A STUDY ON ANAEROBIC BIOLEACHING OF MANGANESE BY USING COMPOST

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This is to certify that the thesis entitled "**A STUDY ON ANAEROBIC BIOLEACHING OF MANGANESE BY USING COMPOST"** submitted in partial fulfilment of the requirements for the award of the degree of **Master of Science in Biotechnology** to the Orissa University of Agriculture and Technology is a faithful record of bonafide and original research work carried out by **Diptymayee padhi** under my guidance and supervision. No part of this thesis has been submitted for any other degree or diploma.

It is further certified that the assistance and help received by him/her from various sources during the course of investigation has been duly acknowledged.

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Declaration

I, Diptymayee Padhi, studying M.Sc. Biotechnology at (college of basic science and humanities, OUAT), hereby declare that I have carried out all the experiments by myself under the guidance of my supervisor. I shall not report or produce the results presented here in any conference/publication/ report or journal without prior permission of my guide Mrs.V.Aishvarya and CSIR-IMMT.

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ABSTRACT

In the present study cow dung compost was used for anaerobic bioleaching of manganese from low grade manganese ores, to find its suitability as a low cost organic carbon source and replace the commercial carbon sources such as glucose and sodium acetate. The study was carried out on low grade Mn ore from Barbil, Odisha, using anaerobic consortia maintained at CSIR-IMMT. It was found that cow dung compost can support the growth of microorganisms present in the consortia, similar to glucose or sodium acetate. The microbial action resulted in Mn dissolution in the culture filtrate and found to increase with incubation time and amount of compost used. It can be said that cow dung compost can be used as a replacement of glucose or sodium acetate to support the growth of anaerobic microorganism and enable Mn bioleaching. Further detailed studies are however necessary to understand how the constituents of compost enable microbial growth and Mn dissolution.

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ABBREVIATION USED

mg	Milligram
ml	milliliter
mM	millimolar
gm	gram
hrs.	hours
min	minutes
%	percentage
PD	pulp density
nm	nanometer
diff.	d difference
conc.	concentration
Fe	iron
Mn	manganese
MSM	mineral salt medium
AAS	atomic absorbance spectroscopy

CHAPTER I

INTRODUCTION

1. INTRODUCTION

In the beginning of the 21century, bioleaching occupies an increasingly important place among the available mining technologies. The situation is now quite different. Today bioleaching is no longer a promising technology but an actual economical alternative for treating specific mineral ores. Examination of the current large-scale bioleaching operations reveals that an important number of them are located in developing countries. The necessary result of two important factors: for one thing, many developing countries have significant mineral reserves and mining constitutes one of their main sources of income; on the other hand, bioleaching is a technique especially suitable for developing countries because of its simplicity and low capital cost requirement [1-6]. It is recognized that microorganisms have contributed to the solubilisation of metal sulphides since ancient times and that Romans benefited from their action long before Christ. It has been pointed out that the copper mine of Río Tinto in Spain was probably the first large-scale operation in which microorganism played a major role [7-8]. The role that microorganisms play in this process was demonstrated only in 1947 when Colmer and Hinkle isolated from acid mine waters bacteria belonging to the *Thiobacillus* genus [9]. Later, *Thiobacillus ferrooxidans* [10]and *Thiobacillus thiooxidans* [11] were isolated and characterised. *Thiobacillus ferrooxidans* has been recently reclassified as *Acidithiobacillus ferrooxidans* [12]. Bioleaching is also successfully applied in gold mining, when the metal is covered with a film of insoluble metal sulphides that difficult the extraction of gold with cyanide solutions. In this case, the sulphide film must be removed in order to obtain satisfactory gold recoveries, and bioleaching is presently the alternative of choice for such pre-treatment step. The current panorama of bioleaching in developing countries is encouraging. It is expected that in the coming years several new commercial-size bioleaching plants will be installed. It is likely that heap leaching will continue to be the choice for low-grade ores and tailings, while tank bioleaching technology will probably increase its application for gold, copper and other base-metal concentrates. The use of thermophilic bacteria and archea will be a major contribution, increasing the leaching rates and metal recoveries and allowing for the treatment of recalcitrant ores such as chalcopyrite.

Most studies were focused on the extraction of manganese and only a few studies included the extraction of ancillary elements [13] investigated manganese, cobalt and nickel release by manganese-reducing bacteria (not identified). Efficient recovery of metals (75%–80% after 48 h of incubation) was achieved during anaerobic leaching at pH 5–6.5 with glucose as the source of organic carbon and temperatures in a range of 30–45 °C. While not directly comparable with other studies, the leach duration was considerably shorter than those reported for fungal production of organic acids [14-15] or the use of RISC-oxidising autotrophs [16]. The release of nickel and cobalt from crushed ferromanganese nodules correlated directly with MnO2 reduction and dissolution when treated with a Bacillus strain [17]. The reduction of manganese(IV) in manganese nodules released simultaneously the ancillary elements copper, nickel and cobalt but not iron. The co-solubilisation of copper, nickel and cobalt was attributed to those elements being bound in the MnO2 matrix, and the poor release of iron as due to the reduction of iron(III) being less favoured than the reduction of MnO2 under the conditions used. A hypothetical anaerobic process utilising manganese(IV)-reducing bacteria was proposed [17] involving the suspension of ground nodules (−2 mm) at optimal solids loading in salts medium containing a specific organic carbon compound that would act as the electron donor as well as supply carbon for the growth of the selected microorganism(s). Ehrlich et al. [17] thought that a mixture of Geobacter metallireducens, a strict anaerobe, and Sh. putrefaciens, a facultative anaerobe that could scavenge any oxygen initially present in the medium, would provide a suitable culture to reduce manganese(IV) and release Mn2+ and other cations to solution.

OBJECTIVE

Overall Project Objective: To study the use of a low cost organic carbon source such as compost in anaerobic bioleaching of low grade Mn ore.

Specific Objectives:

- To collect low cost carbon source i.e cow dung compost.
- Adapt and enrich the anaerobic consortia available at IMMT with the chosen ore and compost.
- To study the growth curve of consortia in presence of compost by protein estimation.
- To study the effect of leaching parameters such as incubation time, concentration of compost and pulp density on Mn solubilization/leaching.

CHAPTER II

REVIEW AND LITERATURE

2.1 BIOLEACHING

Bioleaching is a process of leaching where the extraction of metal from solid minerals into a solution is facilitated by the metabolism of certain microbes which is known as bioleaching microbes. A process described as "the use of microorganisms to transform elements so that the elements can be extracted from a material when water is filtered through it" is known as bioleaching. This is much useful than the traditional leaching like heap leaching, insitu leaching etc. Bioleaching is one of several applications within biohydrometallurgy and several methods are used to recover manganese , copper, zinc, antimony, nickel, gold, silver, and cobaltetc .It is an effective technology for metal extraction from low-grade ores and mineral concentrates. Metal recovery from sulfide minerals is based on the activity of chemolithotrophic bacteria, mainly *Thiobacillus ferrooxidans* and *T. thiooxidans*, which convert insoluble metal sulfides into soluble metal sulfates. Non-sulfide ores and minerals can be treated by heterotrophic bacteria and by fungi. In these metal extraction is occur by production of organic acids and chelating and complexing compounds excreted into the environment. Bioleaching has also some potential for metal recovery and detoxification of industrial waste products. Example; sewage sludge and soil contaminated with heavy metals [18].

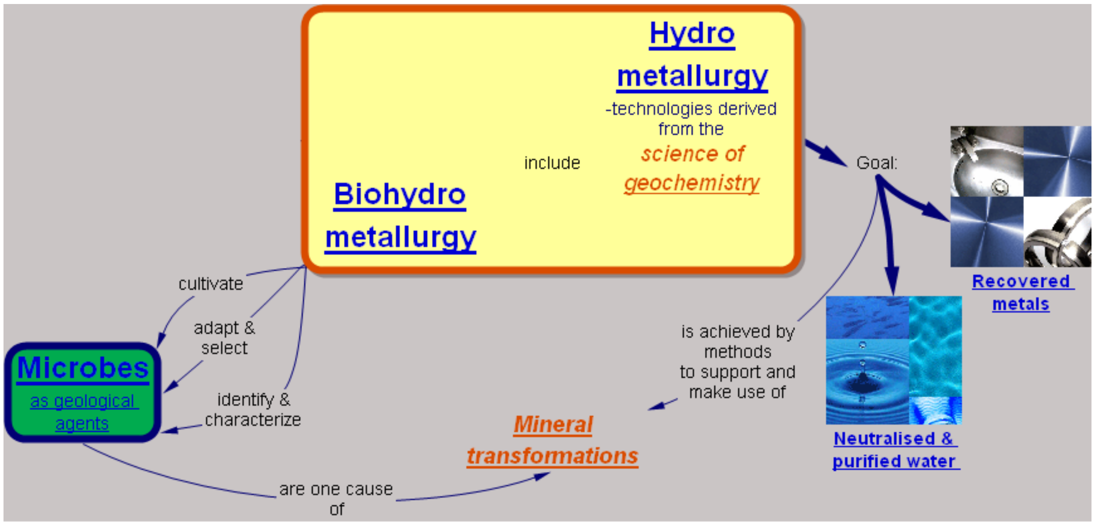


Figure-1 Overall process of bioleaching

2.2 Reductive bioleaching of oxides

Biomining has developed into a vibrant and robust “green technology” and reductive bioleaching fits well into this objective as it has a reduced carbon footprint (the bacteria that carry out the processing fix CO, like green plants). Metal extraction from oxide has not been used but industrial scale biomining of sulphide ores have been applied for dissolution. The Ferredox process for reductive bioleaching of limonitic laterites for recovery of Ni, Co, Cu, Sc, Mn, Cr, Zn is validated in a laboratory scale proof of concept bioreactor. This process of sulphide is for acid generation and food for the bacteria involved. The main operating cost for this process sulphide and analysis of operational expenditure is economically feasible. Besides limonitic laterites oxidized ores comprise polymetallic deep sea nodules and crusts, and supergene sulfide deposits. The technologies for the exploitation of such ores are costly which play a large role in environmental footprint. Biomining seems to be a promising geobiotechnology for processing oxidized ores and potentially waste [19].

2.2.1Heterotrophic leaching

Carbonate and silicate ores limits are set for the use of thiobacilli in the case of oxide. Research is being done on the use of heterotrophic bacteria and fungi for such ores. In this case metals are dissolved by organic acids or complexing or chelating agents produced by the bacteria or fungi [20]. Studies on silicate nickel ores have shown that nickel is dissolved by organic acids produced by microorganisms. The most effective was citric acid. With nickel-tolerant strains of *Penicillium,* up to 80% of the nickel was extracted, depending on the mineralization [21-22]. Various other valuable metals, e.g., gold, titanium, aluminium, chromium, copper, manganese and uranium, can also be leached by heterotrophic microorganisms, however much development remains to be done.

2.2.2 Biobeneficiation

The removal of impurities is a necessary method for the recovery of valuable metals from non sulphideminerals , heterotrophic microorganisms. Quartz sands, kaolins and clays contain iron oxides which lower the quality of these mineral raw materials. The impurities can be removed by chemical as well as by microbiological methods. The latter being based on bacterial and fungal production of organic acids and other chelating metabolic agents. Most of the bacteria active in iron removal are related to the genera *Bacillus* and *Pseudomonas*.

Fungi *Aspergillus* and *Penicillium* were found to be the most effective ones. when oxalic acid and citric acid were the main components in the leach suspension, it gives best results[23].

2.2.3 Heterotrophic aerobic leaching

The most familiar and well-studied microorganisms indigenous to acidic mineral leaching environments are autotrophic sulfur- and iron-oxidizing bacteria such as *Thiobacillus ferrooxidans, Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans*. Some photoautotrophs, such as the thermophilic rhodophyte *Cyanidium caldarium*, may also be present in extremely acidic environments that receive light. Other microorganisms which require pre-fixed (organic) carbon have been isolated from mineral leach dumps and acid mine drainage (AMD) waters. These heterotrophic microorganisms include eukaryotes, such as some fungi and yeasts [24] and protozoa [25], as well as prokaryotic bacteria and archaea. It is somewhat paradoxical, given that heterotrophy is the most widespread form of metabolism among bacteria, that the first acidophilic heterotrophic bacterium which is indigenous and active in mineral leaching environments was isolated and characterized.

2.3 Anaerobic bioleaching

2.3.1 Anaerobic bioleaching of metals from waste activated sludge

Heavy metal contamination of anaerobically digested waste activated sludge hampers its reuse as fertilizer or soil conditioner. Conventional methods to leach metals require aeration or the addition of leaching agents. Metals can be leached from waste activated sludge during the first, acidifying stage of two-stage anaerobic digestion without the supply of leaching agents. These leaching experiments were done with waste activated sludge from the Hoek van Holland municipal wastewater treatment plant (The Netherlands), which contained 342 μg g[26]of copper, 487 μg g [26] of lead, 793 μg g [26]of zinc, 27 μg g [26] of nickel and 2.3 μg g [26]of cadmium. During the anaerobic acidification of 3 g dry weight L [26] waste activated sludge, 80–85% of the copper, 66–69% of the lead, 87% of the zinc, 94–99% of the nickel and 73–83% of the cadmium were leached. The first stage of two-stage anaerobic digestion can thus be optimized as an anaerobic bioleaching process and produce a treated sludge (i.e., digestate) that meets the land-use standards in The Netherlands for copper, zinc, nickel and cadmium, but not for lead[27].

