ADVANCED SUMMARY

Biogeotechnology offers a good alternative for an improvement in the methods of mining and processing of minerals. It is a field of science of metal extraction from ores, concentrates, rocks and solutions under the impact of microorganisms and/or their metabolites. Its components are: (a) Biohydrometallurgy and (b) Biosorption.

Biohydrometallurgy

Biohydrometallurgical processes include bioleaching and biobeneficiation. The former involve microbial dissolution of metal values from ores whereas the latter involves selective microbial removal of undesirable mineral constituents from ores/minerals .The bacteria either function as catalysts for the dissolution or as generators of metabolic products which cause chemical dissolution. The fungi exclusively act as generators of metabolic products that cause chemical dissolution of metal values.

Biosorption of metals from solutions

That various living and dead microorganisms can remarkably accumulate heavy metal ions (and many toxic anions) has been established (Sloan et al., 1983: Sterritt & Lester, 1986: Gadd, 1986). Biosorption has received considerable international attention over the last 25 years. It has been particularly effective in removing metals that are present in low concentrations (i.e., <10 mg/l) and it offers a variety of ways by which the metal(s) can be accumulated.

Microorganisms important for Biogeotechnology

Microorganisms with a potential for bioleaching can be divided into autotrophs (obligate and facultative), mixotrophs, and heterotrophs. The autotrophs used in bioleaching are all chemosynthetic. They get their energy from oxidation of oxidizable inorganic compounds and derive all their cell carbon from carbon dioxide. Mixotrophs also get their energy from the oxidation of an oxidizable inorganic compound but at least a portion of their cell carbon from organic compounds. Heterotrophs get their energy from oxidation of suitable organic compounds and their cell carbon from assimilation of organic compounds. Bacteria are represented in all three nutritional groups but fungi are restricted to heterotrophs. Autotrophs are the most desirable candidates for bioleaching because they are able to grow in absence of organic matter in culture solution, which is unsuitable for growth of ubiquitous interfering mixotrophs and heterotrophs. These mixotrophs and heterotrophs are always associated with an ore and they cannot be removed without sterilization. Heterotrophs with bioleaching potential can grow significantly faster than autotrophs. This is one reason as to why a potential exists for heterotrophs as bioleaching agents.

Autotrophic as well as heterotrophic microorganisms play an important role for the industrial recovery of metals from low grade ore, or in general, from low grade mineral resources. Our mining industry is faced with several problems, most important problem being the depletion of high grade surface ore deposits. Several economic methods are being tried to recover metals from ores with metal content and to recover the small quantities of metal remaining after the processing of richer ones. Bioleaching provides a good solution. It not only reduces the expense of mining at greater depths but also makes a contribution to the control of water, soil and air pollution that results from mining and associated metal recovery processes whereas biobeneficiation helps in removal of undesirable impurities from low grade minerals by which enrichment of low grade material is achieved for further processing.

The world's appetite for minerals is gargantuan and is ever on increase because minerals are basic to national economies. Added to this, the world population is doubling every 35 years causing a proportionate rise of mineral demand. In the years ahead, there is bound to be an extraordinary strain on our resources and a great danger to our environment.

Minerals have fixed location, fixed quantity and fixed quality and these characteristics lead to some of the most challenging problems of mineral industry. Therefore, they require thoughtful attention and mature policy for their exploration, exploitation and use. Occurrences of economic mineral deposits are not prolific. Complexity of geological processes makes most deposits difficult to discover, evaluate and exploit. There is an inherent time lag of a few years to decades between the discovery of a mineral deposit and the time of first production. Mining has to be scientifically planned so as to ensure optimum recovery. Research and development has to be intensified to mine marginal and sub-marginal grade of ores. It is a fact that grade is inversely proportional to the quantity. As the grade lowers, quantity inflates so much so that at much lower concentration, the quantity of mineral commodity available may be infinite. The mining of low grade deposits, gives rise to environmental problems. Mining of such material involves the movement and disposal of large volumes of overburden and the removal and processing of large volumes of rock. This leads to scarring of landscape, modifies the terrain and drainage, presents the problem of waste disposal in the form of sediments and highly mineralised water.

Minerals constitute a fundamental component of a nation's material and economic base. In fact, they have christened various eras of human civilisation and progress, e.g., the Stone Age, Bronze Age, Iron Age and Atomic Age. From the humble beginning of a fruit and meat eater, the man advanced himself by the invention of tools and weapons. And it was from here that minerals began to make their impact on his means of livelihood and progress. Archaeologists have proved that minerals were used in India in pre-vedic times. With the beginning of Industrial Revolution, the role of minerals in the economic development was well established, their production continued to increase at an ever increasing pace and the trend goes on.

