

Growth and development of *Thiobacillus ferrooxidans* for engineering applications

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Abstract. A bioprocessing approach for the extraction of base, nuclear and precious metals from refractory and lean grade ores has been reviewed in this paper. Characteristic morphological features of *Thiobacillus ferrooxidans*, the organism which has been extensively used for biooxidation of sulphide ores have been discussed. Mechanisms of chemoautotrophy and mineral oxidation have been illustrated. The current engineering applications of this microorganism have also been brought out. Various methods for accelerating the growth of *Thiobacillus ferrooxidans* for faster biooxidation and genetic manipulation for development of desired strains have been outlined.

Keywords. *Thiobacillus ferrooxidans*; chemoautotrophy; iron oxidation; sulphur oxidation; bioleaching; genetic manipulation.

1. Introduction

Thiobacillus ferrooxidans, the most widely implicated bacterium in the bioleaching of sulphide minerals was isolated for the first time from the acid drainage of coal mines in 1947 (Colmer & Hinkle 1947). Since then tremendous research activity followed in order to characterize these organisms and investigate their potential in the oxidation of various sulphide minerals (Duncan *et al* 1964; Brierley 1978; Dugan & Apel 1978; Harrison 1982; Norris & Kelly 1982; Groudev 1985; Huber *et al* 1986; McCready 1988).

Its commercial application in the leaching of copper from waste grade ore dumps was demonstrated by the Kennecott Copper Corporation, USA in 1963. During the last three decades, the commercial utility of this microorganism has been proved in the extraction of copper and uranium from waste and lean grade ores as well as tailings. Over 30% of the world's copper is currently estimated to be produced through bioleaching operations. For about a decade, the industrial application of bioprocessing has been further extended to enhance gold recovery from refractory ores and concentrates containing encapsulated gold particles in a sulphide matrix such as pyrite and arsenopyrite (Lawrence 1990).

Commercially, the following techniques have been adopted to bring about biooxidation of the desired mineral.

- Dump/heap leaching,
- solution mining or *in situ* leaching,
- vat leaching and use of bioreactors.

Although it has been proved technically feasible to bioleach almost any sulphide mineral to recover zinc, nickel, cobalt, molybdenum and a variety of other metals, its widespread commercialisation has been currently restricted due to unfavourable process kinetics. Unlike a number of heterotrophs, *Thiobacillus ferrooxidans*, a chemoautotrophic acidophile grows very slowly exhibiting large generation periods ranging between 16 and 20 h. The slowness of their growth coupled with difficulties associated with generation of higher amounts of biomass, limit the universal industrial acceptance of this biotechnology. Current research efforts, therefore, have been directed towards finding ways and means to accelerate the growth of *T. ferrooxidans* with a view to enhancing bioleaching rates.

In this paper different methods developed to augment biomass generation of *T. ferrooxidans* are analysed. The microbiology of the bacterium is brought out with respect to biochemical mechanisms. Engineering aspects of biomass growth and bioleaching technology are illustrated.

2. *Thiobacillus ferrooxidans*

Since *Thiobacillus ferrooxidans* is the most widely implicated organism in bioleaching operations, its microbiology is discussed in detail here. It is a motile organism having a single polar flagellum. It is non-spore forming, gram-negative, rod-shaped (0.5–1.5 μm), aerobic, acidophilic (pH 2–3.5), and essentially mesophilic (optimum temperature 30°C, but also tolerates moderately thermophilic conditions). It divides by binary fission and occurs either singly or in pairs. It derives its carbon from atmospheric CO_2 and obtains its energy for metabolism through the oxidation of ferrous and reduced-valence inorganic sulphur compounds. An electron micrograph of *T. ferrooxidans* with its characteristic morphological features is shown in figure 1. The medium used for growing the organisms under laboratory conditions is illustrated in table 1.

The surface structures of cells of *T. ferrooxidans* differ depending on whether they are grown in liquid or on solid substrates. When grown in a medium containing sulphur, the cells develop peritrichous, rod-shaped, filamentous appendages called pili. The diameter of the pili can vary from 0.7 to 3.0 microns. On the other hand, when grown in 9 K medium, the bacterium does not develop such characteristics. Hence, it can be inferred that pili facilitate cellular contact with the mineral surface for oxidising elemental sulphur to sulphuric acid (Gromova *et al* 1978).

3. Mechanisms of chemoautotrophy

3.1 Sulphur oxidation

The details of the complex mechanisms involved in sulphur oxidation are beyond the scope of this article and hence are considered only in brief here. Oxidation of S^{2-} , S^0 ,

