shown that acidophilic hyperthermophiles tested in reactors have much more limited tolerance for high pulp density than moderate thermophiles or mesophiles (Norries, 1992). The observed accelerating effect at elevated temperature was probably mostly on indirect leaching. Kennecott's patent notwithstanding, commercial bioleaching was initially restricted to copper ores, but reactor-based processes have recently been developed for the extraction of other metals such as Co, Ni and Zn (Briggs and Millard, 1997; Dew and Miller, 1997; Steamson *et al*, 1997; Sandstorm *et al.*, 1997).

Ehrlich reported in 1964 that *T. ferrooxidans* was capable of oxidizing arsenopyrite (Ehrlich, 1964). In his study, he measured mobilization of Fe and As. He did not follow sulfide. The mobilized iron appeared as FeII and FeIII. The mobilized arsenic appeared as arsenite AsIII and as arsenate AsV. Some of the arsenite and arsenate were precipitated by iron. The iron arsenate compound was later shown to be scorodite $FeAsO_4.2H_2O$ (Carlson *et al.*, 1992). Although it seemed possible at the time that the arsenate resulted from oxidation of arsenite by *T. ferrooxidans*, this could not be confirmed by direct testing. However, the thermophilic archeon, *Sulfolobus acidocaldarius* strain BC, is capable of such oxidation (Sehlin *et al*., 1992). Current evidence indicates that the arsenate formed in the presence of *T. ferrooxidans* is the result of chemical oxidation of arsenite by the bacterially generated Fe III.

Transient formation of S^0 was also observed in this recent study (Monroy *et al.*, 1995). The ability of *T. ferrooxidans* to oxidize arsenopyrite led the late Eric Livsey-Goldblatt to propose in 1983 that it be used in biobeneficiating pyritic gold ores in a bioleaching process that he estimated, based on laboratory-scale tests, to be significantly more economical than pyrometallurgical treatment (Livsey- Goldblatt *et al.*, 1983). This has proven to be the case. In pyritic gold ores, pyrite and arsenopyrite encapsulate the gold, making it inaccessible to lixiviants such as cyanide or thiourea. Partial oxidation of the pyrite and arsenopyrite uncover the gold sufficiently for extraction, and at the same time lessens the non-specific, irreversible consumption of cyanide during extraction of the ore.

BIOLEACHING OF NON-SULFIDE ORES

Extension of bioleaching systems to the use of heterotrophic bacteria is of great importance if biologically assisted leaching is to be extended to non sulfide systems. For example, development of a heterotrophic bacterial leaching scheme for low grade lateritic ores could substantially increase world nickel reserves, and the use of heterotrophic bacteria in the leaching of manganese, silver and phosphate ores could also increase world reserves of these important commodities. Such systems pose certain problems, such as those with water recirculation from the bioleaching operations and competition from other heterotophs for energy sources. Also, cultivation of heterotrophs requires one or more organic nutrients to serve as carbon and energy sources. On the other hand, heterotrophs have certain advantages, such as often being faster growing than autotrophs. Further, the use of heterotrophic bacteria may often be more environmentally acceptable than the use of many inorganic chemical leaching agents. As opposed to the enzyme catalyzed oxidations or reductions that are characteristic of the direct action dissolution of minerals by microorganisms such as *T. ferrooxidans,* the dissolutions of non sulfide minerals by heterotrophs are by metabolic products produced by the organisms (Ehrlich, 1993). The action is, thus, in such cases, an indirect one. The metabolic products are usually organic acids such as citric, oxalic, formic, acetic, lactic, succinic, etc. The action by the acids can be by acidolysis and/or by complexation. Additionally, some heterotrophs can also reduce certain metal ions

enzymatically. Ehrlich has compiled a list of some heterotrophs with bioleaching potential, which is partially reproduced in table1 (Ross and Mishra, 1993).

