Bioleaching of metals from ores and electronic scrap

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The Controller of Examination,
University of Agriculture,
Faisalabad.

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Dedicated

TO

MY LOVING PARENTS

Who taught me the first word I spoke
The first alphabet I wrote

AND

First step I walked
I feel I am nothing without them
Whose love and friendship
Encouraged and helped me at every step of life
ACKNOWLEDGEMENT

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4.56. Percent metals solubilization during whole leaching process included preleaching and bioleaching

4.57. Sketch of column bioleaching unit
Declaration

I hereby declare that the contents of the thesis “Bioleaching of metals from ores and electronic scrap” are product of my own research and no part has been copied from any published source (except the references, standard mathematical models/ equations/ formulate/ protocols etc). I further declare that this work has not been submitted for award of any other diploma/degree. The university may take any action if the information provided is found inaccurate at any stage.

Signature of student

2007-ag-18
ABSTRACT

Low grade pyrite, sphalerite, complex Pb-Zn ore, nickel and copper containing sulphide ore’s bioleaching performances of pure un-adapted and metal ion-adapted cultures of different strains of *Sulfobacillus thermosulfidooxidans* as well as their consortium with acidophilic heterotrophs were analyzed in shake flasks studies. Maximum bioleaching potential was observed in case of mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*. Orthogonal experimental array was designed for further co-optimization of process parameters for the enhancement of bioleaching efficiency. Then the technical feasibility to recover valuable metal ions from these ores by bioleaching process was tested in large columns at ambient temperature after optimization of process parameters in several small columns. Different pre-leaching and bioleaching strategies were adopted for maximum dissolution of metal ions from ores during column bioleaching studies. Changes in pH, redox potential, temperature, ferrous, ferric and total iron concentration, microbial growth and percent metals solubelization was observed periodically. Then bioleaching feasibility of electronic scrap by the selected moderately thermophilic strains of acidophilic chemolithotrophic and acidophilic heterotrophic bacteria was tested. These included *Sulfobacillus thermosulfidooxidans* of different strains, *Thermoplasma acidophilum* and an unidentified acidophilic heterotroph (code A1TSB). At scrap concentration of 10 g/L, a mixed consortium of the metal adapted cultures was able to leach more than 81 % of Ni, 89 % of Cu, 7 9 % of Al and 83 % of Zn. Then conical bubble reactor was fabricated locally and effect of hydraulic retention time on bioleaching potential was investigated and after that lab-scale columns with automated pH and temperature control were fabricated locally and bioleaching studies of electronic scrap in a bubble reactor was carried out. In case of column bioleaching studies the tolerance of bacterial cultures to mixed metal ions (Ag⁺, Al³⁺, Cu²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Sn²⁺ and Zn²⁺) was improved markedly after nearly two year adaptation from 12 g/L to 20 g/L. The results from these studies demonstrate that 80 % Zn, 64 % Al, 86 % Cu and Ni 74 % can be recovered from electronic scrap by microbial leaching process using mixed adapted consortium of moderately thermophilic bacteria at column bioleaching level and 4 % Al, 6 % Zn, 5 % Cu and 7 % Ni can be leached out during preleaching. This finding may facilitate on industrial scale implementation of this process for recycling of metals from electronic scrap.
INTRODUCTION

Continuing accumulation of tailing due to worldwide metal rich ore’s depletion, future sustainable developments require measures to reduce the demand for primary resources and to use the non-renewable raw materials economically. Therefore, new resources with the aid of novel technologies must be developed for metals. In addition, improvement of already existing mining techniques can result in metal recovery from sources that have not been of economical interest. Biohydrometallurgical processes offer a possibility to obtain metals from mineral resources not accessible by conventional hydrometallurgical and pyrometallurgical techniques. Metallic compounds are converted into their water-soluble forms in a process called as microbial leaching or bioleaching by bacteria or fungi (Atlas and Bartha, 1997; Fuchs et al., 1996). Traditionally bioleaching can also be termed as biooxidation but there are some small differences by definition (Brierley, 1997). For example, microbially copper sulfide is oxidize to copper sulfate which is soluble in the aqueous phase. The solids remaining are discarded. In biooxidation microbes oxidize host minerals and metals are left in the solid residues in a more concentrated form. Biooxidation is used in gold mining operations to (partly) remove arsenopyrite or pyrite. This process is also termed as “Biobeneficiation”. In this process solid materials are refined to remove unwanted impurities (Groudev, 1983).

The main advantages of bioleaching include:

- The use of microorganisms, water and air, the naturally occurring key components
- The use of simple reactors or modules of reactors
- Simplicity of operation and maintenance
- Low temperature and pressure conditions
- Free from dust and SO₂ hence environment friendly nature
- Lower capital costs

Applications of biohydrometallurgical processes in mineral industries are continuously gaining importance due to their low cost of operation, ease of maintenance and safety of environment. This technology was also successfully used for the processing of sulfidic ores like that of copper, uranium, zinc, gold, silver, cobalt etc. and can prove to be equally beneficial for the recovery of precious metals like beryllium, vanadium,
titanium and zirconium from their respective non-sulfidic ores. Additionally, applying microbial leaching processes, it is possible to recover metals even from cut-off grade ores, dumps and industrial wastes, thus increasing metal recovery considerably. Since this technology can eventually lead to the production of metal in a relatively pure form at the mine site, it bypasses the use of expensive smelters, thus abating the damage to the environment.

Metal cycling by bioleaching is similar to natural biogeochemical processes. Using biohydrometallurgical techniques, the metals can be recovered with increased efficiency particularly from low grade, complex ores and electronic scraps whereas only physico-chemical methods are not much successful in this regard due to certain limitations associated with these processes. In low grade ores of copper and gold conventional treatments are not as successful as the biological methods.

If we observed the world scenario then it becomes clear that bacterial leaching in recent years has been used successfully in many countries to recover metal ions from wide variety of ores. The principle metals recovered are Cu, Ni, Zn, Pb, Co, Au and U. It has been estimated that at least 15 % of world production or 1.1 x 10^6 tons of copper is bioleached annually. Currently it is estimated that 30 % of total copper production in Western United State is the result of bacterial leaching. During 1988 almost three hundred tons of uranium was recovered through in situ bioleaching from the Dennison mine in the Elliot Lake district of Canada that have a value of approximately $ 25 million. In South Africa bioprocessing is commercially being used to recover gold from low grade, sulfide ores. Additional gold-recovery plants have been built at Sao Bento in Brazil (started production in 1990 with a capacity of 150 tons per day). Two mines of gold recovery in West Australia (Harbour Lights, starting in 1991 with 40 tons capacity per day and Wiluna starting in 1993 with 115 tons capacity per day) and enormous commercial plant (720 tons of gold bearing concentrate per day) were constructed during 1994 at a mine in Ashanti, Ghana. Starting in1994 a third west Australian Plant started with a 120 tons capacity per day. Since 1986, 11 commercial bioleaching/biooxidation plants have been commissioned with nine in continuous operation today. Statistics indicate that some 43 minerals are presently being mined in Pakistan, up from 9 in 1947. Various geological regional surveys conducted in the recent past have confirmed the occurrence of ores of copper, aluminum, chromites, gold, platinum, silver, iron, zinc and lead in various regions
of Pakistan. Despite Pakistan is rich in minerals, mineral sector at large plays only a marginal role in the national economy, contributing less than 3% to the gross national product during 1987-88. This is mainly attributed due to the fact that most of these minerals occur as low grade-ores and hence are not fit for extraction of metal values by conventional metallurgical techniques. In this case, biotechnological intervention is an economical technology for valuable metal recovery from low-grade ores. Metal recovery is possible from dumps, cut off grade ores, industrial wastes and even from electronic scraps using microbial leaching.

Electronic and electric products are source of education, communication, health care and transportation all over the world. This revolution will not be abating soon. Technical innovation is contributing in social progress and the way is lead by advance electronics (Fisher et al., 2005). Terazono et al. (2006) have studied electronic waste generation in various parts of world. Studies by Bertram et al. (2002), Jirang and Lifeng (2008) also found that electric and electronic wastes are rapidly increase waste category. But the dark side to this brilliant advancement is the environmental degradation as a product of this progress. With the rapid increasing advancements in electronic technology, electronic products that were formerly thought of as cutting edge are becoming obsolete at a very high rate. By considering obsolete these products are subject to disposal. The disposal process is commonly through the use of landfills, thus becoming electronic scrap. Electronic scrap is waste generated by used electronic products such as obsolete televisions, telephones and computers. Landfills are increasingly reaching their capacity due to these electronic wastes. Also, the disposal of electronic wastes or their products in landfills is just a temporary measure and since these wastes do not degrade properly and remain poisonous forever. Many European countries have implemented strict legislations which ban the use of landfills for non-degradable substances and electronic components. Recycling of electric and electronic wastes are now ultimate goals of Japan and European Union. With legislations in place, land filling will no longer be an option. End of life management of these products in a safe and environmentally manner is the only alternative that we all can arrive at. End of life management deals with the process of managing and trying to dispose, reuse and recycle products that are becoming obsolete or have reached the end of their useful life. End of life Management can be considered as a reactive approach. The main concern with the end of life management is
to reduce the environmental impact of these old and obsolete products and to dispose them in a safe and friendly way. The concept of the end of life management started parallel with life cycle analysis and for all practical purposes can be considered as a part of it. Recycling companies by the help of manual and automated process have tried to cope and to recover valuable metals and glass. But recycling companies still being in its nascent stage do not have the adequate technology, processes, techniques and models to counter this huge volume of electronic scrap. Recycling companies in conjunction with upstream manufacturers are working on end of life management of these electronic components. However, electronic scrap recycling is still limited due to material heterogeneity and requirement of complex equipment (Veit et al., 2005). Among electronic wastes printed circuit boards have quite diverse composition, containing metals, ceramics and polymers. Total metals in electronic scrap around 28- 30 % among which Cu: 10–20 %, Pb: 1–5 %, Ni: 1–3 % and Ag, Pt and Au (0.3-0.4%).Contents of the other materials present include plastics (19 %), Br (4 %), ceramics and glass (4-9%). Organic compounds like phenolic resions, isocyanates, phosgene and acrylic also found in circuit boards (Ludwig et al., 2003). Printed circuits are used in all kinds of electronic circuits, from simple one transistor amplifiers to the largest super computers. Today, printed circuit boards come in all shapes and sizes, small to large. Some boards contain integrated circuits and some bare (unpopulated), which all may be recycled in some capacity. Mechanical and pyrometallurgical electronic waste recycling was investigated by many researchers (Noakes, 1999; Veit et al., 2007, Li et al., 2007). But such processes require high consumption of energy in the smelter feed which is produced by halogenated flame retardants. As a result dioxins and furans are formed and precious metal recovery is not so efficient (Krebs et al., 1997; Menad et al., 1998). Metal recovery using microorganism from wastes could be an economical alternate to traditional methods (Olson et al., 2003; Brandl et al., 2001; Faramarzi et al., 2004; Choi et al., 2004). These studies were conducted with pure culture of mesophilic chemolithotrophic (Acidithiobacillus ferooxidans and Acidithiobacillus thiooxidans) or cyanogenic bacteria (Chromobacterium violaceum). The rates of bioleaching of metals from ores by moderate thermophiles have been demonstrated to be higher than mesophiles and in another case even higher than extreme thermophiles (Das et al. 1999; Deveci et al., 2004). However, no data is available on the use of moderately thermophilic bacteria for leaching of metals from electronic scrap and even no studies are reported toward up scaling of
process up to column level. In this research work an effort has been made to evaluate the potential of different bacterial strains and mixed cultures to solubilize metal ions from printed circuit boards, complex Pb-Zn ores, low grade pyrite, chalcopyrite and sphalerite to figure out strategies for enhanced metal ion recovery and to comprehensively upscale the process after further development of column bioleaching research of the electronic scrap and ores. With a recovery and recycling process these electronic scrap materials and ores could produce potential environmental benefits and economical profits.
CHAPTER 2

REVIEW OF LITERATURE

2.1. HISTORICAL BACKGROUND

Metal extraction from ores is not a new process. Metals are essential parts of daily life from centuries (Rossi 1990; Ehrlich, 1999). Oxidation of elemental sulfur and reduced sulfur compounds resulting in the formation of sulfuric acid was conducted in the 1880s (Winogradsky, 1887), metal sulfides (zinc sulfide) oxidation was not described until 1922 (Rudolf and Helbronner, 1922). In 1947, *Thiobacillus ferrooxidans* (now called as *Acidithiobacillus ferrooxidans*) found in drainage of acid mine (Colmer and Hinkle, 1947). Zimmerley et al., 1958 generated a patent on lixiviant solution of ferric sulfate/ sulfuric acid used for metal extraction.

2.2. MICROORGANISMS INVOLVED IN BIOLEACHING

For many years, in bioleaching of ores, *Acidithiobacillus ferrooxidans* (formerly called *Thiobacillus ferrooxidans*) has been considered to be the most important microorganism (Kelly and Wood, 2000; Lundgren and Silver, 1980; Brierley, 1982). This acidophilic, obligate chemolithotrophic bacterium of the genus Acidithiobacillus (formerly called Thiobacillus) and *Acidithiobacillus thiooxidans*, another bacterium belonging to the same genus, have drawn much interest because of their unique physiological characteristics (e.g. autophilism and acidophilum), environmental impact (e.g., acid and metal pollution) and commercial value e.g., metal recovery by bacterial leaching (Wakao et al., 1990). *Acidithiobacillus ferrooxidans* has been extensively used in studies on pyrite biooxidation process (Karavaiko et al., 1977; Torma and Bosecker, 1982; Konishi et al., 1990). A mixed consortium of *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans* has been considered to be most effective for bioleaching of sulfidic minerals (Dugan and Apel, 1978; Dugan, 1984). *Acidithiobacillus thiooxidans* is probably unable to degrade sulfidic minerals directly but it can increase the rate of dissolution of some metal sulfides and could be active in the removal of sulfur layers which might be forming on the surfaces of minerals being leached by ferric iron or directly oxidized by iron oxidizing bacteria (Kelly et al., 1979). On the contrary, a recent report based on the results from advanced techniques like scanning electron microscopy (SEM) and atomic force microscopy reveals the involvement of *Acidithiobacillus thiooxidans* in the bioleaching of pyrite (Liu et al., 2003). A combination of direct and
indirect mechanism was proposed to be operational for the pyrite oxidation process by these bacteria.

Moderately thermophilic leaching bacteria (optimum growth temperature ~50°C) are members of the genera Acidimicrobium, Sulfobacillus and Ferromicrobium (Clark and Norris 1996; Norris et al. 1996; Johnson and Roberto 1997). Leaching archae bacteria belong to the Sulfolobales. It is a group of highly thermophilic (optimum growth temperature >60°C), S and Fe (II) oxidizers including genera such as Acidianus, Sulfolobus, Sulfurisphaera and Metallosphaera (Kurosawa et al., 1998; Fuchs et al., 1995, 1996; Norris et al., 2000).

The ability of Acidithiobacillus ferrooxidans, Sulfobacillus thermosulfidooxidans and other microorganisms successfully applied to solubilize metal sulfides in their habitats for metals bioleaching from ores. However, the relatively slow process kinetics is the major bottleneck in the widespread use of these microorganisms at commercial level (Beyer, 1986). Acidithiobacillus ferrooxidans is a mesophilic chemolithotrophic prokaryote that obtains energy from the oxidation of Fe (II), S, or sulfur compounds which are partially oxidized (Brierley, 1978; Harrison, 1984; Rawlings, 2002). In contrast, Sulfobacillus thermosulfidooxidans is a moderately thermophilic bacterium having a highly versatile metabolism and may grow as autotroph, heterotroph, mixotroph, or chemolithothroph (Toni and Johnson 1998). Although Sulfobacillus thermosulfidooxidans has considerably higher metal sulfide leaching rates particularly for low grade ores, it is less tolerant to metal ions due to which its leaching activity decreases drastically at increasing concentrations of metal ions in the leach solution. Process could be improved understanding mechanisms of microorganisms attack and solubilization of the ores. In this regard, physiology and molecular biology of sulfur lithotrophy in these bacteria are important in understanding the basic mechanisms at the molecular level and hence to develop valuable strains with an improved leaching efficiency. Then further researches indicate that bioleaching efficiency may also be improved in case of high grade complex ores by successive adaptation of microorganisms with gradually high concentration of metals and by using mixed microbial consortium (Ilyas et al., 2007). For Acidithiobacillus ferrooxidans only a few biochemical investigations of the oxidative dissimilatory metabolism of S compounds were carried out in the past (Valdes et al., 2003, Ramirez et al., 2002, Acosta et al., 2005).
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Direct morphological observations have revealed an increasing contribution of *Leptospirillum* and other similar organisms in the freely suspended population as pyrite oxidation progressed (Norris et al., 1987; Battaglia-Brunet et al., 1998; Helle and Onken, 1998). Dominance of Leptospirilla over *Acidithiobacillus ferrooxidans* has also been reported under certain conditions (specially at pH < 1.8, or a high Fe (III) to Fe( II) ratio, or both) at 20-45 °C (Goebel and Stackebrandt, 1995; Norris, 1998; Rawlings, 1995; Schrenk et al., 1998; Rawlings et al., 1999) in leaching environments. Recently, mesophilic and acidophilic Fe (II) oxidizing archaebacteria, *Ferroplasma acidiphilum* and *Ferroplasma acidarmanus* have also been discovered (Golyshina et al., 2000; Edwards et al., 2000).

Some acidophilic heterotrophs such as representatives of the genus *Acidiphilum* are also known to be active in the pyrite oxidation process (Norris, 1996; Hiraishi et al., 1998). It is likely that they contribute to the stability of the mixed mineral-oxidizing population by using organic excretion products produced by the mineral oxidizers (Harrison 1984). Bacelar-Nicolau and Johnson (1999) have isolated some new strains of mesophilic heterotrophic acidophiles from acid mine drainages and demonstrated that these bacteria were important net contributors to pyrite oxidation. Recently, Kinnunen et al. (2003) have isolated a moderately thermophilic heterotrophic acidophile, closely related to Alicyclobacillus sp., capable of optimally oxidizing ferrous iron at 50°C (Rohwerder et al., 2003; Pronk et al., 1992; Das et al., 1992; Ohmura et al., 2002). Recently, it has also been demonstrated that *Acidithiobacillus ferrooxidans* reduces elemental sulfur in the course of anaerobic hydrogen oxidation (Ohmura et al., 2002). This anaerobic physiology of Acidithiobacillus-like species was used to clean contaminated groundwater and lignite in an anoxic reactor designed (Alfreider et al., 2002). An important aspect of microbial leaching is that mixed bacterial population often occurs in commercial scale operations. Mixed cultures of microorganisms are found to be more effective in bioleaching processes than their respective pure culture. Synergetic effects of several different strains allow the solubilization of ores that are recalcitrant to pure microbial culture (Acevedo and Gentian, 1998; Fournier et al., 1998; Butler and Kempton, 1987). In addition metal solubilization can also occur by the excretion of organic acids such as oxalate, gluconate, citrate, or succinate.

2.3. MECHANISM OF BIOLEACHING
The mechanism of microbial attack on sulfide minerals has long been a controversial issue. Consequently, this topic is still under debate and has taken a new turn after the proposal of a novel mechanism by Sand et al., 1995 and 1999; Shippers and Sand 1999; Sand et al., 2001).

Silverman and Ehrlich (1964) proposed that two independently separate modes, i.e. direct and indirect mechanisms were involved in microbial oxidation of sulfide minerals. In the direct mechanism, the bacteria physically attach to the pyrite surfaces and enzymatically oxidize it to produce ferrous and sulfate ions. In the indirect mechanism, the acidophilic iron oxidizing bacteria rapidly oxidize the ferrous iron, produced during direct microbial attack, to ferric iron, which, by chemical reaction, oxidizes pyrite, producing acid or elemental sulfur.

### 2.3.1. DIRECT MICROBIAL ACTION

The first step in direct oxidation of metal sulfide, MS, is the solubilization of substrate prior to metabolic reaction. This can be achieved through dissociation of MS:

\[
\text{MS} \rightarrow \text{M}^{2+} + \text{S}^{2-} \tag{1}
\]

The released sulfide anion then binds with enzyme system of bacteria, which then oxidize it to sulfate:

\[
\text{Bacteria} \quad \text{S}^{2-} + 2\text{O}_2 \rightarrow \text{SO}_4^{2-} \tag{2}
\]

Consequently sulfide anion is removed from equation (1) and equilibrium is shifted to the right side resulting in acid production followed by further dissolution of metals. Theoretically this process can continue until all substrate (MS) is converted to product (MSO₄). However, in the batch system the accumulation of product may reach at such high levels that these become toxic to microorganisms or iron (111) hydroxy sulfate (jarosite) precipitates on the surface of substrate impending the bacterial action.

The overall reaction of direct mechanism is

\[
\text{Bacteria} \quad \text{MS} + 2\text{O}_2 \rightarrow \text{MSO}_4 \tag{3}
\]

and in case of Sphalerite (ZnS), Galena (PbS) and pyrite (FeS₂) in equation 4,5 and 6 respectively as follows:
2.3.2. INDIRECT MICROBIAL ACTION

Actually the indirect bacterial action covers processes in which the leaching agent is merely produced or regenerated by microorganisms and they have only a catalytic function because they accelerate the oxidation of ferrous which takes place very slowly in the absence of microorganisms. Reduced metal’s indirect oxidation is mediated by Fe (III) generated from the Fe (II) compounds present in the minerals as a result of microbial oxidation. Fe (III) is an oxidizing agent. Fe (III) can oxidize metal sulfides. Fe (III) is chemically reduced to Fe (II). Fe (II) can be microbially oxidized again. Iron has a role as an electron carrier in this mechanism.

The overall reaction is:

\[2Fe^{3+} + MS \rightarrow 2Fe^{2+} + M^{2+} + S\]  \hspace{1cm} (7)

The indirect mechanism for Sphalerite and Galena can be demonstrated as follows.

\[Fe_2(SO_4)_3 + ZnS \rightarrow S + ZnSO_4 + 2FeSO_4\] \hspace{1cm} (8)

\[PbS + Fe_2(SO_4)_3 \rightarrow S + PbSO_4 + 2FeSO_4\] \hspace{1cm} (9)

The ferrous iron produced in equations 8 and 9 is re-oxidised by bacteria to ferric ions:

\[Bacteria\]
\[2FeSO_4 + 0.5O_2 + H_2SO_4 \rightarrow H_2O + Fe_2(SO_4)_3\]  \hspace{1cm} (10)
Consequently in the indirect process bacteria continuously provide the oxidant, Fe$_2$(SO$_4$)$_3$, which is known to be a powerful oxidizing agent.

Elemental sulfur produced during above reactions is oxidized to sulfuric acid by bacteria.

$$\text{Bacteria}$$

\[
S + 1.5 \text{O}_2 + \text{H}_2\text{O} \rightarrow \text{H}_2\text{SO}_4
\]  \hspace{1cm} (11)

Subsequent studies by many investigators further elaborated these two modes of metal sulfide bioleaching (Tuovinen, 1972; Lundgren and Silver, 1980; Brierley, 1982; Torma and Bosecker, 1982; Dutrizac, 1984; Tuovinen, 1986; Khalid et al., 1990; Norris, 1987; Larsson et al., 1994; Jamal et al., 1995; Ahonen and Touvinen, 1995).

Recent studies have presented new theories. Rohwerder et al., (2003) have reviewed mechanisms and fundamentals of metal sulfide oxidation by bacteria. The indirect mechanism via thiosulphate, developed by Sand et al., 1995; Sand et al., 1999; Schippers and Sand, 1999; Sand et al., 2001), is an impotant attempt to explain the bioleaching mechanism. This mechanism called thiosulfate pathway (Schippers et al., 1996), originally conceived for Acidithiobacillus ferrooxidans, has also been applied to Leptospirillum ferrooxidans. Thiosulfate pathway is based on the results obtained from research work on various aspects of metal sulfides and their biooxidation.

**2.3.3. THIOSULFATE MECHANISM**

Thiosulfate mechanism has been characterized from cells attachment to the mineral; physical contact with the surface; excretion of exopolymers; complexation of Fe (III) compounds to glucuronic acid residues; formation of thiosulfate as intermediate in sulfur compounds oxidation.

In the Thiosulfate pathway, solubilization is through Fe (III) attack on the acid-insoluble metal sulfides (e.g. pyrite), with thiosulfate being the intermediate and sulfate the main end product. On cell attachment to metal sulfide surface, the hexi-hydrated Fe (III) indirectly attack on the metal sulfide by the following reaction:

\[
\text{FeS}_2 + 3\text{H}_2\text{O} + 6\text{Fe}^{3+} \rightarrow 7\text{Fe}^{2+} + \text{S}_2\text{O}_3^{-2} + 6\text{H}^+ \hspace{1cm} (12)
\]
\[
\text{S}_2\text{O}_3^{-2} + 5\text{H}_2\text{O} +8 \text{Fe}^{3+} \rightarrow 8\text{Fe}^{2+} + 2\text{SO}_4^{2-} + 10\text{H}^+ \hspace{1cm} (13)
\]
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Valence bonds in these sulfides are provided by metal atom orbitals. That is why such sulfides degradation occurs only by an oxidizing attack with Fe (III) (Tributsch and Bennett, 1981; Gehrke et al., 1995; Crundwell, 1988; Sand et al., 1995; Schippers et al., 1996; Schippers and Sand, 1999; Garcia).