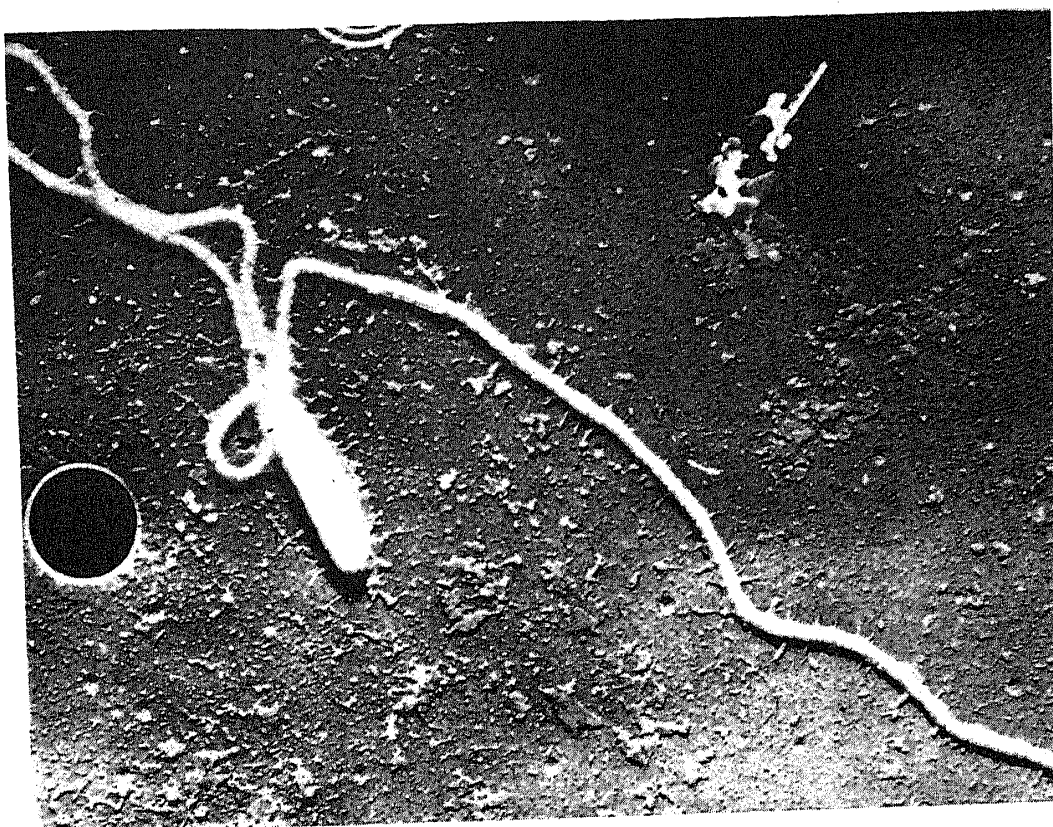
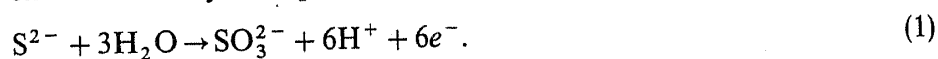


Figure 1. Electron micrograph of *T. ferrooxidans* (20 000 ×).

$S_2O_3^{2-}$ and SO_3^{2-} by the bacteria are considered. A schematic representation of sulphur oxidation by *T. ferrooxidans* is depicted in figure 2.

Sulphide is oxidised directly to sulphite:



The enzyme involved is sulphite reductase. Sulphide can also be oxidised to elemental sulphur (S^0) with the help of enzyme, sulphide oxidase (Parker & Prisk 1953; Kasprzak & Steenkamp 1983).

Table 1. Silverman and Lundgren* medium (9 K medium).

Nitrogen source	$(NH_4)_2SO_4$ - 3 g/l
Growth factors	KCl - 0.1 g/l
	K_2HPO_4 - 0.5 g/l
	$MgSO_4 \cdot 7H_2O$ - 0.5 g/l
	$Ca(NO_3)_2$ - 0.01 g/l
Energy source	$FeSO_4 \cdot 7H_2O$ - 44.8 g/l
Distilled water	1 l
pH	2 - 2.3

*Silverman & Lundgren (1959)

