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Original Article

BIOLEACHING OF ORE USING CHEMOLITHOTROPHIC MICRO-ORGANISMS (Acidithiobacillus Ferrooxidans)

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Abstract

Bioleaching is a process of extracting minerals from ores using microorganisms. The extraction of nickel from low grade ores is today’s need because of gradual depletion of high grade ore. The conventional methods used for extraction of nickel from ore is either Pyrometallurgy or Hydrometallurgy, however both the methods are not free from the environmental pollution problems and economically very expensive, and requires lots of energy. Bioleaching of mineral is the only method considered as most convincing way to solve these problems requires very less energy and is free from environmental pollution and other problems. Generally bioleaching is a process described as being “dissolution of metals from their mineral source by certain and naturally occurring microorganisms” or “use of microorganisms to transform elements so that the elements could be extracted from a material when water is filtered through it”. However, there are some slight differences in definition: Usually, “bioleaching” is described as the conversion of solid metal values into their water soluble forms using microorganisms. Bacterial leaching is the extraction of metals from their ores using microorganisms. The capital costs are low compared to those for a smelter. Environmental pollution caused by mineral processing is a serious problem and on the other hand, microorganisms play crucial roles in biogeochemical cycling of toxic metals and radionuclide. Recent progresses have been made to understand metal–microbe interactions and new applications of these processes to the detoxification of metal and radionuclide contamination have been developed. It also suggests an opportunity to reduce of environmental and air pollution.

Keywords:- Bioleaching, Dissolution of metals, Hydrometallurgy, Microorganisms.

1. INTRODUCTION

Bioleaching is a simple and effective process used for metal extraction from low grade ores and mineral concentrates using the chemolithothrophic bacteria. The extraction of nickel from low grade ore is today’s need because of gradual depletion of high grade ore. The traditional methods used for extraction of nickel are either Pyrometallurgy or Hydrometallurgy. However both the methods are not free from environmental problems. In pyrometallurgical method, the ore is crushed and milled into a fine pulp and then concentrated by flotation using chemical reagents. The concentrate formed is smelted and electrolytically refined; however refining process creates environmental problems. It releases lots of metal ions in their wastes, it also releases lots of sulphur dioxide during smelting which causes environmental pollution. In hydrometallurgical method ore concentrate is leached by chemical methods followed by solvent extraction and electro-wining, however this method is not also free from environmental complexity but also from non-competitive economics. There are many techniques proposed to extract metals but these are not practically suitable, as these requires a very high energy input as well as most of them creates environmental pollution problem, that also rises the cost of environmental protection throughout the world. Biohydrometallurgy or Bioprocessing is a new approach used for extraction of metals this includes bioremediation, biosorption, bioaccumulation and bioleaching. Bio processing of mineral is the only method considered as most convincing way to solve these problems. As these processes are easy to operate, requires less energy and they are free from environmental problems and non-competitive economics of conventional methods. The bacteria most commonly used in bioleaching are of two types, Chemolithotrophic and Heterotrophic. Thiobacillus ferrooxidans is a chemolithotrophic bacterium capable of utilizing ferrous iron as the sole source of energy for its
growth. Due to its capacity to oxidize metal sulphides, this bacterium is one of the most important microorganisms utilized in industrial operations to recover metals, such as Nickel, Uranium and Gold. These organisms cause cytolysis, redoxolysis and acidolysis. Acidolysis is the principle mechanism involved in bioleaching process. In this process organisms produce different acids such as citric, oxalic, and sulphuric acid which helps in metal dissolution process from ores.

By keeping in view this background, in the present study Halophilic Thiobacillus ferroxidans N- 11 is explored for bioleaching of nickel from low-grade ore Bornite.

2. MICRO ORGANISMS USED

For biochemical leaching, both autotrophic and heterotrophic bacterial and fungal species have been used for different ores. Acidophilic bacterial species have been used in refractory gold ore leaching for removal of pyrite matrix. The bacteria belonging to the genus Thiobacillus are aerobic and acidophilic autotrophes which play an important role in the bioleaching of metals from sulphidic minerals. They have been the most extensively studied microorganisms in terms of their physiological and biochemical characteistics. These bacteria derive their energy requirements from oxidation of iron and sulfur compounds. Ferric iron and sulphuric acid produced in the system bring about metal solubilisation. The physiological requirements and the ability of Thiobacillus to oxidize Fe2+ and S determine the bioleaching efficiency.