Sporadic research on Heterotrophic leaching and or beneficiation of silicate, carbonate, and oxide ores has been done on a laboratory scale in the past, but it has not led to industrial applications so far. In the case of silicate and carbonate ores, solubilization of the metal constituents can be achieved by attack with acids and or complexing agents ligands of microbial origin. Examples of such agents are sulfuric acid generated from sulfur by the autotroph *T. thiooxidans*, but more importantly, organic acids and ligands such as 2-ketogluconate generated by some heterotrophic bacteria w69x, and oxalate and citrate generated by fungi (Bosecker, 1986; Kiel, 1977). In the case of metal oxide ores, anaerobic processes in which bacteria reduce the metal oxide and thereby solubilize it may be the most promising for industrial exploitation (Ehrlich, 1991). In such processes, the bacteria use the metal oxide as terminal electron acceptor. The electron donor may be organic carbon, formate, or H, depending on the organism.

An example of a reaction in which MnO is bacterially reduced to Mn^{2+} with acetate as reductant is the following,

$$4MnO_2 + CH_3COO^- + 7H^+ \rightarrow 4Mn^{2+} + 2HCO_3^- + 4H_2O.$$

Since ores are not sterile and cannot be sterilized on a commercial scale, heterotrophic leaching presents some process design challenges that autotrophic leaching with acidophiles does not. The acidophilic autotrophs grow in a highly selective environment that tolerates few if any competitor that can displace them. This is not the case with heterotrophic leaching organisms. For this reason, aerobic, heterotrophic reactor leaching based on the action of microbially produced acidulants and or ligands, should be operated in a two-reactor system in which the first reactor would be the generator in which desired microbes would produce the acidulant ligand in pure culture axenically under optimal growth conditions preferably in a continuous mode. The spent culture solution from this reactor would be bled into a second reactor containing the ore to be leached. Growth of microbes on the ore that might destroy the acidulant ligand could be controlled by ensuring a very low level of residual nitrogen source in

the spent culture medium, a nitrogen source being essential for growth, and by temperature manipulation.

Anaerobic heterotrophic leaching of metal oxides by a reductive process is best carried out in a single reactor. The maintenance of selective growth condition in such a reactor is extremely important. Whereas in autotrophic bioleaching of metal sulfides, conditions of high acidity and the absence of a major organic carbon and energy source are highly selective, in heterotrophic bioleaching of metal oxides, conditions of circumneutral to moderately acid pH and the presence of a general carbon energy source are not sufficiently selective. They can be made more selective by running the leaching anaerobically and using a very specialized carbon energy source. The purpose of anaerobiosis is the exclusion of potentially interfering heterotrophs that are obligate aerobes. The purpose of a specialized carbon energy source, ideally utilizable only by the leaching organisms, is to prevent overgrowth by anaerobic heterotrophs incapable of attacking mineral oxide. Phenol is an example of a specialized carbon energy source that is toxic to many microorganisms but can be used as carbon and energy source by some iron oxide and MnO reducers (Lovley, 1991). Acetate is another specialized carbon energy source. It is non-fermentable except by acetoclastic methanogens, and it is inadequate as a sole source of carbon for many anaerobes because they are unable to convert acetate to essential three-carbon metabolic intermediates such as pyruvate. Acetate can be used as sole carbon energy source by some reducers of iron oxide, MnO_2 and UO2q (Lovley, 1991; Lovley and Philips, 1991). Thus, in designing a heterotrophic leaching process, important considerations are selective conditions in a one-reactor system, or axenic conditions in the first reactor of a two-reactor system. In choosing a carbon energy source for commercial heterotrophic leaching, cost becomes another important consideration. Sugar, in the form of industrial molasses, whether a byproduct of cane- or beet-sugar-production, or of corn-starch-hydrolysis, is a prime candidate, but it is not an especially selective nutrient. If it has to be used, heavy inoculation with active leaching organisms may prevent overgrowth by undesirable competitors. If the carbon energy source is to be an aromatic electron donor, industrial phenolic waste streams from chemical industry might be worth considering. Acetate, a product in a reactor (with an acetogen like *Clostridium thermoaceticum*) growing on sugar e.g., glucose or fructose, as from invert sugar, or corn starch hydrolyzate, but not sucrose or on

some other economic feed stocks could take place at the site of the bioleaching plant (Cheryan *et al.*, 1987). *Cl. Thermoautotrophicum* and some strains of *Cl. thermoaceticum* could also be used to form acetate from CO_2 and H_2 (Gottschalk, 1986).