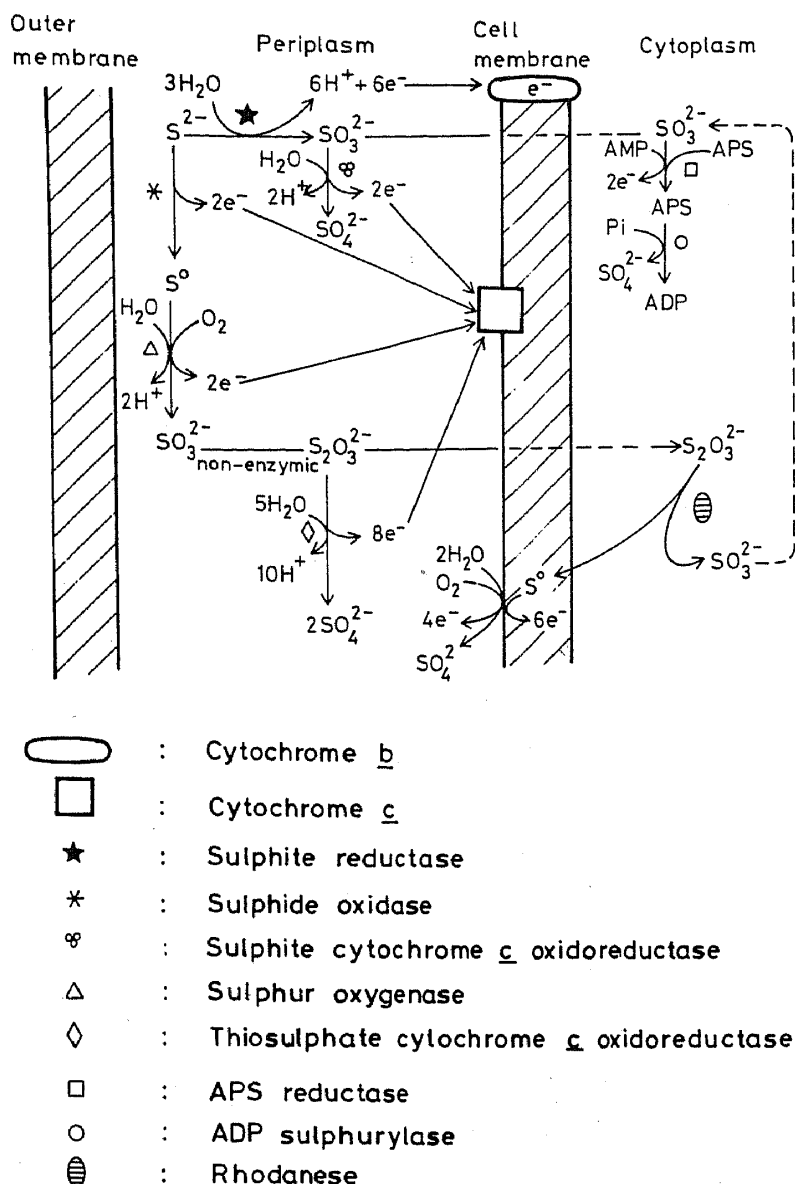


Figure 2. A schematic representation of sulphur oxidation by *T. ferrooxidans*.

Sulphur:



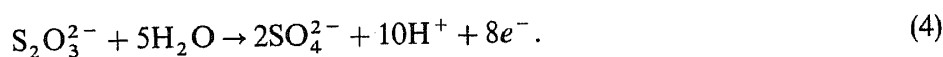
Elemental or colloidal sulphur is insoluble. It is necessary that the cells establish direct physical contact with elemental sulphur before oxidation can occur. Therefore, sulphur is oxidised at the cell surface, dissolved in the cell membrane, or taken up as elemental sulphur by the cells. The enzyme involved has been shown to be an oxygenase (Karavaiko & Pivovarova 1973).

Thiosulphate: All the species of thiobacilli studied oxidise thiosulphate to sulphate. Two mechanisms of oxidation are observed depending on the species of thiobacilli; the

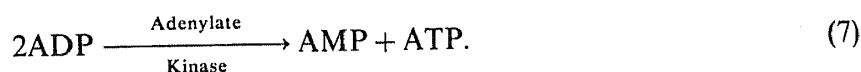
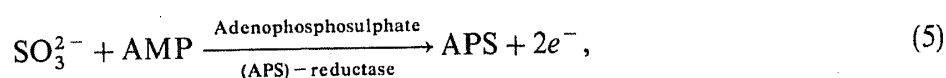
first involves the initial splitting of the S-S bond by enzyme rhodanase with the formation of sulphite and elemental sulphur.



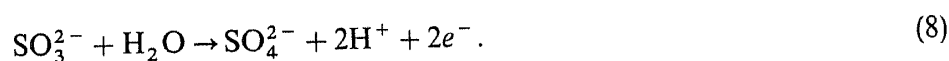
Sulphur is also found to be associated with the membrane and subsequently converted to sulphate. This mechanism involves thiosulphate: cytochrome *c* oxidoreductase (Peck 1960; Charles & Suzuki 1966; Schedel & Truper 1979).



Sulphite: Two mechanisms operate in the oxidation of sulphite depending on the species of thiobacilli. The AMP (adenosine-5-monophosphate)-dependent sulphite oxidation is cytoplasmic.

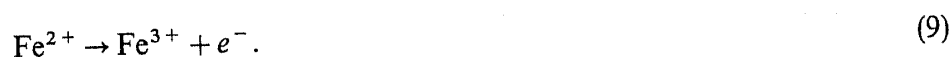


In the second mechanism, the enzyme is extracytoplasmic, namely, sulphite oxidase (sulphite cytochrome *c* reductase). The reaction catalysed by this enzyme is (Peck 1960; Bowen *et al* 1966; Yamanaka *et al* 1981; Toghrol & Southerland 1983; Lu & Kelly 1984).



3.2 Ferrous oxidation

A schematic representation of ferrous iron oxidation by *T. ferrooxidans* is given in figure 3 (Pringsheim 1949; Lundgren *et al* 1974; Black *et al* 1989). The oxidation of ferrous iron does not involve production of protons produced in the enzyme-catalysed reaction:



The ferric iron formed undergoes abiotic hydrolytic reactions which result in the release of protons on the extracytoplasmic side. The most significant reaction at pH 2.0 is:



The cell membrane associated electron transporters involved in the iron oxidation include iron oxidase, cytochrome *c* and a terminal oxidase cytochrome *a*. The soluble electron transport component include cytochrome *c* and a blue copper protein rusticyanin. An iron cytochrome *c* reductase or iron oxidation site has been reported by several authors (Blaylock & Nason 1963; Yates *et al* 1967; Sugio *et al* 1981). It is indicated by several observations that iron oxidation takes place outside the cell membrane, that is, in the