The ability of microorganisms to leach and mobilize metals from solid materials comprises of three principles namely

- Redox reactions
- Formation of organic or inorganic acids
- Excretion of complexing agents

In the process both autotrophic and heterotrophic microorganisms tested for metal removal or substrate degradation are species of Thiobacillus, Bacillus, Pseudomonas, Sulpholobus, Leptosprillum, Acidiphilum, Cyanobacteria, Aspergillus, Penicillium, Rhizopus, Streptomycyes etc.

Specifically, a consortium of microorganisms namely, Acidithiobacillus ferrooxidans, Acidithiobacillus thiooxidans, Leptosprillum ferrooxidans, Sulpholobus spp. And thermophilic bacteria including Sulpholobus hermosusphiloxidans and Sulpholobus briefly are known to be involved in bioleaching. Anaerobes would also be found in leaching areas.

<table>
<thead>
<tr>
<th>Table 1- Micro Organisms, Processes and Areas of Application</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MICROORGANISMS</strong></td>
</tr>
<tr>
<td>Bacteria of the genera Thiobacillus and Leptosprillum</td>
</tr>
<tr>
<td>Thermophilic bacteria similar to Thiobacillus</td>
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<tr>
<td>Thermophilic bacteria of the genus Sulphobacillus</td>
</tr>
<tr>
<td>Acidophilic bacteria of genera Sulphobolus and Acidianus.</td>
</tr>
<tr>
<td>Organotrophic microorganisms and their metabolites (Fungi, Bacteria, Yeast, Algae)</td>
</tr>
</tbody>
</table>

3. BIOLEACHING

Basically it is the dissolution of metals from their ores, concentrates and mineral wastes under the influence of leading to the yield of metal solution of leach liquor containing metals. Such solutions can be processed through solvent extraction and electro winning to get highly pure metal or can be processed to get metal salts. Metals such as nickel, zinc, uranium, nickel, cobalt, gold etc. can be extracted by the process. 15% nickel, 13% uranium and 25% gold are being produced world-wide through bioleaching route. Both oxides and sulphidic ores can be treated by this process.

3.1. Types of bioleaching

- Bench scale bioleaching
- Tank Leaching
- Heap Leaching
- Column Leaching
- Reactor Leaching

3.2. Bioleaching mechanisms

There are two major mechanisms of bacterial leaching. One involves the ferric-ferrous cycle (indirect or non-contact mechanism), while the other involves physical contact of the organism with the insoluble sulphide (direct or contact mechanism) and is independent of indirect mechanism. Originally, a model with two types of mechanisms which are involved in microbial mobilization of metals has been proposed:

3.2.1. Direct mechanism

Microorganisms can oxidize metal sulphides by a direct
mechanism obtaining electrons directly from reduced minerals. Cells have to be attached to the mineral surface and a close contact is needed. Bioleaching of metal sulfides (MS) can be achieved in direct and indirect modes of bacterial metabolism. The direct mechanism is given by:
\[
\text{MS} + 2 \text{O}_2 \rightarrow \text{MSO}_4
\]
Where M is a bivalent heavy metal.

The following equations describe the “direct” mechanism for the oxidation of pyrite:
\[
2 \text{FeS}_2 + 7 \text{O}_2 + 2 \text{H}_2 \text{O} \rightarrow 2 \text{FeSO}_4 + 2 \text{H}_2 \text{SO}_4
\]

3.2.2. Indirect mechanism

The oxidation of reduced metals through “indirect” mechanism is mediated by ferric iron (Fe(III)) originating from the microbial oxidation of ferrous iron (Fe(II)) compounds present in minerals. Ferric iron is an oxidizing agent and can oxidize, e.g., metal sulfides and is chemically reduced to ferrous iron which, in turn, can be oxidized microbial again. In this case, iron has a role as electron carrier. It was proposed that no direct physical contact is needed for oxidation of iron.