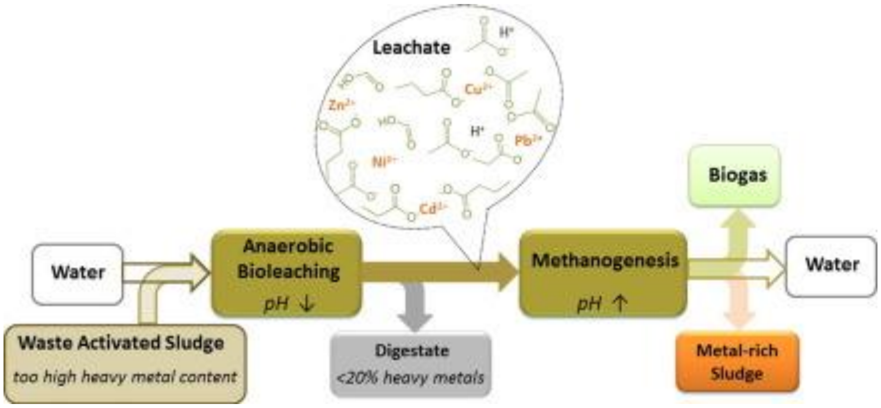


Figure 2; Anaerobic leaching of covellite by *Thiobacillusferrooxidans*

Thiobacilli are a group of gram-negative chemoauthotrophic bacteria that can obtain energy for growth from the oxidation of a variety of inorganic sulphur compounds. *Thiobacillus ferrooxidans*, an obligately autotrophic species, can also oxidise ferrous ion. This organism is very important in many mineral-leaching operations, especially in bacterial leaching of sulphide ores for the recovery of several metals. *T. ferrooxidans* may be involved directly or indirectly in the oxidation of sulphide. *T. ferrooxidans* is generally assumed to be an aerobic organism, so oxygen would be required as the last electron acceptor for bacterial growth. However, the analysis of gases in the pore spaces of leach dumps (one of the most suitable methods in extractive metallurgy) revealed that 0.5 m below the surface level the oxygen concentration is extremely low, while the carbon dioxide concentration is high. Some studies found that, under anaerobic conditions or at extremely low pH values, *T. ferrooxidans* is able to reduce ferric to ferrous ion in the presence of sulphur or sulphide ores. reported that this organism grows anaerobically using ferric ion as the electron acceptor and suggested, in accordance with Brock and Gustafson, that this could be the major biological process during oxidation of metal ores in leaching operations. However, there are no reports showing growth of *T. ferrooxidans* on insoluble sulphides (the common substrates found in metal ores) under anaerobic conditions to con®rm the role of ferric ion as an electron acceptor. This study was conducted to investigate whether *T. ferrooxidans* is able to grow anaerobically on insoluble synthetic copper sulphide in the presence of ferric ion and whether ancient metal recovery could be obtained under these conditions.

Biological wastewater treatment of increasing volumes of municipal wastewater, which most often includes industrial wastewater, has generated tremendous amounts of sludge, which have to be disposed of without threatening the environment. Land application of sludge has been demonstrated to be a feasible and effective means for recovery of beneficial constituents in sludge for plant growth and simultaneously improving soil physical properties. Although wastewater sludge offers good fertilizing characteristics, there have been concerns about heavy metal contamination derived from sewage sludge for land application.

During bioleaching, the highly oxidizing environment and the low pH result in an environment suitable for the dissolution of heavy metals and the digestion of organic matter in the sludge. The generation of phosphorous-rich sludge from treatment plants with efficient biological nutrient removal processes, phosphorous release may also be enhanced through acidification. As a result, the process has a significant loss of nutrients from the sludge through a variety of chemical and biological mechanisms, which may also limit the possibility of sludge application.

2.4 Metal solubilization by microorganisms

Microbial leaching methods are being increasingly applied for metal recovery from low-grade ores and concentrates that cannot be processed economically by conventional methods. As is the case with many biotechnological processes such methods may have been used since prehistoric times and probably the Greeks and Romans extracted copper from mine water more than 2000 years ago. However, it has been known only for about 50 years that bacteria are mainly responsible for the enrichment of metals in water from ore deposits and mines [28]. The solubilization process is called bioleaching and occurs in nature wherever suitable conditions are found for the growth of the ubiquitous bioleaching microorganisms**.**

2.4.1Chemolithotrophic microorganisms

Thiobacillus

The bacteria most active in bioleaching belong to the genus *Thiobacillus*. These are Gram-negative, non-spore forming rods which grow under aerobic conditions. Most thiobacilli are chemolithoautotrophic species which use the carbon dioxide from the atmosphere as their carbon source for the synthesis of new cell material. The energy derives from the oxidation of reduced or partially reduced sulfur compounds, including sulfides, elemental sulfur and thiosulfate, the final oxidation product being sulfate[29-30].The most important role in bacterial leaching is played by *T. ferrooxidans*. This bacterium was first isolated in 1947 by Colmer and Hinkle [28] from acid coal mine drainage. Morphologically the cells are identical to *T. thiooxidans*, but they differ from the latter by the much slower course of the oxidation of elemental sulfur. *T. ferrooxidans* differs from all other thiobacilli by the fact that besides deriving energy from the oxidation of reduced sulfur compounds ferrous iron can be used as an electron donor. In the absence of oxygen *T. ferrooxidans* is still able to grow on reduced inorganic sulfur compounds using ferric iron as an alternative electron acceptor [31]. An excellent overview of the current knowledge of this species was provided by Leduc and Ferroni [32].

Leptospirillum

Leptospirillum ferrooxidans is another acidophilic obligately chemolithotrophic ferrous iron oxidizing bacterium, which was first isolated by Markosyan from mine waters in Armenia[33]. This microorganism tolerates lower pH values and higher concentrations of uranium, molybdenum and silver than *T. ferrooxidans*, but it is more sensitive to copper and unable to oxidize sulfur or sulfur compounds[34-35] . Therefore, by itself, *L. ferrooxidans* cannot attack mineral sulfides. This can only be done together with *T. ferrooxidans* or *T. thiooxidans*.

T. thiooxidans, *T. ferrooxidans* and *L. ferrooxidans* are mesophilic bacteria which grow best at temperatures of 25–35°C.

Thermophilic bacteria

Thiobacillus-like bacteria, so-called Th-bacteria, are moderately thermophilic bacteria and grow on pyrite, pentlandite and chalcopyrite at temperatures in the range of 50°C [36]. Ferrous iron is used as the energy source, but growth is observed only in the presence of yeast extract [37]. Extremely thermophilic bacteria growing at temperatures above 60°C were isolated by Brierley, Norris, Karavaiko and their co-workers [38-39] . *Acidianusbrierleyi*, formerly associated with the genus *Sulfolobus*[40] , is a chemolithoautotrophic, facultatively aerobic, extremely acidophilic *Archaeon* growing on ferrous iron, elemental sulfur and metal sulfides. Under anaerobic conditions elemental sulfur is used as an electron acceptor and is reduced to H_2S. Members of the genus *Sulfolobus* are aerobic, facultatively chemolithotrophic bacteria oxidizing ferrous iron, elemental sulfur and sulfide minerals. The same compounds are used as energy source by *Sulfobacillusthermosulfidooxidans*, a spore-forming facultatively autotrophic bacterium. Growth, however, will only occur in the presence of yeast extract.

2.4.2 Heterotrophic microorganisms

Heterotrophic bacteria and fungi which require organic supplements for growth and energy supply may contribute to metal leaching. As in the case of manganese leaching, metal solubilization may be due to enzymatic reduction of highly oxidized metal compounds [41]or is effected by the production of organic acids (e.g., lactic acid, oxalic acid, citric acid, gluconic acid) and by compounds with at least two hydrophilic reactive groups (e.g., phenol derivatives) which are excreted into the culture medium and dissolve heavy metals by direct displacement of metal ions from the ore matrix by hydrogen ions and by the formation of soluble metal complexes and chelates[42-43] . The heterotrophic microorganisms do not have any benefit from the metal leaching. Among the bacteria, members of the genus *Bacillus* are most effective in metal solubilization, with regard to the fungi the genera *Aspergillus* and *Penicillium* are the most important ones.

2.5 Mechanisms of bioleaching

At the present time bioleaching processes are based more or less exclusively on the activity of *T. ferrooxidans*, *L. ferrooxidans* and *T. thiooxidans* which convert heavily soluble metal sulfides via biochemical oxidation reactions into water-soluble metal sulfates. The most important reaction steps are summarized in a simplified form in Eqs.(1)-(4)given below. In principle metals, can be released from sulfide minerals by direct and indirect bacterial leaching [44].

2.5.1 Direct bacterial leaching

In direct bacterial leaching, there is physical contact between the bacterial cell and the mineral sulfide surface, and the oxidation to sulfate takes place via several enzymatically catalyzed steps.

In this process, pyrite is oxidized to iron(III) sulfate [45] according to the following reactions:

$$4FeS_2 + 14O_2 + 4H_2O \rightarrow 4FeSO_4 + 4H_2SO_4 \quad (1)$$

$$4FeSO_4 + O_2 + 2H_2SO_4 \rightarrow 2Fe_2(SO_4)_3 + 2H_2O \quad (2)$$

The direct bacterial oxidation of pyrite is best summarized by the

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4 \quad (3)$$

Investigations by Torma[46-47] have shown that the following non-iron metal sulfides can be oxidized by *T. ferrooxidans* in direct interaction: covellite (CuS), chalcocite (Cu_2S), sphalerite (ZnS), galena (PbS), molybdenite (MoS_2), stibnite (Sb_2S_3), cobaltite (CoS), millerite (NiS).

Therefore, direct bacterial leaching can be described according to the following reaction

$$MeS + 2O_2 \rightarrow MeSO_4 \quad (4)$$

Where MeS is the metal sulfide.

There is some evidence that the bacteria have to be in intimate contact with the mineral surface. The mechanism of attachment and the initiation of metal solubilization are not completely understood. Obviously the bacteria do not attach to the whole mineral surface but prefer specific sites of crystal imperfection, and metal solubilization is due to electrochemical interactions [48-50].

2.5.2 Indirect bacterial leaching

In indirect bioleaching the bacteria generate a lixiviant which chemically oxidizes the sulfide mineral. In acid solution this lixiviant is ferric iron, and metal solubilization can be described according to the following reaction.

$$MeS + Fe_2(SO_4)_3 \rightarrow MeSO_4 + 2FeSO_4 + S^0 \qquad (5)$$

To keep enough iron in solution the chemical oxidation of metal sulfides must occur in an acid environment below pH 5.0. The ferrous iron arising in this reaction can be reoxidized to ferric iron by *T. ferrooxidans* or *L. ferrooxidans* and as such can take part in the oxidation process again. In indirect leaching the bacteria do not need to be in contact with the mineral surface. They only have a catalytic function because they accelerate the reoxidation of ferrous iron which takes place very slowly in the absence of bacteria. As shown by Lacey and Lowson[51] , in the range of pH 2–3 bacterial oxidation of ferrous iron is about 10^5–10^6 times faster than the chemical oxidation of ferrous iron.

The sulfur arising simultaneously (Eq. (5)) may be oxidized to sulfuric acid by *T. ferrooxidans*, but oxidation by *T. thiooxidans* which frequently occurs together with *T. ferrooxidans* is much faster.

The role of *T. thiooxidans* in bioleaching obviously consists in creating favorable acid conditions for the growth of ferrous iron oxidizing bacteria such as *T. ferrooxidans* and *L. ferrooxidans*.

A well known example of an indirect bioleaching process is the extraction of uranium from ores, when insoluble tetravalent uranium is oxidized to the water-soluble hexavalent stage of uranium:

The lixiviant may be generated by *T. ferrooxidans* by the oxidation of pyrite (Eq. (3)) that is very often associated with uranium ore. Besides the indirect leaching of uranium there is some evidence that *T. ferrooxidans* can oxidize U^{IV} to U^{VI} enzymatically and uses some of the energy of this reaction for the assimilation of CO_2[52].

Altogether, in a classical way, bioleaching is based on the interaction of biological and chemical oxidation processes. Particular importance must be attributed to the cycle of ferrous and ferric iron. In nature and in technical application both mechanisms, the direct and the indirect leaching, will undoubtedly occur in concert.

However, referring to newest publications by Sand and co-workers[53-54]there is some doubt whether a direct leaching mechanism does exist at all. The authors have indications that pyrite is degraded to sulfate via thiosulfate in a cyclic mechanism. The degradation is mediated or at least initiated by the ferric iron being complexed in the exopolymeric compounds of *T. ferrooxidans* and *L. ferrooxidans*. In addition, these iron(III) ions enable the bacteria to attach to the pyrite surface by an electrochemical mechanism. The function of leaching bacteria is thought to be in maintaining a high redox potential by keeping the ferric iron in the oxidized state to optimize the indirect attack on the metal sulfide.