Table 1: Possible heterotrophs for use in bioleaching.

Name	Growth Requirements	Physiological Attributes
	BACTERIA	
Achromobacter delicatulus strain 182-A	Aerobic; Mesophilic	Solubilizes manganese with the aid of organic acids formed in the oxidation of glucose
Bacillus licheniformis	Aerobic; Mesophilic	Solubilizes silicate with exopolysaccharide
Bacillus mucilaginosus	Aerobic; Mesophilic	Solubilizes silicate with exopolysaccharide
Bacillus GJ33 and *Bacillus* 29	Aerobic; Mesophilic	Solubilizes silicate with exopolysaccharide
Pseudomonas sp. 200 and *Clostridium butyricum*	Aerobic; Mesophilic	Reduces Fe(III) enzymatically
Shewanella putrefaciens	Facultative anaerobic; Mesophilic	Reduces Fe(III) and Mn(IV) oxides enzymatically with lactate or H_2 under anaerobic conditions
	FUNGI	
Aspergillus niger	Aerobic; Mesophilic	Can produce oxalic and citric acids that can complex and extract metals from ores
Penicillium simplicissimum	Aerobic; Mesophilic	Can produce oxalic and citric acids that can complex and extract metals from ores

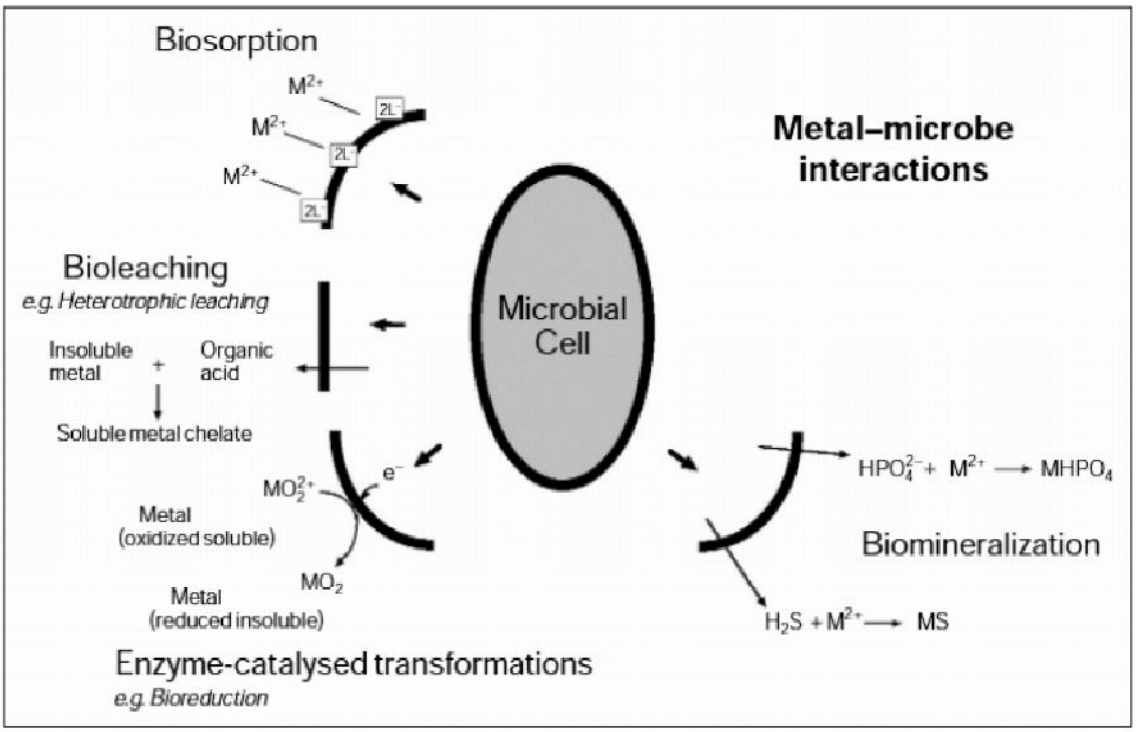

Figure 1: The metal- microbe interactions

Chapter 3

MATERIALS & METHOD

MATERIALS AND METHODS

COLLECTION OF MICROORGANISM

The *Bacillus* strain was procured from IGH hospital and the pure culture was made.