\[
4 \text{FeSO}_4 + \text{O}_2 + 2 \text{H}_2 \text{SO}_4 \rightarrow 2 \text{Fe}_2(\text{SO}_4)_3 + 2 \text{H}_2 \text{O}
\]

The indirect mechanisms can be demonstrated, i.e., for uranium leaching as follows:
\[
\text{UO}_2 + 2 \text{Fe}_2(\text{SO}_4)_3 \rightarrow \text{UO}_2\text{SO}_4 + 2 \text{Fe}_2\text{SO}_4
\]

However, the model of “direct” and “indirect” metal leaching is still under discussion. Recently, this model has been revised and replaced by another one which is not dependent upon differentiation between a “direct” and an “indirect” leaching mechanisms.

3.3. Autotrophic Leaching Mechanisms

Three proposed mechanisms for the action of Acidithiobacillus ferroxidans on sulphide minerals are as follows:

- The indirect mechanism in which the bacteria oxidizes ferrous ions in the bulk solution to ferric ions that leach the minerals.
- The indirect contact mechanism in which the bacteria oxidize ferrous ions to ferric ions within the layer of bacteria and exopolymeric materials and the ferric ions within this layer leach the minerals.
- The direct contact mechanism in which the bacteria directly oxidize the minerals by biological means without any requirements for ferrous or ferrous ions.

For sulfidic ore leaching, Thiobacillus ferroxidans and Thiobacillus thiooxidans were widely used organisms. Groudev reported the mixed culture of Thiobacillus ferroxidans and Thiobacillus thiooxidans could extract more nickel during bioleaching of nickel sulphide than that of individual when used alone.

Wanda et al. reported that 57% of nickel extract from sulfidic ore by using Thiobacillus ferroxidans in 9K medium after 30 days of leaching.

Nickeliferrous limonite constitutes by far the largest known terrestrial reserves of nickel and cobalt. They are also major future sources of chromium and iron. Wood (1985) studied the mineralogical parameters influencing the acid bacterial leaching of sulfidic ores. It included chiefly the mode of nickel occurrence, permeability of the ore, the quantities of acid consuming gangue in the ore etc.

Sukla and Das (1986) reported the leaching behaviour of Sukinda lateritic nickel ore with sulphuric acid. Mineralogically, the Sukinda ore contains goethite, quartz, chromites and manganese in oxide form in the iron matrix and not as a separate mineral. Since nickel is intimately associated with iron in the laterite, any process that recovers nickel from laterite will therefore be likely to recover iron. They also reported that at about 360°C goethite decomposes to hematite.

Sukla and Das (1987) reported on extraction of cobalt from Sukinda laterites by reduction roat and ammonia leaching. Pressure leaching with sulphuric acid and atmospheric acid leaching is associated with goethite.

3.4. Heterotrophic Leaching Mechanisms

Among the heterotrophic bacteria, members of genus Thiothrix and pseudomonas have been found to be effective in the leaching of non-sulfidic mineral ores. Fungi of the genus penicillium and aspergillus have also been used in the mineral leaching.

As reported, microorganisms are able to mobilize metals by the following:

- Formation of organic acid
- Oxidation or reduction reaction
- Extraction of complexing agents
- Chelates formation

3.4.1. Acid production

Glucose/Sucrose → Fungus/microorganism → Citric/Oxalic/Glutanic acid

3.4.2. Acid Leaching

\[\text{NiO} + 2\text{H}^+ \rightarrow \text{Ni}^{2+} + \text{H}_2\text{O}\] (7)

3.4.3. Complexation/Chelation of nickel with citric acid

\[\text{Ni}^{2+} + \text{H}_3(\text{Citrate}) \rightarrow \text{Ni(Citrate)} + 3\text{H}^+\] (8)

3.4.4. Precipitation of Oxalic acid

\[\text{Ni}^{2+} + \text{HO}_2\text{C CO}_2\text{H} \rightarrow \text{Ni(O}_2\text{C CO}_2)_3 + 2\text{H}^+\] (9)

Organic acids however, occupy a central position in the overall process and supply both protons and metal complexing organic acid anion.