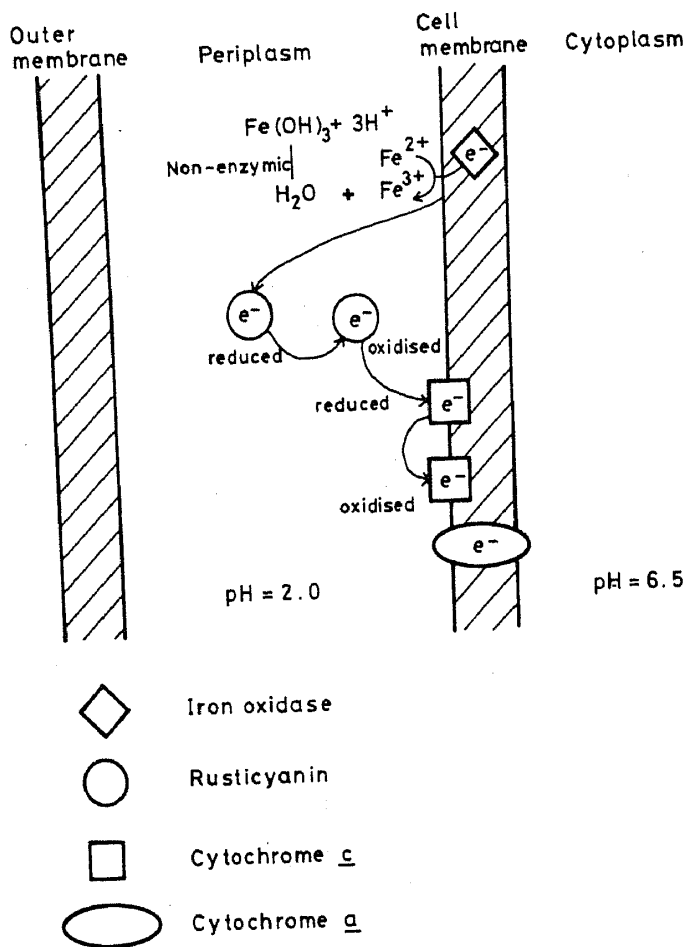


Figure 3. A schematic representation of ferrous oxidation by *T. ferrooxidans*.

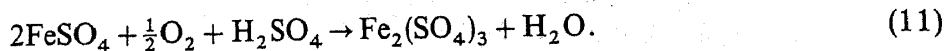
inferred from the fact that iron would be rapidly autooxidised at cytosolic pH values (pH 6.5) and that ferric iron formed at this pH would be insoluble.

4. Mechanisms of mineral oxidation

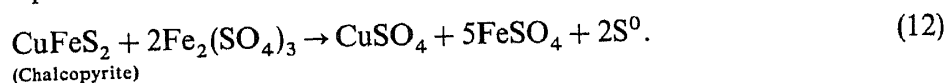
Mechanisms involved in biooxidation of minerals are very complex. When the organisms interact with mineral substrates in an acid medium, a number of reactions occur. The microbe-mineral interactions influence the rate of mineral dissolution by various chemical, biochemical and electrochemical factors. The main leaching mechanisms involved are indirect, direct and electrochemical oxidation.

4.1 Indirect leaching

In this process, the bacteria do not attach to the mineral surfaces. Indirect leaching involves biological regeneration of ferric sulphate.



Ferric sulphate produced, is a strong oxidant and is capable of dissolving a wide variety of sulphide minerals. For example,



The elemental sulphur formed in the reaction can be converted to sulphuric acid by *T. ferrooxidans*.



The sulphuric acid generated, not only maintains the pH at levels favourable to the bacteria but also leaches a variety of copper oxide minerals like azurite [$\text{Cu}_3(\text{OH})_2(\text{CO}_3)_2$], chrysolla ($\text{CuSiO}_3 \cdot 2\text{H}_2\text{O}$) and tenorite (CuO) very effectively.

4.2 Direct leaching

T. ferrooxidans also leaches mineral sulphides by attachment to mineral surfaces. This process can be described by the following reaction,



where *M* is a bivalent metal. Incubation of *T. ferrooxidans* with the mineral substrates results in oxygen consumption and metal solubilization.

Since iron sulphides are invariably present in the natural leaching environments, probably both direct and indirect leaching mechanisms occur simultaneously. Bacterial attachment was found to be governed by particle size of the mineral, period of incubation and by stationary or agitation conditions used during incubation (Natarajan 1992). Some of the researchers attribute enhanced mineral dissolution to the direct mechanism, while others suggest that bacterial assistance in the enhancement of mineral dissolution is due to constant supply of ferric ions. The attachment of *T. ferrooxidans* to a number of sulphide minerals has been studied quantitatively by estimating its protein and nitrogen concentrations.

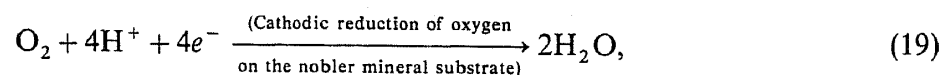
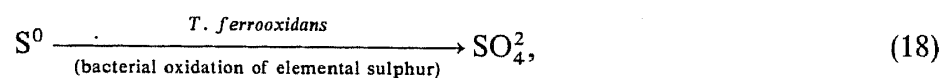
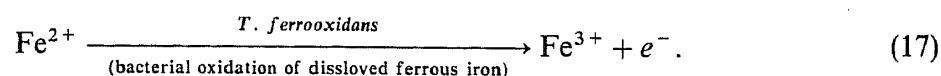
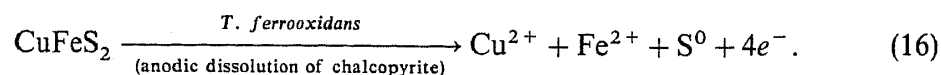
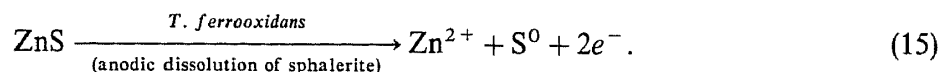
Attachment of *T. ferrooxidans* on to pyrite mineral was observed to be influenced by reducing particle size and increasing incubation periods. It has been also observed that increased bacterial attachment to pyrite mineral surfaces promoted iron dissolution (Murthy & Natarajan 1992).