2.6Factors influencing bioleaching

The leaching effectiveness depends largely on the efficiency of the microorganisms and on the chemical and mineralogical composition of the ore to be leached. The maximum yields of metal extraction can be achieved only when the leaching conditions correspond to the optimum growth conditions of the bacteria.

2.6.1 Nutrients

Microorganisms used for metal extraction from sulfide materials are chemolithoautotrophic bacteria and therefore only inorganic compounds are required for growth. In general the mineral nutrients are obtained from the environment and from the material to be leached. For optimum growth iron and sulfur compounds may be supplemented together with ammonium, phosphate and magnesium salts.

2.6.2 2O_2 and CO_2

An adequate supply of oxygen is a prerequisite for good growth and high activity of the leaching bacteria. In the laboratory this can be achieved by aeration, stirring, or shaking. On a technical scale, particularly in the case of dump or heap leaching, sufficient supply with oxygen may cause some difficulties. Carbon dioxide is the only carbon source required, but there is no need for addition of CO_2.

2.6.3 pH

The adjustment of the correct pH value is a necessary condition for the growth of the leaching bacteria and is decisive for the solubilization of metals. pH values in the range of 2.0–2.5 are optimum for the bacterial oxidation of ferrous iron and sulfide. At pH values below 2.0, a considerable inhibition of *T. ferrooxidans* will occur but *T. ferrooxidans* may be adapted to even lower pH values by increasing addition of acid[55] .

2.6.4 Temperature

The optimum temperature for ferrous iron and sulfide oxidation by *T. ferrooxidans* is between 28 and 30°C[56-57] . At lower temperatures a decrease in metal extraction will occur, but even at 4°C bacterial solubilization of copper, cobalt, nickel and zinc was observed[58] . At higher temperatures (50–80°C) thermophilic bacteria can be used for leaching purposes[59-63] .

2.6.5Mineral substrate

The mineralogical composition of the leaching substrate is of primary importance. At high carbonate content of the ore or gangue material the pH in the leaching liquid will increase and inhibition or complete suppression of bacterial activity occurs. Low pH values, necessary for the growth of the leaching bacteria, can be achieved by external addition of acid, but this may not only cause the formation and precipitation of gypsum but will also affect the cost of the process. The rate of leaching also depends on the total surface of the substrate. A decrease in the particle size means an increase in the total particle surface area so that higher yields of metal can be obtained without a change in the total mass of the particles. A particle size of about 42 µm is regarded as the optimum[64] .

An enlargement of the total mineral surface area can be obtained also by an increase in pulp density. An increase in the pulp density may result in an increase in metal extraction but the dissolution of certain compounds which have an inhibitory or even toxic effect on the growth of leaching bacteria will increase as well.

2.6.6 Heavy metals

The leaching of metal sulfides is accompanied by an increase in metal concentration in the leachate. In general the leaching organisms, especially the thiobacilli, have a high tolerance to heavy metals and various strains may even tolerate 50 g/l Ni, 55 g/l Cu or 112 g/l Zn. Different strains of the some species may show completely different sensitivities to heavy metals. Very often it is possible to adapt individual strains to higher concentrations of metals or to specific substrates by gradually increasing the concentration of metals or substrates[65] .

2.6.7 Surfactants and organic extractants

Surfactants and organic compounds used in solvent extraction generally have an inhibitory effect on the leaching bacteria, mainly because of a decrease in the surface tension and reduction of the mass transfer of oxygen[66-68] . Solvent extraction is currently preferred for the concentration and recovery of metals from pregnant solution. When bacterial leaching and solvent extraction are coupled the solvents become enriched in the aqueous phase and have to be removed before the barren solution is recirculated to the leaching operation.

2.7 Leaching techniques

The bioleaching of minerals is a simple and effective technology for the processing of sulfide ores and is used on a technical scale mainly for the recovery of copper and uranium. The effectiveness and economics of microbial leaching processes depend highly on the activity of the bacteria and on the chemical and mineralogical composition of the ore. Therefore, processes tested on individual types of ores cannot be transferred to other ones. Before a technical application is possible the optimum leaching conditions have to be elaborated for each type of ore.

2.7.1 Submerged leaching

Because the oxygen supply is often inadequate and the surface ratio unfavorable, percolator leaching is not very efficient, fairly slow and series of experiments lasting 100–300 days are not unusual. Therefore, percolator leaching has been substantially displaced by submerged leaching using finegrained material (particle size <100 μm) which is suspended in the leaching liquid and kept in motion by shaking or stirring. Higher rates of aeration and a more accurate monitoring and control of the various parameters favor the growth and the activity of the bacteria so that the reaction times are considerably shortened and the metal extraction substantially increases . Suspension leaching can be carried out in Erlenmeyer flasks or, in a more sophisticated manner, in a bioreactor. Besides mechanically stirred systems an airlift reactor has been proved suitable for the treatment of ore concentrates, industrial waste products and for the biodesulfurization of coal [69-71].

Bacterial leaching of uranium ore in shaking flasks. Extraction of uranium during leaching with *T. ferrooxidans*, *T. thiooxidans*, and a mixed culture of both strains. (Particle size <600 µm, pulp density 5% (w/v)).

2.7.2Column leaching

Column leaching operates on the principle of percolator leaching and is used as a model for heap or dump leaching processes. Depending on their size, the columns may be made of glass, plastic, lined concrete, or steel (Fig 3). Their capacities range from several kilograms to a few tons. At various distances most column systems have devices for taking samples or for installing special instruments for measuring temperature, pH, humidity, oxygen or carbon dioxide. This gives information about what has to be expected in heap or dump leaching and how the leaching conditions can be optimized.

Figure 3 Column leaching facilities.

2.8 Industrial leaching processes

Currently bioleaching is used on an industrial scale for the treatment of low-grade ores which generally contain metal concentrations below 0.5% (w/w). The simplest way of conducting microbial leaching is to pile the material in heaps, allow water to trickle through the heap and collect the seepage water (leachate). Since the bacterial oxidation of sulfides is much slower than other biotechnical processes the leachate is recirculated. There are three main procedures in use: dump leaching, heap leaching and underground leaching (Fig. 4).

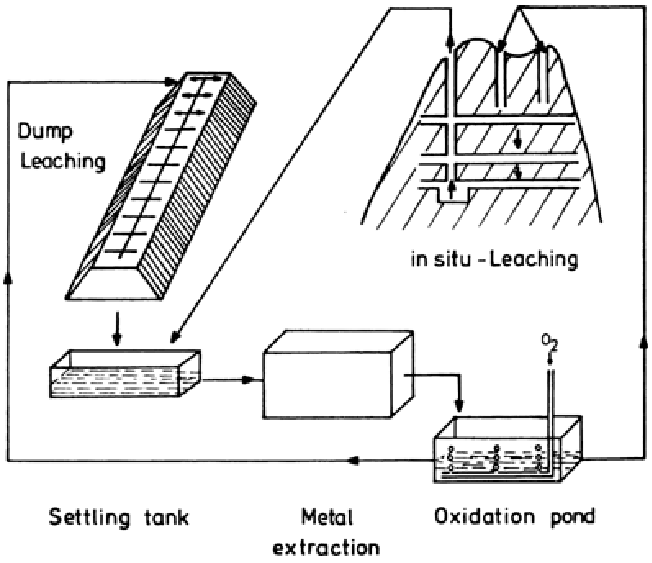


Figure 4 Flow sheet of a dump and in situ leaching process [72].

2.8.1 Dump leaching

Dump leaching is the oldest process. The size of the dumps varies considerably and the amount of ore may be in the range of several hundred thousand tons of ore. The top of the dump is sprinkled continuously or flooded temporarily (Fig. 5). Depending on the ore the lixiviant may be water, acidified water or acid ferric sulfate solution from other leaching operations on the same mining property [51][73]. Before recirculation, the leachate may pass through an oxidation basin, in which the bacteria and ferric iron are regenerated.

Figure 5 (Irrigation of leaching dumps (A) by sprinklers; (B) by flooding.)

2.8.2Heap leaching

This procedure is mainly used for finegrained ores that cannot be concentrated by flotation. The leaching is practised in large basins containing up to 12 000 tons of ore. The procedure is similar to that of dump leaching. In some heap leaching operations, pipes are placed in strategic positions within the heaps during its construction to provide the deeper portions of the heap with sufficient amounts of oxygen.

2.8.3Underground leaching

Underground leaching is usually done in abandoned mines. Galleries are flooded or unmined ore or mine waste in side tunnels are sprinkled or washed under pressure. The water collects in deeper galleries and shafts and is then pumped to a processing plant at the surface. The best known application of this procedure is at the Stanrock uranium mine at Elliot Lake in Ontario, Canada[74] .

Ore deposits that cannot be mined by conventional methods because they are too lowgrade or because they are too small can be leached in situ. Solutions containing the appropriate bacteria are injected into boreholes in the fractured orebody. After a sufficient time for reaction, the leachate is pumped from neighboring wells or collected in drifts. The procedure requires sufficient permeability of the orebody and impermeability of the gangue rock so that any seepage of the pregnant leaching solution is prevented.

2.8.4Tank leaching

Considering the high yields in metal extraction by submerged leaching the change from shake flasks to bioreactors was tested very early. Tank leaching was found to be most effective for the treatment of ore concentrates and more than 80% of the total zinc was extracted from a zinc sulfide concentrate. Tank leaching is more expensive to construct and to operate than dump, heap, or in situ leaching processes. But the rate of metal extraction is much higher and currently this technique is successfully used for bioleaching of refractory gold ores.

llion were produced from the leaching operation. With the present reduction in world demand for uranium the prices are at a low level and Denison Mines have stopped production.

2.9 Manganese

2.9.1 Mineralogy of Manganese

Manganese is the 12th most abundant element on the earth's crust and major deposits are of pyrolusite (MnO_2) in origin. Mn is present as a major and minor constituentin more than 100 naturally occurring minerals. Major manganese components occur in the form of oxides for example, psilomelane [$Ba_, (H_2O)_2Mn5O10$], bimessite[$(Na_{0\ 3}Ca_{0\ 1}K_{0\ 1})(Mn^{4+} , Mn^{3+})_2O_4.I.5H_2O$], pyrolusite [$MnO_2$], vernadite [$Mn^{4+},Fe^{3+},Ca,Na)(O,OH)_2.nH_2O$], manganite [$MnO(OH)$], hausmannite [$Mn^{2+}Mn^{3+}SO_4$], rhodochrosite [$MnCO_3$], and braunite [$Mn^{2+}Mn^{3+}{}_6(O_8SiO_4)$]. Minor compounds of Mn such as pyroxens [$XY(SiAI)_2O_6$] and biotite [$K(Mg,Fe)_3(AISiO_{10})(F,OH)_2$] also occurs in nature. These all are ferromagnesian minerals. Manganese occurs naturally in 0, +2, +3, +4 and +7 oxidation states. Out of this +2, +3 and +4 are common in nature but +2 oxidation state of manganese can be found as soluble inorganic or organic complexes. +3 state of this metal however tends to disproportionate into the more stable +2 or +4 oxidation states. Extraction of minerals from wastes and low grade ores can be carried out using several microorganisms. Deposition of manganese ore is generally of sedimentary source. Deposits of manganese are found in the PaleoproterozoicHotazel formation in South Africa conserving more than four million heaps of manganese ore (13.5 billion metric tons of Mn) creating the world's largest land based economic reserve at Kalahari manganese field. In order of predominance, mainly the cost effective rating, Mn ores are resulted from hydrothermal vents connected through transitional volcanic rocks and are classified into sedimentary rock hosted, volcanic rock hosted, and karst hosted. The entire global production of Mn alloys achieved 17.7 million metric tons in 2011growing to assure demand from steel mills. China remained to be the world's major Mn alloy producing nation. The International Manganese Institute calculates that its alloy production exceeded ten million metric tons in 2011 accounting for almost 58% of global output. In India, Manganese Ore India Limited (MOIL) is the major Mn ore manufacturer, and it reports for 40% of India's entire manufacture. MOIL reported whole resources of Mn ore in the nation as 240 million tons, with reserves category.