McKenzie (1987) studied on the solubilisation of nickel, cobalt and iron from laterites by means of organic chelating acids at low pH.

Alibhai (1991) reported about 55-60% of nickel and cobalt extraction from Greek laterites when strains of indigenous penicillium sp. and Aspergillus niger were used for bioleaching.

Bioleaching of Greek non-sulfidic nickel ores using microorganisms associated leaching process has been reported by Tzeferis (1991). They developed two bioleaching techniques such as:

- Leaching in the presence of microorganisms
- Chemical leaching at high temperature (95°C)

He extracted to the extent of 55-60 % nickel using first technique and the nickel recovery in the second technique were in the range of 70-72%.

Sukla (1995) reported on increased stability of Aspergillus niger in nickel bioleaching. They achieved 95% nickel leaching with ultrasound pretreated strains of Aspergillus niger in 14 days as compared to 92% nickel leaching after 20 days with untreated Aspergillus niger.

Sukla et al. (1995) have reported the use of filamentous
fungus Penicillium for bioleaching of Sukinda lateritic nickel ore. Under optimum conditions, the fungus could leach a maximum of 12.5% nickel.

4. BACTERIAL LEACHING TECHNIQUES

The two major techniques used in leaching are percolation and agitation leaching. Percolation leaching involves the percolation of a lixiviant through a static bed, whereas agitation leaching involves finer particle sizes agitated in a lixiviant. Due to the large scale operations involved in bacterial leaching, percolation leaching is preferred commercially.

The principal commercial methods are in situ, dump, heap and vat leaching. In situ leaching involves pumping of solution and air under pressure into a mine or into ore bodies made permeable by explosive charging. The resulting metal-enriched solutions are recovered through wells drilled below the ore body. Three types of ore bodies are generally considered for in situ leaching surface deposits above the water table, surface deposits below the water table and deep deposits below the water table.

Dump leaching involves uncrushed waste rock which is piled up. These dumps generally contain about 0.1-0.5% Cu, too low to recover profitably by conventional procedures. Some of these dumps are huge, containing in excess of 10 million tonnes of waste rock. Heap leaching requires the preparation of the ore, primarily size reduction, so as to maximize mineral-lixiviant interaction and the leaching of an impermeable base to prevent lixiviant loss and pollution of water bodies. Essentially, both dump and heap leaching involve the application of lixiviant to the top of the dump or heap surface and the recovery of the metal laden solution that seeps to the bottom by gravity flow. The dilute sulphuric acid sprinkled on top percolates down through the dump, lowering the pH and promoting the growth of acidophilic microorganisms. The acid run-off is collected at the bottom of the dump, from where it is pumped to a recovery station. All the above processes are essentially uncontrolled from a biological and engineering standpoint. Beside these processes are slow in nature and require long periods to recover a portion of the metal.

Vat leaching as currently applied to oxide ores in a confined tank. More controls can be brought in for enhanced recovery by the use of bioreactors, though necessarily these involve higher costs. However for ore concentrates and precious metals they are being considered actively.

4.1 NICKEL ORE LEACHING

Nickel ore deposits are of two types:

- Sulfide
- Oxide (Lateritic)

The sulfide ores have been the major source of nickel till date. The laterites are the nonsulfidic ores, highly weathered material rich in secondary oxides of iron, aluminium or both and nearly devoid of bases and primary silicates and may contain abundant quartz and kaolinite. Laterite often contains minor amounts of nickel, cobalt and chromium.

Non-sulfuric ores such as oxides, carbonates and silicates contain no energy source for the microorganisms to utilize. Bioleaching of non-sulfidic ores and minerals may be used for the recovery valuable metals from low grade ores and minerals as well as for the beneficiation of mineral raw materials, recovery of metal from wastes and heavy metal detoxification of soils and solid residues.

Extraction of nickel and cobalt from low grade laterite ores constitutes one of the expensive processes, due to the low grade pf metals present in the ore. The mineralogical concentration and distribution of nickel and cobalt within the ore matrix inhibit the application of beneficiation processes to concentrate the ores. The importance of the low grade laterite ore to the future supply of nickel and cobalt becomes obvious when one considers that 85% of the nickel reserves and a greater proportion of cobalt reserves are in laterite ores.