2.3.4. POLYSULFIDE MECHANISM

Polysulfide and elemental sulfur are the main intermediates in the polysulfide mechanism during the oxidation of galena, sphalerite, chalcopyrite, hauerite, orpiment, or realgar. The presence of iron (III) at the beginning of mineral degradation is an important prerequisite (Sand et al., 1999).

$$2\text{MS} + 3\text{H}_2\text{O} + 2\text{Fe}^{3+} \rightarrow 7\text{Fe}^{2+} + \text{S}_2\text{O}_3^{-2}$$ (14)

Chemolithotrophic bacteria use inorganic energy sources for their growth. In case of non-sulfidic minerals, the metal solubilization is connected with the acid formation as well as with in-situ production of different metabolites that act as chelating and complexing agents. Enzymatic or non-enzymatic iron reduction is involved in this solubilization process. Different heterotrophic bacteria and fungi are also capable of dissolving iron and other metals from oxide minerals. Excretion of extracellular polymeric substances (EPS) by microorganisms adhering to the mineral surface, has also contributed to clarify the mechanism of microbial action Gehrke et al. (1995, 1998; Sand et al., 1999; Liu et al., 2003; Karavaiko et al., 1994).

Interestingly, microbial attachment phenomenon to pyrite has also been presented as a potential evidence for direct mechanism of microbial leaching (Brierley, 1982; Kargi and Weissman, 1984; Kelly and Harrison, 1989, Konishi et al., 1990; Dziurla et al., 1998; Ohmura et al., 1993; Liu et al., 2003; Shrihari et al., 1995; Monroy et al., 1995; Savic et al., 1999; Rahman et al., 1988). But many research found still reasonable doubts on the contribution of the direct mechanism by attached microorganisms to the pyrite dissolution process (Boon and Heijnen, 1993; Nyavor et al., 1996; Sand et al., 2001). Due to these contradictions all proposed mechanisms are still not accepted (Rodriguez et al., 2003).

Another mechanism of sulfide bioleaching, proposed by Tributsch (1999), presents three strategies to bioleaching:
• Indirect bioleaching: Microorganisms are not attached to mineral surface. Their action is only on Fe (III).

• Contact bioleaching: Attachment of microorganism on mineral surface and electrochemical dissolution of mineral surface.

• Cooperative bioleaching: Attachment of microorganisms to mineral surface cooperates with free cells in solution. The oxidizable species liberated by attached bacteria is source of energy for microorganisms present in solution.

• Polysulfide and elemental sulfur are the main intermediates and energy source in polysulfide mechanism.

Conversely, Fowler et al. (1999) argue that Acidithiobacillus ferrooxidans enhances the rate of leaching pyrite above that achieved without bacteria under the same conditions by increasing the pH of the pyrite surface during its attachment, and not by the action of a biological or enzymatic oxidant. Their line of reasoning is based on the data obtained from pyrite bioleaching experiments employing a constant redox apparatus designed to maintain the Fe (II) and Fe (III) concentrations in solution at a constant value. Results of subsequent studies by Rodriguez et al. (2003) support the mechanism proposed by Tributsch (1999).

Certain microorganisms can mobilize metal ions from metal sources by inorganic or organic acids (protons) formation, by redox reactions or complexing agent’s excretion (Bosecker, 1997; Brandl a, b, 2001; Sand et al., 2001; Rawlings, 2002).

2.4. FACTORS INFLUENCING BIOLEACHING

There are several important physicochemical as well as microbiological factors that affect bioleaching process and microorganism. These includes pH, sulphide minerals, temperature, nutrients, O₂ and CO₂, metal toxicity, solid ratio, solid properties etc. (Torma et al., 1972; Brierley, 1978; Murr, 1980; Ferroni et al., 1986; Ballester et al., 1989; Acevedo and Gentina, 1989; Ngubane and Baecker, 1990; Baldi et al., 1992; Ahonen and Tuovinen, 1992; Nagpal et al., 1993; Lindstrom et al., 1993; Boussios and Madgwick, 1994; Lan et al., 1994; Hallberg et al., 1996; Langdahl and Ingvorsen, 1997; Bosecker, 1997; Acevedo et al., 1998; Bacelar-Nicolau and Johnson, 1999; Das et al.,
1999; Deveci, 2002; Akcil and Ciftci, 2003; Deveci et al., 2004). Some examples are as follows

- Pyrite oxidation of *Acidithiobacillus ferrooxidans* was inhibited by yeast extract (Bacelar-Nicolau and Johnson, 1999).

- Arsenic inhibited *Acidithiobacillus ferrooxidans* growth on arsenopyrite and *Sulfolobus acidocaldarius* growth on pyrite (Lan et al., 1994; Hallberg et al., 1996).

- Cu, Ni, U, Th severely effect Fe (II) oxidation by *Acidithiobacillus ferrooxidans* with U and Th were more toxic than Cu and Ni (Le Roux et al., 1997).

- Ag, Hg, Ru, and Mo reduced *Sulfolobus* growth on a Cu concentrate (Mier et al., 1995).

- Mineral bioleaching kinetics is governed by bacterial ability to oxidize Fe²⁺ at high Fe³⁺/Fe²⁺ ratios (Boon et al., 1999).

- It has also been documented that redox potential (Fe³⁺/Fe²⁺ ratios) of the leaching solution affect Fe (III) leaching of sulfide mineral (Boon, 1996; Breed and Hansford, 1999; Ruitenberg et al., 1999).

During bioleaching processes, co-precipitation of metals with mineral phases such as jarosites can reduce leaching efficiencies (Hiroyoshi et al., 1999). There is some evidence that surface-active compounds as well as organic solvents are inhibitory to bioleaching reactions and prevent bacterial attachment (Murr, 1980). The external addition of Tween 20 reduced the oxidation of chalcopyrite by *Acidithiobacillus ferrooxidans* (Torma et al., 1976). In contrast, it was also reported that Tween 80 addition increased the attachment of *Acidithiobacillus ferrooxidans* on molybdenite and the oxidation of molybdenum in the absence of iron (Pistaccio et al., 1994). Chong et al. (2002) have demonstrated that shearing on the surface of a pyrite has detrimental effect on the rate of pyrite biooxidation by *Acidithiobacillus ferrooxidans*. Recently, Ilyas et al (2010) argued that co-optimization of bioleaching process parameters is very important for enhanced bioleaching potential.

### 2.5. APPLICATIONS OF BIOLEACHING

The ability of *Acidithiobacillus ferrooxidans*, *Sulfobacillus thermosulfidooxidans* and other microorganisms to solubilize metal sulfides in their habitats is successfully
applied in bioleaching of metal ions from ores. Continuing accumulation of tailing due to worldwide depletion of metal rich ore deposits, which cannot be further processed economically by conventional processes for the recovery of metals such as copper, aluminum, nickel, uranium and precious metals like gold, platinum, silver and cobalt (Ehrlich, 1987) have lead to the use of newly emerged technologies like biotechnology to devise and practice economically feasible processes. Applications of biohydrometallurgical processes in mineral industries are continuously gaining importance. The increase of interest in bioleaching is the consequence of interdisciplinary studies. Metallurgical industries recognize that the use of microorganisms in certain hydrometallurgical processes presents a potential solution for the problems faced in many countries where the continuous depletion of high grade ore deposits have created a need to look for low priced and environmentally friendly methods for metal recovery from low-grade resources, where conventional methods are not effective handled by conventional processes (Torma and Bosecker, 1982).

Biohydrometallurgical processes depend on microorganism’s activity and offer a possibility to obtain metal ions from mineral resources where conventional techniques failed (Bosecker, 1997; Brierley, 1978; Torma and Banhegyi, 1984). Bioleaching with fungi such as *Aspergillus niger*, *Penicillium simplicissimum* and bacteria such as *Acidithiobacillus thiooxidans*, *Acidithiobacillus ferrooxidans* were grown electronic scrap presence. Both fungal strains mobilized Sn and Cu by 65 %, and Al, Pb, Ni, and Zn by more than 95 % (Brandl et al., 2001).

Bioleaching studies of the complex Pb-Zn ore using mesophilic (at 30 °C), moderate (at 50 °C), and extreme thermophilic (at 70 °C) strains of acidophilic bacteria has also been undertaken. The results showed that the ore was readily amenable to the selective extraction of zinc and lead using the acidophilic strains of bacteria [i.e., majority of lead (>98 %) reported to the residue]. Regarding the dissolution of zinc, moderate thermophiles displayed superior kinetics as compared with the other two groups of bacteria (Deveci et al., 2004) and these results were further confirmed by the studies with complex Pb/Zn ore/concentrate (Ilyas et al., 2008). Ahonen and Tuovinen (1992) have investigated the feasibility of bacterial oxidation of sulfide minerals at suboptimal temperatures in bench scale column reactors. Zhen et al (2009) also after optimization of column bioleaching experiments in small reactors have studied the leaching feasibility at
upscale level of sulfide ore containing high level of magnesium ions as main gangue minerals (Zhen et al., 2009) for a period of 312 days including 60 days acid pre-leaching stage and 252 days bioleaching stage. They have optimized initial pH of pre-leaching 0.8, particle size 10 mm, irrigation rate of pre-leaching 50 g/L, air flow 80 mL/min or 68 L/m2/h while pH, particle size, irrigation rate and air flow rates for bioleaching were optimized at 1.8-2.2, 10 mm, 50 mL/min or 42 L/m2/h ,200–300 L/h. So far no report in literature is available about upscale column bioleaching studies of sulfide ores containing high level of iron, carbonaceous materials and ores those are oxidized at periphery. So present studies were carried out to check the technical feasibility of these types of ores as it will be beneficial for heap scale implementation of process in future.

2.6. BIOLEACHING OF ELECTRONIC SCRAP

Electronic waste is being generated two to three times faster than other waste streams (Grossman, 2006; U.S. EPA., 2008). Electronic scrap is presently reused, remanufactured, recycled, incinerated or disposed of in landfills (Ilyas et al., 2007; Cui and Zhang, 2008). The united state environmental protection agency estimates that 500 million computers were discarded between 2000 and 2007, 2 million tons of tech trash ended up in landfills, and only 400 thousand tons were recycled (U.S. EPA., 2008). Estimates by the Natural Resources Defense Council indicated that 130 thousand computers were discarded daily and that one million cell phones were discarded in 2006 (NRDC., 2008).

Waste electronic and electric equipment contains non degradable and hazardous material contents (EC., 2000; Cui and Forssberg., 2003; Niu and Li., 2007). Major reason for electronic scrap recycling is the recovery of precious metals (Sum., 1991; Dalrymple et al., 2007; Shuey and Taylor, 2005; Chiang et al., 2007).

In this regard biotechnology served as a most promising technology in metallurgical processing. Bioleaching and biometallurgy are potential break breakthrough for the materials and minerals industries (Faramarzi et al., 2004; Brandl et al., 2001a).

The number of studies carried out in bioleaching of electronic scrap is limited. In Cu bioleaching from printed circuit board scrap by Acidithiobacillus ferrooxidans Fe2(SO₄)₃, formed oxidizes Cu contained in PCBs to Cu(II) following the Reaction (Choi et al., 2004; Li et al., 2008).
Metal precipitation during bioleaching was observed and analyzed by various authors. The presence of various metal ions was confirmed by Atomic Absorption Spectroscopy (AAS) analysis (Brandl et al., 2001; Choi et al., 2004; Ilyas et al., 2007).

Santhiya and Ting (2004) applied bioleaching process to a spent refinery processing catalyst for the recovery of metal ions using *Aspergillus niger*. The metal recovery was 62.8 % for Ni, 58 % for Al and 78.9 % for Mo.

Column bioleaching feasibility and capability of metal ions from electronic scrap by the selected moderately thermophilic strains of mixed adapted consortium of acidophilic heterotrophic bacteria and acidophilic chemolithotrophic bacteria was checked during this process. These included *Sulfobacillus thermosulfidooxidans* and *Thermoplasma acidophilum* and proved that tolerance of bacterial cultures to mixed metal ions (Al$^{3+}$, Ag$^{+}$, Cu$^{2+}$, Fe$^{3+}$, Ni$^{2+}$, Pb$^{2+}$, Zn$^{2+}$ and Sn$^{2+}$) could be improved markedly after gradual adaptation of nearly two year from 12 g/L to 20 g/L. During whole leaching process included acid pre-leaching operation of about 27 days and bioleaching operation of about 28 days about 80 % Zn, 64 % Al, 86 % Cu and 74 % Ni was leached out. This might be helpful for further implementation of process on commercial level (Ilyas et al., 2010).
CHAPTER 3

MATERIALS AND METHODS

3.1. SOURCE OF ORE SAMPLES

Pyrite, sphalerite, complex Pb-Zn ore, Fe, Ni and Cu containing sulphide ores were obtained from different sources. A detailed list of ores and their origin is tabulated below (Table 3.1).

Table 3.1. Ores and their origin

<table>
<thead>
<tr>
<th>Ore</th>
<th>Origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyrite</td>
<td>Saindak, Pakistan</td>
</tr>
<tr>
<td>Sphalerite</td>
<td>Saindak, Pakistan</td>
</tr>
<tr>
<td>Pb-Zn</td>
<td>Duddar, district Balochistan, Pakistan</td>
</tr>
<tr>
<td>Huangshan-ore</td>
<td>Anhui province, China</td>
</tr>
<tr>
<td>Gansu-ore</td>
<td>Gansu province, China</td>
</tr>
<tr>
<td>Daye- ore</td>
<td>Hubei province, China</td>
</tr>
</tbody>
</table>

3.2. ANALYSIS OF ORE SAMPLES

Finely powdered samples (1.0 g each) of sphalerite, pyrite, Pb-Zn ore, Huangshan, Daye and Gansu ores were refluxed separately with 100 mL of aqua regia in round bottom flasks for one hour. After filtration, the solutions were cooled at room temperature. The concentration of dissolved iron from pyrite, iron and zinc ions from sphalerite, lead, zinc and iron from Pb-Zn ore, iron, copper, zinc and nickel from Huangshan ore, magnesium, copper, nickel and zinc from Gansu ore and copper, zinc, cobalt, nickel and iron ions from Daye ore were determined by atomic absorption spectrophotometer (VarienAA10/20) and the percentage of these metal ions in the samples was calculated.

3.3. PREPARATION OF ORE SAMPLES FOR BIOLEACHING STUDIES

Statistically representative samples of the respective ore were taken and large pieces were crushed in a jaw-crusher separately. Then all pieces were ground to relatively smaller particles using disc-grinding machine (FRITSCH Pulverisette, Germany). The final grinding of the ores was carried out using ring grinder (FRITSCH Pulverisette,
MATERIALS AND METHODS

Germany). In order to separate the particles according to their sizes, an ASTM Sieves were used. Various mesh size fractions of ores were separated and the ore particles that range from 100-270 mesh were used in the experiments.

3.4. MICROORGANISMS USED IN BIOLEACHING EXPERIMENTS

Microorganisms used in this study were collected from culture collection of National Institute for Biotechnology and Genetic Engineering (NIBGE), Pakistan, isolated by us and collected from culture collection of Wuhan Institute of Technology, China. The origin and salient characteristics of these microorganisms are tabulated below (Table 3.2).

Table 3.2. Microorganisms and their origin

<table>
<thead>
<tr>
<th>Microorganisms</th>
<th>Source</th>
<th>Salient Characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Sulfobacillus thermosulfidooxidans</em></td>
<td>NIBGE</td>
<td>Moderate thermophile (optimum growth temperature 45°C)</td>
</tr>
<tr>
<td>(MT-13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acidophilic heterotroph</td>
<td>NIBGE</td>
<td>Moderately thermophilic, acidophilic heterotroph. (optimum growth temperature 45°C)</td>
</tr>
<tr>
<td>(Code: A1TSB)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Sulfobacillus thermosulfidooxidans</em></td>
<td>Isolated by our self</td>
<td>Moderate thermophile (optimum growth temperature 45°C)</td>
</tr>
<tr>
<td>(RDB)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Thermoplasma acidophilum</em></td>
<td>Wuhan Institute of Technology</td>
<td>Moderately thermophilic, acidophilic heterotroph. (optimum growth temperature 45°C)</td>
</tr>
<tr>
<td><em>Acidithiobacillus thiooxidans</em></td>
<td>NIBGE</td>
<td>Mesophile (optimum growth temperature 30°C)</td>
</tr>
<tr>
<td><em>Acidithiobacillus ferrooxidans</em></td>
<td>NIBGE</td>
<td>Mesophile (optimum growth temperature 30°C)</td>
</tr>
<tr>
<td><em>Leptospirillum ferrooxidans</em></td>
<td>Isolated by our self</td>
<td>Mesophile (optimum growth temperature 30°C)</td>
</tr>
</tbody>
</table>

3.5. COLLECTION, ISOLATION AND ENRICHMENT OF MICROBIAL SAMPLES
MATERIALS AND METHODS

Liquid samples were obtained in sterile bags from different locations of Dexing copper mine, in Jiangxi province, China and Reko Diq, Pakistan. Collected samples were further treated within next 24 hours. Soil samples were mixed with autoclaved distilled water in the ratio of 50 % (w/v) and slurries were made while liquid samples were filtered through a hyper filtration membrane (0.22-µm, BioBasic Inc., Canada) with a vacuum pump and grow on acidophilic basal salt media. For the solid state growth, the culture was grown on 1.8 % agar plates. About 3 % volume of these samples was inoculated into autoclaved basal salt medium. The flasks were incubated at temperatures of the origin of the samples, in an orbital shaker adjusted at 180 rotations per minute. For the growth of uncontaminated culture, samples aliquots and enriched liquid microbial cultures were streaked in triplicate on the solid growth media plates and incubated at their optimum growth temperatures after screening studies.

3.5.1. Microbial growth studies

Initial growth optimization studies were conducted at different pH (1-9) and temperatures values (20-70 °C). Rich growth was obtained at their growth medium with optimum growth temperature and pH and then mean time of microbial generation (td) and their specific growth rates (μ) were calculated.

The growth was regularly monitored by taking absorbance at 650 nm. Doubling time was calculated and specific growth rates were derived from the doubling time using this expression, doubling time = 0.693/specific growth rate, from equation. The isolated bacterial cultures were phenotypically characterized.

3.5.1. Evolutionary analysis of isolated cultures

DNA isolation of liquid bacterial culturas (about 50 ml) was carried out by using the GenomicPrep™ Cells and Tissue DNA isolation Kit (Amersham Pharmacia Biotech.). After isolation the samples were stored at -20 ºC for further use. Amplification of 16S ribosomal DNA of isolates, by Polymerase chain reaction (PCR), was carried out using the forward (AGAGTTTGATCCTGGCTCAG) and reverse primers (ACGG(ACT)TACCTTGTTACGACT T) and set of conditions optimized by Ghauri et al (2003). Forward primer (FD1), Reverse primer (rP1). The PCR product was then purified using Rapid PCR purification system (Marligen Bioscience, USA). Amplified 16S ribosomal DNA fragment of 1,500bp size obtained from each isolated culture was
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partially sequenced commercially (GeneLink™, Hawthorne, New York). These gene sequences were compared with other sequences in the GenBank databases using (National Center for Biotechnology Information) Basic Local Alignment Search Tool (www.ncbi.nlm.nih.gov). The sequences of 16S ribosomal RNA genes were first analyzed using the basic local alignment search tool (BLAST) at the national center for biotechnology information (NCBI) website: http://www.ncbi.nlm.nih.gov/BLAST/.

Related sequences were preliminarily aligned with the default setting of Clustal X (2.0) (Hippe, H., 2000) Phylogenetic and molecular evolutionary analyses were conducted using MEGA version 4.0 (Huaqun et al., 2008) Accession numbers of the partial 16S ribosomal DNA sequences for isolated cultures were obtained by depositing partial sequences to GenBank of NCBI.

3.6. GROWTH MEDIA

Liquid media described below were used for obtaining growth of the microorganisms used in this study.

3.6.1. Growth of Sulfobacillus thermosulfidooxidans

Iron-tryptone soya broth (FeTSB) liquid growth medium, developed by Johnson et al. (1987), was used to obtain the growth of Sulfobacillus thermosulfidooxidans. The FeTSB medium composed of (g/L): MgSO₄, 7H₂O, 0.50; (NH₄)₂SO₄, 0.15; KCl, 0.05; KH₂PO₄, 0.05; Ca(NO₃)₂, 0.01 and TSB, 0.25. The pH of liquid medium was adjusted to 2.0 using H₂SO₄ and then sterilized by autoclaving at 121 °C and 15 psi for 15 min. Filter sterilized ferrous sulfate solution (fresh prepared) was poured to the solution to an ultimate concentration of 50 mM, before inoculation and after inoculation flasks were adjusted in orbital shaker having temperature 45°C.

3.6.2. Growth of acidophilic heterotrophs

The acidophilic heterotrophs were grown in the same medium but supplemented with glucose (1 % w/v) instead of ferrous sulfate, as energy source at pH 2.0.

3.6.3. Growth of iron oxidizers

The liquid media for the growth of iron oxidizers was based upon the concentration described by Leathen et al. (1951) and later modified by Postgate (1956). Its composition was (g/L): MgSO₄, 7H₂O, 0.50; (NH₄)₂SO₄, 0.15; KCl, 0.05; KH₂PO₄,
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0.05; Ca(NO₃)₂, 0.01. The solution pH was adjusted to 2.0 using sulfuric acid and autoclaved at 121 °C and 15 psi for 15 min. Filter sterilized ferrous sulfate solution (fresh prepared) was added to the solution to a final concentration of 50 mM, before inoculation and after inoculation flasks were adjusted in orbital shaker having temperature 28 °C.

3.6.4. Growth of sulphur oxidizers

The culture of Acidithiobacillus thiooxidans used was maintained on the medium of Vogler and Umbreit (1941), which had the following composition: (g/L) NH₄Cl, 0.3; KH₂PO₄, 3.0; CaCl₂, 0.25; MgSO₄. 7H₂O, 0.5; FeSO₄, 0.01 and distilled water, 1,000 ml (initial pH 4.5 and temperature was 28 °C). Elemental sulfur, sublimed, was included as the energy source. 100 ml of the basal liquid medium were dispensed in 250-mL Erlenmeyer flasks. Additions of sterile supplements were then made as desired sulfur was weighed out, sterilized by tyndalization and was added before inoculation and the flasks were then inoculated and after inoculation were adjusted in orbital shaker having temperature 28 °C.

3.6.5. Harvesting and washing of cells

After obtaining rich growth, the cell mass of bacterial cultures was centrifuged at 10,000 rotations per minute, for 20 minutes. The cell pellet was washed twice with sterilized distilled water having pH adjusted at 2.0 with 2.0 M sulfuric acid and finally it was suspended in sterilized (autoclaved) distilled water and preserved at 4 °C for inoculation for further experiments.

3.6.6. Solid medium

The above mentioned liquid media were supplemented with 0.5 % (w/v) agarose to prepare solid media.

3.7. BIOLEACHING STUDIES

3.7.1. Preliminary bioleaching study

Preliminary bioleaching studies were carried out with pure cultures and different mixed cultures of all above mentioned microorganisms on pyrite and sphalerite ores. Then the strain and combination with preferentially good bioleaching potential were selected for further studies.
3.7.2. Bioleaching of metals from different ores

After preliminary study, bioleaching studies on different ore were carried out in three different ways:

A. Leaching with un-adapted cells of *Sulfobacillus thermosulfidooxidans*:

For bioleaching of ores, two Erlenmeyer flasks of 250 mL capacity were taken separately, one was experimental and the other acted as control. Fe-TSB medium (100 mL) was added in each flask and pH was adjusted to 2.0 with 2.0 M H₂SO₄. The aerobic condition of the system was maintained by plugging (with cotton plugs) the flasks. All flasks were autoclaved at 121 °C and 15 psi pressure for 15 minutes. Then 1.0 g of sterilized ore was added under aseptic conditions. The pH of each flask was monitored daily and was maintained at 2.0 in the successive days with 2.0 M H₂SO₄. After adjustment of initial acid demand and pH 2.0, the experimental flask was inoculated with 1.0 mL inoculum of unadapted cells of *Sulfobacillus thermosulfidooxidans* aseptically. The control flask was not inoculated, while other conditions were kept same as that of the experimental flask. All the flasks were incubated in shaker (Kuhner, Switzerland) at 45 °C temperature and 180 rpm shaking speed.

B. Leaching with adapted cells of *Sulfobacillus thermosulfidooxidans*:

The cells were adapted to metal ions by continual sub culturing in the liquid medium having gradually increasing concentrations of metal ions. Microbial cells were called metal ions adapted after their growth was to a same extent as in case of un-adapted cells. The cell mass of the adapted cultures was approximately the same as that of unadapted cells in the absence of metal ions. (~0.019 mg/L cell dry mass). Metal-adapted cells were used as inoculums in this experiment. Other procedure was same as described in the preceding section (A).

C. Leaching of ores with mixed culture:

A mixed consortium (1:1 v/v) containing cells of *Sulfobacillus thermosulfidooxidans* and acidophilic heterotrophs, adapted to metal ions, was used as inoculums in these experiments, while other procedure is same as mentioned above.