4.3 Electrochemical leaching

Principles of electrochemistry are applicable to mineral dissolution in leaching processes (Natarajan 1990). Many natural minerals behave as electrodes in the presence of a leaching medium. Thus in leaching systems containing more than one mineral, galvanic interactions essentially come into play. When two sulphide minerals establish contact in a leaching medium, a galvanic cell will be formed; the more active mineral in the couple will undergo corrosion while the nobler (less active) one will be cathodically protected. Considering the example of a chalcopyrite-pyrite couple, chalcopyrite has the lower electrical rest potential and becomes the anode while pyrite behaves as a cathode. As a result, the chalcopyrite phase undergoes rapid dissolution while the pyrite phase remains essentially unaffected. *T. ferrooxidans* further catalyses the above

electrochemical reactions by continuously oxidising the film of elemental sulphur formed on mineral surfaces which would otherwise form a physical hindrance to diffusion of copper and iron salts away from the reacting phase. Prediction of the electrochemical behaviour of sulphide minerals in multiple combinations is more difficult.

Typical reactions illustrating galvanic interaction mechanisms are given below:



Thus, the galvanic dissolution of an active mineral (chalcopyrite, sphalerite etc.) when contacted with a nobler mineral like pyrite, in the presence of *T. ferrooxidans*, is increased several fold (Natarajan 1988, 1992; Jyothi *et al* 1989)

5. Current engineering applications

Microbial leaching is rapidly becoming an important process tool for the treatment of especially low and lean grade ore reserves. The role of microorganisms in the leaching of a number of minerals has long been established. The microorganisms, in most of the cases occur indigenously to the various mining operations. From a metallurgical point of view, as the grade of the ore becomes lower and lower, the technology for metal extraction becomes more and more difficult and expensive. The mineral industries are seeking development of modified extraction processes and lowering of costs. Dump/heap leaching, *in situ* solution mining and bioreactor leaching with the help of *T. ferrooxidans* have been attractive innovations in this direction.

5.1 Copper

On a commercial scale, dump/heap leaching operations at the mine site for lean and waste mined copper ores are being employed in many parts of the world. Biological copper leaching is practised in many countries such as USA, Russia, Chile, Peru, Australia, Spain, Canada and Mexico.

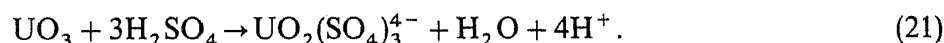
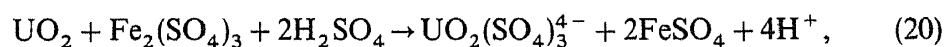
Engineering layouts for leaching operations all over the world are remarkably uniform. Essentially, copper ore mined from open pits is segregated. Higher grade material is concentrated to produce a feed for smelting, while the lower-grade ore is subjected to leaching. The ore is piled on until a dump/heap of suitable dimension accumulates, the top of the dump is levelled and then the leach solution is flooded or

sprayed onto the dump. Bacterial colonization occurs mainly in the top layers. The temperature generally rises to 80°C–90°C in the central regions of the dump due to exothermic reactions. In this case indirect leaching by ferric sulphate prevails. Leach solutions enriched with copper exit at the base of the dump and are conveyed to a central recovery facility. The barren solution is then recycled to the leach dumps.

In situ leaching is yet another popular technique in this direction. It facilitates extraction of deeper low value mineral reserves without the need for mining and expensive operations. It involves breaking the ore body with powerful explosives to make the deposit penetrable to solution. Leach solution and air are then injected under pressure. The resulting copper-enriched solutions are then recovered below the ore body. Copper is recovered by conventional methods. *In situ* mining eliminates environmental pollution problems associated with large scale mining (Ehrlich 1963; Duncan *et al* 1964; Brierley & Lockwood 1977).

5.2 Uranium

Uranium leaching using bacteria has been implemented since the 1960s in Canada. The process involves intermittent spraying or flooding of worked-out stopes and tunnels of underground mines with water or dilute sulphuric acid solution at regular intervals. The activity of *T. ferrooxidans* is limited to oxidation of pyrite and ferrous ions because it does not directly interact with uranium minerals. Uranium leaching proceeds according to the following reactions:



Uranium bearing solution drains to the lower portions of the mine and accumulates in sumps. The solution is then pumped to the surface for uranium recovery (Harrison *et al* 1966; Tuovinen & Kelly 1974; Ferroni *et al* 1986; McCready 1988).

5.3 Coal desulphurization

Although bioprocessing has not been applied to coal on an industrial scale, a number of possible applications have been examined in the laboratory and on pilot scales.

A preliminary economic analysis has shown that microbial desulphurization compares favourably with alternative chemical coal-cleaning methods. The parameters governing rates of pyrite dissolution in coal are particle size, pulp density, solution pH and temperature, inoculum size and characteristics of the leaching bacteria. In some instances, leaching rates are enhanced by supplemental nutrients and carbon dioxide. Fine coal slurries could be subjected to oxidation by *T. ferrooxidans* at a pH of 2.0–2.5 and temperatures in the range of 25°C–35°C.

The use of *T. ferrooxidans* in coal desulphurization involves surface modification of the pyrite. It has been observed that pyrite is more easily separated from coal during froth flotation following an initial exposure to these bacteria, because pyrite is rendered more hydrophilic by selective attachment of the bacteria; flotability increases with increase of hydrophobic character. Coal cleaning by this method would avoid the long

retention period required for microbial dissolution of pyrite (Dugan & Apel 1978; Kargi 1982; Monticella & Finnerty 1985; Huber *et al* 1986).