GROWTH KINETIC STUDY

The bacterium was cultured with 200ml of Bromfield medium in 250ml standard Erlenmeyer shake flask A 10% v/v of an active inoculum was added to Bromfield medium and incubated at 37ºC on a rotary shaker at 240 rpm. The composition of Bromfield Medium is given in Table 2. The bacterial growth pattern was studied at 630 nm. To avoid the lag phase the culture was kept overnight around 12 hrs. For the estimation of biomass, the absorbance of the media was studied with respect to time with the help of UV/VIS spectrophotometer. The absorbance values were taken at a time interval of one hour until there was no change in absorbance value which indicated the on-set of stationary phase.

Table2. Composition of Bromfield medium

Sl no.	Constituents	Amount (g/L)
1	$(NH_4)_2SO_4$	0.25
2	KH_2PO_4	0.25
3	$MgSO_4$	0.7
4	Carbon source	20
5	Yeast extract	1.0
6	pH	6.5

COLLECTION OF SLIME AND ANALYSIS

The sample was collected from the Jodha mines of iron ore and finely ground.

ANALYSIS OF SLIME

The slime was analyzed with the help of XRF (X- Ray Fluorescence) for the percentage of iron oxide and impurities like alumina and silica.

EFFECT OF TIME

The effect of time on the activity of cells and on slime bioleaching was studied in the batch system. All experiments were carried out in 250ml Erlenmeyer Flasks containing 250-ml of the medium, 10% and 20% (v/v) inoculum (of maximum biomass concentration of 2.0 × 108 cells/ml) and 3gram of initial slime. The aerobic condition of the system was maintained by putting non-absorbent cotton to the mouth of the flasks. The flasks were incubated in an incubator maintained at 37°C along with constant shaking of 230 rpm. The initial pH was adjusted to 7 using 1M HCl and 1N NaOH. Flasks were taken out on a regular basis, that is, after 5, 7, 9 and 10 days of inoculation, respectively followed by analysis the extent of leaching.

EFFECT OF pH

The experimental procedures, as stated in effect of time, were performed accordingly with varying initial pH like 6.8, 7.0, 7.2, and 7.4 respectively. The initial pH was maintained constant throughout the incubation by adding NaOH because due to progress on bioleaching process, the pH declined gradually. Samples were withdrawn after 10 days (optimum time) of inoculation and analysed.

EFFECT OF INOCULUM SIZE

The experimental procedure was carried out now with varying initial innoculum size like 10%, 20%, 30% and 40% respectively. Samples were withdrawn after 7 days of incubation and analysed.

ANALYSIS OF THE COMPOUND AFTER BIOLEACHING

The compounds after bioleaching were centrifuged and the clear supernatant obtained was analyzed for iron content with the help of AAS (Atomic Absorption Spectrophotometer) (Beaty, 1998).

Chapter 4

RESULTS & DISCUSSION

RESULTS AND DISCUSSION

ANALYSIS OF IRON ORE SLIME

The XRF results for the analysis of slime showed a 56% of iron oxide, 6.33% of Alumina and 6.88% of silica.

GROWTH KINETIC STUDY

Figure 2 shows the growth curve of *Bacillus*. *Bacillus* has shown a decent growth rate when incubated in Bromfield media. The absorbance pattern was recorded after 12 hrs. The stationary phase reached after 6 hours of incubation.