3.8. SAMPLING PROCEDURE
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During the period of the bioleaching studies, 3.0 ml of sample was taken from the experimental and control flasks periodically and kept at -20 °C for further use and for inactivation of microbial cultures. Filtration of the samples was carried out with filter paper (Whatman No. 1) for the removal of ore particles and then samples were centrifuged at 10,000 rpm for 12 minutes for the removal of microbial cell mass. After suitable dilutions, the obtained clear solutions were then analyzed for different metal ions by atomic absorption spectrophotometry. The volume in each flask was maintained by adding equal volume of autoclaved distilled water of pH 2. In this way, at least 10 samples were collected on alternate days for pyrite while for chalcopyrite and Pb-Zn ore, samples were collected after two days. Same procedure was adopted for electronic scrap except that the samples were taken after an interval of 3 days.

3.8.1. Sample Preparation for Analysis

1.5 mL aliquot of each sample was taken in test tube and centrifuged at 10,000 rotations per minutes for 10 min. Then pellet was removed and 1mL of supernatant was taken in falcon tubes and 8 mL water and 1mL concentrated HCl was added to obtain total volume of 10 ml. Samples were further diluted as required and analyzed on atomic absorption spectrophotometer for metal ion concentration.

3.9. ORTHOGONAL EXPERIMENTAL ARRAY

For co optimization of process parameters and further enhancement of bioleaching efficiency, an orthogonal experimental array design was constructed that consisted of L25 orthogonal array (Dehghan et al., 2009). The extent of effect of individual factors and the best co-optimization combination of these parameters for bioleaching of metal ions from sulphide ore with moderately thermophilic bacterial culture was studied.

Investigational considerations and their levels were chosen on the basis of previous experimentation and inoculum size was kept at 1.0 ×10⁷ /mL. In the orthogonal array design, factors were chosen on the basis of some previous information, due to experimentation, of bioleaching process. Based on these experimental conditions reported by other researchers for the bioleaching of low grade sulfide ores, initial experiments were carried out and noteworthy issues that may influence the metal ions dissolution were recognized as pulp density, initial pH, particle size, temperature and agitation as shown in Table 3.3.
Table.3.3. Quantitative value of coded parameter levels

<table>
<thead>
<tr>
<th>Coded factors</th>
<th>Parameters</th>
<th>Levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>A</td>
<td>Temperature-A (°C)</td>
<td>57</td>
</tr>
<tr>
<td>B</td>
<td>Initial pH-B</td>
<td>1.8</td>
</tr>
<tr>
<td>C</td>
<td>Particle size-C (μm)</td>
<td>50</td>
</tr>
<tr>
<td>D</td>
<td>Agitation-D (rpm)</td>
<td>100</td>
</tr>
<tr>
<td>E</td>
<td>Pulp Density-E (%)</td>
<td>1</td>
</tr>
</tbody>
</table>

The most essential factors were selected for optimization studies, using a 5-leveled orthogonal array with L_{25} matrix as shown in Table 3.4, that designate five parameters each with 5 levels, as it is the most appropriate for the situations being examined and every test was repeated twice under the similar situation to observe the effects of noise sources of the laboratory medium in the bioleaching process.

Levels were chosen in the light of previous results. In the planned scheme, possible associations among variables were not consider in the matrix, the attention was reserved on the key influences of the five most significant factors. Low, medium and high ranges of the factors were selected.

Arrangement of the tests was attained systematically by putting parameters into columns of arrays, L_{25}, selected as the test sketch, but the arrangement of tests was prepared unsystematic to prevent noise sources that may affect results.
Table 3.4 $L_{25}(5^5)$ Experimental work plan

<table>
<thead>
<tr>
<th>Experiment.No.</th>
<th>Parameters and their levels</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
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<tr>
<td>2</td>
<td>1</td>
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<td>3</td>
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<td>24</td>
<td>5</td>
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<tr>
<td>25</td>
<td>5</td>
</tr>
</tbody>
</table>

In $L_{25}$ orthogonal array, a table of integers was obtained whose column elements mention the range of the levels of column factors. Each row of orthogonal design represents a particular run as shown in Table 3.4, which is a specific set of factor levels, to be tested (Safarzadeh *et al*., 2008).
3.9.1. Bacterial leaching studies

Experiments on bioleaching of metal ions from sulphide ores were conducted in 250 mL Erlenmeyer shake flasks having 100 mL Fe-TSB medium in each flask at different levels of various factors (temperature, pH, pulp density, particle size and agitation). After sterilization by autoclaving, twenty five batch bioleaching tests were run with different combinations of factors and levels for a period of 30 days. All flasks (experimental and control) were weighed before sampling and any decrease in volume due to evaporation during incubation was compensated by adding corresponding volume of sterilized distilled water. During leaching experiments, 3.0 mL of sample was taken periodically from each flask and its redox potential, ferrous, ferric, total iron concentration and pH was noted. The sample was then filtered through filter paper to take away solid particles and centrifugation was carried out at 10,000 rpm for the removal of bacterial cell mass and then supernatant was analyzed for nickel, copper, cobalt, zinc and iron concentration.

3.10. COLUMN BIOLEACHING OF HUANGSHAN ORE

3.10.1. Mineral characteristics

The ore for column bioleaching studies was collected from south Anhui province of China. Mineralogical analysis of the ore sample indicated the presence of 8.7 % Pyrrhotite, 2.0 % Marcasite, 1.6 % Pentlandite, 0.8 % Chalcopyrite, 0.7 % Pyrite, 0.5 % Wurtzite and 0.2 % Sphalerite as main sulphide minerals. The 50 % fayallite, 32 % hematite, 2 % chlorite, 4 % magnetite, were the main gangue minerals.

3.10.2. Chemical leaching of ores

Finely powdered samples (1.0 g each) of low grade Huangshan iron ore were refluxed separately with 100 mL of aqua regia in round bottom flasks for one hour. The sample solutions were cooled at room temperature and then filtered. The dissolved metal ions from ore samples were analysed by atomic absorption spectrophotometer (Varien AA10/20) and the percentage of these metals in the samples was calculated.

3.10.3. Preparation of ore samples for bioleaching studies

Statistically representative samples of the respective ore were taken and large pieces were crushed in a jaw-crusher separately. Then all pieces were ground to relatively smaller particles using disc-grinding machine (FRITSCH Pulverisette, Germany). The final grinding of the ores was carried out using ring grinder (FRITSCH Pulverisette,
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Germany). In order to separate the particles according to their sizes, an ASTM Sieves were used. Ore sample used as column charge were screened to different particle sizes and the fine ore particle size was controlled less than 0.1 mm (mass fraction).

3.10.4. Microorganisms

Mixed adapted consortium of acidophilic moderately thermophilic bacteria was used in these studies. These included Sulfobacillus thermosulfidooxidans strain RDB and Thermoplasma acidophilum. Culture conditions were same as described in Section 3.6.1.

3.10.5. Pre-strategies for bioleaching operation

Due to high level of fayallite, hematite and magnetite present in the ore, as a main gangue, ore was pre-treated with acid, periodic bleeding of the ore was carried out and the adaptation of the mixed consortium of moderate thermophiles to iron ion was carried out by sequential sub culturing and regularly rising iron ion concentration in the liquid medium.

3.10.6. Leaching parameter determination

Several small columns (height 30 cm; diameter 8 cm) were used at ambient temperature to investigate leaching parameter for the extraction of Fe, Cu, Zn and Ni ions. The charge was pre-leached with concentrated H₂SO₄ to adjust the pH in the range of 2-2.2 before inoculation and on different on-solution pH values and particle sizes, the metal extraction rates, permeability and duration of stabilization of pH at required range was investigated. After this screening study the permeability of columns and metal extraction rates with selected tests were checked and from these the best one was chose for further up scale column bioleaching studies. Irrigation rate and aeration rate were also optimized. pH of the effluents was stabilized at pH 2-2.2 and then the irrigation of the pre-leaching was stopped. The bioleaching system was composed of the pre-leaching solution containing 10 % (v/v) inoculums with cell density of about 3×10⁷ cell/mL and pro rata nutrient.

The pH was stabilized 2.0-2.2. Periodic bleeding of a portion of the pregnant leaching solution was carried out to control excessive concentration of iron ions based on the tolerance of the mixed consortium of the moderate thermophiles. Effluents both from pre-leaching operation and bioleaching operation were sampled and analyzed to
determine metal dissolution and acid balance. When analysis indicated the termination of bioleaching operation, irrigation was stopped to prepare for final analysis.

3.10.7. Bioleaching studies in large columns

When optimized leaching parameters were determined by small columns, two large columns of height of 85 cm with an internal diameter of about 15 cm were used to verify the above mentioned optimized parameters. One column was designed as experimental column and the other was designed as abiotic control. These columns were made of 30 µm thick stainless steel. A high density polyethylene support shield with multiple 80 µm holes was placed on 800 µm high supports permitting air to be introduced below the shield and disbanded consistently over the ore in the column. A coating of support rock, low acid-consuming minerals and sized at 100-120 µm, was putted in the bottom of the column before the 80 kg charge was loaded in the column. The column bioleaching was carried out under purely ambient temperature conditions. The changes in temperature were noted throughout the leaching operation. Two thermocouples were installed in the center of the two columns to measure temperature within the charge and the other one thermocouple was installed to measure the ambient air temperature.

Before inoculation the charge was pre-leached by sulfuric acid. Each column was fed with acidic solutions. Solution was dispersed to the periphery of the column feed using a simple garden sprinkler head. Solution for column moved down through the ore sample by force of gravity and recirculated through a side loop with a peristaltic pump. Two containers, each with a capacity of 18 L, collect the effluent from the column and on-solution injecting in the column. Irrigation of the pre-leaching was closed when the pH of the effluent was adjusted at pH 2–2.2. Subsequently, the columns were singled out to perform bioleaching in the next stage. The on-solution level was kept at a adequate height by forcing the air upwards through the column charge. Clean air was supplied through a rotameter and the optimized air flow rate was adjusted. Periodic bleeds of a portion of the ore was carried out to control the iron ion concentration in the tolerance range of the bacteria.

Samples of effluents from pre-leaching and bioleaching operation were collected periodically and analyzed for metal ions concentration and the acid balance. When analysis of the bioleaching solution sowed the termination of bioleaching operation, irrigation was closed to permit the column effluents to be drained off, and the column
contents were then washed to remove remaining metal ions. The column charge was washed first with dilute H$_2$SO$_4$ solution and then with distilled water. The column was then unloaded and the residue was dried and prepared for final analysis.

3.10.8. Analytical techniques

Free bacteria in solution were counted by direct counting with phase contrast microscope, using a counting chamber (Neubauer). Soluble metal ions (Al, Zn, Cu and Ni) in the control and experimental solutions were analysed using an atomic absorption spectrophotometer (Varian AA-400). The ferrous, ferric and total iron concentration in the solution was determined by spectrophotometric method using 1–10 ortho phenanthroline. The pH of the leaching solutions (experimental and control) was checked at with a pH meter calibrated with a low pH buffer. The redox potential of the leaching solution (experimental and control) was monitored with a Platinum electrode in reference to a saturated Ag/AgCl electrode.

3.11. COLUMN BIOLEACHING OF GANSU ORE

3.11.1. Mineral characteristics

The low grade sulphide ore used in the column bioleaching test was obtained from Gansu province of China. Mineralogical analysis of the ore sample showed that the main sulfide minerals were 7.2 % Polydymite, 0.8 % Pentlandite, 0.75 % Chalcocite, 0.7 % Pyrite, 0.8 % Wurtzite and 0.3 % Sphalerite. The 30 % dolomite, 22 % forsterite, 5 % diopside, 2 % talc were the main gangue minerals.

3.11.2. Chemical leaching of ores

Finely powdered samples (1.0 g each) of low grade nickel containing sulphide ore were refluxed separately with 100 mL of aqua regia in round bottom flasks for one hour. The sample solutions were cooled at room temperature and were filtered through filter paper (Whatman No.42). The dissolved metal ions from ore samples were determined by atomic absorption spectrophotometer (Varien AA10/20) and the percentage of these metals in the samples was calculated.

3.11.3. Preparation of ore samples for bioleaching studies
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Preparation of ore samples for bioleaching studies was carried out similarly as mentioned in section 3.10.3.

3.11.4. Microorganisms

Mixed adapted consortium of acidophilic moderately thermophilic bacteria was used in these studies. These included *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*. Culture conditions were same as described in Section 3.10.4.

3.11.5. Pre-strategies for bioleaching operation

Due to high level of dolomite, forsterite, diopside and talc present in the ore, as a main gangue, ore was pre-treated with acid, period bleeding of a portion of ore was carried out and the adaptation of the mixed consortium of moderate thermophiles to mixed metal ions was carried out through sequential sub culturing and repeatedly increasing the concentration of metal ions in the medium.

3.11.6. Leaching parameter determination

Several small columns (height 30 cm; diameter 8 cm) were used at ambient temperature to investigate leaching parameter for the extraction of Mg, Cu, Ni and Zn ions. Other procedure was same as described in section 3.10.6.

The pH was stabilized at 2.0-2.2. Periodic bleeding of a portion of the pregnant leaching solution was carried out to control excessive concentration of magnesium ions based on the tolerance of the mixed consortium of the moderate thermophiles. Effluent both from pre-leaching and bioleaching were sampled and analyzed to determine the concentration of dissolved metal ions and acid balance. When analysis indicated the termination of bioleaching operation, irrigation was stopped to prepare for final analysis.

3.11.7. Bioleaching studies

When optimized leaching parameters were determined in small columns, two large columns of same dimensions as describes above in section 3.10.7 were used to perform bioleaching operation except that the inoculum was of the mixed moderate thermophiles that were adapted to mixed metal ions (Mg, Cu, Ni and Zn). Bioleaching potential of bacterial cultures, Changes in total iron, ferrous, ferric concentration, pH,
redox potential were monitored and periodic bleeding was also carried out to maintain magnesium concentration in columns.

3.12. COLUMN BIOLEACHING OF Daye ORE

3.12.1. Mineral characteristics

The low grade sulphide ore used in the column bioleaching test was obtained from Hubei province of China. Mineralogical analysis of the ore sample showed that the main sulfide minerals were 8.2 % bornite, 0.3 % Pentlandite, 0.8 % chalcocite, 0.8 % linnaeite, 0.3 % Wurtzite and 0.3 % sphalerite. The 20 % smithsonite, 3 % talc, 25 % sphaerocobaltite, 10 % Azurite was the main gangue minerals.

3.12.2. Chemical leaching of ores

Finely powdered samples (1.0 g each) of low grade copper containing sulphide ore were refluxed separately with 100 mL of aqua regia in round bottom flasks for one hour. The solutions were allowed to cool at room temperature and were filtered through filter paper (Whatman No.42). The dissolved metal ions from ore samples were determined by atomic absorption spectrophotometer (varienAA10/20) and the percentage of these metals in the samples was calculated.

3.12.3. Preparation of ore samples for bioleaching studies

Preparation of ore samples for bioleaching studies was carried out similarly as mentioned in section 3.10.3.

3.12.4. Microorganisms

Mixed adapted consortium of acidophilic moderately thermophilic bacteria was used in these studies. These included *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*. Culture conditions were same as described in Section 3.10.4.

3.12.5. Pre-strategies for bioleaching operation

Due to high level of carbonaceous materials as main gangue and oxidation of ore from surface, ore was pre-treated with acid and before loading to column; charge was mixed with 10 % pyrite as an energy source. For enhanced bioleaching potential adaptation of the mixed consortium of moderate thermophiles to mixed metal ions was
performed through serial sub-culturing and gradually increasing the concentration of metal ions in the medium.

3.12.6. Acid consumption by low grade sulphide ore of copper

Before inoculating columns the pH of ore was stabilized for the estimation of acid consumption and preparation of the ore surface for bacterial attack in the next bioleaching operation. Each column was washed with autoclaved distilled water. Then autoclaved basal salt solution of pH 2.0 was added in each column. The pH of the effluent was maintained after recirculation of required amount of concentrated sulfuric acid. During this whole process permeability of columns, slumping, solution accumulation and preferential flows were checked and parameters were optimized in a way to avoid these.

3.12.7. Bioleaching studies

First leaching parameters were optimized in small columns and then two large columns of same dimensions as describes above in section 3.10.7 were used to perform bioleaching operation except that the inoculum was of the mixed moderate thermophiles that was adapted to mixed (Co, Zn, Cu, Ni and Fe) metal ions and periodic bleeding was not carried out. Bioleaching potential of bacterial cultures, changes in total iron, ferrous, ferric concentration, pH, redox potential was monitored also.

3.13. SOURCE AND DESCRIPTION OF ELECTRONIC SCRAP SAMPLES

Electronic scrap, in the form of printed circuit boards, was obtained from an electronics shop in Faisalabad, Pakistan. No physical/mechanical separation process was used before its transportation to the laboratory. For experimental use, the scrap was crushed and then ground to fine powder of 50 to 150 μm particle size by using ring mill grinder.

3.13.1. Analysis of electronic scrap samples

For metal ions analysis, the electronic scrap sample (1.0 g) was dissolved in 100 mL of aqua regia by refluxing in a round bottom flask for 1 h. The sample solution was then allowed to cool at room temperature and the final volume was made up to 100 mL. The concentrations of dissolved metal ions (i.e. Ag⁺, Al³⁺, Cu²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Sn²⁺ and Zn²⁺) were determined by atomic absorption spectrophotometer (Varian AA-120) and the
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data were analyzed for calculating the concentrations of different metal ions in the electronic scrap.

3.13.2. Preparation of electronic scrap for bioleaching studies

Finely ground unwashed and washed electronic scrap samples were used in the bioleaching experiments. Washed sample was prepared by suspending 10 g of electronic scrap in 100 mL of saturated solution of sodium chloride. The mixture was stirred for 10-12 min and then the heavier particles were allowed to settle down at the bottom. The denser part, which settled down at the bottom, was separated, washed and dried to constant weight, after the floating material was decanted off. The sample prepared in this way was referred to as “Washed sample” and 1.0 g of this washed electronic scrap sample was used in bioleaching studies. The samples were sterilized by Tyndalization in successive days prior to bioleaching studies.

3.13.3. Microorganisms

Acidophilic moderately thermophilic and mesophilic bacteria were used in these studies. These included Sulfolobus thermosulfidooxidans strain RDB, Sulfolobus thermosulfidooxidans strain MT-13, an acidophilic heterotroph (A1TSB) and Thermoplasma acidophilum. Sulfolobus thermosulfidooxidans sample was collected from Reko Diq copper ore deposits, Pakistan. After isolation, purification, 16S rDNA gene amplification, sequencing and checking homology (98 %) by NCBI blast search, it was submitted to GenBank for accession number (GQ228448) while Thermoplasma acidophilum was obtained from culture collection of Wuhan Institute of Technology, Wuhan, China and its origin was Xinjing coal deposits in China. Sulfolobus thermosulfidooxidans strain MT-13, Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans were obtained from culture collection of National Institute for Biotechnology and Genetic Engineering (NIBGE), Pakistan. The acidophilic heterotroph (A1TSB) was obtained from coal heap established for biodesulfurization at Askari Cement, Nizampur, Distt. Nowshera, Pakistan. Approximately 10-11 g of coal samples were suspended in 100 mL of sterilized liquid FeTSB medium (described in Section 3.6.1) in 250 mL Erlenmeyer flasks. After 7 days of incubation in shaking incubators, a 10 mL aliquot from each flask was transferred to fresh medium. After three successive transfers, 0.1 mL of each culture was spread on solid media plates, which were then incubated at 45 °C to get bacterial growth in the form of colonies. Pale white colonies of acidophilic heterotrophic
bacteria (A1TSB) were obtained along with the brown colonies of iron oxidizing bacteria. Single isolated colonies were picked with a wire loop and inoculated into sterilized liquid medium for acidophilic heterotrophs (Section 3.6.1). The cultures were further purified by repeated transfers to solid and liquid media, alternatively.

**3.14. CULTURE CONDITIONS**

**3.14.1. Growth of microorganisms**

Iron-tryptone soya broth (FeTSB) liquid medium, developed by Johnson *et al.* (1987), was used to obtain the growth of *Sulfobacillus thermosulfidooxidans*. The FeTSB medium composed of (g/L): MgSO₄·7H₂O, 0.50; (NH₄)₂SO₄, 0.15; KCl, 0.05; KH₂PO₄, 0.05; Ca(NO₃)₂, 0.01 and TSB, 0.25. The solution pH was adjusted to 2.0 using sulfuric acid and autoclaved at 121 °C and 15 psi for 15 min. Filter sterilized ferrous sulfate solution was added to the solution to a final concentration of 50 mM, before inoculation and after inoculation flasks were adjusted in orbital shaker having temperature 45°C.

The acidophilic heterotrophs were grown in the same growth medium but supplemented with glucose (1 % w/v) instead of ferrous sulfate, as energy source at pH 2.0.

The liquid media for the growth of iron oxidizers was taken from Ghauri (1961), which was based upon the concentration described by Leathen *et al.* (1956) and later modified by Postage (196). Its composition was (g/L): MgSO₄. 7H₂O, 0.50; (NH₄)₂SO₄, 0.15; KCl, 0.05; KH₂PO₄, 0.05; Ca(NO₃)₂, 0.01. The solution pH was adjusted to 2.0 using sulfuric acid and autoclaved at 121 °C and 15 psi for 15 min. Filter sterilized ferrous sulfate solution (fresh prepared) was added to the solution to a final concentration of 50 mM, before inoculation and the flasks were then inoculated and after inoculation were adjusted in orbital shaker having temperature 28 °C.

The culture of *Acidithiobacillus thiooxidans* used was maintained on the medium of Vogler and Umbreit (1941), which had the following composition: NH₄Cl, 0.3 g; KH₂PO₄, 3.0 g; CaCl₂, 0.25 g; MgSO₄. 7H₂O, 0.5 g; FeSO₄, 0.01 g; distilled water, 1,000 mL (initial pH 4.5 and temperature was 28°C). Elemental sulfur, sublimed, was included as the energy source. 100 mL of the basal medium were dispensed in 250-mL Erlenmeyer flasks. Additions of sterile supplements were then made as desired sulfur was weighed
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out, sterilized by tyndalization and was added before inoculation and the flasks were then inoculated and after inoculation were adjusted in orbital shaker having temperature 28 °C.

3.14.2. Harvesting and washing of cells

After obtaining rich growth, the cell mass of cultures was harvested by centrifugation at 10,000 rpm for 20 min. The cell pellet was washed twice with autoclaved distilled water having pH adjusted at 2.0 with 2.0 M sulfuric acid and finally it was suspended in sterilized distilled water and preserved at 4 °C for inoculation in the further experiments.

3.14.3. Solid medium

The above mentioned liquid media were supplemented with 0.5 % (w/v) agarose to prepare solid media.

3.15. ADAPTATION OF MICROORGANISMS

To obtain metal adapted bacterial cultures, 100 mL of the above mentioned media were prepared in 300 mL Erlenmeyer flasks and sterilized by autoclaving. Stock solutions (1 M) of each of Ag⁺, Al³⁺, Cu²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Sn²⁺ and Zn²⁺ ions were prepared by dissolving appropriate amounts of the salts of these metals in distilled water. The metal solutions were sterilized by passing through membrane filters (0.2 μm nitrocellulose) and appropriate volume of each of these solutions was dispensed into the above said media in flasks to obtain 10 mM final concentration of each metal ion. The flasks were inoculated with 1 mL of inoculum containing 1×10⁷ cells/mL and incubated at their optimum temperatures in a shaking incubator at 180 r/min. While in the logarithmic phase of bacterial growth, 1 milliliter of microbial culture from each shake flask was shifted to fresh liquid medium having 20 mM of each metal ion. In this way further step wise shifts were made to the liquid media containing next higher metal ions concentrations i.e., 30, 40, 50, 100, and 200 mM. Finally the bacterial cells were harvested by centrifugation and inoculum was prepared as described earlier in this section.

3.16. SHAKE FLASK BIOLEACHING STUDIES

3.16.1. Bioleaching of unwashed electronic scrap
MATERIALS AND METHODS

Studies on bioleaching of metal ions from unwashed electronic scrap were conducted in 250 mL Erlenmeyer flasks containing 100 mL respective media having their optimum pH 1.8-2.2 adjusted with sulfuric acid. The flasks were autoclaved at 121 °C and 15 psi pressure for 15 min. Then 1.0 g of sterilized (tyndalization) unwashed electronic scrap sample was added to each flask under aseptic conditions. The pH of each flask was monitored daily and added 2.0 M H₂SO₄ in order to obtain a stable pH of 2.0 in the successive days. After adjustment of initial acid demand, the experimental flasks were inoculated with 1.0 mL inoculum (1×10⁷ cells/mL) of adapted cells of Sulfobacillus thermosulfidooxidans strain MT-13, Sulfobacillus thermosulfidooxidans strain RDB, Acidithiobacillus thiooxidans, Acidithiobacillus ferrooxidans, aseptically. Uninoculated controls were run in parallel. All the flasks were weighed and incubated in shaking incubator (Kuhner) at their growth temperature and 180 rpm shaking speed.

3.16.2. Bioleaching of washed electronic scrap

Experimental set up used for the bioleaching of washed electronic scrap was similar as described above. The experimental flasks were either inoculated with unadapted or metal ion adapted cultures. Two more experiments were designed for the bioleaching of metal ions from washed electronic scrap. In these experiments, the leaching medium was supplemented with elemental sulfur (1.0 % w/v) in case of both strains of Sulfobacillus thermosulfidooxidans, which was added at the time of inoculation. The pH optimization step was omitted for this experiment in case of Sulfobacillus thermosulfidooxidans.