India like, most of the developing countries has indeed smaller stock of capital and comparatively high numbers of unskilled labour. All the three basic factors of production, i.e., land, labour and capital are inadequately developed in these countries. Considering these constraints, in developing countries, the role of minerals occupies a greater important place. They are the basis of automobile age, which is the dominant characteristic of industrial economy, requiring huge quantities of steel, chrome, glass, lead, zinc and plastics. The other main characteristics of the industrial society is massive use of minerals as sources of energy - coal, oil, gas and uranium.

In general, India has good reserves of coal, manganese, iron ore, mica and chrome ores. Coal and manganese occupy an important position in the mineral industry of India. Coal, a mineral fuel, meets a large part of growing energy requirements of the country whereas manganese, a metallic mineral, is earning valuable foreign exchange for our country. But the utilization of coal has a large impact on the environment. A major disadvantage associated with the use of coal as energy, is emission of sulphur dioxide into the environment during the combustion. Problems with the existing technology concerning costs, efficiency, applicability and waste disposal have led to increased research efforts on microbial processes which have potential of solving some of these problems. Hence, our investigation was undertaken to assess the possibility of reducing the sulphur content by the action of *Thiobacillus ferrooxidans*, the process otherwise known as biobeneficiation.

So far as manganese is concerned, the bulk of the world's reserves exist in the form of low grade ores. The increasing demand of manganese in stainless steel industry and consequent depletion of existing high grade ore reserves, encouraged the investigation of possible alternative technologies that could utilize the abundant low grade manganese ore reserves. Microbially assisted leaching or bioleaching comprises one of these possible alternatives. Thus, *Penicillium citrinum*, a native microorganism isolated from the top soil of manganese ore mine, was utilised for leaching of manganese ore in the present study.

Extensive research has been carried out to highlight both the processes of biohydrometallurgy-biobeneficiation as well as bioleaching. Therefore, this thesis has been divided into two sections to have a better insight into the problem. Section-1 comprises of biobeneficiation of coal or microbial desulphurisation of coal whereas Section –2 deals with the bioleaching of manganese ore.

For microbial desulphurisation of coal, three coal samples, one from Assam, one from Poland and one lignite sample from Rajasthan were used. *Thiobacillus* cultures were isolated from the three coal samples. Growth studies of the *Thiobacillus* isolates were performed in shake flasks in three different conditions: (1) in the absence of coal in the growth media, (2) in the presence of coal in the growth media, and (3) in the presence of increased cell concentration and absence of coal in the growth media. Growth was determined in terms of cell concentrations, specific growth rate (μ), maximum specific growth rate (μ_{max}), Fe^{3+}/Fe^{2+} ratio, total iron concentration, average iron oxidation rate, oxidation rate constant (k), pH and redox potential values. It was observed that under all conditions, *Thiobacillus* isolated from Rajasthan lignite (Tf-R) showed best growth in comparison to other two cultures isolated from Assam coal and Polish coal respectively, (Tf-A and Tf-P). Also, it was seen that under all experimental conditions, Tf-R showed best growth in presence of increased cell concentration in the media. The specific growth rate for Tf-R was 0.0115 h^{-1}, the maximum specific growth rate was 0.0154h^{-1} with a generation time of 60.3 h. Also, the average iron oxidation rate was the highest (68.21 mg/l.h) with a rate constant of 0.0185 h^{-1}. Simple first order kinetics were observed during iron oxidation reaction. Tf-R, when grown in bioreactor of 2l capacity, showed a specific growth rate of 0.0181 h^{-1} and a generation time of 38.3 hours. The average iron oxidation rate was 77.96 mg/l.h with a first order rate constant of 0.0128 h^{-1}.

Further studies were carried out to desulphurise the three coal samples with their respective native strains. The effect of various parameters like initial pH, pulp density, media composition and particle size of coal samples were studied to optimise the conditions for best removal of sulphur. The lignite was found to be the most suitable coal for microbial desulphurisation. The conditions optimised for best removal of total sulphur in three coals (91.87% in lignite, 63.13% in Polish coal and 9.44% in Assam coal) were : initial pH, 1.5 (2.5 in case of Assam coal); particle size, 45 microns; pulp density, 2% (w/v); incubation period, 30 days; Temperature, 35°C with shaking at 140 rpm.

Simple first order kinetics were observed in the study of microbial desulphurisation of lignite sample. The first order rate constant for lignite desulphurisation in shake flask was found to be 0.05 day^{-1}. The same lignitic coal, when used for desulphurisation in bioreactor with Tf-R, showed a removal of 71.5% of total sulphur and 88.4% of pyritic sulphur in only 7 days. The first order rate constant was found to be 0.17 day^{-1}.

It was attempted to combine the two processes (froth flotation and bacterial leaching) for better removal of pyritic as well as total sulphur from high sulphur bearing Assam coal. In our initial investigation, it was attempted to float the coal with light diesel oil and then bioleaching of the flotation concentrate was done using various autotrophic and heterotrophic microorganisms. Very poor results were observed as there was no removal of sulphur. As the light diesel oil formed a coating on coal surface, the pyrite was not accessible for the microbes to attack, hence poor removal was obtained.