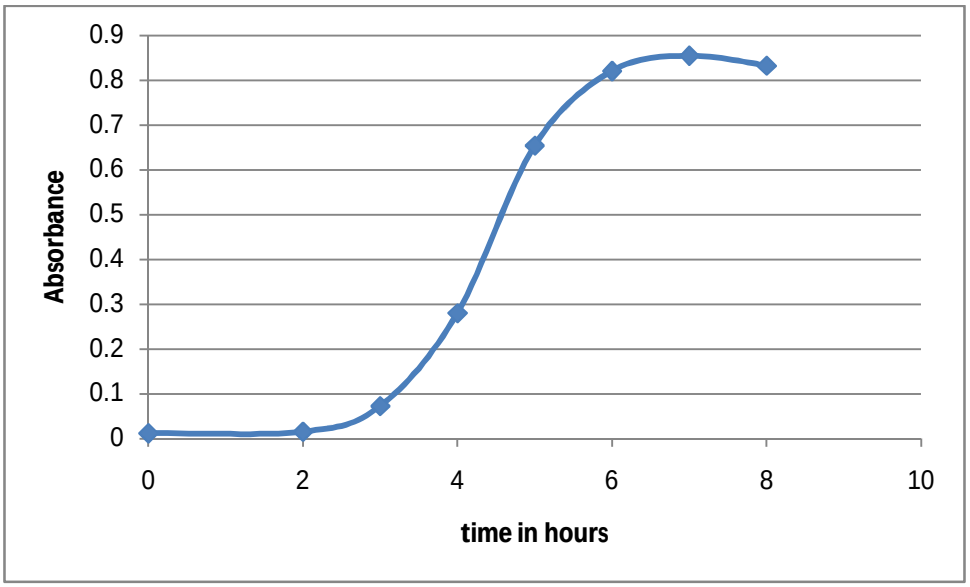

Figure 2: Growth curve of *bacillus*

BIOLEACHING EXPERIMENT

Effects of various parameters on bioleaching of slime are discussed.

EFFECT OF TIME

Figure 3 shows the optimum time of leaching of slime with Bacillus strain. In this study, bioleaching is analyzed after 5, 7 and 9 days. The *bacillus* showed maximum growth on the fifth day. After this time, the bioleaching gradually declines. Therefore 5 days is taken as optimum bioleaching time. The percentage of iron in the slime was found to be 58.65%. This time the pH was not adjusted in order to check whether, the addition of NaOH to the system neutralized the solutions and affected the bioleaching. It was found that when the pH was adjusted the results were better than the results obtained by not adjusting the pH. The reason behind this can be attributed to the death of bacteria due to low pH.

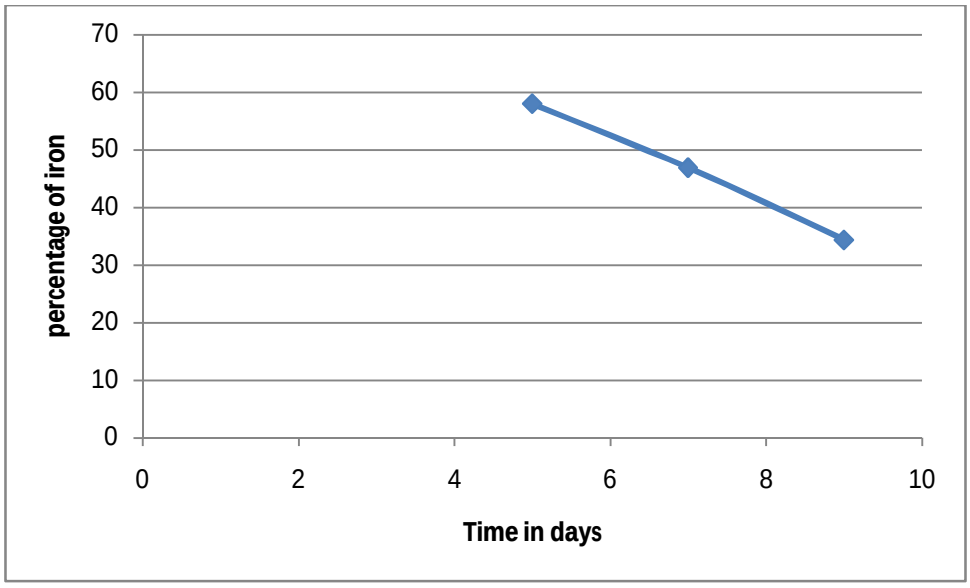

Figure 3: Effect of time on bioleaching of slime.