3.16.3. Bioleaching of washed electronic scrap with mixed cultures

In other experiments, a metal-adapted mixed consortium (1:1 v/v) of Sulfobacillus thermosulfidooxidans strain MT-13 and acidophilic heterotrophs (A1TSB), Sulfobacillus thermosulfidooxidans strain RDB and Thermoplasma acidophilum, Sulfobacillus thermosulfidooxidans strain RDB and acidophilic heterotrophs (A1TSB), Sulfobacillus thermosulfidooxidans strain MT-13 and Thermoplasma acidophilum and Acidithiobacillus thiooxidans and Acidithiobacillus ferrooxidans were used for metal bioleaching studies.

3.16.4. Sampling procedure and sample analysis
MATERIALS AND METHODS

All flasks were weighed before sampling and any decrease in volume due to evaporation during incubation was compensated by adding corresponding volume of sterilized distilled water. During the course of the leaching experiments, 3.0 mL of electronic scrap sample was taken from each flask (experimental and control) periodically and its pH was noted. Then sample was filtered through filter paper (Whatman No. 1) to remove solid particles and centrifuged at 10,000 rpm for 10 min to remove bacterial cell mass. Supernatant was analyzed for zinc, copper, nickel and aluminum concentration while the cell pellet was preserved for determining the total protein content. Metal ions concentration in the leach liquor was measured by atomic absorption spectrophotometer (Varian AA-120). Hydrochloric acid was added to the samples (experimental and control) to a final concentration of 6 M, before analysis on atomic absorption spectrophotometer. Total protein content of the samples was determined by Bradford method (Bradford, 1976) after dissolving the cell pellet in 1.0 M NaOH solution. At the end of the leaching experiments, a part of white precipitates was recovered by decantation from the flask with mixed microbial consortium. These white precipitates were washed properly with distilled water, dried to constant weight and then analyzed for metal contents by atomic absorption spectroscopy.

3.17. PROTEIN ESTIMATION

Growth of the microorganisms during bioleaching studies was monitored by determining total protein content in the culture flasks periodically. Dry biomass was calculated from the total protein by multiplying it with a factor of 0.019.

3.17.1. Reagent preparation

Chelating reagent was prepared instantaneously before use by mixing following stock solutions A, B, C in the proportion of 100:1:1 (v: v: v), respectively.

Solution A (w/v):
2 % Na2CO3 was prepared in distilled water.

Solution B (w/v):
1 % CuSO4.5H2O, prepared in distilled water.

Solution C (w/v):
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2 % Sodium Potassium tartrate, prepared in distilled water.

3.17.2. Folin Phenol Reagent:

Commercially available reagent was diluted 1:2 (v/v) in distilled water before use.

3.17.3. Standards:

Stock solution of standard protein BSA containing 4mg/ml protein was prepared in distilled water. Working standards were prepared by diluting the stock solution with distilled water.

In 0.2 mL of standard solution, 1 mL of freshly mixed chelating reagent was added and the mixture was allowed to stand at room temperature for 10 minutes. Then 0.1 mL of diluted folin reagent was added and vortex immediately to ensure homogeneity and mixture was allowed to stand at room temperature for 30 minutes. Absorbance was noted at 750 nm against reagent blank on UV-Visible double beam spectrophotometer (CARY 1) and a standard curve of absorbance as a function of initial protein concentration was plotted.

3.17.4. Sample preparation for protein estimation:

An aliquot of 1.5 mL of sample, collected at different time interval (electronic scrap), was taken in eppendorf tubes. Sample was centrifuged at 10,000 rpm for 3 minutes. The pellet was suspended in distilled water of pH 2.5 and solution was vortex (several bursts) until homogeneity. The mixture was again centrifuged at 10,000 rpm for 3 minutes and pellet was suspended in 1N NaOH solution and was vortex and then kept in boiling water bath for 10 minutes. The mixture was again centrifuged at 10,000 rpm for 3 min. Then 0.2 mL of the supernatant was taken in a test tube and 1ml of freshly prepared complex forming reagent was added and solution was allowed to stand at room temperature for 10 minutes. Then 0.1 mL of diluted Folin phenol reagent was added and solution was again allowed to stand at room temperature for 10 minutes after vortex. Then absorbance was noted at 750 nm and the unknown protein concentration was determined from the standard curve.

3.18. BIOLEACHING OF ELECTRONIC SCRAP IN A BUBBLE REACTOR

3.18.1. Source and pre- treatments of electronic scrap
MATERIALS AND METHODS

Electronic scrap, in the form of printed circuit boards, was obtained from local electronic waste supplier, Wuhan, China. No mechanical/physical separation process was used before its transportation to the laboratory. Chemical analysis of metals was also carried out in the same way as in section 3.13.1. Finely ground washed samples of electronic scrap (as a charge) were used in the bubble reactor bioleaching experiments. Washed samples of electronic scrap were prepared as mentioned in section 3.13.2.

3.18.2. Microorganisms

Mixed adapted consortium of acidophilic moderately thermophilic bacteria was used in these studies. These included *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*. Culture conditions and adaptation studies were performed in the same way as described in section 3.14.

3.18.3. Bubble reactor bioleaching studies

Two conical glass bubble reactors were fabricated locally. The column was designed as narrowed from top to downward to fully stir and suspend the slurry. An air current of about 4.7 l h⁻¹ was used as a stirring system. The top of the column reactor had an internal diameter of about 25 cm and a total height about 47 cm. The carrier volume capacity was about 5 L. The basal salt media with a pulp density of about 10 % (w/v) was autoclaved and fed pumped through a peristaltic pump. The pH of the feed slurry and process temperature were kept at 2.0 and 45 °C, respectively. Then a mixed consortium of mixed metal adapted cells of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* was inoculated in the bubble column reactor for further initiation of bioleaching operation. After screening studies the selected feed rates were 0.035, 0.038, 0.041, 0.050 l h⁻¹, and thus the hydraulic retention time for the fluidized bed volume were 40, 80 and 120 h, respectively. After pre-leaching stage of 10 days, aliquots of effluent solution were taken from the reactor to analyze the concentration of Al, Cu, Ni and Zn. The changes in pH and redox potential were also monitored at periodic intervals.

3.19. COLUMN BIOLEACHING STUDIES OF ELECTRONIC SCRAP

3.19.1. Source of electronic scrap samples
Source of electronic scrap was same as mentioned in section 3.18.1. No mechanical/physical separation process was used before its transportation to the laboratory. For experimental use, the electronic scrap sample was crushed and then ground to fine powder of 100 to 120 μm particle size by using small ring mill grinder.

3.19.2. Analysis of electronic scrap samples

For metal analysis, the electronic scrap sample (1.0 g) was dissolved in 100 mL of aqua regia by refluxing in a round bottom flask for 1 hour. The solution was then allowed to cool at room temperature and the volume was made up to 100 mL. The concentrations of dissolved metal ions (i.e. Ag⁺, Al³⁺, Cu²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Sn²⁺ and Zn²⁺) were determined by atomic absorption spectrophotometer (Varian AA-400) and the data were analyzed for calculating the concentrations of different metal ions in the electronic scrap.

3.19.3. Preparation of electronic scrap for column bioleaching studies

Finely ground washed samples of electronic scrap were used in the bioleaching experiments. Washed samples of electronic scrap were prepared by suspending 50 g of electronic scrap in 500 mL of saturated solution of sodium chloride. The mixture was stirred for 10 min and allowed to stand till heavier particles settled down at the bottom. The floating material was decanted off and the denser part, which settled down at the bottom of every sample, was separated, washed and dried to constant weight. Then the samples were sterilized by tyndalization and homogenized prior to bioleaching. The samples treated in this way were referred to as “Washed charge” and this washed charge was used in further column bioleaching studies.

3.19.4. Microorganisms

Mixed adapted consortium of acidophilic chemolithotrophic (moderately thermophilic) bacteria was used in bioleaching studies. These included *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* and the conditions were same as in section 3.14. The adaptation of moderately thermophilic bacterial cultures to mixed metal ions (Ag⁺, Al³⁺, Cu²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Sn²⁺ and Zn²⁺) was performed through serial sub culturing in the logarithmical phase of growth and by gradually increasing the concentration of mixed metal ions at the same time. Finally, the cell mass was harvested by centrifugation and inoculum was prepared as described in 3.14.
3.19.5. Column bioleaching studies

Two columns of 58 cm long with an internal diameter of 13 cm were used in the bioleaching studies that were fabricated locally. A high density polyethylene support shield with multiple holes (10 mm) was used for permitting air to be introduced below the plate and dispersed uniformly over the washed charge in columns. A layer of support scrap sized at 10–15 mm was placed in the bottom of the column before 10 kg washed charge was loaded. Temperature of column was maintained at 45 °C with water jacket around the column and thermocouple installed inside the charge.

Because electronic scrap was alkaline in nature so before inoculation the column charge was pre treated with sulfuric acid for pH stabilization. Each column (experimental and control) was fed with two liters autoclaved distilled water of pH 2.0 that was applied to the surface of the column charge by using a garden sprinkler head and was allowed to pass through the washed charge by gravity and recirculated through a side pipe with a peristaltic pump, pH of effluent was monitored continuously and concentrated sulfuric acid was added gradually. After stabilization of pH in columns, preleaching was stopped. Effluent of both columns was allowed to drain off and the column contents were rinsed with dilute sulfuric acid and autoclaved distilled water of pH 2. Finally, all the solution was removed to perform bioleaching operation. During column bioleaching studies each column was fed with two liters of autoclaved FeTSB liquid medium having pH 2.0, adjusted with sulfuric acid 2M. Then column B was inoculated with respective inocula 10 % (v/v) with a cell density of about 10⁷ cells/mL and the rate of flow was adjusted to 50 mL/min while column A was considered as aseptic control.

In both columns, Solution level was maintained at a sufficient height and clean air was provided through a rotameter with the flow rate of 150–200 L/h. Effluents from preleaching and bioleaching operation were collected and analyzed periodically to determine the solution concentration, metal dissolution and acid consumption. When analysis of bioleaching solution indicated that bioleaching had ended the solution in columns was allowed to drain off completely. Column charge was rinsed with dilute sulfuric acid followed by distilled water. Residues were dried and prepared for final analysis.

3.19.6. Sampling procedure
MATERIALS AND METHODS

Effluents of pre-leaching and bioleaching were first filtered through filter paper (Whatman No. 1) to remove solid particles and then centrifuged at 10,000 rpm for 10 min to remove bacterial cell mass and supernatant was then analyzed for copper, zinc, aluminum and nickel concentration.

3.20. ESTIMATION OF SOLUBLE IRON SPECIES

Various soluble iron species Fe$^{2+}$, Fe$^{3+}$ and total iron were determined by 1-10 phenanthroline method using UV-Visible Double Beam spectrophotometer (CARY1).

3.20.1. Ferrous (Fe$^{2+}$) ion estimation

Sample aliquots (0.5 mL) of leachate from each flask were taken in 25 mL measuring flasks. Then added successively 1mL of 1-10 phenanthroline solution (0.5 %), 2 mL of sodium acetate solution (10 %) and the volume was made up-to mark with distilled water (pH adjusted to 2.0 with H$_2$SO$_4$). Samples were allowed to stand for 15 minutes for colour development and then the absorbance was measured on spectrophotometer at 510 nm (wave length) against reagent blank. Standard solutions used for iron estimation were 2, 4, 6, 8 and 10 ppm.

3.20.2. Total iron estimation

In order to determine the total iron, the procedure was same as that in case of ferrous iron estimation but in this case 1 mL of hydroxylamine hydrogen chloride was added prior to the addition of 1-10 phenanthroline. It reduced ferric (Fe$^{3+}$), if present, to ferrous (Fe$^{2+}$) because 1-10 phenanthroline can only form complex with ferrous iron.

3.20.3. Ferric (Fe$^{3+}$) ion estimation

The amount of ferric (Fe$^{3+}$) present in the medium was measured by subtracting ferrous (Fe$^{2+}$) concentration from total iron. The data obtained from all these experiments were plotted in the form of graphs and ferrous ion oxidation rates, sulfur oxidation rates and specific growth rates were determined from the maximum slopes of the plots.

3.20.4. Analytical techniques

Free bacterial cells in solution were counted by direct counting, using a counting chamber (Neubauer) with phase contrast microscope. Soluble metal ions (Al, Zn, Cu and Ni) in the leached solutions were measured using an atomic absorption spectrophotometer...
(Varian AA-400). The ferrous, ferric and total iron concentration in the solution was determined by spectrophotometric method using 1–10 ortho phenanthroline. The pH of the experimental solution and abiotic control was checked, at room temperature, with a pH meter standardized with a low pH buffer. The redox potential of the leaching solution was measured with a platinum electrode in reference to a saturated Ag/AgCl electrode.
RESULTS AND DISCUSSION

4.1. SOURCE OF MICROBIAL SAMPLES

Isolated microbial samples were collected from Dexing copper mine in Jiangxi province of China and Reko Diq copper ore deposits, Pakistan (Table 4.1).

Table 4.1. Source of isolated microbial samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Site name</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>LRP</td>
<td>2.5</td>
<td>38</td>
<td>Liquid from mine site</td>
</tr>
<tr>
<td>2</td>
<td>LM</td>
<td>2.0</td>
<td>50</td>
<td>Liquid from rock pile</td>
</tr>
</tbody>
</table>

4.2. PHYSICO-CHEMICAL ANALYSIS OF COLLECTED SAMPLES

Physico-chemical analysis of mine samples was carried out due to diverse pH, temperature, metal contents and ions. A detailed description is given in Table 4.2.

Table 4.2. Physico-chemical analysis of samples

<table>
<thead>
<tr>
<th>Site</th>
<th>As (mg/L)</th>
<th>Rb (mg/L)</th>
<th>Zn (mg/L)</th>
<th>Pb (mg/L)</th>
<th>Cr (mg/L)</th>
<th>Mg (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRP</td>
<td>9.7</td>
<td>0.7</td>
<td>40.25</td>
<td>2.16</td>
<td>0.50</td>
<td>51.0</td>
</tr>
<tr>
<td></td>
<td>10.35</td>
<td>0.40</td>
<td>53.42</td>
<td>2.68</td>
<td>1.19</td>
<td>52.4</td>
</tr>
<tr>
<td>LM</td>
<td>245.2</td>
<td>19.3</td>
<td>427.8</td>
<td>8.70</td>
<td>47.17</td>
<td>5.90</td>
</tr>
<tr>
<td></td>
<td>148</td>
<td>39.63</td>
<td>308.7</td>
<td>10.50</td>
<td>43.14</td>
<td>5.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>Cu (mg/L)</th>
<th>Co (mg/L)</th>
<th>Fe (mg/L)</th>
<th>Ni (mg/L)</th>
<th>Al (mg/L)</th>
<th>Ca (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRP</td>
<td>245.2</td>
<td>19.3</td>
<td>427.8</td>
<td>8.70</td>
<td>47.17</td>
<td>5.90</td>
</tr>
<tr>
<td></td>
<td>148</td>
<td>39.63</td>
<td>308.7</td>
<td>10.50</td>
<td>43.14</td>
<td>5.63</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Site</th>
<th>Cd (mg/L)</th>
<th>Sn (mg/L)</th>
<th>Be (mg/L)</th>
<th>Ag (mg/L)</th>
<th>K(mg/L)</th>
<th>Si (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRP</td>
<td>7.9</td>
<td>0.23</td>
<td>0.20</td>
<td>0.01</td>
<td>0.2</td>
<td>21.4</td>
</tr>
<tr>
<td></td>
<td>5.65</td>
<td>0.14</td>
<td>0.17</td>
<td>0.2</td>
<td>0.3</td>
<td>27.4</td>
</tr>
</tbody>
</table>
Morphological characteristics and growth studies of enriched bacterial isolates are given in Table 4.3.

**Table 4.3. Morphological characteristics of various isolates and growth studies of enriched bacterial isolates**

<table>
<thead>
<tr>
<th>Site</th>
<th>Isolate code</th>
<th>Colony colour</th>
<th>Elevation</th>
<th>Margins</th>
<th>Cell shape</th>
<th>Cell Size (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>LRP</td>
<td>JCM17</td>
<td>brownish</td>
<td>Flat</td>
<td>Entire</td>
<td>Rods</td>
<td>0.65</td>
</tr>
<tr>
<td>LM</td>
<td>RDB</td>
<td>Cream</td>
<td>Flat</td>
<td>Entire</td>
<td>Rods</td>
<td>0.85</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Isolate code</th>
<th>Specific growth Rate (µ)</th>
<th>Mean generation Time td (h)</th>
<th>Optimum growth pH</th>
<th>Optimum growth Temperature (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCM17</td>
<td>0.22</td>
<td>3.5</td>
<td>1.75</td>
<td>35</td>
</tr>
<tr>
<td>RDB</td>
<td>0.30</td>
<td>3.2</td>
<td>2.0</td>
<td>45</td>
</tr>
</tbody>
</table>

**4.3. PCR AMPLIFICATION OF THE 16S rDNA**

Bacterial samples were PCR amplified using conditions earlier developed by Ghauri et al. (2003). A product of 1,500 bp was obtained in case of all isolates. Nucleotide blast search of partial sequences of the 16S ribosomal DNA for various isolates showed that isolates had variable percent identity (92-98 %) with *Sulfobacillus thermosulfidooxidans* and *Leptospirillum ferrooxidans*. Accession numbers of all isolates were obtained by submitting them in GenBank after comparing with other isolates of closest known homology as shown in Table 4.4.

**Table 4.4. Isolates and their accession number**

<table>
<thead>
<tr>
<th>Isolates (Accession no.)</th>
<th>Nature of samples</th>
<th>% similarity to closest match</th>
<th>Organism</th>
</tr>
</thead>
<tbody>
<tr>
<td>JCM17(GQ202139)</td>
<td>Liquid</td>
<td>92</td>
<td><em>Leptospirillum ferrooxidans</em></td>
</tr>
<tr>
<td>RDB (GQ228448)</td>
<td>Liquid</td>
<td>98</td>
<td><em>Sulfobacillus thermosulfidooxidans</em></td>
</tr>
</tbody>
</table>
4.4. PRELIMINARY BIOLEACHING STUDIES

Preliminary bioleaching studies of sphalerite ore was carried out with different wild cultures and different mixed consortium of these microorganisms with 10% pulp density as shown in Fig.4.1.

Fig.4.1. Preliminary bioleaching studies of sphalerite ore with different wild cultures and different mixed consortium of these microorganisms

(A; *Sulfobacillus thermosulfidooxidans* strain RDB, B; *Sulfobacillus thermosulfidooxidans* strain MT 13, C; *Acidithiobacillus thiooxidans*, D; *Leptospirillum ferrooxidans*, E; *Sulfobacillus thermosulfidooxidans* strain MT 13 + *Thermoplasma acidophilum*, F; *Sulfobacillus thermosulfidooxidans* strain MT 13 + A1TSB, G; *Sulfobacillus thermosulfidooxidans* strain RDB + *Thermoplasma acidophilum*, H; *Sulfobacillus thermosulfidooxidans* strain RDB + A1TSB, I; *Leptospirillum ferrooxidans* + *Acidithiobacillus thiooxidans*).

The wild cultures and combinations (mixed consortium) with preferentially high bioleaching rate were selected for further studies. It can be seen from Fig.4.1 that bioleaching potential of *Sulfobacillus thermosulfidooxidans* was comparatively better then other cultures that might be attributed due to superior kinetics of moderate thermophiles as compared to mesophiles (Deveci *et al.*, 2004).
Percent metal solubilization ability of different unadapted mixed consortiums was also investigated as shown in Fig. 4.1. Mixed consortium of *Sulfobacillus thermosulfidooxidans* with heterotrophs leached out all the metals at higher rates as compared to those attained from other consortium and among these, the consortium of *Sulfobacillus thermosulfidooxidans* strain- RDB with *Thermoplasma acidophilum* was found to have higher bioleaching rates then others. That might be due to the excretion of extracellular metabolites by chemolithoautotrophs, use of these metabolites as a carbon source by chemolithoheterotrophs and mutual stability of both strains based on their indigenous nature (Butler and Kempton, 1987).

4.5. ORTHOGONAL EXPERIMENTAL ARRAY FOR PROCESS PARAMETERS OPTIMIZATION

Initially bioleaching experiments were conducted at temperature 45°C, pH 2.5, agitation of 180 rpm and particle size 50-150μm. After adjustment of initial acid demand, the experimental flasks were inoculated with 1.0 mL inoculum (1×10^7 cells/mL) of *Sulfobacillus thermosulfidooxidans* aseptically. The percent metals recovery was very less, as can be seen from preliminary study, so an orthogonal experimental array was designed for optimization of process parameters prior to bioleaching studies while size of inoculum was kept at 1×10^7 cells/mL on the basis of some previous research (Ilyas et al., 2007; Olson et al., 2003). Corresponding bioleaching efficiencies with two replications obtained under the candidate conditions of parameters are displayed in Table 4.5.

Empirical models describing the experimental results were developed using data collected from the 25 batch runs and were generated using the orthogonal array method. Model parameters were estimated using a second-order model of the form given below;

\[ E(Y) = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \sum_{j=1}^{k} \beta_{ij} X_i X_j \]

where \( E(Y) \) is the expected value of the response variable, \( \beta_0, \beta_i, \beta_{ij} \) are the model parameters, \( X_i \) and \( X_j \) are the coded factors (A, B, C, D = X_1, X_2, X_3, X_4) being studied, and \( k \) is the number of factors being studied (Xu et al., 2004). Generalized algebraic modeling system (GAMS; GAMS Development Corp.) was used to optimize the second order statistical empirical models.
Table 4.5. Percentage (%) bioleaching er of metals and experimental factors

<table>
<thead>
<tr>
<th>Runs</th>
<th>Experimental Factors</th>
<th>Bioleaching Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>B</td>
</tr>
<tr>
<td>1</td>
<td>1.8</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>1.8</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>120</td>
</tr>
<tr>
<td>4</td>
<td>1.8</td>
<td>200</td>
</tr>
<tr>
<td>5</td>
<td>1.8</td>
<td>270</td>
</tr>
<tr>
<td>6</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>100</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>120</td>
</tr>
<tr>
<td>9</td>
<td>2</td>
<td>200</td>
</tr>
<tr>
<td>10</td>
<td>2</td>
<td>270</td>
</tr>
<tr>
<td>11</td>
<td>2.5</td>
<td>50</td>
</tr>
<tr>
<td>12</td>
<td>2.5</td>
<td>100</td>
</tr>
<tr>
<td>13</td>
<td>2.5</td>
<td>120</td>
</tr>
<tr>
<td>14</td>
<td>2.5</td>
<td>200</td>
</tr>
<tr>
<td>15</td>
<td>2.5</td>
<td>270</td>
</tr>
<tr>
<td>16</td>
<td>3</td>
<td>50</td>
</tr>
<tr>
<td>17</td>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>18</td>
<td>3</td>
<td>120</td>
</tr>
<tr>
<td>19</td>
<td>3</td>
<td>200</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
<td>270</td>
</tr>
<tr>
<td>21</td>
<td>3.5</td>
<td>50</td>
</tr>
<tr>
<td>22</td>
<td>3.5</td>
<td>100</td>
</tr>
<tr>
<td>23</td>
<td>3.5</td>
<td>120</td>
</tr>
<tr>
<td>24</td>
<td>3.5</td>
<td>200</td>
</tr>
<tr>
<td>25</td>
<td>3.5</td>
<td>70</td>
</tr>
</tbody>
</table>

(Bioleaching efficiency (%) of all metals ions is mean response of two replications with an error of ± 0.5).
RESULTS AND DISCUSSION

Fig.4.2a. Effects of controllable factors on mean responses for percent metal ions dissolution.

The obtained data were then analyzed by Origin pro 7.5 software to evaluate the effect of individual parameter on the optimization criteria. Maximum amount of percent metals dissolution was defined as optimization criterion. In the view of above data, mean response calculations were performed to achieve the defined criterion. For efficient realizing of the shake flask experimental conditions related to each response factor, the corresponding conditions were displayed in each row of that table. Taguchi designing recommends analyzing the mean responses for each run and uses graphs of marginal means of each factor, as shown in Figs. 4.2 a, b, c, d, and e, but these graphs can only be used to show the trends of each factor more precise, more understandable and it is incorrect to use these graphs for predicting other values that were not experimented. The usual strategy is to examine the graphs and note the maximum value. In Fig. 4.2, the effects of these controllable factors (parameters) on mean responses for metals of sulphide ore are displayed.

Bioleaching process, which causes solubilization of heavy metals and acidification, is mainly governed by pH of the leaching medium. The temporal change in pH relate to the buffering capacity of the leaching medium. The optimum pH for ores bioleaching process with moderately thermophilic culture of *Sulfobacillus thermosulfidooxidans* was found to
be 1.8 as shown in Fig.4.2a. At pH values in the range of 2.5-3.5, the decrease of percent recovery of metals could be due to the formation of hydronium jarosite on ore particles.