5.4 Gold

The most important application of biotechnology to gold recovery is the treatment of refractory ores and concentrates to increase gold dissolution during cyanide leaching (Natarajan 1993). In many such ores, finely divided gold is encapsulated in pyrite or arsenopyrite minerals and thus cannot be dissolved by the cyanide solutions unless the ore is very finely ground. An alternative to grinding is to use bacteria to selectively dissolve enough of the sulphides to expose the gold, and then to leach with cyanide by the normal process. The use of bacteria keeps the cost of sulphide mineral dissolution at a reasonable level, and since it is only necessary to dissolve a fraction of the pyrite to expose the gold, the leaching time can be kept to a few days.

Large scale application of bioleaching of refractory gold ores and concentrates before cyanidation has been demonstrated at the Fairview mine in South Africa in 1986. This industrial plant treats 18 t/day of high grade gold concentrates. The San Bento plant in Brazil was commissioned in 1990 to treat 300 t/day of low grade concentrate. A large number of commercial biotreatment plants have since been commissioned for processing of refractory gold concentrates in Australia and Ghana (Livesey-Goldblatt *et al* 1977, 1983; Griffin & Luinstra 1989; Hackl *et al* 1989; Morin & Ollivier 1989; Lawrence 1990; Torma Oolman 1992).

6. Methods for accelerating growth of *T. ferrooxidans* for faster biooxidation

T. ferrooxidans is a very slow-growing organism. Its generation time varies between 16 and 20 h. Therefore obtaining a large biomass for industrial purposes using the classical methods is very time-consuming and complicated. Under normal growth conditions in 9 K medium, it takes upto 30–40 h to obtain a cell mass of about 10^8 cells/ml. Hence there is a quest for developing methods which would produce large amounts of biomass in a shorter time, with a view to enhance leaching rates. Some methods developed for the enhancement of the biomass of *T. ferrooxidans* are illustrated below.

6.1 Bacterial film oxidation (BACFOX process)

This method is proposed to raise the number of bacteria in the medium during heap and underground leaching. The method comprises the following procedures.

Cells of *T. ferrooxidans* are placed in the form of a film on a corrugated surface of bioactive material. The corrugated material coated with the film of bacteria is submerged in ferrous sulphate solution saturated with air. Oxidation of Fe^{2+} takes place. Bacteria bind with the precipitated jarosite; the film containing bacteria and jarosite may be grown on various materials like glass, plastic etc. Livesey-Goldblatt *et al* (1977) reported that the best results are obtained in the media where bacterial film was fixed on corrugated plastic. Maximum specific rate of ferrous to ferric oxidation was observed to be 7.5 g/h/m² of bacterial film. A schematic representation of the BACFOX process is depicted in figure 4.

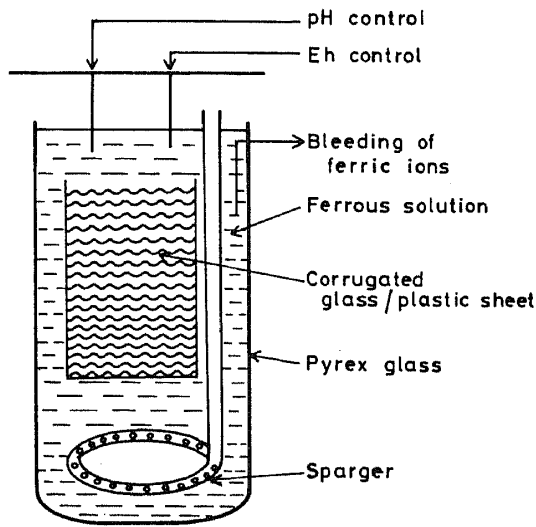


Figure 4. Bacterial film oxidation (BACFOX).

6.2 Biomass production by using electrical energy

Another way to increase the cell concentration in the culture and at the same time to overcome all the above mentioned drawbacks of the classical methods is offered by the method proposed by Kinsel & Umbreit (1964). Several researchers have proposed similar methods (Kovrov *et al* 1978; Denisov *et al* 1980; Yunker & Radovich 1985; Natarajan 1992b). The main feature of this method is combining two processes, namely, the microbiological oxidation of Fe^{2+} and electrochemical reduction of Fe^{3+} to Fe^{2+} . The method comprises electrochemical reduction of bacterially generated ferric ions

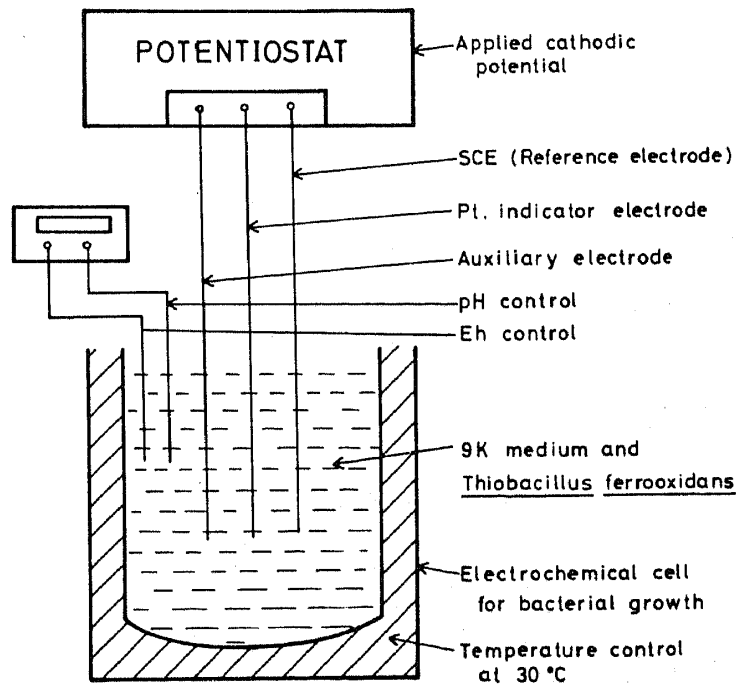


Figure 5. A schematic representation of biomass production using applied DC potentials.