Lateritic nickel ore, or ores produced by the weathering of parent rock, constitute 75% of the world’s nickel reserves and it is necessary to utilize these for the extraction of metal values. The complexity of the ores has led to the development of a variety of possible extraction techniques. Four of these namely matte smelting, productions of ferronickel, sulfuric acid leaching at elevated pressures and reduction followed by ammonia leaching are in commercial operation. Pyrometallurgical methods for the production of nickel require a large amount of energy whereas hydrometallurgical methods need less energy but sophisticated technology. Therefore it has become necessary to develop new hydrometallurgical methods. In this respect, the microbial leaching techniques for extraction of metal values are worth mentioning. Ores that are subjected to leaching process of lateritic ores are:

- Saprolite
- Weathered Saprolite
- Limonite
- Nontronite

These ores represent the various layers in the lateritic bedrock. The limonite consists of mainly goethite, a hydrated iron oxide such as alpha-FeO(OH), HFeO2, or Fe2O3.H2O. This continues to a nontronite rich zone. Saprolite is the next layer, which is distinguished because it is rich in magnesium silicate.

5. GROWTH KINETICS AND EFFECTS OF PHYSICOCHEMICAL PARAMETERS ON THE GROWTH OF THIOBACILLUS FERROOXIDANS

There is evidence that the growth of T.ferrooxidans and oxidation of ferrous iron are tightly coupled. A direct relationship between ferrous iron oxidation and O2 uptake/CO2 fixation has been shown. It was also found that the rate of ferrous iron oxidation by T.ferrooxidans was directly related to concentration of nitrogen. It is also proved that the presence of toxic metals could produce a similar effect. Many studies show that the growth of T.ferrooxidans and its ability to oxidize metal ions are dependent on pH, temperature, and concentrations of ferrous and ferric ions. In addition the availability of oxygen and CO2 are the other important factors which influence the metabolism of the bacteria.

5.1 pH

With ferrous ion as the energy source, T.ferrooxidans grows in an environment with pH between 1.0 - 6.0. Lag periods are observed before the activity of the bacteria is
increased with a pH value of 1.2, pH values in the range of 1.5-3.5 did not influence the growth of bacteria but values less than 1.5 or greater than 3.5 affected the growth. The activity of T. ferrooxidans is also reported to be independent of pH in the pH range of 1.9-2.4. pH values between 1.0 and 4.0 inhibited growth strongly. Bacteria must be equipped with an efficient mechanism that allows them to grow with fluctuating pH values. Whatever be the mechanism, we must maintain the cytoplasmic pH at a constant value. With acidophile, resistance to pH has been south in the structural and compositional peculiarities of the cell wall or the cytoplasmic membrane or both. The Thiobacilli contains huge amounts of cyclopropane fatty acids. It has been suggested that these constituents within the cell membrane function by increasing rigidity and decreasing membrane permeability. T.ferrooxidans has a remarkable ability to adapt to different environmental conditions and to produce spontaneous phenotypic variants. Recent genetic studies suggest that this is due to the transposition of mobile DNA sequences.

5.2. Temperature
Most strains of T.ferrooxidans characterized with respect to temperature are mesophilic with temperature optima between 30oC-40oC. The optimum temperature is said to be pH dependent showing a lower optimum temperature with decreasing pH. The maximum temperature for bacteria to grow is also pH dependent, being 45oC over the pH range of 2.5-3.5 and 35oC at a pH of 1.5. The discrepancy between the optimal values for temperature as well as pH, suggests the possibility that different strains, as employed in different studies exhibit optimum conditions that are strain dependent. The optimal values may be derived in terms of growth rate, oxidation rate of ferrous iron etc. The relatively low and stable ambient temperatures in underground mines employing biohydrometallurgical processes in temperate climates have generated an interest in psychrophilic or psychrotrophic strains of T.ferrooxidans. Psychrotrophic members of this population were numerically superior to mesophilic members and the former had wide temperature ranges for growth. The relationship of growth and iron oxidizing ability of T. ferrooxidans with temperature has usually been correlated with an Arrhenius equation. The values of activation energy calculated on the basis of ferrous iron oxidation in growing cultures of bacteria are usually higher than those which are the results of short term measurements of oxidation rate in the presence of cell suspension.