These jarosite are causes diffusion barrier of reactants and products, which leads to declined kinetics of the ferrous and sulfur oxidation reactions. Low bioleachability obtained at higher pH values are mainly because of cessation of bacterial growth and sulfur oxidation reactions due to the presence of high concentration of heavy metal ions as well as protons (Chen, et al., 2001; Garcia et al., 1993).

Particle size influenced the surface area of ore particles; it is considered an important factor in determining the kinetics of the bioleaching reactions (Ahonen and Tuovinen, 1995). Increased surface area increases the specific contact area between microorganisms, liquid phase and substrate. According to Fig.4.2 b, particle size of 120μm was considered to be optimum. However, a further decrease in particle size adversely affected the activity of the microbial cells. This might be due to the presence of very fine particles, which may apparently damaged the structure of cells, thereby resulting in their rendered ability to oxidize the ore particles.

![Graph](image)

**Fig.4.2 b.** Effects of controllable factors on mean responses for percent metal ions dissolution.
Fig.4.2 c. Effects of controllable factors on mean responses for percent metal ions dissolution.

Pulp density is among one of the physical parameter that strongly affect the metal dissolution. Main limitations that are associated in industrial scale operation are high pulp density and insufficient stirring resulting in reagent starvation and mass transfer resistance. While the declined metal solubilization at smaller pulp densities are attributed due to less accessibility of the substrate. Hence, it is important to maintain optimum pulp density in order to gain maximum bioleachability rates. Optimum pulp density was considered to be 10 % as shown in Fig. 4.2 c.
Fig. 4.2d. Effects of controllable factors on mean responses for percent metal ions dissolution.

The optimum temperature was considered to be 47 °C as in Fig. 4.2 d. Like all chemical and biochemical processes, the rates of bioleaching reactions are also temperature dependent. Hence, it is considered as important environmental factor that influences bacterial growth activity in bioleaching processes (Ahonen and Tuovinen., 1990).

Shaking is a major parameter in bioleaching processes. Optimum velocity of shaking is required for homogeneous solid suspension, proper aeration, temperature and pH uniformity, mass and heat transfer (nutrients, oxygen and carbon dioxide). High shaking velocity reduced the solubilization of metals due to excessive mixing phenomenon that led to high turbulence of ore particles with the microbial cells, which seriously affected the bacterial growth and ultimately resulted in reduced levels of bioleachability (Witne and Philips, 2001; Shi and Fang., 2005). At low shaking, oxygen and mass transfer limitations led to reduced solubilization of metals.
Fig. 4.2e. Effects of controllable factors on mean responses for percent metal ions dissolution

Hence, a shaking of 180 rpm for a pulp density of 10% was considered optimum as in Fig. 4.2e. So the optimized conditions for enhanced metals solubilization were found to be, particle size 120 μm, pH 1.8, pulp density 10%, agitation 180 rpm and temperature 47 °C.

To see whether the process parameters are statistically significant, statistical analysis of variance (ANOVA) was performed. The F-value (a ratio of the squared deviations to the mean of the squared error) for each factor indicates that which parameter has a significant effect on the bioleaching potential. According to these results, temperature, initial pH and agitation has the significant effect on mean response for metals bioleaching process. The F-value for these parameters is greater than the F-value obtained from the table at 95.0% confidence interval. This means that the variance of all these factors (parameters) is significant compared with the variance of error. All of them showed meaningful effect on responses for metals (Table 4.6).

Finally, using Taguchi method, results for all combinations of levels were predicted and these predictions were confirmed by experimentation. Then under optimized process conditions bioleaching was carried out with Daye copper ore and further bioleaching of ores was carried out under already defined process conditions.
**RESULTS AND DISCUSSION**

Table 4.6. Summery of statistical results

<table>
<thead>
<tr>
<th>P-Value</th>
<th>Zn</th>
<th>Co</th>
<th>Cu</th>
<th>Fe</th>
<th>Ni</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.003</td>
<td>0.009</td>
<td>0.009</td>
<td>0.005</td>
<td>0.003</td>
</tr>
<tr>
<td>B</td>
<td>0.045</td>
<td>0.048</td>
<td>0.050</td>
<td>0.041</td>
<td>0.045</td>
</tr>
<tr>
<td>C</td>
<td>0.036</td>
<td>0.030</td>
<td>0.038</td>
<td>0.032</td>
<td>0.037</td>
</tr>
<tr>
<td>D</td>
<td>0.001</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
<td>0.002</td>
</tr>
<tr>
<td>E</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Fig.4.3. Percent metal ions solubilization under optimized conditions of bioleaching

The initial value of pH of the bioleaching medium was adjusted at 1.8 in case of shake flask studies that was checked every day and raise in pH was adjusted by adding 2M sulfuric acid. At the 4\textsuperscript{th} day when no further increase in pH was observed, flasks were inoculated. After inoculation, the pH remained stable for almost three days that may be attributed to delayed growth phase of bacterias and then started dropping down that was due to the hydrolysis of the metal ions that may released in the medium due to bacterial
action. No further effectible change in the pH of the medium was observed. While in case of abiotic control the increased value of pH was observed as in Fig.4.3.

During this whole process about Co 68%, Zn 72%, Ni 81%, Cu 78%, and Fe 70% was leached out fig.5.

Then the bioleaching process was carried out with metal ions adapted culture of *Sulfobacillus thermosulfidooxidans* under already optimized conditions. Further enhancement in percent metals solubilization was observed (Co 72 %, Zn 76%, Cu 84%, Fe 75% and Ni 86 %) as in fig.6. The percent bioleachability of metals in control flasks were insignificant.

4.6. SHAKE FLASK EXPERIMENTS WITH DIFFERENT SULFIDIC ORES

Studies were undertaken for bioleaching of metals from ores and electronic scrap. The results are presented below.

4.6.1. Chemical analysis of Pyrite

Chemical analysis of the pyrite ore used in these studies was carried out to determine the concentrations of various metals present in the materials. Pyrite contained about 42 % iron as main metal (Table.4.7).

<table>
<thead>
<tr>
<th>Ores</th>
<th>Metal ions concentration % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fe</td>
</tr>
<tr>
<td>Pyrite</td>
<td>42</td>
</tr>
<tr>
<td>Sphalerite</td>
<td>10</td>
</tr>
<tr>
<td>Pb-Zn ore</td>
<td>7</td>
</tr>
</tbody>
</table>

(All values are mean response of two replications with an error of ± 0.03).

4.6.2. Bioleaching of Pyrite

Experiments were conducted on bioleaching of iron from pyrite ore using pure un-adapted and adapted cultures of *Sulfobacillus thermosulfidooxidans* as well as their consortium with acidophilic heterotrophs. Maximum pyrite leachability was exhibited by the mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and
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*Thermoplasma acidophilum* which leached out about 60 % of iron from pyrite in 120 h. Whereas, pure adapted culture leached out a maximum of 53 % of iron from pyrite and the un-adapted culture solubelized about 30 % iron with biomass production rates of 0.0033 gL⁻¹h⁻¹, 0.0025 gL⁻¹h⁻¹ and 0.0022 gL⁻¹h⁻¹ of mixed, adapted and pure cultures. While un-adapted, pure adapted and mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB leach out about 29 %, 37 % and 47 % of iron in the same time period as shown in Fig.4.4.

![Fig.4.4. Bioleaching of iron from pyrite](image)

(A1; Mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB + *Thermoplasma acidophilum*, A2; Mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT 13 + A1TSB B1; Adapted culture of *Sulfobacillus thermosulfidooxidans* strain RDB, B2; Adapted culture of *Sulfobacillus thermosulfidooxidans* strain MT 13, C1; wild culture of *Sulfobacillus thermosulfidooxidans* strain RDB, C2; wild culture of *Sulfobacillus thermosulfidooxidans* strain MT 13).

In case of pure un-adapted, adapted cultures and mixed adapted cultures, biomass production rates (in terms of total cellular protein) were 0.0017 gL⁻¹h⁻¹ and 0.0019 gL⁻¹h⁻¹,
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0.0028 gL⁻¹h⁻¹ respectively as can be seen from Table 4.8. It may be mentioned that these results provide an estimate of the growth that do not give exact measurement of the biomass in these flasks, because the readings were only for planktonic cells, while their may be the cells attached to the particulate matter like ore body or scrap surface that were not accounted for. Furthermore, the plot for mixed adapted consortium of microorganisms should not be regarded as a true growth curve of these cultures due to presence of two different types of bacterial cultures. Only about 12-13 % of iron was solubelized in 120 h in the sterile control flasks.

Table 4.8. Growth (determined as total cellular protein) of pure unadapted, pure adapted and mixed adapted cultures of *S. thermosulfidooxidans* during bioleaching of Pyrite

<table>
<thead>
<tr>
<th></th>
<th><em>S. thermosulfidooxidans</em> strain RDB</th>
<th><em>S. thermosulfidooxidans</em> strain MT-13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure unadapted culture</td>
<td>0.0025</td>
<td>0.0017</td>
</tr>
<tr>
<td>Pure adapted culture</td>
<td>0.0025</td>
<td>0.0019</td>
</tr>
<tr>
<td>Mixed&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0033</td>
<td>0.0028</td>
</tr>
</tbody>
</table>

(a; Mixed adapted consortium of *S. thermosulfidooxidans* strain RDB + *Thermoplasma acidophilum*, b; *S. thermosulfidooxidans* strain MT 13 + A1TSB, All values are mean of two replications with an error of ± 0.002).

4.6.3. Chemical analysis of sphalerite

Chemical analysis of the sphalerite ore used in these studies was carried out to determine the concentrations of various metals present in the materials. Sphalerite contained about 10 % iron and 40 % Zn as main metals (Table.4.7).

4.6.4. Bioleaching of sphalerite

As in case of pyrite, maximum leachability of sphalerite was obtained from same mixed consortium, which leached out about 67 % zinc in 504 hours. The pure un-adapted
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and adapted cultures leached out 45 % and 50 % zinc respectively in the same durations (Fig.4.5). In case of iron, mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasmia acidophilum* solubilized about 50 % iron in 432 hours, adapted and un-adapted cultures solubilize 38 % and 32 % iron (Fig. 4.6). Biomass production rates 0.0043 gL⁻¹h⁻¹, 0.0038 gL⁻¹h⁻¹ and 0.0034 gL⁻¹h⁻¹ for mixed adapted, adapted and pure cultures as can be seen from Table 4.9.

![Fig.4.5](image)

**Fig.4.5.** Bioleaching of Zn from sphalerite

(A1; Mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB + *Thermoplasmia acidophilum*, A2; Mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT 13 + A1TSB, B1; Adapted culture of *Sulfobacillus thermosulfidooxidans* strain RDB, B2; Adapted culture of *Sulfobacillus thermosulfidooxidans* strain MT 13, C1; wild culture of *Sulfobacillus thermosulfidooxidans* strain RDB, C2; wild culture of *Sulfobacillus thermosulfidooxidans* strain MT 13).
Results and Discussion

Fig. 4.6. Bioleaching of Fe from sphalerite

(A1; Mixed adapted consortium of *Sulfolobus thermosulfidooxidans* strain RDB + *Thermoplasma acidophilum*, A2; Mixed adapted consortium of *Sulfolobus thermosulfidooxidans* strain MT 13 + A1TSB B1; Adapted culture of *Sulfolobus thermosulfidooxidans* strain RDB, B2; Adapted culture of *Sulfolobus thermosulfidooxidans* strain MT 13, C1; wild culture of *Sulfolobus thermosulfidooxidans* strain RDB, C2; wild culture of *Sulfolobus thermosulfidooxidans* strain MT 13).

While un-adapted, pure adapted and mixed adapted consortium of *Sulfolobus thermosulfidooxidans* strain MT-13 and A1TSB leach out about 41%, 45% and 61% of zinc and 32%, 36% and 48% of iron in the same time duration in according to their biomass production rates 0.0039 gL⁻¹h⁻¹, 0.0035 gL⁻¹h⁻¹ and 0.0033 gL⁻¹h⁻¹ of mixed adapted, adapted and pure cultures (Table 4.9). But 20% zinc and 17% iron was leached out in sterile control flask.
Table 4.9. Growth (determined as total cellular protein) of pure unadapted, pure adapted and mixed adapted cultures of *S. thermosulfidooxidans* during bioleaching of sphalerite

<table>
<thead>
<tr>
<th></th>
<th><em>S. thermosulfidooxidans</em> strain RDB</th>
<th><em>S. thermosulfidooxidans</em> strain MT-13</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pure unadapted culture</td>
<td>(gL⁻¹h⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Pure adapted culture</td>
<td>(gL⁻¹h⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Mixed (^a) adapted culture</td>
<td>(gL⁻¹h⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Pure unadapted culture</td>
<td>(gL⁻¹h⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Pure adapted culture</td>
<td>(gL⁻¹h⁻¹)</td>
<td></td>
</tr>
<tr>
<td>Mixed (^b) adapted culture</td>
<td>(gL⁻¹h⁻¹)</td>
<td></td>
</tr>
<tr>
<td>0.0034</td>
<td>0.0038</td>
<td>0.0043</td>
</tr>
<tr>
<td>0.0033</td>
<td>0.0035</td>
<td>0.0039</td>
</tr>
</tbody>
</table>

(a; Mixed adapted consortium of *S. thermosulfidooxidans* strain RDB + *thermolysma acidophilum*, b; *S. thermosulfidooxidans* strain MT 13 + A1TSB, All values are mean of two replications with an error of ± 0.002).

### 4.6.5. Chemical analysis of Pb-Zn ore

Chemical analysis of the Pb-Zn ore used in these studies was carried out to determine the concentrations of various metals present in the materials. Pb-Zn ore contained about 7 % Fe and 7.2 % Pb and 10 % Zn as main metals (Table.4.7).

### 4.6.6. Bioleaching of Pb-Zn ore

Comparison of the bioleaching performance of pure un-adapted and adapted cultures of *Sulfobacillus thermosulfidooxidans* strain RDB as well as it consortium with acidophilic heterotroph (*Thermoplasma acidophilum*) was studied. The extraction of Zn with mixed consortium was rapid about 68 % while with pure adapted and un-adapted it was 59 % and 47 % respectively, in 360 h as in Fig.4.7. On the contrary, the leaching rate in sterile flask was insignificant. Similarly mixed consortium leached out about 54 % iron as compared to pure un-adapted and adapted cultures that leached out 45 % and 51 % iron in 288 hours as in Fig.4.8. However, only 12 % of the total was leached out in the control flasks. Maximum Pb dissolution (59%) was obtained with mixed consortium, while pure adapted and un-adapted cultures extracted out 55 % and 30 % Pb, respectively (Fig.4.9).
RESULTS AND DISCUSSION

Fig.4.7. Bioleaching of Zn from Pb-Zn ore

(A1; Mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB + *Thermoplasma acidophilum*, A2; Mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT 13 + A1TSB B1; Adapted culture of *Sulfobacillus thermosulfidooxidans* strain RDB, B2; Adapted culture of *Sulfobacillus thermosulfidooxidans* strain MT 13, C1; wild culture of *Sulfobacillus thermosulfidooxidans* strain RDB, C2; wild culture of *Sulfobacillus thermosulfidooxidans* strain MT 13).

Their dry biomass also showed the same pattern with the mixed consortium having the highest biomass production rate of 0.0038 gL⁻¹h⁻¹. The biomass rates of pure un-adapted and adapted cultures were 0.0030 gL⁻¹h⁻¹ and 0.0023 gL⁻¹h⁻¹ respectively. While un-adapted, pure adapted and mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB leach out about 40%, 55% and 58% of zinc, 38%, 43% and 49% of iron and 26%, 52% and 57% of lead in the same time duration. Lead was detected in the precipitate. The rates of mixed adapted, pure adapted and pure un-adapted cultures were 0.0026 gL⁻¹h⁻¹, 0.0022 gL⁻¹h⁻¹ and 0.0020 gL⁻¹h⁻¹ respectively as can be seen from Table 4.10a and b.
RESULTS AND DISCUSSION

Fig. 4.8. Bioleaching of Fe from Pb-Zn ore

(A1; Mixed adapted consortium of *Sulfo*bacillus *thermosulfidooxidans* strain RDB + *Thermoplasma acidophilum*, A2; Mixed adapted consortium of *Sulfo*bacillus *thermosulfidooxidans* strain MT 13 + A1TSB B1; Adapted culture of *Sulfo*bacillus *thermosulfidooxidans* strain RDB, B2; Adapted culture of *Sulfo*bacillus *thermosulfidooxidans* strain MT 13, C1; wild culture of *Sulfo*bacillus *thermosulfidooxidans* strain RDB, C2; wild culture of *Sulfo*bacillus *thermosulfidooxidans* strain MT 13).

Table 4.10a. Growth of wild, adapted and mixed adapted cultures of chemolithotrophs during bioleaching of Pb-Zn ore

<table>
<thead>
<tr>
<th></th>
<th>a Pure unadapted culture (gL⁻¹h⁻¹)</th>
<th>a Pure adapted culture (gL⁻¹h⁻¹)</th>
<th>b Mixed adapted culture (gL⁻¹h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1</td>
<td>0.0020±0.001</td>
<td>0.0022±0.001</td>
<td>0.0026±0.002</td>
</tr>
</tbody>
</table>

(S. *thermosulfidooxidans* strain MT-13; S. *thermosulfidooxidans* strain MT-13+A1TSB)
RESULTS AND DISCUSSION

Fig. 4.9. Bioleaching of Pb from Pb-Zn ore

(A1; Mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB + *Thermoplasma acidophilum*, A2; Mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT 13 + A1TSB B1; Adapted culture of *Sulfobacillus thermosulfidooxidans* strain RDB, B2; Adapted culture of *Sulfobacillus thermosulfidooxidans* strain MT 13, C1; wild culture of *Sulfobacillus thermosulfidooxidans* strain RDB, C2; wild culture of *Sulfobacillus thermosulfidooxidans* strain MT 13).

Table 4.10b. Growth of pure, adapted and mixed adapted cultures of chemolithotrophs during bioleaching of Pb-Zn ore

<table>
<thead>
<tr>
<th></th>
<th>Pure culture (gL⁻¹h⁻¹)</th>
<th>Pure adapted culture (gL⁻¹h⁻¹)</th>
<th>Mixed adapted culture (gL⁻¹h⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>a</td>
<td>0.0023±0.001</td>
<td>0.0030±0.001</td>
<td>0.0038±0.002</td>
</tr>
</tbody>
</table>

(\(^a\) *S. thermosulfidooxidans* strain RDB; \(^b\) *S. thermosulfidooxidans* strain RDB + *Thermoplasma acidophilum*)
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4.6.7. Chemical analysis of low grade Huangshan ore

Chemical analysis of the low grade Huangshan ore used in these studies was carried out to determine the concentrations of various metals present in the materials. Fe, Cu, Ni and Zn were present as main metals (Table 4.11).

Table 4.11. Chemical analysis of Huangshan ore

<table>
<thead>
<tr>
<th>Metals</th>
<th>Metal ions Concentration % (W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>17 ± 0.06</td>
</tr>
<tr>
<td>Cu</td>
<td>6.0 ± 0.04</td>
</tr>
<tr>
<td>Ni</td>
<td>2.95 ± 0.03</td>
</tr>
<tr>
<td>Zn</td>
<td>1.2 ± 0.04</td>
</tr>
</tbody>
</table>

4.6.8. Bioleaching of low grade Huangshan ore

Because the bioleaching efficiency of mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* with their respective heterotrophs was far high then the adapted and wild cultures of these microorganisms so the comparison of bioleaching performance of mixed adapted cultures of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* with *Sulfobacillus thermosulfidooxidans* and A1TSB was made. During shake flask bioleaching studies of low grade nickel containing sulphide ore having high amount of fayallite, hematite and magnetite as main gangue, mixed adapted consortium of, *thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* solubilized 83 %, 85 %, 55 % and 81 % of Ni, Cu, Fe and Zn while mixed consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB solubelized 79 %, 80 %, 43 % and 78 % of Ni, Cu, Fe and Zn as can be seen in Fig.4.10.
Fig. 4.10. Bioleaching of low grade Huangshan ore

(A; mixed adapted consortium of *S. thermostufidoxidans* strain RDB and *Thermoplasma acidophilum*, B; mixed consortium of *Sulfobacillus thermostufidoxidans* strain MT-13 and A1TSB)

4.6.9. Chemical analysis of Gansu ore

Chemical analysis of the Gansu low grade ore used in these studies was carried out to determine the concentrations of various metals present in the materials. Ni, Zn, Mg and Cu were present as main metals (Table 4.12).

Table 4.12. Chemical analysis of Gansu ore

<table>
<thead>
<tr>
<th>Metals</th>
<th>Metal ions concentration % (W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ni</td>
<td>5.2 ± 0.03</td>
</tr>
<tr>
<td>Zn</td>
<td>2.8 ± 0.03</td>
</tr>
<tr>
<td>Mg</td>
<td>28 ± 0.05</td>
</tr>
<tr>
<td>Cu</td>
<td>3 ± 0.04</td>
</tr>
</tbody>
</table>

4.6.10. Bioleaching of low grade Gansu ore
Similarly shake flask bioleaching studies of nickel containing sulphide ore (with high amount of magnesium in main gangue) were carried out with mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB and mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *thermoplasma acidophilum*.

![Graph showing metal solubilization](image)

**Fig. 4.11. Bioleaching of low grade Gansu ore**

(A; mixed adapted consortium of *S. thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*, B; mixed consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB)

After consumption of initial acid demand flasks were inoculated. The mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* showed enhanced bioleaching potential. With low grade nickel containing sulphide ore having high amount of dolomite, diopside, talc and forsterite as main gangue, mixed adapted consortium of *thermosulfidooxidans* strain RDB and *thermoplasma acidophilum* solubilized Ni 85 %, Cu 80 %, Zn 79 %, and Mg 57 % while mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB solubilized Ni 78 %, Cu 75 %, Zn 73 %, and Mg 42 % as shown in Fig. 4.11.

**4.6.11. Chemical analysis of Daye copper ore**
Chemical analysis of the Daye ore used in these studies was carried out to determine the concentrations of various metals present in the materials (Table 4.13).

**Table 4.13. Chemical analysis of Daye ore**

<table>
<thead>
<tr>
<th>Metals</th>
<th>Metal ions concentration % (W/W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>7.2 ± 0.04</td>
</tr>
<tr>
<td>Cu</td>
<td>8.0 ± 0.04</td>
</tr>
<tr>
<td>Ni</td>
<td>1.2 ± 0.06</td>
</tr>
<tr>
<td>Co</td>
<td>0.3 ± 0.03</td>
</tr>
<tr>
<td>Zn</td>
<td>0.8 ± 0.04</td>
</tr>
</tbody>
</table>

4.6.12. Bioleaching of low grade Daye ore

Shake flask bioleaching studies of copper containing sulphide ore were carried out with mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB and mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*. Before starting bioleaching studies about 1% of pyrite was added as an energy source due to the peripheral oxidation of this ore. After consumption of initial acid demand flasks were inoculated. mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* solubelized 78, 82, 85, 85, 77 % of Co, Zn, Cu, Ni and Fe and mixed consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB solubelized 74, 79, 80, 73, 62 % of Co, Zn, Cu, Ni and Fe as shown in Fig.4.12.

In all the ores bioleaching processes, a mixed consortium of the metal adapted cultures of the above-mentioned bacteria was found to exhibit the maximum metal leaching efficiency. It is likely that the acidophilic heterotrophs contribute to the stability of the mixed mineral-oxidizing population by consuming organic excretion products produced by the mineral oxidizers (Harrison 1984). This group of organisms uses cell lysates and extracellular metabolites from autotrophs as carbon source resulting in the removal of an inhibitory excess of carbon and stimulating, therefore, growth and iron
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In all the flasks where high bioleaching rates of metal ions were observed, concomitantly bacterial biomass production rates were also high indicating high growth rates. It shows that the metal bioleaching capability of the bacteria was associated with their growth. It is due to the reason that from the oxidation of metals, chemolithotrophic bacteria obtain energy for their growth (Rohwerder et al. 2003).

![Fig.4.12. Bioleaching of Daye ore](image)

(A; mixed adapted consortium of *S. thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*, B; mixed consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB)

As compared to inoculated flasks, dissolution of metals (due to acid leaching) was significantly low in the un-inoculated control flasks in all the experiments. It showed that the bioleaching of metals is not merely an acid leaching process; rather it involves some enzymatic factors that enhance the metal leaching rates. This finding is not fully in agreement with the arguments that certain microorganisms are able to mobilize metals
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from solid materials (minerals, ores, wastes) by the formation of organic or inorganic acids (protons), by oxidation and reduction reactions; and by the excretion of complexing agents (Bosecker et al. 1997; Rawlings 2002; Brandl et al. 2001).

A decrease in the bioleaching activity was observed at the late stages of bioleaching of metals from ore. It might be due to the reason that during bioleaching processes, co-precipitation of metals with mineral phases such as jarosites reduced the leaching efficiencies as reported by Hiroyoshi et al. [1999]. In addition, the precipitation of compounds present in the leachates on the minerals to be leached can make the solid material inaccessible for bacterial leaching.