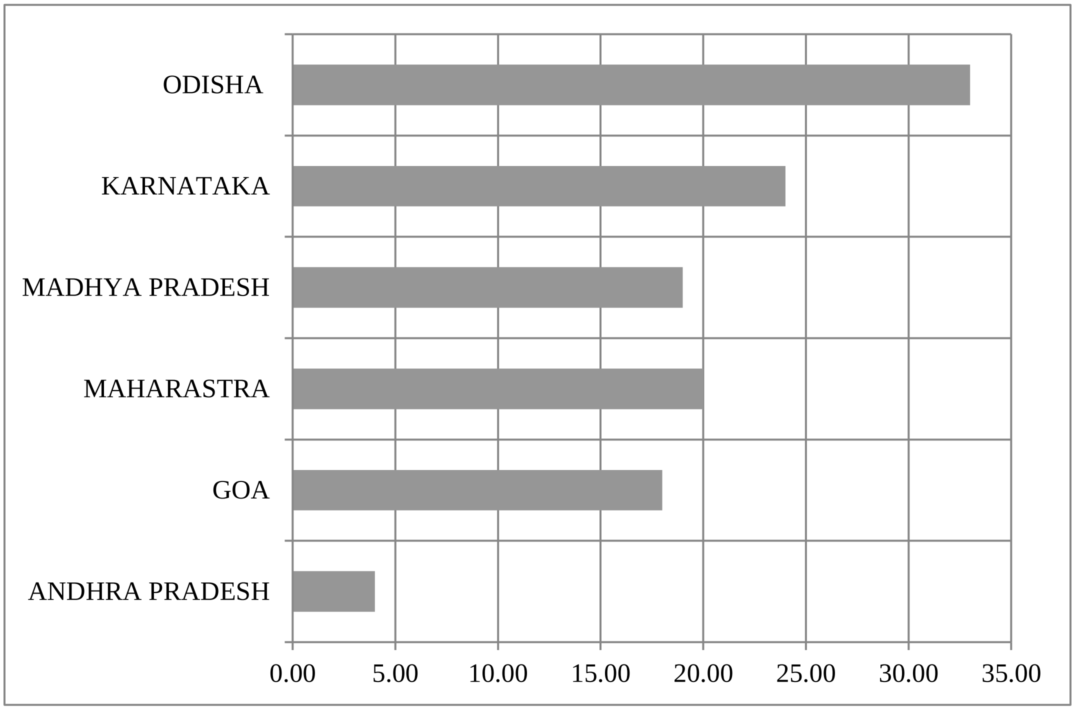


Figure 6 Distribution of Manganese Resources in India

Table 1- forms of manganese

Name	**Ion**	**Example minerals**
manganese(II)	Mn^{2+}	Rhodochrosite, Rhodonite
manganese(III)	Mn^{3+}	Bixbyite
manganese(IV)	Mn^{4+}	Pyrolusite

This list of minerals containing Manganese is built from the mindat.org locality database. This is based on the number of localities entered for mineral species and is therefore slanted towards minerals interesting to collectors with less coverage of common rock-forming-minerals so it does not give an undistorted distribution of Manganese mineral species. It is more useful when comparing rare species rather than common species.

TABLE-2 List of Major Manganese Mineral forms

Name	Formula	Crystal System
Pyrolusite	$Mn^{4+}O_2$	Tetragonal
Rhodochrosite	$MnCO_3$	Trigonal
Spessartine	$Mn^{2+}_3Al_2(SiO_4)_3$	Isometric

PYROLUSITE RHODOCHROSITE

SPESSARTINE

Fig.7. Major mineral forms of manganese

2.9.2 Application of Manganese

Mn finds its essential application in the manufacturing chemical, tannery, glass, and battery industries. Compounds of Manganese are globally distributed. Its extensive utility as large as 90% of the total mined manganese. Then comes the batteries and chemicals which consume 55 of the mined manganese. In addition to this, the rest are used in ferro alloys, catalysts, glassmaking, wet galvanic cell. At industrial scale, pyro and hydrometallurgical processes are associated with several environmental impacts [75]. Keeping in view of environmental problems associated with conventional processes, extraction of manganese through bioleaching or biohydrometallurgy processes with the help of different kinds of microorganisms has been studied extensively by several workers. Commercial applications of manganese already in practice are as follows.

Table: 3 Commercial uses of Manganese Compounds

Compounds	**Application**
Manganese(II) Oxide	Fertilizers feed additive, Mn (II) salt
Native ore	Oxides in chemical processes, absorbent for H_2S and SO_2 ferrite lower grade batteries
Chemical Manganese Dioxide	Dry cell batteries, Oxidant in organic synthesis, constituent in oxidation catalyst
Electrolytic Manganese Dioxide	Dry cell batteries, Ferrites

2.9.3MANGANESE IN HEALTH AND BIOLOGY

- In humans, a manganese deficiency causes defective ovulation and ovarian degeneration in females and testicular degeneration in males. increased infant mortality occurs when the mother lacks manganese during pregnancy.
- It can be use in medicine to help support the immune system, regulate your blood sugar, and maintain bone regulation and reproduction.
- The existence of manganese in the body is vital to processes on the cellular level. Without it, enzymes that are vital to life are disrupted and can cause complications in health.

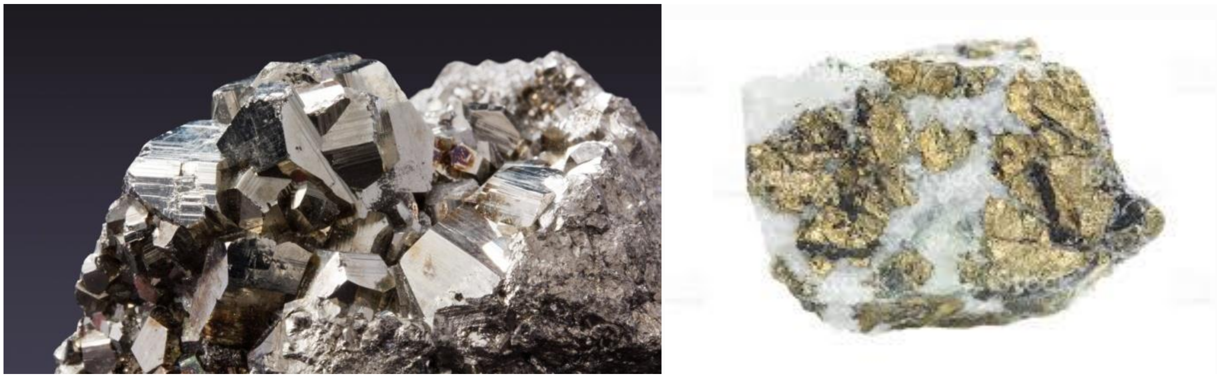
- For example, manganese aids in the formation of connective tissue in our bodies, without it or with minimal amounts, ligaments and muscles for example are less flexible and injuries can occur more readily.
- However, if too much manganese is consumed then health problems such as weakness, drowsiness and even paralysis may occur.

2.10 Mineralogy of Iron

Iron is the 4th most abundant element on the earth's crust, making up to a maximum mass of 5.1%. It can be found in different layers of mineral depositions. Fe has oxidation states ranging from -2 to +6, +2 and +3 being common. Iron is an important member in biogeochemical cycle of sulphur, phosphorous and carbon. Vast majority of oxidised forms of iron is reduced anaerobically, however understanding the complexity of formation of sedimentary iron is still to be understood. The redox reaction occurring in nature thus converting Fe(II) to Fe(III) and vice versa has an important role during geochemical cycles of iron. Siderite ($FeCO_3$), vivianite ($Fe^{2+}Fe^{2+}_2(PO_4)_2.8H_2O$) or iron sulphide are some Fe(II), for formed under and only in anoxic habitats having weak acidic to neutral conditions. In the presence of oxygen, ferrous iron is stable only under acidic conditions; Fe(III) ions are readily formed at neutral p^H however ferric iron, forms minerals that are widespread in both oxic and anoxic habitats. This list of minerals containing Iron is based on the number of localities entered for mineral species and is therefore slanted towards minerals interesting to collectors with less coverage of common rock-forming-minerals so it does not give an undistorted distribution of Iron mineral species. It is more useful when comparing rare species rather than common species.

Table-4 List of Iron Minerals

NAME	FORMULA	CRYSTAL SYSTEM
PYRITE	FeS_2	ISOMETRIC
CHALCOPYRITE	$CuFeS_2$	TETRAGONAL
MAGNETITE	$Fe^{2+}Fe^{3+}_2O_4$	ISOMETRIC



PYRITE CHALCOPYRITE

MAGNETITE

Fig.8. Mineral forms of Iron

following are some most common iron ores found on earth crust,

Table-5 List of some important ores of iron

Name	Formula	Crystal System
Hematite	Fe_2O_3	Trigonal
Goethite	α-$Fe^{3+}O(OH)$	Orthorhombic
Limonite	(Fe,O,OH,H_2O)	

HEMATITE

GEOTHITE

LEMONITE

Fig.9.Important ores of iron

2.11 Standard Methods of Manganese Production

Exhaustion of high and medium grade ore deposits brings the economic and eco-friendly methodologies into view to treat lean grade ores. Physicochemical methods of mineral benefication such as flotation, acid dissolution, magnetic amd gravity separation are often energy-intensive, costly and environmentally not suitable. Conventional benefication techniques involve the use of various toxic reagents, which could be safely replaced by environment-friendly biological reactants. Role of commensal and non-pathogenic microbes has been well recognised in both mineral benefication as well as extraction.

2.12 Biological Recovery of Mn& Fe

Catalysis is the sole player for sustaining life on earth. Several categories of microorganisms' bio-catalyse the minerals, thus the concept of bio-mineralisation evolved. Kennecott Utah Company was the first ever to apply bioleaching technology at commercial scale by developing their mine at Bingham Canyon located near Salt Lake City, Utah. Blue coloured run off solutions were observed from waste piles harbouring copper containing sulphide minerals, presuming the presence of any agent to be involved in this mysterious phenomenon, microbiological methods were tested and subsequently confirmed for the presences of metal utilizing microbes. Biological agents have been demonstrated for the effected recovery of metals from low grade ores. For example, microbially induced flocculation or flotation of minerals, remediation of toxic chemicals discharged from mineral processing operations, degradation of cyanide and so forth have proven as promising alternatives. Leaching of metals using microorganisms is the preliminary step for metal extraction in biometallurgical role. Recovery techniques like electro winning, selective precipitation, solvent extraction, and froth flotation are used in downstream separation of solubilized metals. Unlike conventional techniques, microbial processes would be more energy efficient, cost effective and environmentallybening. Bioleaching the use of microorganisms to recover precious and base metals from mineral ores and concentrates, has developed into a successful and expanding area of biotechnology. While careful considerations are made in the design and engineering of bioleaching operations, microbiological aspects have been subjected to far less security and control.