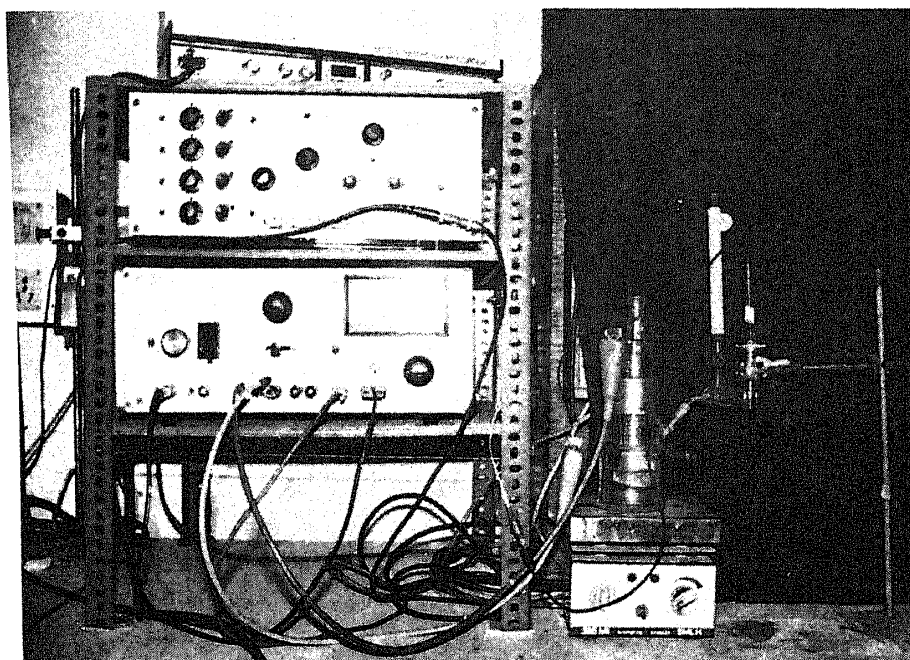


Figure 6. Experimental set-up of electrochemical cell.

directly in the culture. Figure 5 shows a scheme of reactions taking place in an electrochemical cell that provides the energy source for *T. ferrooxidans*. The energy for cell growth comes from the electrochemical cell's electrodes, while the energy providing substrate (ferrous ion), serves merely as a carrier of energy from the cathode to the bacterial cell. The Fe^{2+} is not supplied from outside but is formed in the culture on the anode surface. Figure 6 shows a typical laboratory experimental set-up of electrochemical instrumentation.

The electrochemical reduction of ferric ion is coupled with continuous cultivation of *T. ferrooxidans*. The concept involves the correlation of two mutually opposing reactions, namely: the bacterial oxidation of Fe^{2+} and the electrochemical reduction of Fe^{3+} . Most useful for practical application is the method of maintaining a constant cathode potential. The optimal bacterial biomass concentration during continuous cultivation ranges from 5 to 10 g/l (dry weight).

Application of negative DC potentials in the range of -500 mV to -1000 mV to a bacterial culture containing ferric ion, effectively converts ferric ion to the ferrous state, promoting bacterial activity and growth. Under a negative applied potential of -500 mV the cell number of *T. ferrooxidans* increased from 2.5×10^7 cells/ml to about 1.3×10^8 cells/ml within a period of about 60 h (Natarajan 1992b). Subsequent imposition of -500 mV to a fully grown culture containing 1.3×10^8 cells/ml promoted significant growth of the bacteria (Natarajan 1992). It has been reported that the generation time of *T. ferrooxidans* can be reduced from 16 h to 10 h with a ten-fold increase in biomass when an applied potential of -500 mV is maintained (figure 7). Electrobiorecovery also enables faster and selective dissolution of the desired mineral phase from complex sulphides, unlike the case with only bioleaching, as depicted in figure 8. Zinc dissolution from binary mixtures containing either pyrite or chalcopyrite in addition to sphalerite was observed to be the highest at an applied potential of -500 mV in the presence of *T. ferrooxidans* (Natarajan 1992b).

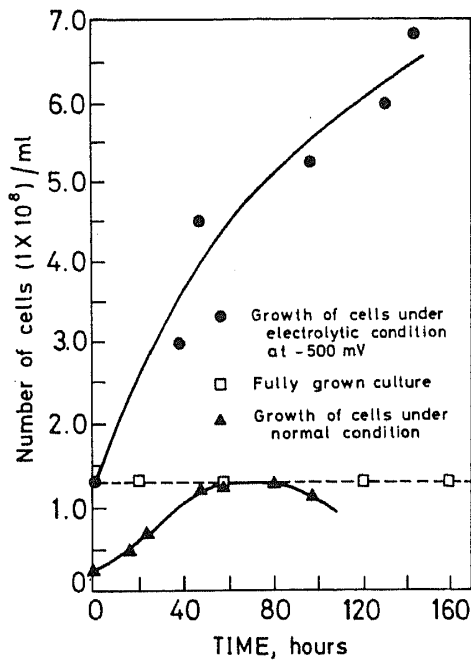


Figure 7. Variation in number of cells per ml with time in the growth of *T. ferrooxidans*.