4.7. Column Bioleaching of Huangshan ore

4.7.1. Adaptation of the mixed bacteria to iron ion

Due to high level of fayallite, hematite and magnetite present in the ore, as a main gangue, ore was pre-treated with acid and the adaptation of the mixed consortium of moderate thermophiles to iron ions was performed through serial sub-culturing and gradually increasing iron ion concentration in the medium. The tolerance of the mixed bacteria to iron ions was also measured before and after adaptation and was found to improve markedly after adaptation.
Fig. 4.13. Effects of iron ions on the growth of the mixed bacteria before adaptation

Before adaptation the mixed bacterial consortium was able to tolerate about 25 g/L iron, reproduced very slowly with a lag phase of about 7 days with 28 g/L iron and died with 33 g/L iron concentration during an incubation period of 40 days as depicted in Fig. 4.13.

Fig. 4.14. Effects of iron ions on the growth of the mixed bacteria after adaptation

It can be seen from Fig. 4.14 that after one year adaptation the mixed consortium was able to tolerate up to 35 g/L iron, reproduced very slowly under 38 g/L iron ion and died during 40 d incubation at a concentration of 40 g/L iron ions.

4.7.2. Bioleaching parameters optimization in small columns

The pre-leaching of the Huangshan ore at different particle sizes and on solution pH values was investigated in small columns and the results are listed in Table 4.14.

It can be seen from Table 4.14 that some tests were aborted because of solution accumulation in columns. These columns failed to perform bioleaching at next stage and
Table 4.14. Bioleaching parameters optimization in small columns

<table>
<thead>
<tr>
<th></th>
<th>pH 0.2</th>
<th>pH 0.4</th>
<th>pH 0.6</th>
<th>pH 0.8</th>
<th>pH 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 2 mm</td>
<td>-(3d) *</td>
<td>-(5d) *</td>
<td>-(8d) *</td>
<td>-(12d) *</td>
<td>-(14d) *</td>
</tr>
<tr>
<td>≤ 5 mm</td>
<td>-(6d) *</td>
<td>-(8d) *</td>
<td>-(13d) *</td>
<td>-(18d) *</td>
<td>-(20d) *</td>
</tr>
<tr>
<td>≤ 7 mm</td>
<td>-(10d) *</td>
<td>-(15d) *</td>
<td>-(23d) *</td>
<td>-(29d) *</td>
<td>-(33d) *</td>
</tr>
<tr>
<td>≤ 10 mm</td>
<td>-(15d) *</td>
<td>-(27d) *</td>
<td>-(33d) *</td>
<td>-(35d) *</td>
<td>+ (40d; Fe 30%, Cu 2.0%, Ni 2.3%, Zn 1.0%) **</td>
</tr>
<tr>
<td>≤ 12 mm</td>
<td>-(23d) *</td>
<td>-(32d) *</td>
<td>-(39d) *</td>
<td>+ (44d; Fe 45%, Cu 3.8%, Ni 6.3%, Zn 3.5%) **</td>
<td>+ (65d; Fe 35%, Cu 2.9%, Ni 3.3%, Zn 2.0%) **</td>
</tr>
<tr>
<td>≤ 15 mm</td>
<td>-(27d) *</td>
<td>-(40d) *</td>
<td>+ (55d; Fe 37%, Cu 2.0, Ni 5.1, Zn 2.2%)</td>
<td>(73d; Fe 40%, Cu 2.5, Ni 5.3, Zn 2.2%)</td>
<td>+ (77d; Fe 36%, Cu 2.5%, Ni 5.3, Zn 2.3%) **</td>
</tr>
<tr>
<td>≤ 20 mm</td>
<td>-(39d) *</td>
<td>+(67d; Fe 41%, Cu 1.9%, Ni 2.0, Zn 2.0%)</td>
<td>+(80d; Fe 35%, Cu 3.5%, Ni 4.3, Zn 3.0%)</td>
<td>-(130d) ***</td>
<td>-(130d) ***</td>
</tr>
<tr>
<td>≤ 30 mm</td>
<td>-(45d) *</td>
<td>+(85d; Fe 39%, Cu 2.8%, Ni 4.0, Zn 3.2%)</td>
<td>- (130d) ***</td>
<td>-(130d) ***</td>
<td>-(130d) ***</td>
</tr>
</tbody>
</table>

(*: The pulverization of the ore by sulfuric acid led to an accumulation of solution in column and test was aborted after pre-leaching operation. +**: The permeability of the ore kept well, no solution accumulation was found in the column during pre-leaching operation, considered for verification test. *** pH of ore does not stabilize at 2-2.2 after 130 days of pre-leaching operation yet the permeability of ore kept well, test was aborted due to economic reasons)
RESULTS AND DISCUSSION

Table 4.15 also showed that some tests took more than 130d to stabilize the pH at 2-2.2. These tests were deleted also due to economic reason. Other tests were performed for bioleaching and the results are listed in Table 4.15.

Table 4.15. Permeability and metal extraction of charge after bioleaching operation

<table>
<thead>
<tr>
<th>Column number</th>
<th>Metal Extraction (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>particle size ≤ 10, mm Pre-leaching pH 1.0</td>
<td>+ (120d; Fe 23%, Cu 18%, Ni 20, Zn 15%)</td>
</tr>
<tr>
<td>particle size ≤ 12, Pre-leaching pH 0.8</td>
<td>+ (120d; Fe 83%, Cu 78%, Ni 72, Zn 75%)</td>
</tr>
<tr>
<td>particle size ≤ 12, Pre-leaching pH 1.0</td>
<td>+ (210d; Fe 82%, Cu 70%, Ni 78, Zn 75%)</td>
</tr>
<tr>
<td>particle size ≤ 15, Pre-leaching pH 0.6</td>
<td>+ (250d; Fe 85%, Cu 72%, Ni 75, Zn 75%)</td>
</tr>
<tr>
<td>particle size ≤ 15, Pre-leaching pH 0.8</td>
<td>+ (310d; Fe 85%, Cu 68%, Ni 76, Zn 60%)</td>
</tr>
<tr>
<td>particle size ≤ 15, Pre-leaching pH 1.0</td>
<td>+ (340d; Fe 84%, Cu 59%, Ni 79, Zn 64%)</td>
</tr>
<tr>
<td>particle size ≤ 20, Pre-leaching pH 0.4</td>
<td>+ (370d; Fe 83%, Cu 65%, Ni 81, Zn 65%)</td>
</tr>
<tr>
<td>particle size ≤ 20, Pre-leaching pH 0.6</td>
<td>+ (380d; Fe 84%, Cu 62%, Ni 80, Zn 72%)</td>
</tr>
<tr>
<td>particle size ≤ 30, Pre-leaching pH 0.4</td>
<td>+ (395d; Fe 85%, Cu 57%, Ni 74, Zn 75%)</td>
</tr>
</tbody>
</table>

It can be seen from Table 4.14 and 4.15 that the optimized particle size was 12 mm and on solution pH was controlled at 0.8 based on permeability of column, leaching time and percent metal ions solubilization. The main gangue minerals fayallite, hematite and magnetite were easily pulverized by sulfuric acid. Smaller particle size required shorter time to extract metals however leads to rapid pulverization of the ore. Larger particle size leads to better permeability however requires longer time so both of these tests were excluded. The optimized leaching conditions depend on the balance between these leaching parameters. Irrigation rate and aeration rate were also optimized. The
RESULTS AND DISCUSSION

Optimized irrigation rates were 65-70 L/m²/hr and 45-48 L/m²/hr for pre-leaching and bioleaching process respectively. The optimized aeration rate was 2-3 L/h/kg ore.

4.7.3. Experimental test

When the leaching parameters were optimized by small column reactors, experimental test and abiotic control were performed using large column reactors.

4.7.4. Pre-leaching

The pre-leaching pH and irrigation rate of on-solution were adjusted at pH 0.8 and 65-70 L/m²/hr, respectively. The effluent’s redox potential and pH of the experimental test and abiotic control are shown in Fig.4.15 and 4.16.

![Fig.4.15. Changes in redox potential of effluent during pre-leaching](image)

It can be seen from Fig. 4.15 that the effluent’s redox potentials ranged from 360 to 395 mV and showed no marked difference between the experimental test and the abiotic control. The results indicated that the pre-leaching stage was a typical chemical leaching process (Zhen et al., 2009). Fig. 4.16 also showed that the pH values of the experimental test and the abiotic control steadily decreased from pH 4.9 to pH 2.2 over 30 days and stabilized at pH 2.0-2.2 after 44 days of sulfuric acid pre-leaching. The permeability of the charge kept well. Results showed that no solution accumulation and
preferential flows were observed in both columns. Fig. 4.17 shows the trends of acid consumption during pre-leaching.

Fig.4.16. Changes in pH of effluent during pre-leaching

Fig.4.17. Acid consumed during pre-leaching
4.7.5. Bioleaching

Hydrogen ions play an important role in the energy supply of microorganisms in bioleaching systems. Biological oxidation of inorganic sulfur, sulfide and reduced iron form (Fe\(^{2+}\)) requires the presence of hydrogen ions as well as electrons. When chemolithoautotrophic bacteria and other species of the kind are present, acid is generated in situ by the biological oxidation of sulfur and metal sulfides (Kelly and Touvinen., 1988). This acid lowers the pH of the minerals in the column while releasing the metal of the value from the low grade minerals. Fig.4.18 presents a pattern of pH changes recorded during the column leaching experiments. Initially the pH value of on-solution was adjusted at 1.85-2.2 and irrigation rate was controlled at 45-48 L/ (m\(^2\)/h).

![Fig.4.18. Changes in pH during column bioleaching](image)

Figs.4.19 and Fig.4.20 show the total iron (Fe), ferrous (Fe\(^{2+}\)), ferric (Fe\(^{3+}\)) concentrations and the redox potential of the effluent during the 215 day bioleaching operation whilst 4.21 and 4.22 show the temperature changes and bacterial population during bioleaching operation.

Production of Fe\(^{3+}\) ions during oxidation of iron containing sulfide minerals resulted in more positive values of redox potential of leach suspension (Nemati and Web, 1997).
During the first 32 days, the concentration of the ferrous ion increased linearly and that of the ferric ion increased very slowly, redox potential also increased slowly.
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coinciding with a slow increase in column temperature and a low concentration of the mixed moderate thermophiles. This trend indicated that the initial phase was dominated mainly by acid-leaching and was indicative of delayed growth phase of the moderate thermophiles. However, from day 33 bacteria started to proliferate more rapidly and redox potential of solution rose which was further confirmed by increase in ferric to ferrous ratio and total iron solubilization. From day 69 to 182, high value of the ferric ion concentration, high value of the redox potential, increased temperature of the charge and high value of the mixed moderate thermophiles indicated that this phase was a typical bioleaching process and kept consistent with the logarithmic and the stationary phase of the microorganisms.

Fig.4.21. Changes in temperature during column bioleaching

At the last 33 days, the concentration of the ferric ion and the redox potential decreased coinciding with a decrease in column temperature and there was a quick depletion of microbial counts in the effluents. Such a pattern could be due to deceleration phase or adsorption-desorption phenomenon of microorganisms on mineral surface. If more bacteria are attached to the minerals in column then there will be less bacteria released free in the effluents. In such cases the rates of leaching should have been augmented but the experimental findings are not supporting this reasoning and the rates are also diminished at these points. So these trends indicated that the last phase was a
RESULTS AND DISCUSSION

regressive bioleaching process and kept consistent with the deceleration phase of the microorganisms. This regressive trend was also indicative of the termination of bioleaching operation.

![Graph showing bacterial population during column bioleaching](image)

**Fig.4.22.** Bacterial population during column bioleaching

The effluent was bled periodically to control the levels of iron ion and autoclaved basal media was added to maintain volume. Zigzag curves in above figures indicate the periodic bleeding of the effluent in both columns. In abiotic control, redox potential ranged from 320 to 415 mV, the concentration of the ferrous ions was relatively high then the concentration of the ferric ion and the temperature in the charge was nearly the same as that of ambient temperature.

It can be seen from fig. 4.23 that about 79 % Ni, 82 % Cu and 75 % Zn was solubilize within 215 days of bioleaching process from the Huangshan low-grade ore. It can also be observed from the results that iron dissolution was controlled by both acid and bioleaching processes but with the domination of acid leaching process. While in case of other metals major contribution was of bioleaching. In abiotic control about 11% of Ni, 13% Cu, 15% Fe and 10% Zn was leached out as shown in Fig.4.23.
Fig. 4.23. Percent metal ions dissolution during the whole leaching operation

4.8. Column Bioleaching of low grade Gansu ore

4.8.1. Strategies for bioleaching operation

Excessive metal ions have toxic effect on the growth of microorganisms due to inactivation of enzymes by complexation with protein (Ilyas et al., 2010). Because this low grade ore contain high amount of dolomite, diopside, talc and forsterite as main gangue so three strategies were adapted, adaptation of mixed moderate thermophiles with different mixed metal ions along with magnesium, acid pre-leaching due to the ease of dissolution of these gangue materials in acid and the periodic bleeding of the portion of effluent to prevent the excessive accumulation of magnesium ions.

4.8.2. Adaptation of microorganisms for metal ions

The effects of different metal ions on the growth of the mixed bacteria before and after adaptation are shown in Fig. 4.24a and b.
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**Fig.4.24a.** Effect of mixed metal ions on the growth of the consortium of moderately thermophiles before adaptation

In this figure, \( \text{LgC} \) is the log concentration of the bacteria in the solution (cell/mL). It can be seen from Fig. 1A that before adaptation the bacterial culture reproduced well with 5 g/L mixed metal ions (\( \text{Mg}^{2+}, \text{Cu}^{2+}, \text{Ni}^{2+} \) and \( \text{Zn}^{2+} \)) and gave a lag phase of 4 days with 10g/L mixed metal ions, gave a lag phase of 10 days with 15 g/L mixed metal ions little reproduced and then died during 42 days incubation period with 18 g/L mixed metal ions. While after adaptation of nearly one year and three months, bacterial cultures reproduced well with 18g/L mixed metal ions and gave rich growth after a lag phase of 5 days with 20 g/L metal ions, grew with 23 g/L mixed metal ions after a lag phase of 9 days and died with 25 g/L mixed metal ions during an incubation period of 42 days as depicted in Fig.4.24b. It might be due to the physiological changes that facilitated the bacteria to cope with the high metal concentration (Silver, 1996) as similar phenomena was investigated by other researchers in case of mesophiles (Zhen et al., 2009).
Fig. 4.24b. Effect of mixed metal ions on the growth of the consortium of moderately thermophiles after adaptation

4.8.3. Pre-leaching

Column pre-leaching of low grade ore at different particle sizes and on solution pH values was carried out and results are depicted in Table 4.16. Some tests were not considered to continue during the pre-leaching operation due to slumping, decrepitation and solution accumulation in the columns while some were aborted due to economical feasibility issues.

The best result was obtained at initial pH value of 0.8 and 12 mm particle size as depicted in Table 4.16. This test showed highest % extraction of magnesium ions (32.0%) along with dissolution of copper ions 5.2 %, Nickel ions 8.3% and Zinc ions 7%. Column permeability was also good and column was singled out for bioleaching operation at the next stage. Chemical content of the bleeds from the effluents was Ni 0.50-0.55 g/L, Zn 0.7–0.8 g/L, Cu 0.68–0.79 g/L and Mg 38-40 g/L.
RESULTS AND DISCUSSION

Table 4.16 Column pre-leaching of ore at different particle sizes and on solution pH values

<table>
<thead>
<tr>
<th>Particle Size of ore</th>
<th>pH 0.2</th>
<th>pH 0.4*</th>
<th>pH 0.8*</th>
<th>pH 1.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤ 5 mm</td>
<td>-(10d) *</td>
<td>-(15d) *</td>
<td>-(28d) *</td>
<td>-(30d) *</td>
</tr>
<tr>
<td>≤ 10 mm</td>
<td>-(25d) *</td>
<td>-(32d) *</td>
<td>-(45d) *</td>
<td>-(38d) *</td>
</tr>
<tr>
<td>≤ 12 mm</td>
<td>-(33d) *</td>
<td>-(42d) *</td>
<td>+ (Mg 32 %, Cu 5.2%, Ni 8.3%, Zn 7%) **</td>
<td>+ (Mg 28 %, Cu 4.2%, Ni 8.0%, Zn 3%)</td>
</tr>
<tr>
<td>≤ 15 mm</td>
<td>-(37d) *</td>
<td>-(50d) *</td>
<td>+ (Mg 25 %, Cu 5%, Ni 5.7%, Zn 5%)</td>
<td>+ (Mg 26 %, Cu 5.2%, Ni 6.8%, Zn 5%)</td>
</tr>
</tbody>
</table>

−*: The pulverization of the ore by sulfuric acid led to an accumulation of solution in column and test was aborted after 26 days pre-leaching operation. +**: The permeability of the ore kept well, no solution accumulation was found in the column during 52 days pre-leaching operation, maximum value of metal ions leached during pre-leaching operation (error is ≤ ±0.08).

Although the ferrous and ferric iron concentrations of the effluents were not investigated during pre-leaching process, the redox potential of the effluents ranged from 310–415 mV as depicted in Fig.4.25.

The pH value of the effluents steadily decreased from pH 6.2 to pH 2.5 over 45 days and stabilized at pH 1.8-2.0 after 50 days sulfuric acid pre-leaching operation as shown in Fig.4.26. Here pre-leaching has two fold objectives one was to provide rough estimate of acid consumption and second was to prepare ore surface for bacterial attack.
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Fig. 4.25. Changes in redox potential during pre-leaching

Fig. 4.26. Changes in pH of effluent during pre-leaching
4.8.4. Column Bioleaching

After pre-leaching, bioleaching operation was carried out and during whole bioleaching studies the pH of on solution was controlled at 1.8-2.2 with concentrated sulfuric acid.

Figs. 4.27, 4.28 and 4.29 show the pH, total iron (Fe Total), ferrous (Fe\(^2^+\)), ferric (Fe\(^3^+\)) concentrations and redox potential of the effluents during the 195 day bioleaching operation whilst Fig. 4.30 depicted the temperature changes during bioleaching studies.

![pH Changes during Column Bioleaching](image)

**Fig. 4.27.** Changes in pH during column bioleaching

Although the pH of the on-solution was controlled at pH 1.8-2.2, the pH of the effluent ranged from 1.8 to 2.5. At the start of bioleaching operation an increase in pH values was observed and this rise in pH might be due to the fact that initially bacteria were unable to produce acid through oxidation of sulfide moiety of ore at the rate required for neutralization of acid consuming material present in the ore (Rahman, 1998). A more consistent decrease of pH value was observed from day 28 (Fig. 4.27) indicating that acid was continuously consumed. Hence, the sulfuric acid consumption was 220 kg per ton of the ore during the bioleaching stage. It can be seen from Fig.4.28 that total iron and ferrous concentrations increased linearly while ferric concentrations increased slowly during the initial 27 days.
Figs. 4.28. Changes in total iron, ferrous and ferric during column bioleaching

Fig. 4.29 shows the redox potential of the effluent also increased slowly from 395 to 456 mV coinciding with a slow increase in column temperature Fig. 4.30 and of the
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delayed growth phase of bacteria. This trend indicated that the initial 27-days leaching stage was typically chemical acid leaching.

![Changes in temperature during column bioleaching](image)

**Fig.4.30.** Changes in temperature during column bioleaching

However, during the second 19 days from day 27 to day 46, the concentrations of ferric ions increased and ferrous ions decreased in a linear pattern and the redox potential rose to 570 mV coinciding with an increase in column temperature from 40 to 45 °C. The trend indicates that the second 19-days leaching stage was initial stage of bioleaching actually. During the third 120 days from day 46 to day 166, the concentrations of Fe$^{3+}$ and Fe$^{2+}$ ranged from 4.2 to 10.8 g/L and from 0.18 to 1.0 g/L respectively and the redox potential ranged from 575 to 680 mV coinciding with a stabilized increase in column temperature from 43-50 °C which was indicative of rich bacterial activity and dominant bioleaching operation. However, during the last 29 days, Fe$^{3+}$ concentration decreased and Fe$^{2+}$ increased while the redox potential decreased from 655 to 478 mV and the column temperature decreased also from 50 to 42 °C indicative of regressive bioleaching. From above discussion it is evident that an optimum value of redox potential and iron solubilization is essential to attain maximum bioleachability of metals as reported by many other authors (Hiroyoshi et al., 1997; Rubio et al., 1994; Konishi et al., 1992, Garcia 1993). Consequently, there may be a relationship between optimum ferric ion
RESULTS AND DISCUSSION

concentration and bioleaching efficiency. The presence of ferric ions in excess of its optimal concentration resulted in the formation of insoluble compounds i.e (Verbaan and herberts 1988) that ultimately decrease the bioleaching efficiency through making the passive layers around sulfide minerals.

During the 195 days of column bioleaching studies, the effluent was bled periodically to control the level of magnesium in the main gangue and volume was compensated by the addition of autoclaved basal media.

Residue analysis was carried out after dismantling the column at the termination of the bioleaching test. The metal solubilization calculated from the intermediate samples analyses of the effluent during the whole leaching period is shown in Fig. 5. Magnesium solubelization was mostly due to chemical leaching as investigated by Zhen et al (2009) in case of bioleaching of similar type of ore with mesophiles but as compared to those studies the mixed consortium of moderate thermophiles show higher potential for percent dissolution of copper in a relatively short bioleaching period. The linear dissolution of nickel and zinc ions was controlled by both chemical acid leaching and bioleaching. The chemical composition of the bleeds from the effluent were Ni 2.5-3.0 g/L, Zn 3.8–4.6 g/L, Cu 5.5–6.3 g/L and Mg 12-22 g/L. The metal dissolution calculated from the sample analyses of the residue after dismantling the column were Ni 81%, Cu 78%, Zn 76%, and Mg 52%.

4.9. Column Bioleaching of low grade sulphide ore of copper

4.9.1. Pre-strategies for bioleaching operation

Copper containing low grade sulphide ore was oxidized at surface so for carrying out bioleaching operation it was first mixed with 3% pyrite as an energy source for the healthy growth of microorganism. Secondly adaptation of microorganisms with mixed metal ions was carried out. Thirdly due to the presence of magnesite, siderite and dolomite with lesser amount of sphaerocobaltite as main gangue, acid pre-leaching was carried out.

4.9.2. Acid consumption by low grade sulphide ore of copper

Before inoculating columns the pH of ore was stabilized. This experiment has two fold objectives; 1) It gave an estimate of the acid consumption by the ore 2) It prepared the ore surface for bacterial attack under optimum conditions in the next stage. Each column was washed with autoclaved distilled water. These washings were found to contain negligible amount of iron and dissolved copper. Then autoclaved basal salt
solution of pH 2.0 was added in each column. After 96 hour the pH of the effluent was raised to 6.3 due to solubelization of basic materials mainly carbonates present in ore. Medium was recirculated again and again with the addition of concentrated sulfuric acid and pH of effluent was monitored continuously. After 672 hour of recirculation pH started to decrease steadily and after 960 hour of recirculation 42.5ml of concentrated sulfuric acid was consumed and pH was maintained at almost 2.0 in each column as shown in Fig. 4.31.

**Fig.4.31.** Changes in pH during pre-leaching

It could be seen from Fig. 4.32 that effluent's redox potential remained in the range of 340 to 450 mV. This indicates that preleaching process was a typical acid leaching process. During this whole process permeability of columns remained well and no slumping, solution accumulation and preferential flows were found in columns and 5.5% Fe, 2.2% Zn, 5.2% Cu 3% Ni and 2.8% Co was leached out.
4.9.3. Adaptation of microorganisms to metal ions

Effects of metal ions on the growth of moderately thermophilic chemolithotrophic bacteria before and after adaptation are shown in Fig.4.33a and b. Before adaptation bacterial culture reproduced after a lag phase of 11 days with 20 g/L mixed metal ions and died during 37 days incubation period with 25 g/L mixed metal ions. While after adaptation of nearly one year’s bacterial cultures reproduced well with 20 g/L mixed metal ions and gave rich growth after a lag phase of 7 days with 25 g/L metal ions. With 28 g/L metal ions, bacterial cultures grew slowly with a lag phase of 12 days and died with 30 g/L mixed metal ions during an incubation period of 37 days as depicted in Fig.4.33b. It might be due to physiological changes, occur when the microorganisms were acclimatized to tolerate increasing concentrations of metal ions by repeated sub-culturing on metal containing media that facilitated the bacteria to cope with the high metal concentration in the media during bioleaching and further contribute toward the enhancement of process efficiency.
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Fig.4.33a. Effect of mixed metal ions on the growth of the consortium of moderately thermophiles before adaptation

Fig.4.33b. Effect of mixed metal ions on the growth of the consortium of moderately thermophiles after adaptation
4.9.4. Column bioleaching studies

Fig.4.34 shows the changes in pH during 212 days of bioleaching period. Initially some increase in pH was observed during first 32 days of bioleaching that was consistent with the delayed growth of bacteria and depicts that this stage is mostly contributed to chemical acid leaching instead of bioleaching. Then from day 33, pH started decreasing due to the hydrolysis of the metal ions that were released in the medium due to microbial action and continued up to day 160 with pH 2.0 Trend of pH indicates that this stage was typically bioleaching stage instead of chemical acid leaching. Then pH started to decrease very slowly up to pH 1.87 from day 161 to 185 and no significant change in pH was observed after the day 212 and pH remained 1.85 till the termination of bioleaching operation. So pH remained in the range of 1.85 to 2.6 throughout the bioleaching operation. While in a biotic control pH remained almost in the range of 3.3 to 3.9.