2.13 Role of Microorganisms in Bioleaching of manganese

Chemolithotrophic microorganisms are often get energy from the inorganic sources. To release the metal from the ore matrix, biomineral processing use te inherent capacity of microorganisms. Bioleaching method implement microbial consortium which extract the metal from oxides. Sulphuric minerals also used by reduction or oxidative dissolution. The process of the biooxidation of mineral takes place in highly aerated, continous flow, stirred tank reactors or in irrigated dump or heap reactors. Continuous flow and stored tank reactors are provided with an open, nonsterile environment. These are characterized by homogeneous and constant growth conditions where the selection is for rapid growth, and consequently tank consortium seems to be dominated by 2or 3 species of organism. The heterogeneous growth environments that changes with the age of the heap provided by heap reactors. And these tend to be colonized by a much greater variety of microorganisms. Heap microorganisms grow as a structure of biofilms that are not subject to washout and the major challenge is to provide sufficient biodiversity for optimum performance throughout the life of a heap. The theoretical and pragmatic aspects of assembling microbial consortium is to differently processed mineral ores and concentrates. Bioleaching is nothing but the extraction of metals with the help of microorganism. Microbial technology is used to recovery of metal from low to high grade ores. And it also can be used in the concentrator tailings, newly mined run-of-mine material, etc. industrial grade non-renewable mineral resources can be depleted and it necessitates the extraction of minerals lean grade ores, overburdens and tailings, thus, making metals extraction process use various technique which is costly for the mineral processing. Commercially used microorganisms are usually divided into 2 broad groups. First one is chemolithotropic bacteria and the second one is heterotrophic microbes such as bacteria, fungi, and yeast. The microorganisms of chemolithotropic grow are generally acidotrophiles and can sustain their life at pH2 or below, including bacteria and Eucharea which can only be found at extreme condition such as low pH, high temperature, subzero temperatures, high salt concentration etc.Miningusing microbes has promising eco-friendly impacts on environment than other physico-chemical processes. Consumption of energy is at the minimal stage as compared to roasting and smelting and does not produce sulphur dioxide or other harmful gases. The increasing demand for Manganese at the international platform has led to development of several novel technologies for recovery and processing of Manganese and a better way of utilization of well-established technologies such as pyro and hydrometallurgical processes. India being at top scales as steel producer in the world in return requires high grade (>35%) manganese to process. The complicacy of the economic criteria for extraction, processing and utilization of metals in various products of waste materials and its components leads to the evolution of the green chemistry where the utilization of various microorganisms for recovery of metals is done. Mineral excavation by industries are associated with several deleterious effects on environment and on ecosystem as well. Unskilled management of the wastes such as mine tailings, low grade ores, sediments from lagoons generated by industrial processes develops into accumulation and unwanted mobilization leading to pollution. A distinguishing definition between fermentation and anaerobic metabolism affects the study. Mn (II)-oxidising microbes have an integral role in the biogeochemical cycling of manganese, iron, nitrogen, carbon, sulphur, and several nutrients and trace metals. There is great interest in mechanistically understanding these cycles and defining the importance of Mn(II) oxidising bacteria in modern and ancient geochemical environments. Linking Mn(II) oxidation to cellular function, although still enigmatic, continues to drive efforts to characterize manganese biomineralization. Recently, complexed-Mn (III) has been shown to be a transient intermediate in Mn (II) oxidation to Mn (IV), suggesting that the reaction might involve a unique multi-copper oxidate system capable of a two-electron oxidation of the substrate. Tebo et.al reported that the application of synchrotron based X-ray scattering and spectroscopic techniques can significantly help the understanding of the oxidation state of a biogenic and abiotically synthesized Mn oxides which can provide a new blueprint for the structural signature of Mn oxides. Manganese and iron reduction in marine sediments are known to play important roles in the biogeochemical cycles of many elements, including carbon, sulphur, phosphorous and several trace elements. These reduction reactions affect these cycles on a variety of time scales, ranging from those as short as seasonal time scales (e.g., nutrient cycling in coastal ecosystems), to those as long as thousands to tens of thousands of years (e.g., glacial-interglacial transformations in deep sea sediments). A study by Burdige, types of manganese and iron reduction that are known to be occurring in nature has been discussed well. Some light has also been put on the occurrence of these processes in different sedimentary environments. Geochemical transformations by many important microbes are influenced by minerals including energy generation, nutrient acquisition, cell adhesion and biofilm formation. Ecological microbial flora obtains essential nutrients from mineral surfaces. The properties of mineral surfaces (e.g. microtopography, surface composition, surface charge, hydrophobicity) also play an integral role in colonization and biofilm formation, and the ecology of proximal microbial populations in, on, around, mineral substrates. The anaerobic environment is certainly governed by biogeochemical cycles since the absence and presence of oxygen proves to be fatal for the survival of obligate anaerobic microorganism. The formation of manganese oxides under both aerobic and anaerobic environment have been studied well. One such bacterium, *Alteromonasputrefaciem* MR-1 studied by Myers and Nealson, has a coupling reaction to manganese oxide to reduce into Mn^{2+} under anaerobic condition only. They have also reported the fact that the concerned microorganism has a dynamic feature of utilizing other various metals as terminal electron acceptor. Certain microorganisms require carbon source as electron donor however if coupled to oxygen atom, can act as an electron acceptor. In an attempt to study the Mn leaching capacity of bacterial flora of low grade Mn ore, Ghosh isolated 4 taxonomically different strains and started their Mn solubilizing capacity. As per the study, *Bacillus anthrasis*MSB 2, *Acetobacter sp.* MSB 5, *Lysinibacillus sp.* MSB 11, and *bacillus sp.* MMR-1 had a solubilisation efficiency of 40, 96, 97.5 and 48.5% respectively, similar studies were carried out by sanket et.al. four morphologically distinct and taxonomically different acidophilic bacterial strains were isolated and were studied for their Mn solubilizing capacity, *Bacillus cerus*MSB3, isolated by the same author was found to have the highest solubilisation capacity. However, whether reduction of oxidation was indistinct. In both the studies, two different conclusions regarding the type of microorganisms could be deduced. It was also suggested that the ability of acidophilic microorganisms to solubilize heavy metals supported by five basic mechanisms which include: enzymatic conversion, metal effluxing, reduction in sensitivity of cellular targets, intra or extracellular sequestration, and permanently barrier exclusion. Such ecological studies undoubtedly will provide insights into Mn biogeochemical processes occurring in leaching environments. To recover Mn from low grade ores, a study carried by Das et.al using an established strain *Staphylococcus epidermidis*(MTCC-435), reported the potentiality of the microorganism to leach Mn at laboratory scale. Under optimized conditions, a maximum recovery of 80% of Mn was reported by the authors. This suggests that under optimized conditions, microorganisms with capability of leaching up to such higher extent can be explored further for scaled up studies.

2.14 Significance of Mn in Biological Processes

The unutilized mined low grade ores are usually left out or dumped to a greater depth on a cultivable land which in turn becomes toxic at subsequent times to the environment, human health and to the biodiversity of flora and fauna as well. Studies by Cordova et.al, neurodegenerative diseases such as Parkinson's disease is highly correlative of excessive exposures to manganese. Major routes of exposure are predicted to be through air in contact and water bodies resourceful for daily purposes. Being an important element on earth with several industrial applications, Mn is an essential trace element for nutrition but also a toxicant when cells et exposed to high concentration. Mn is an essential metal found in a variety of biological tissues and is necessary for normal functioning of a variety of physiological processes including amino acid, lipid, protein and carbohydrate metabolism. Increased environmental exposure of Mn containing products towards large set of population justifies need for an extensive study for a correct assessment of the potential health risk caused due to this metal. Mitochondrial dysfunction leading to impaired cerebral energy metabolism and possibly decreased production of ATP.

2.15 Modes of Microbial Action on Minerals

Many microbes possess inherent ability to mineralize the metals as a part of their metabolism. Several physiologically important reactions in these microorganisms enable them to grow and reproduce using metals. Microorganisms are said to be acting upon minerals via I) Bioaccumulation II) Acidolysis III) complexolysis IV) Redoxolysis. The native microbes (eubacteria, fungi, euarchea) of mining deposits are capable of utilizing metal ions as a part of their metabolism. They utilize ions by breaking the complex form of metal into soluble form. However, it cannot be stated that they nullify the toxic effect of metals although they alter the chemical properties hence dissociating the complexes into ionic form which is related into the leach liquor. In cases, such as acidophilic microorganisms, protons are pumped out of the cell to maintain the cytoplasmic p^H which helps them to survive at very low p^H. Certain microbial action is yet to be understood hence few nonspecific mechanisms have been prophesized to be involved which leads to formation of new mineral phases. The nonspecific mechanisms are said to be resulting in the entrapments of toxic metals within the complex forms. The indigenous floras are native to the respective mining deposits and thus need to be explored for beneficiation of precious metals.

2.16 Cow Dung Compost

Traditionally cow dung has been used as a fertilizer, though today dung is collected and used to produce biogas. This gas is rich in methane and is used in rural areas of India/Pakistan and elsewhere to provide a renewable and stable source of electricity. According to the International Energy Agency, bioenergy (biogas and biomass) have the potential to meet more than a quarter of world demand for transportation fuels by 2050.

Biomass has become an increasingly important energy source in Denmark over the last 25 years. Being a carbon neutral energy source, it has already helped make a significant contribution to the reduction of Danish carbon emissions. The conversion of more biomass at power stations will help Denmark reach its target of 30% renewable energy by 2020. Today, biomass accounts for approximately 12% of world energy consumption.

Yet the potential of using biogas has so far been unexploited, especially in the form of livestock manure in the agriculture system. Denmark is well known for its farming industry; approximately 65% of the land is used for agriculture, emitting 18% of all greenhouse gases here, through methane and nitrogen. So, farming has an important part to play in the transition to a fossil fuel free society. The Danish government now wants up to 50% of livestock manure to be made into this green energy supply.

Fig.10.Cow dung compost used in the present study

Since the compost is a rich natural source of organic carbon material, it can form a useful substitute to conventional carbon sources applied in bioleaching. The same has been studied in the present work in a systematic manner. Cattle manure is basically made up of digested grass and grain. Cow dung is high in organic materials and rich in nutrients. It contains about 3 percent nitrogen, 2 percent phosphorus, and 1 percent potassium (3-2-1 NPK). In addition, cow manure contains high levels of ammonia and potentially dangerous pathogens. For this reason, it's usually recommended that it be aged or composted prior to its use as cow manure fertilizer.

CHAPTER III

MATERIAL AND METHODOLOGY

3.1. RAW MATERIALS

The ferromanganese ore samples (low-grade ore) from a manganese mine deposits in Odisha, India, were collected and stored at room temperature until further studies were carried out. The chemical composition of the ore was done by acid digestion. Prior to experiments the ore was prepared by crushing, grinding and sieving. A size fraction of -150 microns was used for the experiments. The chemical composition of the ore is as follows:

Mn- 15%, fe- 14%

Fig.11.Raw material

The metal content in the sample was analyzed by acid digestion using atomic absorbance spectrometry, protein estimation by Bradford method for analysis of growth of organism in culture, Scanning electron microscope was used to observe the surface morphology of the collected ore sample.

3.2 MICROORGANISM

Anaerobic Consortia available only at **IMMT**, was used as inoculums for the bioleaching experiments. Consortia is a group of microorganisms used in the bioleaching process.

3.3 MEDIA

The optimum growth and survival of microorganism, environmental factors like temperature, pH, and nutrition are the major required parameters. For the present study MSM was used as the growth and enrichment media. Mineral salt media having composition of (g/l) KH_2PO_4-0.8, K_2HPO_4 3.0, KCl 0.2, NH_4Cl 1.0, $MgCl_2$ 0.2 , $CaCl_2$ 0.1 ,yeast extract 0.05 , glucose and sodium acetate 10mm. The experiments were carried out in duplicates. MSM should be prepared by using Millipore water.

Table 6- composition of mineral salt medium

CHEMICALS	QUANTITY(g/l)
KH_2PO_4	0.8
K_2HPO_4	3
KCl	0.2
NH_4Cl	1.0
$MgCl_2$	0.2
$CaCl_2$	0.1
yeast extract	0.05
glucose and sodium acetate	10mM

3.4 SUBCULTURE METHOD

Prepare MSM as per required volume. Before using, media should be autoclaved at 121°C, 15psi with fe/Mn and Mn nodule ore (take 1gm ore for 100ml culture, and also some glass wares like measuring cylinder, volumetric flask (as per required), glucose and sodium acetate (10 mM), tip(1ml). Before the inoculation laminar hood should be sterilized under UV light for 15 mins. At the time of inoculation 12 ml of bacterial inoculums was added to volumetric flasks containing approx. 70ml media. After inoculation make up the volume of flask up to 100ml. Then add 1ml of paraffin oil for maintaining O_2 free atmosphere in the culture media. Repeating the sub culturing process helps to maintain activity of the organism present in consortia. Same

procedure was followed for subculture process by using low cost carbon source instead of glucose and sodium acetate. For experiments, the same culture procedure was followed. The parameters such as time, carbon source, concentration of compost, pulp density etc. were studied by varying the same.

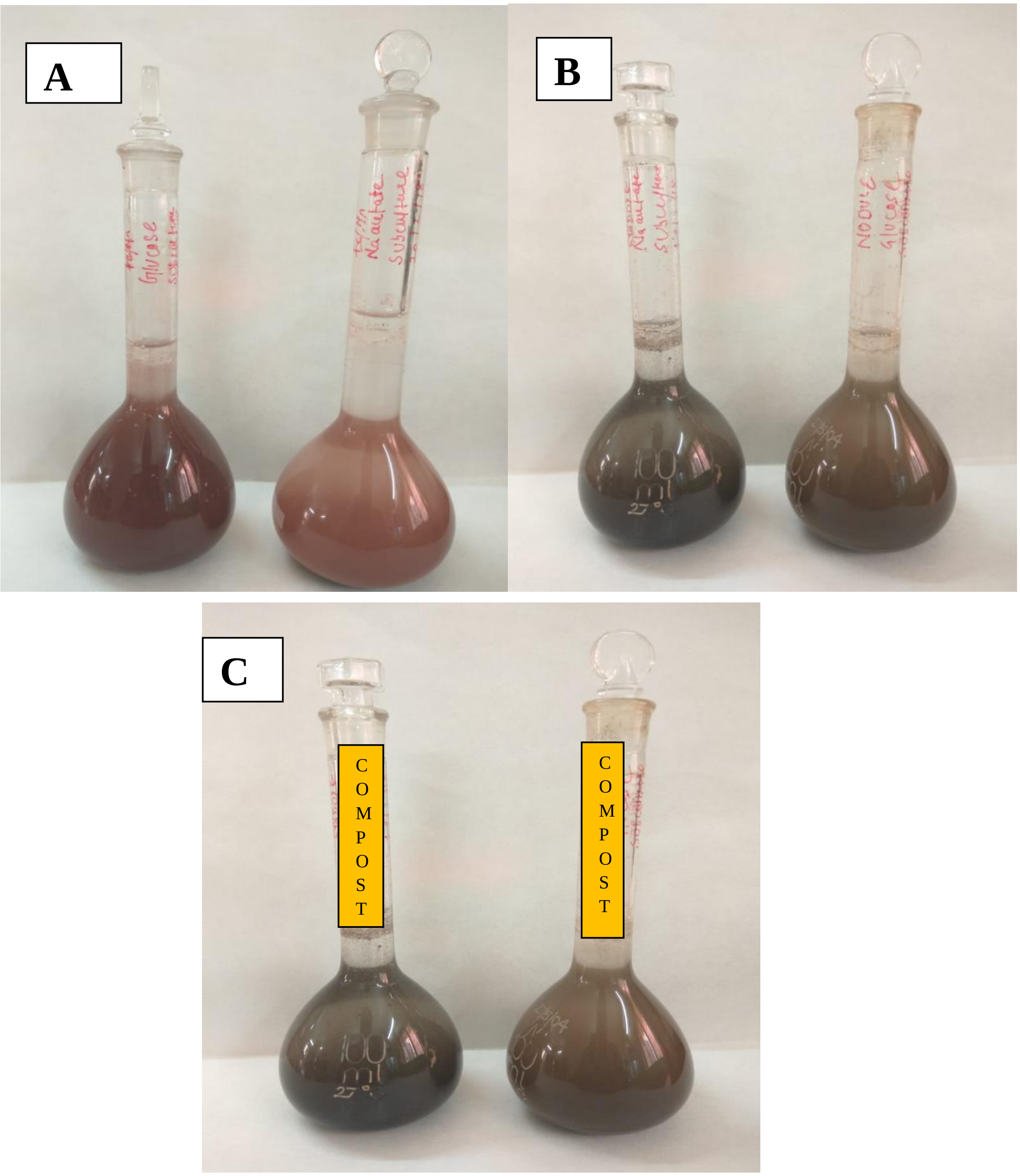


Fig.12.Subculture of (A)ferromanganese ore, (B)nodule by using glucose and sodium acetate and (C)subculture of ferromanganese by using compost

3.5 CURRENT WORK

Leaching of low grade manganese ore was studied by using compost as the carbon source. Leaching was studied by varying parameters such as ratio of ore and compost, concentration of compost, variation of pulp density on leaching process.