Results were encouraging when it was attempted to reduce sulphur from the same coal by conditioning with *Thiobacillus ferrooxidans* followed by froth flotation. Around 60% of the pyritic sulphur as well as 23.2% of total sulphur could be reduced using the two procedures - bacterial conditioning followed by flotation.

For bioleaching of manganese ore, a low grade ore containing 27.26% of manganese collected from Joda East manganese mines of TISCO, Orissa, was used. Eight types of microorganisms were isolated from the top soil of manganese mine area, out of which one fungus was selected for all bioleaching studies on the basis of its maximum efficiency for manganese solubilization in comparison to other strains. It was identified as *Penicillium citrinum*. Growth studies of the isolate were carried out in shaking as well as in static conditions. Growth was measured in terms of final pH, strength of total acid produced by the fungus and the biomass dry weight of the fungus. It was observed that growth of *P. citrinum* was better in static condition where the strength of the total acid produced during the potential growth phase was 0.1N and the corresponding biomass dry weight was 11.22 g/l. In case of shaking conditions, the maximum strength of acid produced was 0.08 N and the biomass dry weight was 9.04 g/l. First order rate constants were derived for growth of the fungus (in terms of biomass dry weight). The rate constant for the fungus in shaking condition was 0.13 day^{-1} and in static condition, it was 0.18 day^{-1}. Though the growth of *P. citrinum* was observed to be better in static condition, shaken cultures were used for bioleaching of manganese ore because of the following reasons :

(a) *P. citrinum* is an aerobic organism and needs oxygen supply; shaking provides aeration to the organism.

(b) Shaking cultures provided more uniform type of growth and the leached material is not much accumulated by the fungal mycelia.

(c) The ageing of static cultures was rapid.

Leaching of the manganese ore was carried out in three methods :

(a) In-situ leaching with *P. citrinum*

(b) Leaching with culture filtrate of *P. citrinum*

(c) Chemical leaching with various acids.

During in-situ leaching, effect of various parameters like particle size, pulp density, sucrose concentration, inoculum size and duration on bioleaching of manganese ore was studied. The conditions optimised for maximum recovery of manganese (64.58%) were: particle size, 45 microns; pulp density, 2% (w/v); sucrose concentration, 10% (w/v); inoculum size, 10% (w/v) and duration, 30 days.

That leaching of manganese ore is connected mainly with the action of secreted metabolites (basically organic acids) was proved while using the culture filtrate of *P. citrinum*. It was found that nearly 19-20% of manganese was solubilized within 30 days. Further, to prove that the reduction of MnO_2 took place with organic acids, the ore was leached with various concentrations of citric, oxalic and sulphuric acid. Manganese was precipitated when citric acid was used for leaching. As manganese oxide had negligible solubility in sulphuric acid, as low as 1.15% of manganese was extracted with 0.5M of sulphuric acid in 30 days. Maximum recovery of manganese (38.7%) was achieved with oxalic acid (0.5M) in 30 days. Kinetic analyses were performed in three cases: (a) in-situ leaching (only soluble manganese was taken for kinetic analyses), (b) culture filtrate leaching, and (c) oxalic acid leaching of manganese ore. It was found that the Mn^{2+} ions (as manganese citrate and manganese oxalate) formed a product layer surrounding the manganese ore. The reaction was controlled by diffusion of reactants through the permeable product layer, following the equation :

$$[1 - (1 - \alpha)^{1/3}]^2 = 2kt/r_o^2 = k't$$

where 'α' is the fraction of manganese reacted and k and k' are rate constants, 't' is time and 'r_o' is initial radius of the particle. The rate constant for in-situ leaching was 0.0002 day^{-1}, for culture filtrate leaching 0.0001 day^{-1} and for oxalic leaching, it varies from 0.000007 day^{-1} to 0.0009 day^{-1} according to different strength of oxalic acid. Bioleaching of two other low grade ores containing 17-25% of manganese ore and high grade ore containing 62.75% of manganese was done using *P. citrinum*. The total recovery of manganese (49-56%) in case of two low grade ores was comparable to the total recovery of manganese (60.1%) achieved with Joda ore under similar experimental conditions. Hence, it was concluded that since *P. citrinum* was an inhabitant of active manganese mine area, it could effectively leach manganese ores from other manganese mine sites also.

The distribution of manganese leached out from ore was established by bioaccumulation and adsorption studies. It was found that nearly 8% of leached manganese was accumulated by the biomass of *Penicillium citrinum*, 50% of leached manganese was adsorbed on the manganese ore itself and the rest was soluble manganese.

Hence, it was concluded that manganese ore could be effectively leached with *Penicillium citrinum*.