Fig.4.34. Changes in pH during column bioleaching studies

Iron species present in the effluents of two columns were monitored and results have been presented in Fig.4.35.
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Fig. 4.35. Changes in total iron, ferrous and ferric during column bioleaching

Fig. 4.36. Changes in redox potential during column bioleaching
It can be seen from Fig. 4.35 that ferric concentration increased very slowly during first 32 days but total iron and ferrous concentration at the start increased in a quite linear pattern and redox potential of effluents also increased very slowly as shown in Fig. 4.36, coinciding with low growth of bacterial cultures and domination of acid leaching stage.

Then from day 33 to day 56 the ferric ions concentration started to increase while ferrous ions concentration started to decrease linearly and from day 66 to day 185 the concentration of ferric ions increased up to 1690 mg/L (Fig. 4.35) and high value of redox potential 652 mV was observed (Fig. 4.36). This trend was attributed to bioleaching stage instead of acid leaching and indicative of good bacterial activity as seen from Fig. 6. Then from day 186 to 212 the concentration of ferric ions start to decrease little and value of redox potential decreased from 682 to 485 mV respectively, indicative of regressive bioleaching and deceleration phase of bacteria.

The metal ions solubilization calculated from the analysis of the effluents during the whole leaching period is shown in Table 4.17.

Table 4.16. Percent metal ions dissolution in experimental test and abiotic control during the whole leaching operation

<table>
<thead>
<tr>
<th>Test/Control</th>
<th>Metals solubilized</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Co</td>
</tr>
<tr>
<td>Pre-leaching (intermediate solution analysis) (%)</td>
<td>2.5/2.5</td>
</tr>
<tr>
<td>Bioleaching (intermediate solution analysis) (%)</td>
<td>72/7</td>
</tr>
<tr>
<td>Whole stage (Sample analysis of residue) (%)</td>
<td>81/26</td>
</tr>
<tr>
<td>Periodic bleeds of pre-leaching (PLS) (g/L)</td>
<td>0.42/0.41</td>
</tr>
<tr>
<td>Periodic bleeds of bioleaching (PLS) (g/L)</td>
<td>4.0/0.045</td>
</tr>
</tbody>
</table>

These dissolution trends of metals were different in preleaching and bioleaching operations. Only a small quantity of zinc, copper, cobalt and nickel dissolution was
RESULTS AND DISCUSSION

observed during the pre acid consuming operation. These dissolution trends of nickel and zinc were quite linear while copper and cobalt dissolved slowly but iron dissolves more than other metals during this pre leaching period.

During whole bioleaching operation about 74, 79, 84, 78, 51 % of Co, Zn, Cu, Ni and Fe were leached out with mixed consortium of metal-adapted cultures of *Sulfobacillus thermosulfidooxidans* and the acidophilic heterotrophs. These results indicated that dissolution of iron was contributed to both acid leaching and bioleaching but dissolution of copper, nickel, cobalt and zinc were mainly contributed to bioleaching.

High leaching rates in present study were possibly due to the synergistic effects of acidophilic heterotrophs on the growth of *Sulfobacillus thermosulfidooxidans*. Nickel, copper, zinc, Cobalt and Iron leaching rates no longer increased from 186 to 212, indicating the termination of leaching operation. During preleaching and bioleaching operation, the temperature of column charge was remained in the range of 40-50 °C.

In case of uninoculated controls the percent leachabilities were significantly low about 4.0, 3.8, 5.2, 4.5 and 5.2 % for Co, Zn, Cu, Ni and Fe, respectively. This pattern shows that the leaching of metal ions from the low grade sulphide ore of copper due to chemical oxidation was insignificant and major contribution is of bioleaching for dissolution of metals.

4.10. Shake flask bioleaching studies of electronic scrap

4.10.1. Chemical analysis of electronic scrap

Chemical analysis of electronic scrap used in these shake flask bioleaching studies was carried out to determine the concentrations of various metals present in the material. The major metal ions in the electronic scrap/printed circuit boards were found to be zinc (8.0%), iron (8.0%) and copper (8.5%) with considerable amounts of nickel (2.0%) and lead (3.15%) as well. In addition to these metal ions, small amounts of precious metals were also present (Table 4.18). Metallic content constituted about 30% of the electronic scrap/printed circuit boards, rest of the material being non-metallic content. Heterogeneity of material was observed when comparison was made among the concentrations of different metal ions obtained in these studies and those reported by Brandl *et al* (2001) and Veit *et al* (2002).
Table 4.18. Chemical analysis of electronic scrap for metal content

<table>
<thead>
<tr>
<th>Metals</th>
<th>Metal ions conc. % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>8.0±0.05</td>
</tr>
<tr>
<td>Cu</td>
<td>8.5±0.05</td>
</tr>
<tr>
<td>Ni</td>
<td>2.0±0.06</td>
</tr>
<tr>
<td>Pb</td>
<td>3.15±0.05</td>
</tr>
<tr>
<td>Ag</td>
<td>0.0029±0.0004</td>
</tr>
<tr>
<td>Au</td>
<td>0.00124±0.0003</td>
</tr>
<tr>
<td>Al</td>
<td>0.71±0.02</td>
</tr>
<tr>
<td>Zn</td>
<td>8.0±0.05</td>
</tr>
</tbody>
</table>

The minor difference in the concentration of metal ions in various electronic scrap samples may be attributed, to some extent, to the analytical methods used in the experiments, but the major differences in the concentrations of some metal ions particularly aluminium and zinc seems to be mainly dependent upon the origin of the material. Scraps of such varied natures may need different strategies to be adopted for microbiological processing.

4.10.2. Preliminary bioleaching studies

Preliminary bioleaching studies were carried out with different pure cultures and different mixed consortium of these microorganisms using unwashed electronic scrap and pulp density of 10% as shown in Fig. 4.37.

The pure cultures and combination (mixed consortium) with preferentially good bioleaching potential were selected for further studies. It can be seen from Fig. 4.37 that bioleaching potential of *Sulfobacillus thermosulfidooxidans* was comparatively better then other cultures that might be attributed due to superior kinetics of moderate thermophiles as compared to mesophiles (Das *et al*. 1999; Deveci *et al*., 2004).

Percent metal solubilization ability of different mixed consortiums of unadapted bacterial cultures was also investigated as shown in Fig. 4.38.
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Fig. 4.37. Percent metal solubilization after 18 days of bioleaching of electronic scrap with different pure bacterial cultures (A: with *Sulfobacillus thermosulfidooxidans* strain MT-13. B: *Sulfobacillus thermosulfidooxidans* strain-RDB. C: with *Acidithiobacillus ferrooxidans*. D: with *Acidithiobacillus thiooxidans*. E: with heterotroph A1TSB. F: with *Thermoplasma acidophilum*).

Mixed consortium of *Sulfobacillus thermosulfidooxidans* with heterotrophs leached out all the metals at highest rates as compared to those attained from other consortium and among these, the consortium of *Sulfobacillus thermosulfidooxidans* strain- RDB with *thermoplasma acidophilum* was found to have higher bioleaching rates then others. First reason in this regard might be due to the excretion of extracellular metabolites by chemolithoautotrophs and use of these metabolites as a carbon source by chemolithoheterotrophs and second reason may be attributed to mutual stability of both strains (Butler and Kempton, 1987).
Fig. 4.38. Percent metal solubilization after 18 days of bioleaching of electronic scrap with different mixed consortium (A: Mixed consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and *T. acidophilum*. B: *Acidithiobacillus ferrooxidans* and *Acidithiobacillus thiooxidans*. C: *Sulfobacillus thermosulfidooxidans* strain MT-13 and heterotroph A1TSB. D: *Sulfobacillus thermosulfidooxidans* strain-RDB and heterotroph A1TSB. E: *S. thermosulfidooxidans* strain-RDB and *Thermoplasma acidophilum*).

4. 10.3. Metal bioleaching studies

In this work the results are presented for 10% scrap concentration, which exhibited the highest metal bioleaching rates, on the basis of our previous experiments that were conducted at lower (5% w/v) and higher (20% w/v) electronic scrap concentrations.

In a previous study by Brandl *et al.* (2001a), a harmful effect of electronic scrap was observed on the growth of *Acidithiobacillus ferrooxidans* that can generally tolerate high metal ions concentrations. In the present study, purpose behind washing the electronic scrap was to remove its non-metallic component and hence to investigate if this part of the electronic scrap exerts any toxic effect on the activity of the microorganisms.
used in this investigation. As the potential toxic components, i.e. organic compounds and plastics, have lower specific gravity than metallic part, a high density solution (saturated NaCl solution) was used to separate these components without changing their Physico-chemical properties. It is worthy to mention that there was no change in the metal ions concentration of scrap/printed circuit boards before and after washing.

Initial metal bioleaching studies were conducted with unadapted cultures and unwashed electronic scrap. Lag phases of 5 to 7 days were observed under these conditions with mixed consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and heterotroph A1TSB while lag phases of 3 to 5 days were observed under these conditions with mixed consortium of *Sulfobacillus thermosulfidooxidans* strain-RDB and *Thermoplasma acidophilum*. Therefore, cultures and/or washed electronic scrap metal adapted were used in the subsequent experiments.

Using unadapted cells or unwashed electronic scrap resulted in reduced metal bioleaching rates, the former being more drastic. Enhanced bioleaching rates were observed when washed electronic scrap was subjected to bioleaching operation with metal-adapted cultures. It might be due to the fact that when the microorganisms were acclimatized to tolerate increasing concentrations of metal ions by repeated sub-culturing on metal containing media, there were physiological changes that facilitated the bacteria to cope with the high metal ion concentrations in the media during bioleaching.

Many genetic systems are known in microorganisms particularly in bacteria for acquiring resistance against toxic metals and maintaining intracellular homeostasis of essential metal ions (Silver, 1996). Among these, the well-studied genetic mechanisms of metal resistance in bacteria include the presence of metal binding proteins (Olafson *et al.*., 1988; Robinson *et al.*., 1990) and heavy metal efflux systems (Nies and Silver, 1995). Surprisingly higher metal leaching rates were observed when bioleaching studies were conducted in the presence of sulfur, though the purpose of adding sulfur in the medium was to generate sulfuric acid in situ for omitting the pH stabilization step. As discussed in the following sections, this might be due to the enhanced growth of the microorganisms in the presence of sulfur, which acted as an additional energy source for their growth. After 18 days of bioleaching under different experimental conditions, percent bioleachabilities of metal ions were observed.
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Maximum percent bioleachability was obtained for washed electronic scrap with mixed consortium of metal adapted culture of *Sulfobacillus thermosulfidooxidans* strain RDB and acidophilic heterotrophs (*Thermoplasma acidophilum*). Among metals, copper exhibited maximum bioleachability (92 % w/w) while 86, 83 and 84 % of Ni, Al and Zn were leached out, respectively, and with mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB about 89, 81, 79 and 83 % of Cu, Ni, Al and Zn were leached out after 18 days (Fig.4.39).

![Graph showing metal solubilization](image)

**Fig.4.39.** Percent metal solubilization after 18 days of bioleaching of electronic scrap under different conditions (A: Unwashed ES with adapted cells of *S. thermosulfidooxidans* strain MT-13, B: Washed ES with unadapted cells of *S. thermosulfidooxidans* MT-13, C: Washed ES with adapted culture of *S. thermosulfidooxidans* MT-13, D: Washed ES with sulfur and adapted cells of *S. thermosulfidooxidans* MT-13, E: Washed ES with adapted mixed culture of *S.thermosulfidooxidans* MT-13 and A1TSB, F: Washed ES with adapted mixed culture of *S.thermosulfidooxidans* strain-RDB and *Thermoplasma acidophilum*).

Mixed consortium of metal-adapted cultures of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* leached out all the metals at highest yield as compared to those attained under other experimental conditions.
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(Fig.4.40). For chemolithoautotrophic bacteria such as *Acidithiobacillus ferrooxidans*, it is considered that the acidophilic heterotrophs contribute to the stability of the mixed mineral-oxidizing population by consuming organic excretion products produced by the other mineral oxidizers (Harrison, 1984).

![Figure 4.40](image)

**Fig.4.40.** Percent metal solubilization after 18 days of bioleaching of electronic scrap under different conditions (A: Unwashed ES with adapted cells of *S. thermosulfidooxidans* strain MT-13, B: Washed ES with unadapted cells of *S. thermosulfidooxidans* MT-13, C: Washed ES with adapted culture of *S. thermosulfidooxidans* MT-13, D: Washed ES with sulfur and adapted cells of *S. thermosulfidooxidans* MT-13, E: Washed ES with adapted mixed culture of *S. thermosulfidooxidans* MT-13 and A1TSB, F: Washed ES with adapted mixed culture of *S. thermosulfidooxidans* strain-RDB and *Thermoplasma acidophilum*).

This group of organisms uses cell lysates and extracellular metabolites from autotrophs as carbon source resulting in the removal of an inhibitory excess of carbon and therefore stimulating growth of chemolithoautotrophs (Butler and Kempton, 1987; Fournier *et al.*, 1998). Contrarily, *Sulfobacillus thermosulfidooxidans*, the bacterium used in the present studies requires an initial supply of fixed carbon source for its optimum growth (Ghauri and Johnson, 1991). In addition, several acidophilic heterotrophs can also
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contribute to metal solubilization by the excretion of organic acids such as citrate, gluconate, oxalate, or succinate (Johnson, 1998).

It may be mentioned that pure culture of acidophilic heterotroph was unable to leach out metals from electronic scrap with significant level. Therefore, the enhancement of metal leaching rate in this case was possibly due to synergistic effects of acidophilic heterotrophs on the growth of *Sulfobacillus thermosulfidooxidans*.

White precipitates observed in the experimental flasks during bioleaching of electronic scrap with *Sulfobacillus thermosulfidooxidans* MT-13 and *Sulfobacillus thermosulfidooxidans* RDB. Although it was not possible to separate all these precipitates from the remaining electronic scrap, a small portion of these was decanted from the flask in which mixed microbial consortium was used for bioleaching and the composition of these precipitates was determined by atomic absorption spectrophotometry (Table 4.19 and 4.20).

**Table 4.18. Concentrations of various metals in the white precipitates formed during bioleaching of electronic scrap with adapted mixed culture of *S.thermosulfidooxidans* MT-13 and A1TSB as determined by atomic absorption spectroscopy**

<table>
<thead>
<tr>
<th>Element</th>
<th>Content (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>0.7±0.05</td>
</tr>
<tr>
<td>Copper</td>
<td>2.0±0.08</td>
</tr>
<tr>
<td>Iron</td>
<td>2.0±0.07</td>
</tr>
<tr>
<td>Lead</td>
<td>20.0±0.08</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.25±0.005</td>
</tr>
<tr>
<td>Tin</td>
<td>6.9±0.05</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.09±0.006</td>
</tr>
</tbody>
</table>

It is evident from the analysis of these precipitates that lead and tin were concentrated in the white precipitates along with some other metals like copper, iron and aluminium. In a previous investigation by Brandl *et al.* (2001a), lead and tin were not detected in the leachate; rather these metals were speculated to be precipitated as lead sulphates and tin oxide respectively. Our results obtained from atomic absorption
RESULTS AND DISCUSSION

spectrophotometric analysis confirm the presence of both of these metals in the precipitates.

Table 4.19. Concentrations of various metals in the white precipitates formed during bioleaching of electronic scrap with adapted mixed culture of *S. thermosthodioxidans* strain RDB and *T. acidophilum* as determined by atomic absorption spectroscopy

<table>
<thead>
<tr>
<th>Element</th>
<th>Content (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aluminium</td>
<td>0.63±0.05</td>
</tr>
<tr>
<td>Copper</td>
<td>1.75±0.05</td>
</tr>
<tr>
<td>Iron</td>
<td>1.75±0.05</td>
</tr>
<tr>
<td>Lead</td>
<td>23.0±0.07</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.23±0.005</td>
</tr>
<tr>
<td>Tin</td>
<td>7.2±0.06</td>
</tr>
<tr>
<td>Zinc</td>
<td>0.08±0.005</td>
</tr>
</tbody>
</table>

The leaching mechanism of copper from printed circuit boards/electronic scrap by *Acidithiobacillus ferrooxidans* has been suggested to be similar to that of metal sulfides (Choi *et al.*, 2004). Previously, it was documented that certain microorganisms are able to mobilize metals from mineral, ores and wastes (solid material) by the formation of organic or inorganic acids (protons), by oxidation and reduction reactions, and by the excretion of complexing agents (Bosecker, 1997; Brandl, 2001a). Some other researchers have shown that sulfuric acid is the main inorganic acid found in leaching environments formed by sulfur-oxidizing microorganisms such as Acidithiobacillus species (Rawlings, 2002; Sand *et al.*, 2001).

A series of organic acids have also been reported to be produced by bacterial (as well as fungal) metabolism resulting in organic acidolysis, complex and chelate formation (Brandl, 2001b; Burgstaller and Schinner, 1993).

On the basis of our results from shake flask bioleaching of electronic scrap, it might be speculated that similar bioleaching mechanism was operational in case of *Sulfobacillus thermosthodioxidans* in the present studies. In case of abiotic controls the
maximum percent leachability (0.2%) was exhibited by nickel in the presence of sulfur. Under all other experimental strategies and for other metal ions the percent bioleachability was significantly lower than this value. This shows that the bioleaching of metal ions from the electronic scrap due to only chemical oxidation was insignificant.

4. 10.4. Changes in pH during bioleaching of metals from electronic scrap

It is documented, previously, that electronic scrap is alkaline in nature, resulting in increase in the pH of the medium (Brandl et al 2001a). As described in earlier studies, electronic scrap has a complex composition and it has not yet been studied particularly which component contributes towards its alkalinity. However, it is very worthy to mention that this alkaline nature of electronic scrap was not exhibited when it was stirred with distilled water. This phenomenon was only observed when the electronic scrap was subjected to bioleaching in the medium having initial pH of 2.0, depicting that some alkaline materials were mobilized from the electronic scrap due to the action of acid in the medium.

In the present shake flask study, pH profile of the medium was studied under three different bioleaching conditions viz unwashed ES, washed ES and washed ES with addition of sulfur to the medium. With unwashed ES, pH of the medium rose up to 3.4 in 3 days in case of *Sulfobacillus thermosulfidooxidans* MT-13 and A1TSB while rose up to 3.3 in case of adapted mixed culture of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*, in spite of the fact that sulfuric acid was added after every 24 h to the medium to bring the pH down to 2.0. It was only at the day 3 in case of mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* and at the 4th day in case of mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB when no further increase in the pH was observed, flasks were considered ready for inoculations (Fig.4.41). The increase in pH with washed ES was not as high as in case of the unwashed ES, showing that the non-metallic part of the ES also contributed towards its alkalinity. Under both of these above mentioned conditions, inoculations were made when no further increase in pH was observed. After inoculation, the pH started dropping down due to the hydrolysis of the metal ions that were released in the medium due to microbial action. No significant changes in the pH were observed after the day 6 in both flasks. In contrast to the above
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mentioned conditions, pH did not rise up in the flasks in which sulfur was added as an additional energy source along with ES.

![Graph showing pH changes during bioleaching](image)

**Fig.4.41.** Changes in pH of the medium during bioleaching of electronic scrap by metal adapted mixed culture of *S. thermosulfidooxidans* under different conditions

In this case the washed electronic scrap, sulfur and the inoculum were added simultaneously on the same time and the bacteria started producing sufficient sulfuric acid by the oxidation of sulfur right from the beginning of the reaction, which prevent to increase the pH of the medium further.

Such strategy has also been successfully implemented by Salo-zieman *et al* (2006), where they coupled the biologically elemental sulfur oxidation with the leaching of nickel from acid-consuming ore to overcome the high acid demands.

**4. 10.5. Determination of microbial growth during bioleaching of electronic scrap**

The biomass production rates (in terms of total cellular protein), Corresponding to the bioleaching rates, were also high in flasks in which mixed consortium of adapted cells of *Sulfobacillus thermosulfidooxidans* and acidophilic heterotrophs were used for
bioleaching of metals from electronic scrap. While biomass production rates in other flasks were comparatively low (Figs. 4.42 and 4.43).

**Fig. 4.42.** Growth (determined as total cellular protein) of *S. thermosulfidooxidans* (MT-13) during bioleaching of metals from electronic scrap under different experimental conditions. (Unw- unwashed scrap, W.S- washed Scrap, Unadapt.C- unadapted culture, Adapt. C- adapted culture, Mix. Adapt. C- Mixed adapted culture).

But among the *Sulfobacillus thermosulfidooxidans* strain MT-13 and *Sulfobacillus thermosulfidooxidans* strain RDB, the biomass production rates of later were higher then former as shown in Fig.4.42 and Fig.4.43. These results indicated that the metal ions bioleaching efficiency of the bacteria was associated with their active growth as they obtained energy for their growth from the oxidation of metal ions. It may be mentioned that these growth results provide only a rough estimate of the growth and do not give an exact measurement of the biomass in the shake flasks, as these readings are only for planktonic cells, while cells attached to the particulate matter were not accounted for. Furthermore, the plots for mixed adapted cultures should not be regarded as true growth curves due to presence of two varied types of bacteria. The results of the experiments with unwashed adapted cells + ES and unadapted cells + washed ES give a comparison between the toxic effects of metallic and non-metallic components of the scrap on microbial activity.
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Fig. 4.43. Growth (determined as total cellular protein) of *S. thermosulfidooxidans* (RDB) during bioleaching of metals from electronic scrap under different experimental conditions. (Unw- unwashed scrap, W.S- washed Scrap, Unadapt.C- unadapted culture, Adapt. C- adapted culture, Mix. Adapt. C- Mixed adapted culture).

In case of unwashed electronic scrap and adapted cells, the major toxic constituent was the non-metallic part, as the metal adapted cells were used in this experiment. Whereas in case of washed electronic scrap and adapted cells, the non-metallic part was removed from the electronic scrap and metal unadapted cells were used for bioleaching. Therefore, the major factor that shows inhibitory effect in this case was the metallic part of the scrap. The growth curves for electronic scrap bioleaching either with unadapted cells or unwashed electronic scrap with single culture show that both, the metal components as well as the non-metal moiety, have inhibitory effect on growth of the bacteria used in these studies. In case of unwashed electronic scrap and adapted cells, the metal leaching rate as well as the microbial growth is better than washed electronic scrap and unadapted cells (Figs. 4.42 and 4.43), which shows that the metal component of the scrap exerted higher inhibitory effect as compared to the non-metal component.

Similarly, biomass production rate in case of washed electronic scrap and adapted cells was 4.7mg/L/day, which is slightly higher than that observed (4.5 mg/L/day) in case of
unwashed electronic scrap and adapted cells of *Sulfobacillus thermosulfidooxidans* strain MT-13. Significantly higher biomass production rate (5.5mg/L/day) was observed when the bioleaching medium was supplemented with elemental sulfur depicting enhanced growth rate in the presence of sulfur due to the presence of additional energy source. Even higher then these biomass production rates were observed but with same pattern in case of *Sulfobacillus thermosulfidooxidans* strain RDB.

4.11. Bioleaching of Electronic scrap in a Bubble reactor

4. 11.1. Source and Chemical analysis of electronic scrap

Electronic scrap was collected from electronic shop in Wuhan, China. Chemical analysis of scrap showed the concentration of metals as mentioned in Table.4.21.

<table>
<thead>
<tr>
<th>Metals</th>
<th>Metal ions conc. % (w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fe</td>
<td>8.0±0.03</td>
</tr>
<tr>
<td>Cu</td>
<td>8.9±0.03</td>
</tr>
<tr>
<td>Ni</td>
<td>2.0±0.04</td>
</tr>
<tr>
<td>Pb</td>
<td>3.15±0.05</td>
</tr>
<tr>
<td>Ag</td>
<td>0.0030±0.0004</td>
</tr>
<tr>
<td>Au</td>
<td>0.0013±0.0003</td>
</tr>
<tr>
<td>Al</td>
<td>0.75±0.03</td>
</tr>
<tr>
<td>Zn</td>
<td>8.2±0.03</td>
</tr>
<tr>
<td>Sn</td>
<td>0.00065±0.0002</td>
</tr>
</tbody>
</table>

4. 11.2. Effect of hydraulic retention time (HRTs) on percent metals dissolution

Firstly, the bubble column reactor was run for 20 days with a series of different HRTs (180, 160, 140, 120, 100 and 80). The steady-running parameters, including pH, redox potential, microbial population and metals recovery, were obtained from the reactor for an HRT of 140 h and after a start up of 10 days. The reactor was subsequently operated for 40 days with HRTs of 140, 130, 120 h and 100h, respectively.
Figure 4.44 shows that the metal recovery ratio decreased as the HRT decreased from 140HRT to 130HRT and onward. With each change of HRT, average metals recovery ratios of 16, 20, 28 and 36% for Al, 30, 35, 43 and 58 % for Ni, 48, 57, 60 and 72 % for Zn and 70, 75, 80 and 85 % for Cu were achieved for HRTs of 100, 120, 130 and 140h, respectively, when the running of the reactor had stabilized.