3.6 PROTEIN ESTIMATION BY BRADFORD METHOD

Protein estimation was done by the Bradford's protein estimation assay. Before calculate the concentration of protein present in given sample, we should know the standard value of a known sample that is standard curve should be done by using BSA for find out the concentration of protein in unknown sample.

Standard curve for Protein estimation:

Prepare a Bradford reagent by following the method of Bradford protein assays. Then make a standard curve by using BSA (0 to 10µg) as a protein sample. Prepare different concentration of protein sample in their duplicates, then add 3ml of Bradford reagent in each sample. After 15mins of incubation note down the absorbance of sample at 595 nm. Find out R^2 value i.e 0.97 (1.00 is accurate) from plotted standard curve by which the concentration of unknown sample can be identify. To find out the concentration of unknown sample use the formula **Y= MX+C**, where x is the concentration protein.

$Y=0.0016x+0.1327$ (equation-1)(according to R^2 value given in standard curve)

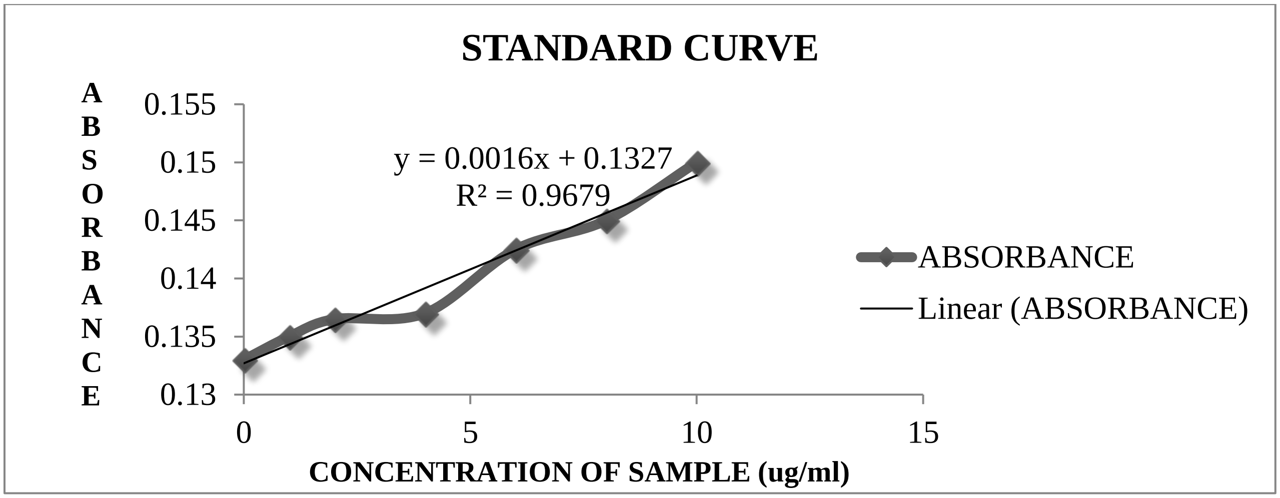


Fig.13.Standard curve

Protein estimation in experimental sample:

To know the concentration of unknown sample, collect the pellet by centrifugation at 650*10rpm for 15mins, then add 5ml of NaOH(0.1N) to the collected pellet, shake them or vortex them properly. After addition of NaOH put the sample on water bath at 100°C for 45mins to 1 hr. Before putting the sample in water bath mark the level of sample in test tubes.

After 1hr cool the test tubes containing sample completely. Then make the volume of test tubes according to their mark by Millipore water. Again, shake it well by a vertex mixture and filter the sample. Take 300µl of filtered sample from filtration and add 3ml of Bradford reagent. After shake by using a vertex, incubate them for 15-20 min then note down their absorbance at 595nm by using spectrophotometer, then find out the concentration f protein present in sample by using formula (equation 1) respect to the standard curve.

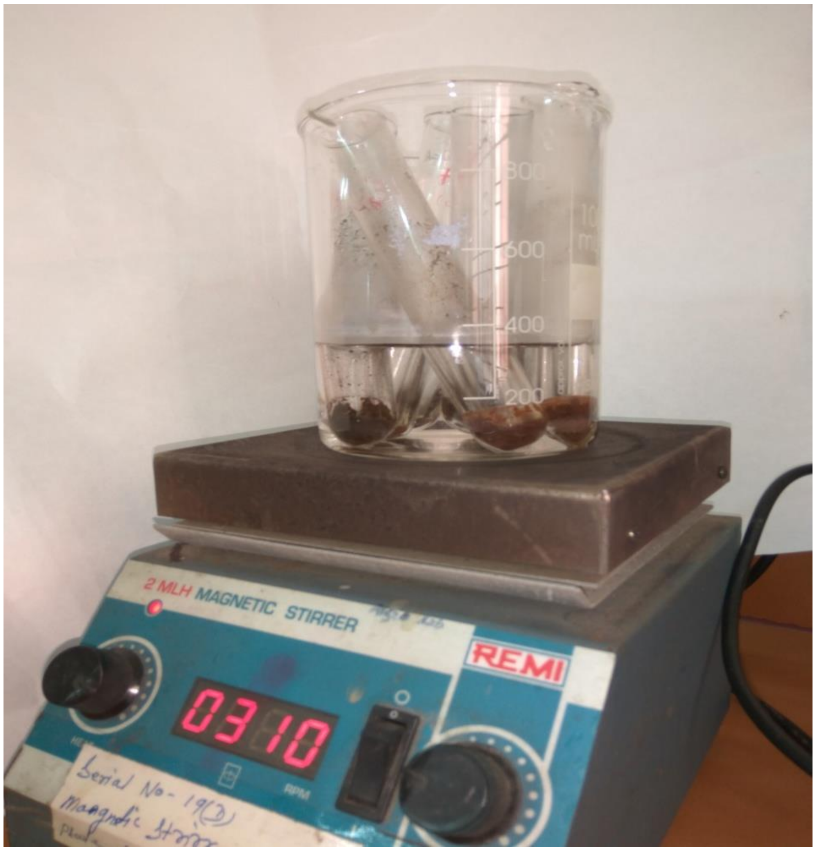


Fig.14.Sample in waterbath for 1hr

Fig.15.After 1hr collection of sample filtrate

Fig.16. Filtration of stock Bradford reagent before use in experiment

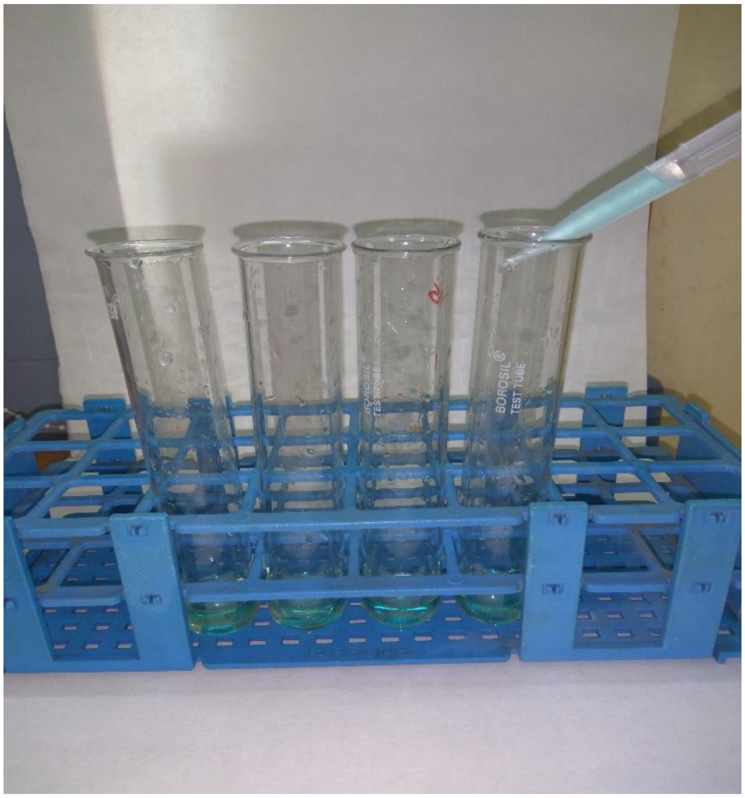


Fig.17 Addition of Bradford reagent

3.7ANALYSIS OF MANGANESE IN SAMPLE

Analysis of manganese was done by Atomic Absorption Spectrometer (AAS, Perkin Elmer). Before going to the analysis of manganese by using AAS, some steps should be follow properly. 1st take required sample, then harvest it. Filtrate the sample by using a filter paper and collect the pellet with liquid filtrate which was known as culture filtrate. After filtration pellet are mixed with acid i.e 0.1N CONC.H_2SO_4. Mixed acid and pellet should be shake well in magnetic stirrer For 4 hrs. for better digestion. After 4hrs of stirrer filter the acid mixed sample and dry the solid filtrate in hot air oven for 2 hrs. at 80^0c, and at that time collect the liquid filtrate for analysis. Both culture filtrate and acid wash filtrate should be taken for analyzed the presence of manganese in sample. According the original sample if analysis give high value then dilution should be done on both. For dilution of sample a formula should be follow-

Volume of given sample * dilution factor = final volume of sample

Before make up the volume as per required amount, 1ml conc. HCL should be added in sample for a better digestion of material present sample.

After AAS analysis note down the values and then calculate the manganese present in sample by the formula;

Metal value in (g/L) = [AAS value (in ppm) *dilution

3.8 Comparison of carbon sources with time

In this experiment, the carbon source used for bioleaching was changed. Glucose, Sodium acetate and cow dung compost were used for the study. The media was prepared as described earlier, and the cultures were inoculated in test tubes containing 30ml MSM each, with 10% (v/v) inoculum (3ml) and 1% pulp density (0.3 g ore). A concentration of 10mM of glucose and sodium acetate was taken for the study. In case of compost, 1g was taken for every 1g of ore.

In test tube experiment glucose and sodium acetate are used as carbon source. 20 test tubes with their duplicates were used. Ore contained in test tubes should be properly plugged to avoid the entry of moisture at the time of autoclave. Add 2 ml of paraffin oil after make up the volume of tubes. Before covering the tubes with clean film heat their mouth in spirit lamp to avoid the contamination of culture. Incubate the test tubes in dark at room temperature for the given period. The tubes were periodically manually shaken in a gentle manner. The protein content in the test tubes was estimated by completely harvesting it as described in section in3.6.

3.9 Effect of Low cost carbon source with time

Prepare the MSM for 20 test tubes. Take equal amount of compost and ore in each test tubes i.e 0.3 gm. As before described follow the procedure of protein estimation and AAS analysis for 10 days to know the growth curve and amount of leached manganese.

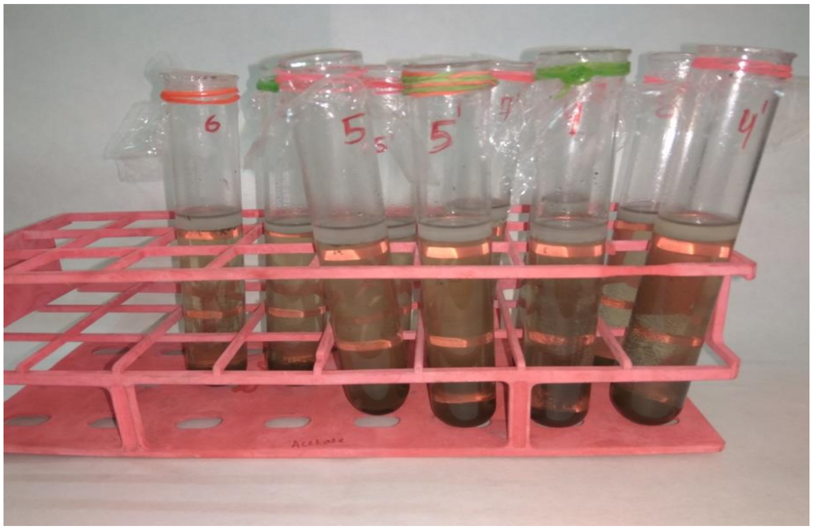


Fig.18.Sample with compost culture in test tubes

CHAPTER IV

RESULT AND DISCUSSION

In this chapter, we discuss the result of the experiments conducted for the bioleaching of low grade ferromanganese ore under anaerobic conditions. Here we discuss about the result of 6 experiments with the effect of different parameters. Also, we discuss about the effect of carbon source and the absence of carbon source in bioleaching process.