EFFECT OF pH

The effect of pH on bioleaching of slime is shown in Figure 4. The optimum pH was found to be 7.2 – 7.4 at which the maximum bioleaching was found to be respectively. The percentage of iron in the slime was found to be 79.64%.

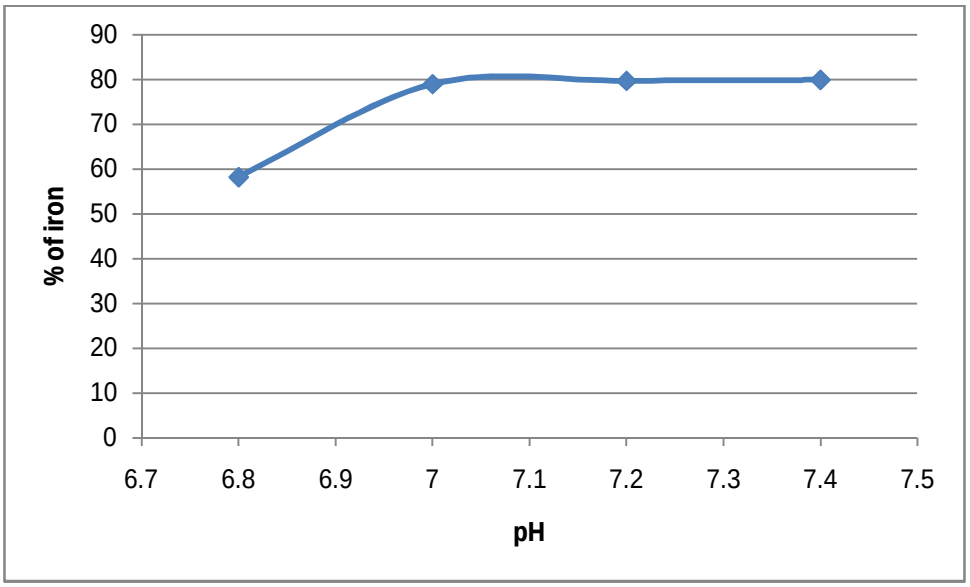

Figure 4: Graph showing the percentage of iron against pH.

EFFECT OF INOCULUM SIZE

The optimum inoculum size was found to be 20% at which maximum bioleaching was found. The percentage of iron present in the slime was found 79.64%.

Chapter 5

CONCLUSION

CONCLUSION

- The following experiments showed that the bioleaching of the slime collected from the Joda mines was feasible using the heterotrophic bacteria, *Bacillus.*
- For the slime to be used in the making of steel, the iron content has to be increased to more than 62%. In our experiments we have tried to recover the maximum amount of iron during the bioleaching process, which was found to be 79%.
- The conditions optimized during this experiment were pH, time and inoculum size at which maximum iron was recovered from slime. The optimum pH was found to be 7.2 to 7.4. The optimum time for which a solution of 250 ml can be left for maximum bioleaching to occur was found to be 5 days. The optimum inoculum size at which maximum bioleaching was observed was found to be 20%. All these parameters were optimized by taking 3 gm of slime into 250 ml of Bromfield media, set at 240 rpm shaking and a temperature of 37^0 C.
- It was also seen that the proper maintenance of pH is important for the growth and maintenance of the *bacillus*, without which, the proper growth of the microorganisms will be affected and hence it will affect the bioleaching process eventually stopping it from further continuation. The neutralization of the pH doesn't have an effect on the bioleaching.
- Thus we can conclude that leaching of slime with the help of bacteria is a bio-friendly, cost effective and pollutant free process and can be of great help to the steel industries.

FUTURE PROSPECTS

The future prospects of this experiment include:

- Identification of the strains of Bacillus best suited for bioleaching.
- The effect of mixed cultures on bioleaching, and their comparison with the effect of single stains on bioleaching.
- On the basis of the present work large scale bioleaching of slime can be carried forward with the help of a bioreactor.

Chapter 6

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