Cu, Zn, Ni and Al recovery ratios decreased by 5, 12, 5, 4 % when 120 HRT is shifted to 100HRT. However, the metals recovery ratios increased by 5, 3, 8, 8 % with the increase in HRT of 130 from 120 h. while about 5, 2, 12 and 8 % increase in metal ions recovery was observed when moved from 130 to 140h. In addition, the process efficiency significantly declined with every decrease in HRT, and the reactor slowly recovered to attain a new steady state: 20 days from stage 3 to stage 4, compared with 16 days from stage 2 to stage 3 and10 days from stage 1 to stage 2. This evidence indicates that a reactor inoculated with mixed consortium is not suited to running at HRTs that are too low due to its assailability to a high slurry feed and loading rate. For the investigated HRTs, the reactor is suited to running at an HRT of 140 h with maximum percent metal ions dissolution.

Fig.4.44. Percent metal ions solubilization with different HRTs in a bubble reactor
4. 11.3. Changes in pH and redox potential at different stages

Changes in pH of the effluent are shown in Fig. 4.45 as a function of time. pH remained in the range of 1.7 to 2.2 during the whole process stages, except at the start of stage 1. At the start of stage 1 from day 10 to day 15, the pH value of the effluent solution slightly increased from 2.0 to 2.5 than that of the feeding medium that was may be due to the nature of electronic scrap.

![Fig.4.45](image)

**Fig.4.45.** Changes in pH of the effluent at different stages of bioleaching operation in a continuous flow bubble reactor (Stage-1 depicts the bioleaching operation at 140 HRT, Stage-2 depicts the bioleaching operation at 130 HRT, Stage-3 depicts the bioleaching operation at 120 HRT and Stage-4 depicts the bioleaching operation at 100 HRT).

Electronic scrap has been documented to be alkaline in nature, resulting in increase in the pH of the medium (Brandl et al, 2001a). Increase in pH at the start may also consistent with the delayed growth of bacteria and redox potential of effluents also increased very slowly as shown in Fig. 4.47, coinciding with low growth of bacterial cultures.
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Fig. 4.46. Changes in redox potential of the effluent at different stages of bubble reactor

Then from day 16, pH started decreasing due to the hydrolysis of the metal ions that were released in the medium due to microbial action and continued up to day 40 with pH 1.72 and the value of redox potential also reached to 610 mV. This was indicative of rich bacterial activity and high bioleaching efficiency.

At the start of stage 2 after attaining steady state the pH value was 2 but at the end it drop down to 1.87 and the value of redox potential reached to 520 mV while at the start of stage 3 the value of pH was 2 after attaining the steady state that decrease up to 1.97 and the value of redox potential was up to 437 mV and in case of stage 4 a little increase in pH was observed from 2 to 2.2 after the reactor was in steady state with the value of redox potential drop down to 380 mV.

4. 11.4. Microbial Population for various HRTs

A free cell concentration of $4.2 \times 10^9$ cells l$^{-1}$ was obtained at stage 1 with an HRT of 140 h, $3.5 \times 10^9$ cells l$^{-1}$ at stage 2, $2.8 \times 10^9$ cells l$^{-1}$ at stage 3 and $0.65 \times 10^9$ cells l$^{-1}$ at stage 4 as shown in Fig. 4.47.
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Fig. 4.47. Changes in microbial population at different stages of bubble reactor

The cell concentration decreases with decreasing HRT. This suggests that the most serious consequence of low HRT is the entrainment of bacteria with the effluent (Coughlin et al., 2002) and rather limited time exposure to bacterial activity at low HRT and high feed flow so due to a low concentration of biomass and the short process time for a high feed flow, low bioleaching performance at low HRT was observed. According to different groups of researchers, stirred tank reactors showed promise as a technology for extracting metal from concentrate ore because of their high capability/volume ratio, high microorganism growth activities, and comparatively high leaching rates. However, the intensity of turbulence or shear produced to achieve the desired level of agitation may affect the microorganism performance (Rawlings et al., 1999; Thomas, 1993). Same type of phenomenon might exist in case of electronic scraps also (Alejandra et al., 2007).

However, formation of ferric iron precipitation should be considered for high HRTs, a moderate HRT of 140h is more beneficial for improving the bioleaching efficiency than low/high HRTs as shown in Fig.4.47 in a continuous flow bubble reactor.

A sketch of bubble reactor that was fabricated locally has been given in Fig.4.48.
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Fig. 4.48. Sketch of bubble reactor bioleaching unit

K: Acid, O: Base, M, L peristaltic pumps, B: feeding inlet with pump and inoculation bath, D: air regulator, E: air outlet, I: effluent outlet, N: water jacket with thermostat and pump.

This is a continues flow bubble reactor and such a reactor can be better utilized because of reduced delays when filling and discharging slurry compared with batch process reactors in industrial operations and will be an important contribution toward the upscale implementation of process.

4.12. Column bioleaching studies

4.12.1. Chemical analysis of electronic scrap

Chemical analysis of electronic scrap used in column bioleaching studies was carried out to determine the concentrations of various metal ions present in the material. The major metal ions in the present electronic scrap were found to be iron (8.0%), zinc (8.2%) and copper (8.9%) with considerable amounts of nickel (2.0%) and lead (3.15%) as well. In addition to these metal ions, small amounts of precious metal ions were also present as shown in Table 4.21. It was checked that electronic scrap used in column
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bioleaching must be of similar chemical composition as was in shake flask studies to avoid the heterogeneity of material and to check the shake flask strategies on up scale level. However the minor difference might be attributed, to some extent, to the analytical approaches used. Variation in electronic scrap concentration was observed when comparison was made among the concentrations of different metal ions obtained in these studies and those reported by other researchers (Brandl et al. 2001a and Veit et al. 2006). A large difference in the concentrations of some metal ions particularly aluminium and zinc seems to be mainly dependent upon the origin of the material. Materials of such varied nature may need to adopt different strategies for microbiological processing.

4. 12.2. Pre-leaching

Electronic scrap was considered to have complex composition, material heterogeneity and of alkaline nature (Brandl et al., 2001a). It was also investigated in our preliminary shake flasks studies that this alkaline nature was not exhibited further when the scarp was stirred with distilled water (Ilyas et al., 2007). This phenomenon was only observed when the electronic scrap was subjected to bioleaching in the medium having initial pH of 2.0, depicting that, due to the action of acid in the medium, some alkaline materials were mobilized from the scrap. So before carrying out bioleaching, the pH of the scrap was stabilized in pre-leaching period. Fig. 51 depicts the acid consumption of solution.

pH and redox potential of effluents during pre-leaching stage can be seen in Fig.4.49 and Fig.4.50. It has been found from Fig. 4.49 that first pH increased from 2 to 5.8 and then, after 192 h of recirculation, started to decrease steadily and reached at 2.0 after 552 h. After 648 h of recirculation pH was stabilized at 2.0 and about 32 mL concentrated H₂SO₄ was consumed in both columns as in Fig. 4.51.

It could be seen from Fig. 4.50 that effluents redox potential remained in the range of 318 to 390 mV. This indicated that pre-leaching process was a typical acid leaching process. During this whole pre-leaching process, permeability of columns remained well.

No solution accumulation, slumping and preferential flows were found in both columns. Other metallic elements leached out during the pre-leaching operation were 7% Ni, 5% Cu, 6% Zn and 4% Al.
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Fig. 4.49. Changes in pH during column pre-leaching studies of electronic scrap

Fig. 4.50. Changes in redox potential during column pre-leaching studies of electronic scrap
4.12.4. Adaptation of microorganisms to metal ions

Metal ions beyond the range of bacterial cultures are toxic due to the fact that metal ions do chelation with protein molecules which render them inactive, for example, inactivation of enzymes.

The effects of mixed metal ions on the growth of moderately thermophilic bacterial cultures, before and after adaptation, are shown in Fig. 4.52 and Fig. 4.53. Before adaptation, mixed bacterial cultures reproduced well with 1 g/L mixed metal ions (i.e. Ag⁺, Al³⁺, Cu²⁺, Fe³⁺, Ni²⁺, Pb²⁺, Sn²⁺ and Zn²⁺) and gave a lag phase of 7 days with 4 g/L mixed metal ions, grew very slowly with 8 g/L and died out during 56 days incubation period with 12 g/L mixed metal ions.

While after nearly 2 years adaptation, bacterial cultures reproduced well with 8 g/L mixed metal ions and gave rich growth after a lag phase of 6 days with 12 g/L metal ions. With 16 g/L metal ions, mixed bacterial cultures grew slowly and died with 20 g/L mixed metal ions during an incubation period of 77 days as depicted in Fig. 4.53.
Fig.4.52. Effect of mixed metal ions on the growth of the consortium of moderately thermophiles before adaptation

Fig.4.53. Effect of mixed metal ions on the growth of the consortium of moderately thermophiles after adaptation
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It might be due to the fact that when the microorganisms were acclimatized to tolerate increasing concentrations of mixed metal ions by repeated sub-culturing on metal containing media, there were physiological changes that facilitated the bacteria to cope with the high metal concentration in the media during bioleaching.

Many genetic systems are known in microorganisms particularly in bacteria for acquiring resistance against toxic metals and maintaining intracellular homeostasis of essential metal ions (Silver, 1996). Among these, the mostly studied genetic mechanisms of metal resistance in bacteria include the presence of metal binding proteins (Olafson et al., 1988; Robinson et al., 1990) and heavy metal efflux systems (Nies and Silver, 1995).

4. 12.5. Column bioleaching studies

Washed electronic scrap, as a column charge, was used in these column bioleaching studies. Fig. 4.54 shows the significant changes in pH during 280 days of bioleaching operation. Initially pH rose up during first 42 days of bioleaching that was consistent with the delayed growth of bacteria and depicts that this stage is mostly contributed to chemical acid leaching operation instead of bioleaching operation.

Then after day 42, pH started dropping due to the hydrolysis of the metal ions that were released in the medium due to bacterial action and continued up to day 196 with a decrease in pH up to 1.6. Trend of pH indicated that this stage was typically bioleaching stage instead of chemical acid leaching stage. Then pH started to drop very slowly up to pH 1.55 from day 196 to 238 and no further significant change in pH was observed after the day 238 and pH remained up to 1.55 till the dismantling of bioleaching operation. So throughout the bioleaching operation pH remained in the range of 1.57 to 2.7. While in uninoculated control pH remained almost in the range of 3.3 to 3.5.
Iron species present in the effluents of two columns were monitored and results have been depicted in Fig. 4.55.

It can be seen from Fig. 4.55 that total iron and ferrous ions concentration at the start increased in a quite linear pattern but ferric ions concentration increased very slowly during first 42 days and redox potential of effluents also rise very slowly as shown in Fig. 4.54, coinciding with poor growth of bacterial cultures and dominant contribution of acid leaching stage. Then from 42th day to 70th day the ferrous ions concentration started to drop down while ferric ions concentration started to rise up linearly and from day 70 to day 196 the concentration of ferric ions rose up to 1490 mg/L as shown in (Fig. 4.55) and value of redox potential 618 mV also increased (Fig. 4.54). This trend was typically attributed to bioleaching stage instead of acid leaching. Then from day 196 to 238 the concentration of ferric ions increased to 1795 mg/L and the value of redox potential also increased to 665 mV which was indicative of rich bacterial activity and efficient bioleaching rate. However, during the last 42 days, value of redox potential and concentration of ferric ions drop down from 1795 mg/L to 1170 mg/L and from 665 to 460 mV respectively, indicative of regressive bioleaching stage and deceleration phase of bacteria.
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Fig. 4.55. Total iron, ferrous and ferric ions concentration changes during column bioleaching studies

The metal dissolution calculated from the analysis of the effluents during the whole leaching period is shown in Fig. 4.56. These dissolution trends of zinc, aluminum, copper and nickel were different in pre-leaching operation and bioleaching operation. Only a small quantity of zinc, aluminum, nickel and copper dissolution was observed during the pre-leaching operation. These dissolution trends of zinc and nickel were quite linear while aluminum and copper dissolved slowly.

During whole bioleaching operation, about 64, 80, 86, 74 % of Al, Zn, Cu and Ni were leached out with mixed consortium of metal-adapted cultures of *Sulfobacillus thermosulfidooxidans* and the *Thermoplasma acidophilum*. While during our optimization studies with mixed consortium of pure cultures of *Sulfobacillus thermosulfidooxidans* and the acidophilic heterotrophs about 53, 44, 60, 39 % of Zn, Al, Cu and Ni were leached out.
These results indicated that percent dissolution of aluminum was contributed to both acid leaching and bioleaching but percent dissolutions of copper, nickel and zinc were mainly contributed to bioleaching. This enhanced dissolution might be due to mutual stability of acidophilic chemolithoautotrophs and heterotrophs because former produce organic metabolites during growth and later use these metabolites as an energy source (Harrison, 1984; Butler and Kempton, 1987; Fournier et al., 1998).

In addition, several acidophilic heterotrophs can also contribute to metal dissolution by the excretion of organic acids such as citrate, gluconate, oxalate, or succinate (Johnson, 1998).

Fig. 4.56. Percent metals solubilization during whole leaching process included pre-leaching and bioleaching
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Fig. 4.57. Sketch of column bioleaching unit

So, high bioleaching rates in present study were possibly due to the synergistic effects of acidophilic heterotrophs on the growth of *Sulfobacillus thermosulfidooxidans*. Nickel, copper, aluminum and zinc leaching rates no longer increased from day 280 to day 322, indicating the termination of leaching operation. During preleaching operation and bioleaching operation, the temperature of column charge was kept at 45 °C with an error of ±0.2. Fig. 4.57 shows very simple column bioleaching unit that was fabricated locally and installed in our own laboratory.

Residues were analyzed after the dismantling of the experimental column at the termination of bioleaching operation and the presence of some metals particularly Sn and Pb were confirmed by later mineralogical analysis that consisted of iron 2.5±0.07, aluminum 0.65±0.08, copper 1.88±0.05, lead 27.0±0.08, tin 74±0.07, nickel 0.22±0.005 and zinc 0.10±0.01%.

The leaching mechanism of copper from printed circuit boards/electronic scrap by *Acidithiobacillus ferrooxidans* has been suggested to be similar to that of metal sulfides.
RESULTS AND DISCUSSION

(Choi et al., 2004). Previously, it was documented that certain microorganisms are able to mobilize metals from mineral, ores and wastes (solid material) by the formation of organic or inorganic acids (protons), by oxidation and reduction reactions, and by the excretion of complexing agents (Bosecker, 1997; Brandl, 2001a). Some other researchers have shown that sulfuric acid is the main inorganic acid found in leaching environments formed by sulfur-oxidizing microorganisms such as Acidithiobacillus species (Rawlings, 2002; Sand et al., 2001). A series of organic acids have also been reported to be produced by bacterial (as well as fungal) metabolism resulting in organic acidolysis, complex and chelate formation (Brandl, 2001b; Burgstaller and Schinner, 1993). On the basis of our results, it can be speculated that similar bioleaching mechanism was operational in case of Sulfolobus thermosulfidooxidans in the present studies and up scaling of this process up to column reactor level is feasible as metals can be leached out efficiently. So this finding may be helpful for industrial level recycling of electronic scrap by bioleaching.

In case of abiotic controls the percent bioleachability was significantly low about 4.4, 6.85, 6.2, and 7.64% for aluminum, zinc, copper and nickel, respectively. This pattern showed that the leaching of metal ions from the electronic scrap due to chemical oxidation was insignificant and major contribution is of bioleaching for dissolution of metals in case of electronic scraps.
SUMMARY

Purpose of the present work was to investigate the bioleaching potential of various wild, adapted and mixed consortiums of microorganisms for extraction of metals from low grade ores and electronic scrap up to column level. Preliminary experiments were conducted on bioleaching of low grade sulphide ore with pure cultures of chemolithoautotrophs, chemolithoheterotrophs and different mixed consortium of these microorganisms. After selection of appropriate microorganism and mixed culture further shake flask studies were carried out for bioleaching of pyrite, sphalerite, Pb-Zn complex ore, nickel containing sulphide ore with high amount of iron in main gangue, nickel containing sulphide ore with high amount of magnesium in main gangue, copper containing sulphide ore and electronic scrap using pure un-adapted and adapted cultures of *Sulfobacillus thermosulfidooxidans* strain RDB as well as its consortium with acidophilic heterotroph (*Thermoplasma acidophilum*).

Maximum pyrite leachability was exhibited by the mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*, which leached out about 60% of iron from pyrite in 120 h. Whereas, pure adapted culture leached out a maximum of 53% of iron from pyrite and the un-adapted culture solubilize about 30% iron with biomass production rates of 0.0033 gL⁻¹h⁻¹, 0.025 gL⁻¹h⁻¹ and 0.022 gL⁻¹h⁻¹ of mixed, adapted and pure cultures. While un-adapted, pure adapted and mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB leach out about 29%, 37% and 47% of iron in the same time period. In case of pure un-adapted, adapted cultures and mixed adapted cultures, biomass production rates (in terms of total cellular protein) were 0.017 gL⁻¹h⁻¹ and 0.019 gL⁻¹h⁻¹, 0.0028 gL⁻¹h⁻¹ respectively. It may be mentioned that these results provide an estimate of the growth that do not give exact measurement of the biomass in these flasks, because the readings were only for planktonic cells, while there may be the cells attached to the particulate matter like ore body or scrap surface that were not accounted for. Furthermore, the plot for mixed adapted consortium of microorganisms should not be regarded as a true growth curve of these cultures due to presence of two different types of bacterial cultures. Only about 12-13% of iron was solubilize in 120 h in the sterile control flasks.

As in case of pyrite, maximum leachability of sphalerite was obtained from same mix consortium, which leached out about 67% zinc in 504 hours. The pure un-adapted
and adapted cultures leached out 45 % and 50 % zinc respectively in the same durations. In case of iron, mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* solubilize about 50 % iron in 432 hours, adapted and un-adapted cultures solubilize 38 % and 32 % iron with biomass production rates 0.0043 gL⁻¹h⁻¹, 0.0038 gL⁻¹h⁻¹ and 0.0034 gL⁻¹h⁻¹ for mixed adapted, adapted and pure cultures. While un-adapted, pure adapted and mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB leach out about 41 %, 45 % and 61 % of zinc and 32 %, 36 % and 48 % of iron in the same time duration in according to their biomass production rates 0.0039 gL⁻¹h⁻¹, 0.0035 gL⁻¹h⁻¹ and 0.0033 gL⁻¹h⁻¹ of mixed adapted, adapted and pure cultures. But 20 % zinc and 17 % iron was leached out in sterile control flask.

Comparison of the bioleaching performance of pure un-adapted and adapted cultures of *Sulfobacillus thermosulfidooxidans* strain RDB as well as it consortium with acidophilic heterotroph (*Thermoplasma acidophilum*) was studied in case of complex Pb-Zn ore. The extraction of Zn with mixed consortium was rapid about 68 % at while with pure adapted and un-adapted it was 59 % and 47 % respectively, in 360 h. On the contrary, the leaching rate in sterile flask was insignificant. Similarly mixed consortium leached out about 54 % iron as compared to pure un-adapted and adapted cultures that leached out 42 % and 51 % iron in 288 hours. However, only 12 % of the total was leached out iron in the control flask. Maximum Pb dissolution (59 %) was obtained with mixed consortium, while pure adapted and un-adapted cultures extracted out 55 % and 30 % Pb, respectively. Their dry biomass also showed the same pattern with the mixed consortium having the highest biomass production rate of 0.0038 gL⁻¹h⁻¹. The rates of pure un-adapted and adapted cultures were 0.0030 gL⁻¹h⁻¹ and 0.0023 gL⁻¹h⁻¹ respectively. While un-adapted, pure adapted and mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB leach out about 40 %, 55 % and 58 % of zinc, 38 %, 43 % and 49 % of iron and 26 %, 52 % and 57 % of lead in the same time duration. Lead was detected in the precipitate. The rates of pure un-adapted, adapted and mixed adapted cultures were 0.0020 gL⁻¹h⁻¹, 0.0022 gL⁻¹h⁻¹ and 0.0026 gL⁻¹h⁻¹ respectively.

Similarly shake flask bioleaching studies of nickel containing sulphide ore (with high amount of iron in main gangue), nickel containing sulphide ore (with high amount of magnesium in main gangue), copper containing sulphide ore were carried out with mixed...
adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB and mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*. After consumption of initial acid demand flasks were inoculated and in copper containing sulphide ore pyrite was added as an additional energy source for the growth of microorganisms due to oxidation of ore, may be from periphery. The mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* showed enhanced bioleaching potential. Similarly with low grade nickel containing sulphide ore having high amount of dolomite, diopside, talc and forsterite as main gangue, mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* solubelized Ni 85 %, Cu 80 %, Zn 79 %, and Mg 57 % while mixed consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB solubilize Ni 78 %, Cu 75 %, Zn 73 %, and Mg 42 %. Similarly with low grade nickel containing sulphide ore having high amount of fayallite, hematite and magnetite as main gangue, mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* solubilize 83 %, 85 %, 55 % and 81 % of Ni, Cu, Fe and Zn while mixed consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB solubilize 79 %, 80 %, 43 % and 78 % of Ni, Cu, Fe and Zn. While with copper containing oxidized sulphide ore, mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* solubilize 78, 82, 85, 85, 77 % of Co, Zn, Cu, Ni and Fe and mixed consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB solubilize 74, 79, 80, 73, 62 % of Co, Zn, Cu, Ni and Fe. Then based on shake flask studies the column bioleaching experiments were conducted with mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum*. Technical feasibility of extraction of valuable metals from last three ores by above mentioned consortium of microorganisms was tested. Different preleaching strategies were applied for enhanced recovery of metals in a next stage bioleaching operation including adaptation of microorganisms with high amount of iron in case of ore containing high amount of fayallite, hematite and magnetite as main gangue, adaptation of mixed microbial consortium with mixed metal ions in for improving its metal tolerance level, due to the ease of dissolution of these gangue materials in acid and the periodic bleed of the portion of effluent. Changes in pH, redox potential, iron concentration and dissolved metals concentration was monitored during whole bioleaching operation. After optimization of process parameters in small columns bioleaching operation in large
columns was started and permeability of column was keep in notice. Overall it was concluded that after adapting some pre and bio leaching strategies like acid leaching, mixing of additional energy source, periodic bleeding and adaptation of microbial consortium to high metal ions concentration can enhance the potential of microbial process remarkably and in future heap scale implementation of bioleaching process for, oxidized, depleted and high gangue material containing ores will be feasible.

The bioleaching of metals from electronic scrap were carried out by the moderately thermophilic strains of acidophilic chemolithotrophic and acidophilic heterotrophic bacteria viz. *Sulfobacillus thermosulfidooxidans* strain RDB, *Sulfobacillus thermosulfidooxidans* strain MT-13, *Thermoplasma acidophilum* and an unidentified acidophilic heterotroph (code A1TSB) isolated from local environments. Among the strategies adapted to obtain enhanced metal leaching rates from electronic scrap, the mixed consortia of the metal adapted cultures of the above-mentioned bacteria cultures were found to exhibit the maximum metal leaching efficiency. Initially metal bioleaching experiments were conducted with unwashed electronic scrap and unadapted bacterial cultures. Among metals, copper exhibited maximum bioleachability (92 % w/w) while 86 %, 83 % and 84 % of Ni, Al and Zn were leached out, respectively, after 18 days and with mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB 89 %, 81%, 79 % and 83 % of Cu, Ni, Al and Zn were leached out, respectively, after 18 days. Although Pb and Sn were also leached out, they were detected in the precipitates formed during bioleaching. In case of uninoculated controls the maximum leachability (0.2 %) was exhibited by nickel in the presence of sulfur. Under all other experimental conditions and for other metal ions the percent leachabilities were significantly lower than this value. pH profile of the medium was studied under three different conditions of bioleaching viz unwashed ES, washed ES and washed ES with addition of sulfur to the medium. In case of unwashed ES, pH of the medium rose up initially with both mixed consortia, in spite of the fact that sulfuric acid was added after every 24 h to the medium to bring the pH down to 2.0. It was only at the day 3 in case of mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* and at the day 4 in case of mixed adapted consortium of *Sulfobacillus thermosulfidooxidans* strain MT-13 and A1TSB when no further increase in the pH was observed. The increase in pH with washed ES was not as high as in case of the unwashed ES, showing that the non-metal part of the ES also contributed towards its
alkalinity. Under both of the above mentioned conditions, inoculations were made when no further increase in pH was observed. Based on shake flak studies mixed consortium of *Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* was used for further column bioleaching studies of electronic scrap with the adaptation of mixed consortium to mixed metal ions for little longer duration. The results from these studies demonstrate that Zn (80%), Al (64%), Cu (86%) and Ni (74%) can be recovered from electronic scrap by microbial leaching using mixed adapted consortium of moderately thermophilic bacteria i.e.*Sulfobacillus thermosulfidooxidans* strain RDB and *Thermoplasma acidophilum* at column bioleaching level and Al (4%) Zn (6%), Cu (5%) and Ni (7%) can be leached out during preleaching. This finding may facilitate on industrial scale implementation of this process for recycling of metals from electronic scrap. Pre-adaptation of the microorganisms to high metal ion concentrations and using mixed consortia of acidophilic chemolithotrophs and acidophilic heterotrophs greatly enhances the metal solubilization rates as they can survive under high concentration of metal ions. Acid pre-leaching of electronic scrap reduced the time required to stabilize the pH at a level of (pH 1.8–2.0) and provide optimum conditions for bacterial attack on scrap surface. But studies also provide evidence for the precipitation of Pb and Sn along with other metal ions confirmed by later mineralogical analysis. So some additional strategy is still needed to prevent precipitation or recover these metals from precipitates
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