4.1 EFFECT OF DIFFERENT CARBON SOURCES IN BIOLEACHING PROCESS:

Here glucose and sodium acetate were used as carbon source in culture process. By following the procedure of protein estimation, we can compare and observe the difference between the growth curve of two carbon source used in culture process.

Table.7-concentration of protein in presence of sodium acetate

DAYS	CONCENTRATION OF PROTEIN(μg/ml)
0	8.3125
4	3.3125
5	19.5625
6	23.3125
13	29.25
15	17.687
18	13.625

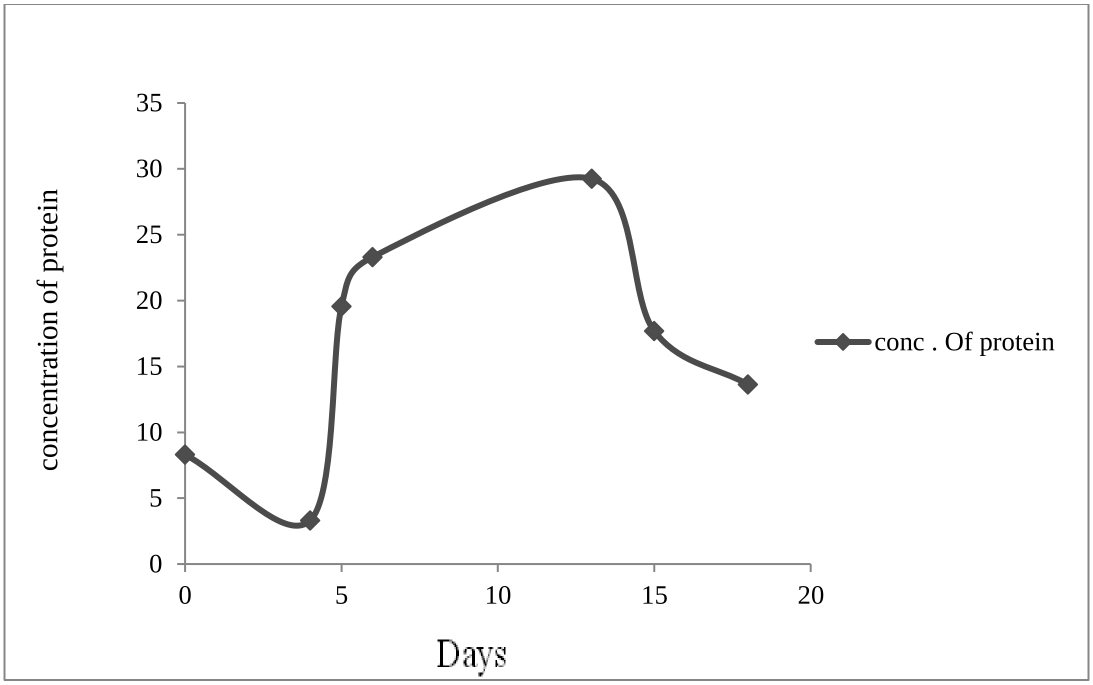


Fig.19.Growth curve in presence of sodium acetate

Table 8- Concentration of protein in presence of glucose

DAYS	CONC. OF PROTEIN (µg/ml)
0	15.5
4	20.187
5	23.625
6	27.687
11	29.562
13	30.187
18	41.75

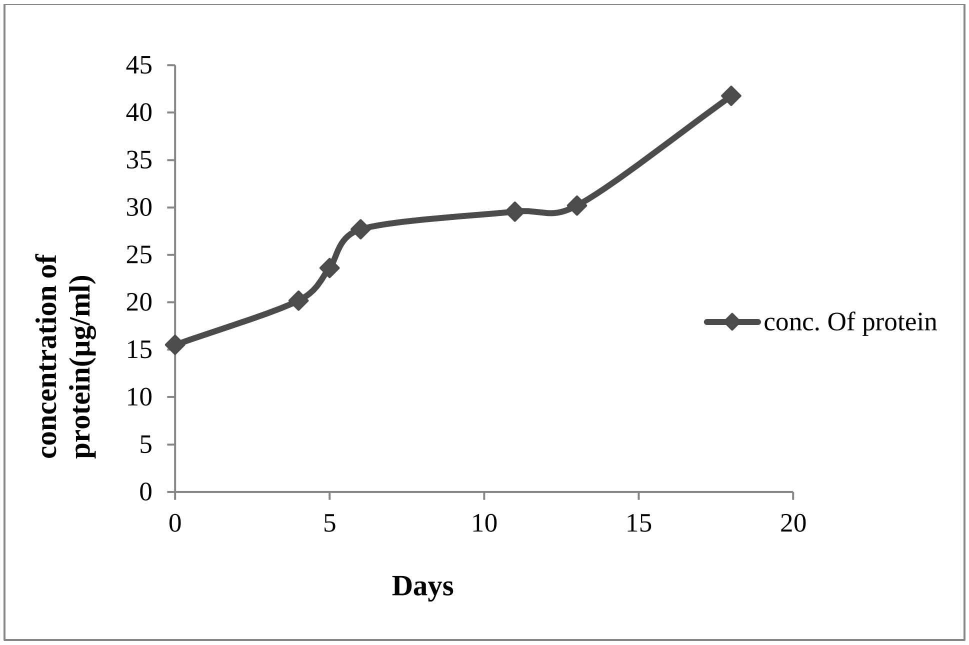


Fig.20.Growth curve in presence of glucose

OBSERVATAION –

Here glucose and sodium acetate were used as carbon source as per standard culture process. Glucose contained culture tube gives slightly better result in their growth curve and leaching process rather than tube contained with sodium acetate as carbon source.This could be due to the reason that many facultative anaerobes can utilize glucose, while only acetate utilizing organisms can grow on sodium acetate. The growth of glucose dependent culture shows that the log phase continues till 20 days, while that of acetate culture enters steady phase after 12 days.

4.2 EFFECT OF COMPOST WITH TIME

Compost contains simple organic carbon compounds by which they support the growth of organisms present in the culture. In presence of compost, sample culture maintain pH level at which microorganisms can grow properly.

Table 9- pH value of sample with the incubation time.

DAYS	pH value
5	8.85
10	7.815
17	8.135
20	8.15
25	8.175
30	8.235
35	8.245
40	8.415

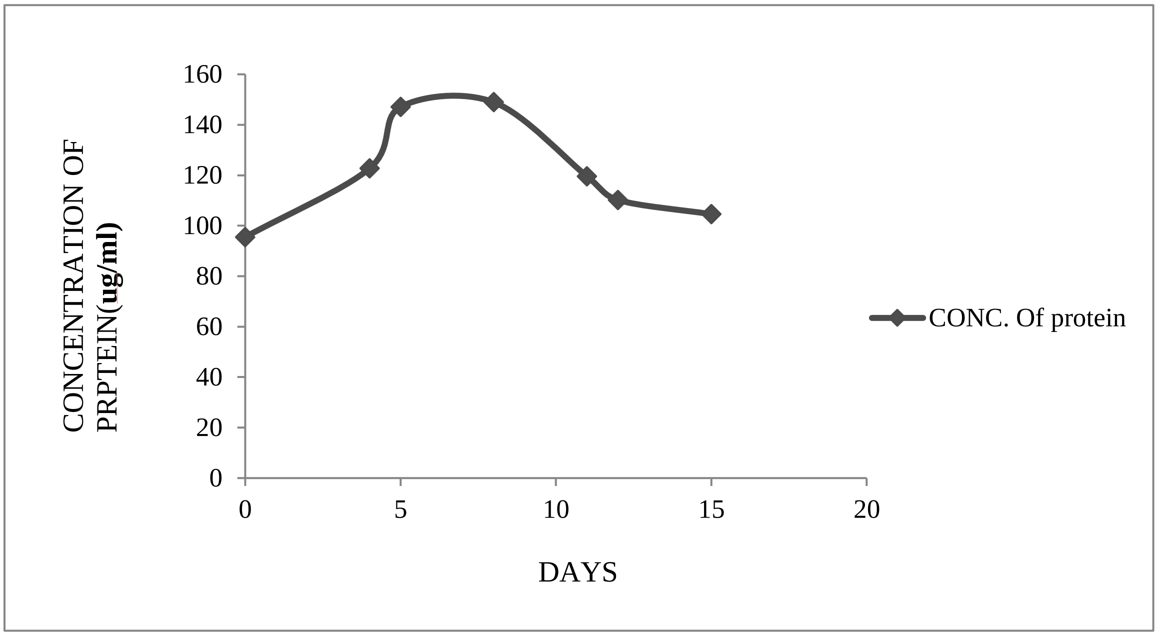


Fig.21. Growth curve in presence of compost

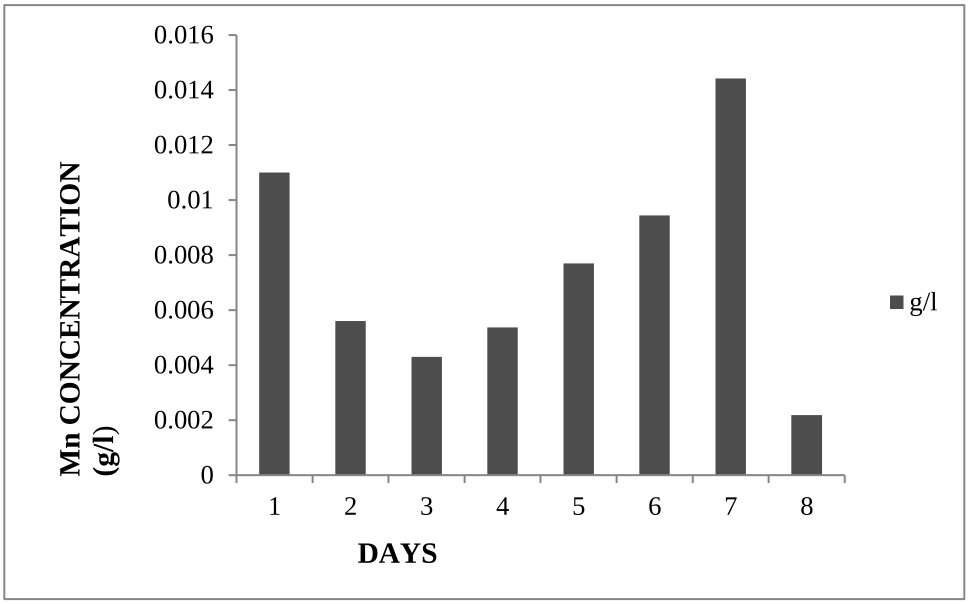


Fig.22.Manganese concentration in sample analyzed by AAS

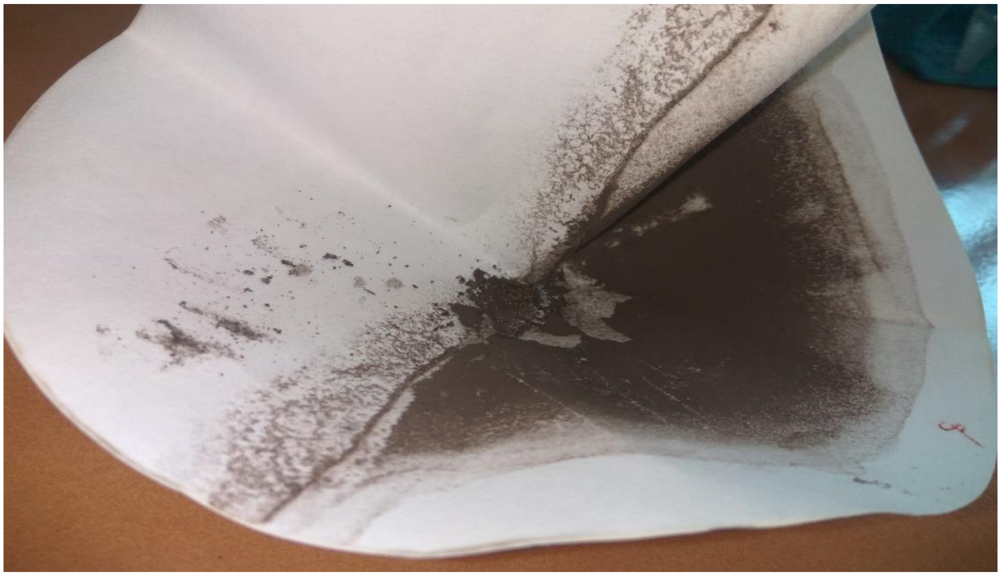


Fig.23. Collection of residue

OBSERVATION-

- Low cost carbon source that is compost, have ability that they can support the growth of culture organism and enrich in MSM medium.
- It can be observed that low cost compost can support the growth of consortia in presence of Mn ore, and can be used as replacement for glucose or acetate.
- The Mn dissolution started after 5-6 days of inoculation, after which an increasing trend was observed. The log phase started from 4 or 5 days as seen in growth curve, and matches with increasing dissolution of Mn after day 5.