5.3. Concentration of Ferrous Iron
The growth of T.ferrooxidans and its ability to oxidize is significantly influenced by the concentration of ferrous iron. It is reported that a decline in the growth of the bacteria due to the exhaustion of ferrous iron in the substrate. It is reported that increasing the ferrous iron concentration up to 5.6 kg/m3 enhanced the oxygen uptake rate by employing higher concentrations of ferrous iron resulted in lower oxidation rates. Higher concentrations of ferrous iron showed an inhibitory effect on growth. The Monod equation is the most widely used expression to correlate the growth of T.ferrooxidans as a function of ferrous iron concentration. The values for maximum specific growth rate and substrate saturation constant have been determined.

5.4. Carbon Dioxide and Oxygen
T.ferrooxidans is a chemosynthetic and as such an obligate aerobe with a strict requirement for CO2 as its source of carbon for growth. In the view of limited solubility of CO2 at the pH required for the optimum growth of T.ferrooxidans cultures could be predicted to become limited by CO2 stability. It has been shown that the limitation of CO2 in growing cultures of T.ferrooxidans shifts the exponential kinetics of ferrous iron oxidation to linear relationship. The importance of CO2 availability in achieving optimal growth rates and maximum cell yield has been shown. Significantly higher cell yields were obtained as the level of CO2 were increased although growth rate remains unaffected. The level of CO2 that supports the maximal rate of cell growth is in the range of 7-8%. An increase beyond 8% results in the inhibition culture growth. Microbial growth relates to substrate utilization, the electron transfer efficiencies and the free energies of the microbial reactions. It was reported that O2 is not a limiting substrate for bacterial growth in experiments carried out at 30oC in 500 ml flasks containing 200 ml broth shaken at 240 rpm. It has been reported that oxygen becomes a limiting substrate when the concentration of dissolved oxygen is less than 0.29 mg/l and T. ferrooxidans does not grow in cultures with dissolved oxygen concentrations less than 0.2 mg/l.

5.5. Applied Potential
Recent studies have indicated that the yield of T.ferrooxidans can be significantly increased through electrochemical means. It was demonstrated that enhanced yields could be attained due to the in situ electrochemical reduction of soluble ferric iron in the growth medium. The effect of applied D.C potentials both in the +ve and –ve range on the activity and growth of T.ferrooxidans has also been investigated. Application of +ve potentials up to 1000 mv in an acidic medium inhibited bacterial activity because of electrolytic generation of nascent oxygen in the absence ferrous iron. Employing –ve potentials in the range -500 to -1000 mv to bacterial culture containing ferric iron promoted the activity and growth of T.ferrooxidans through electrochemical conversion of ferric iron to ferrous iron.

6. EXPERIMENTAL SET-UP

![A Generalized Flow Sheet of Bioleaching Process](image)

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7. EXPERIMENTAL PROCEDURE

7.1. Ore Analysis

The lateritic nickel ores so obtained are reported to have nickel associated with goethite matrix and cobalt occurred primarily in manganese mineral phases. The ore is a complex, soft, and agglomerate of highly porous fine particles of very high surface area. The ore is ground and sieved to different sizes ranging from +44 to –350 BSS and is used for study of nickel leaching. The raw ore is treated with some chemical to get better leaching.

7.2. Procedure of Ore Analysis

To know the percentage of nickel in the lateritic ore, chemical analysis in the following procedure is to be done. Lateritic nickel ore of 1 g is taken and added to 50 ml of concentrated hydrochloric acid in a beaker. Then the mixture is heated in fume cupboard until the residue turns white. Then the mixture is cooled and filtered and kept in a clean conical flask which is washed prior to it with distilled water several times and the volume was made up to 250 ml by adding distilled water. Only 10 ml of the filtrated solutions was pipetted out into a 100 ml volumetric flask. This solution was made up to 100 ml by adding further distilled water. This solution is regarded as 10 times dilution. Then the diluted samples are to be taken for Atomic Absorption Spectrophotometer (AAS) analysis.