4.3 EFFECT OF CONCENTRATION OF COMPOST

After 19 days of incubation collect the sample of 10 test tubes with their duplicates and after the complete harvest of sample following by the protein estimation and analysis procedure note down the concentration of protein and AAS values and then calculate the manganese present in sample by the formula;

Metal value in (g/L) = [AAS value (in ppm) *dilution factor]/1000.

Table 10- Different ratio of ore and compost

SAMPLE NO.	ORE: COMPOST
1	0.3:0.3
2	0.3:0.09
3	0.3:0.18
4	0.3:0.36
5	0.3:0.045
6	0.3:0.6
7	0.3:1.2
8	0.3:1.8
9	0.3:2.4
10	0.3:3

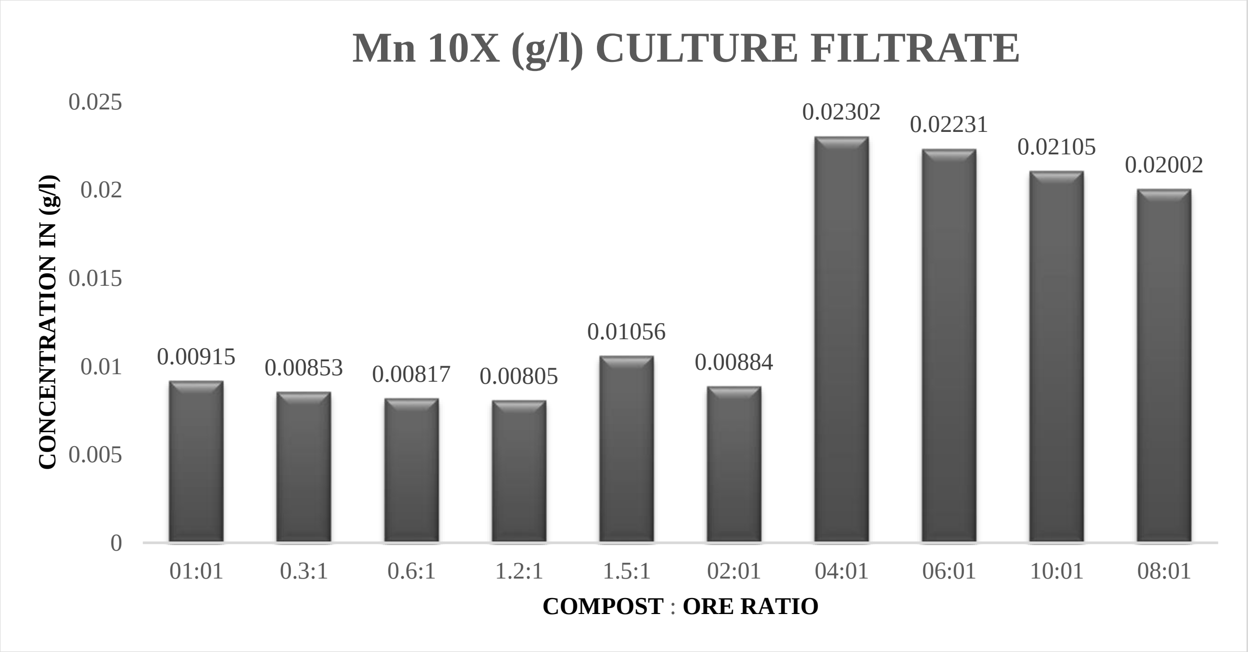


Fig.24. Manganese present in culture filtrate by using different concentration of compost present in samples

OBSERVATION-

In this experiment, we observe the effect of different concentration of compost on the leaching of manganese. The amount of manganese present in sample is different for different concentration of compost. Maximum Mn dissolution was observed in culture filtrate at 1:4 ration of ore: compost, beyond which the effect was negligible.

4.4 VARIATION OF PULP DENSITY

Pulp density is the ratio of weight of solids to volume of slurry.

For this experiment 3 different pulp densities were studied- 1, 2 & 5 (%w/v) of pulp (ore and compost). Following the procedure of analysis by AAS we can compare and calculate the manganese present in the different percentage of pulp density.

Table 11: composition of ore and compost

Density (in %)	Ore: compost (in gm)
1(A)	2.5:10
2(B)	5:20
5(C)	12.5:50

Continue the procedure of protein estimation and AAS from day 1 to day 9 then done it for 5 days of difference up to 40 days. After 40 days of this experiment harvest all these sample.

Fig.25. **Culture filtrate of 3 pulp density samples**

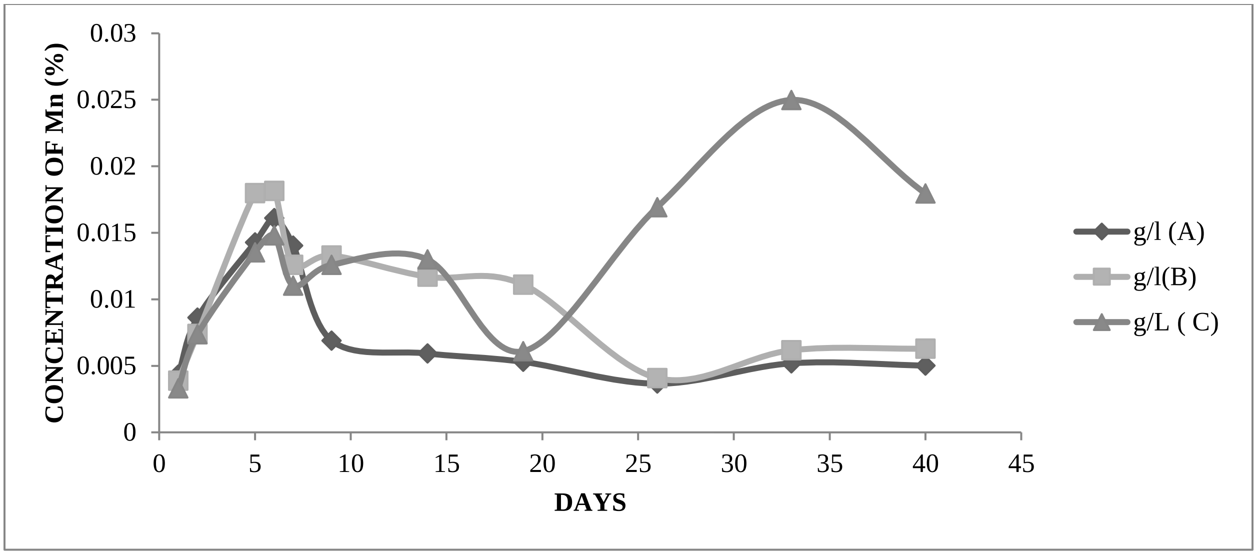


Fig.26.Manganese analysis from culture filtrate of variation in pulp density

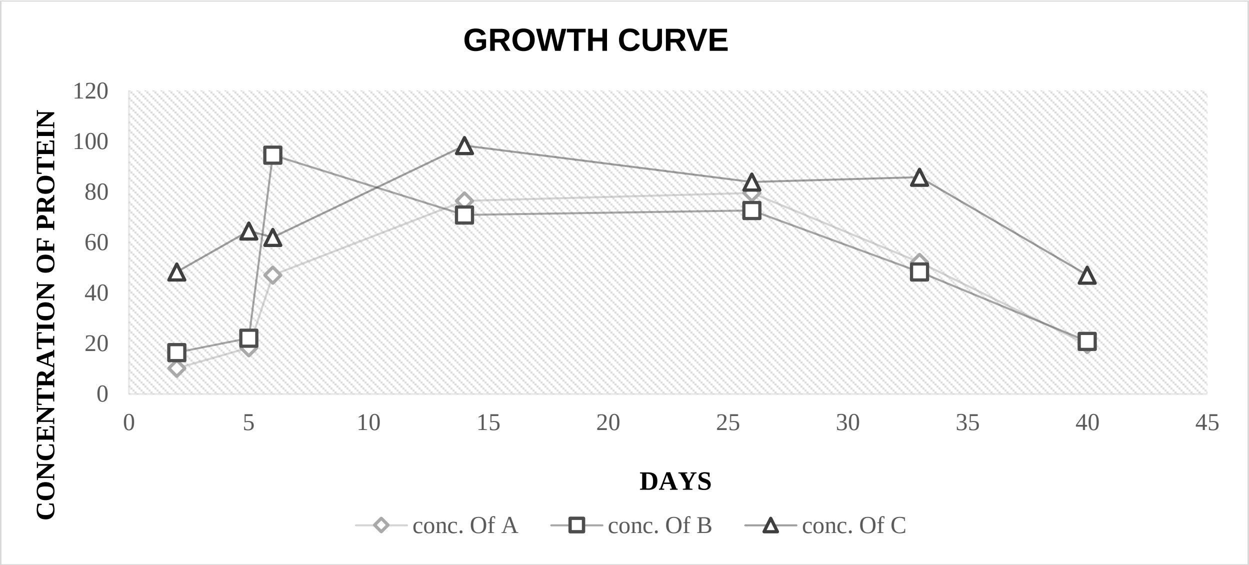


Fig.27. growth curve given by variation in pulp density

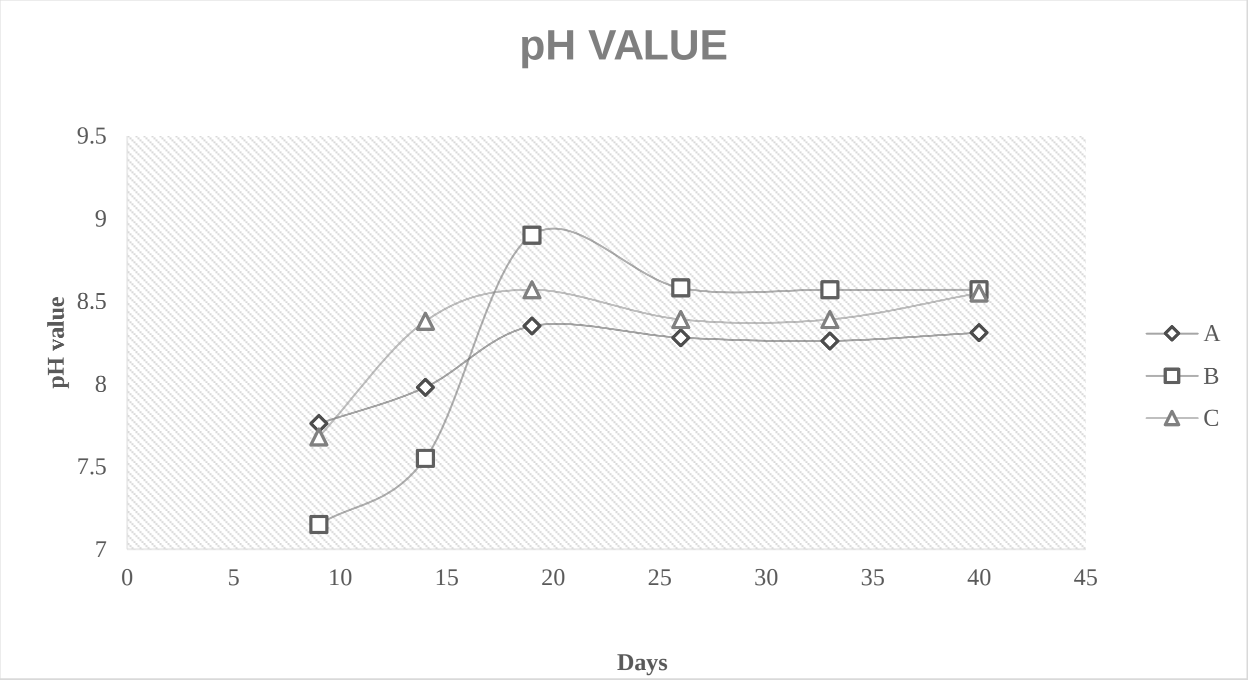


Fig.28. pH value of 3 different pulp density samples

OBSERVATION -

- The growth curve signifies that at higher compost concentrations as well as higher pulp density of ore, the consortia was well supported by the compost as shown by the protein content.
  - The consortia was at log phase from 5-15 days, after that remained in steady phase.
  - The pH of culture remained between 7.5 and 8.5 throughout the incubation period.

CHAPTER 5

CONCLUSION

From the present study, it can be observed that Cow dung compost can be effectively used as a low cost organic carbon source in place of glucose and sodium acetate in anaerobic bioleaching of Mn. Which is cheaply available in our surrounding . From the protein estimation of samples by using Bradford method it can be observed, that compost can support the growth of microorganisms present in the consortia similar to glucose or acetate. The microbial action resulted in Mn dissolution in the culture filtrate and found to increase with incubation time and amount of compost used. Maximum Mn dissolution was observed at a ratio of 1:4 of ore: compost. Higher pulp density of ore supported by corresponding required amount of compost (at 1:4) was also found to give increased Mn leaching with time in the culture filtrate. This could be due to humic substances present in the compost. From the study, it can be said that cow dung compost can be used as a replacement of glucose or sodium acetate to support the growth of anaerobic microorganism and enable Mn bioleaching. However further detailed studies are required to establish the constituents of compost enabling the microbial growth and Mn dissolution.

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