7.3. Mineralogical Analysis

Mineralogical analysis of the lateritic nickel ores are analyzed using high resolution synchrotron based X-ray Diffractometer and by optical microscopy. The lateritic nickel ore will reveal the presence of goethite, a hydrated iron oxide (alpha-FeOOH) and the leached residues will show some jarosite peaks. This step is just required to demonstrate the structure of the mineral sample.

7.4. Microbiological Analysis

Laboratory stock cultures of Acidithiobacillus ferrooxidans strain are used for experiments. This organism is strictly aerobic, gram negative rod, flagellated and chemoautotrophic, which derives its energy for metabolism from the oxidation of inorganic iron and reduced sulfur compounds. This bacterium is non-sporulating rod (0.4 - 0.5 micrometer wide and 1 - 2 micrometer long.) in nature with round ends usually occurring in single or in pairs. This organism has the ability to achieve optimum growth under strongly acidic conditions.

The strain is grown in 9K+ medium of Silverman and Lundgrend containing (NH4)2SO4 (3 g/l), KCl (1 g/l), MgSO4.7H2O (0.5 g/l), FeSO4.7H2O (44.2 g/l). The pH of the medium is adjusted to 1.5-2 with dilute sulfuric acid. The bacterial growth is assessed by monitoring the iron oxidation rate and also by the appearance of a reddish brown colour in the medium due to the formation of ferric iron. The genus Acidithiobacillus represents a versatile group of chemolithotrophic organisms. The organism most studied is Acidithiobacillus ferrooxidans. Thiobacilli are members of the division of Proteobacteria close to junction between the beta and gamma sub-division.

7.5. 9k+ Medium Preparation And Inoculation Of Acidithiobacillus Ferrooxidans Strain

500 ml conical flask was taken with 300 ml of distilled water in it which was previously autoclaved and the media constituents were poured into it. The concentrations of the chemicals for the media are given below in g/l. The appropriate amounts for 300 ml was calculated and added to the distilled water. pH of the medium was found to be approximately 3.5 now. The pH meter so used was standardized by using pH buffer tablets of standard Ph like 4, 7, 9. Then the pH was adjusted to 2 with dilute sulphuric acid using the pH meter. For the control flask, the entire procedure is same but 10 ml of mercuric chloride (HgCl2) was added as bactericide. Then 10 ml of active culture of
Acidithiobacillus ferrooxidans with approximately 2.8x10^6 cells/ml were added to the other flask and the both the flasks were kept in rotary shaker cum incubator at 30oC-35oC and speed was adjusted to 130rpm-150 rpm. 2 ml of sample was taken from each flask to estimate the ferrous and total iron concentration for zero hr. analysis. Simultaneously, ferrous and total iron estimation was done at regular intervals to see the generation time and activity of the strain.

8.2. Sample Calculations
For ferrous iron estimation and total iron estimation, for 10% pulp density at 10 days residence time, burette reading was 2.6. Strength of K2Cr2O7 used was 0.1 N and volume taken was 2 ml.

So, ferrous iron and total iron estimations are:

\[
(2.6 * 0.1 * 55.85) / 2 = 7.261 \text{ grams/liter}
\]

For a pulp density of 2% and a residence time of 5 days, the mean value from the AAS was found to be 0.598. So according to the formula,

\[
X = (0.598*12.5*100)/ (2*10^6) = 0.00934375
\]

% NICKEL EXTRACTED = (0.00934375/0.57) * 100 = 1.639%

Similarly, we can proceed for all other observations.

REFERENCES

### Table 2: Medium Composition

<table>
<thead>
<tr>
<th>COMPOSITION</th>
<th>CONCENTRATION (g/l)</th>
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</thead>
<tbody>
<tr>
<td>Ammonium Sulfate</td>
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<tr>
<td>Dipotassium Hydrogen Phosphate</td>
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<tr>
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<td>Magnesium Sulfate</td>
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<td>Ferrous Sulfate</td>
<td>44.0</td>
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