6.3 Oxidation in packet-bed reactors

Bacterial biomass can also be enhanced by using *T. ferrooxidans* in a fixed-film bioreactor containing glass beads, activated carbon particles or ion-exchange resin (Grishin & Tuovinen 1988). Both batch and continuous flow modes of operation have been studied for the biological oxidation of ferrous sulphate to ferric sulphate. Various packed-bed reactors have been designed to improve the rate of iron oxidation. Three

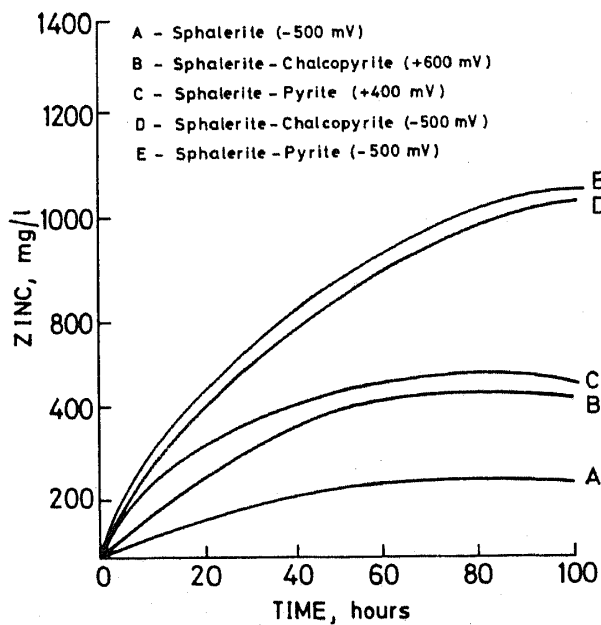


Figure 8. Zinc dissolution from binary mixtures containing pyrite or chalcopyrite with sphalerite under applied potential.

parameters were taken into consideration; namely, providing a larger surface area for attachment of *T. ferrooxidans* to the support matrices, to reduce loss of biomass and the use of a low pH (between 1.3 to 1.5) to provide more extensive adsorption of the bacteria and also to eliminate ferric precipitation.

The activated-carbon packed-bed reactor had the highest level of biomass, amounting to 32 mg of protein per ml of interstitial liquid or 128 micro g/cm² of matrix surface. The ability of the glass beads to retain biomass was about 50 times less.

6.4 Growth in solid medium

Growing *T. ferrooxidans* on a solid medium so far has been a very time-consuming and tedious process; the solidifying agent, agar-agar, neither forms a stable gel when sterilised at low pH values nor properly solidifies due to hydrolysis. To overcome these problems, a new medium has been designed by Khalid *et al* 1993. They have used bacterial polysaccharide, 'Gellan Gum' or 'Gelrite' as solidifying agent for acidophilic thiobacilli. This medium has been devised for estimating the growth of *T. ferrooxidans* on the solid substrate. The plating efficiency was determined to be $92.1 \pm 5.8\%$. Dark brown, circular, well-differentiated colonies developed within 72–96 h. The cell count was approximately 7×10^6 cells/ml for ATCC 13661 strain of *T. ferrooxidans*. Dry biomass of 650 mg/l can be obtained by this method which corresponds to 320 mg total protein/l. A linear relationship has been found which gave a value where 0.5 g dry mass/l was equivalent to 2.8×10^6 cells/ml of the ATCC 13661. This medium can be employed to purify cultures of *T. ferrooxidans* strains which have frequently been reported to have been associated with other acidophiles. The high plating efficiency also indicates that this method can be used for selection of mutants resistant to toxic metals and also for genetic investigation of *T. ferrooxidans*.

6.5 Genetic manipulation for development of special strains

The necessity to develop special strains of *T. ferrooxidans* which possess both high metal and temperature tolerance as well as better leaching capability becomes very evident because accumulation of toxic metal ions such as arsenic, copper and zinc hinders bacterial activity. Development of metal-tolerant, preadapted bacterial strains is thus necessary to obtain efficient bacterial activity during leaching. Adapted strains are found to be more efficient in gold liberation compared to the unadapted wild strains. One of the principal objectives of genetic manipulation is to develop techniques to introduce the desired characteristics into the leaching bacteria. Attempts have already been made to detect the plasmid DNA in *T. ferrooxidans*. Methods of plasmid isolation and identification from *T. ferrooxidans* have been worked out. The possibility of cloning them into *Escherichia coli* has also been evaluated. However, the development of genetically engineered *T. ferrooxidans* is still far-fetched (Groudeva *et al* 1980; Cox & Boxer 1986; Rawlings *et al* 1986; Yates & Holmes 1987; Holmes *et al* 1988; Yates *et al* 1988; Holmes & Yates 1990).

Much progress has been made in understanding the pathways of ferrous and sulphur oxidation, energy coupling, and carbon assimilation. However, physiological descriptions do not at present appear to be leading toward a coherent taxonomic analysis of the thiobacilli.

The ribosomal ribonucleic acid (rRNA) of *T. ferrooxidans* is independent of growth conditions and metabolic status. So they are useful for comparing even physiologically disparate species. 5S rRNA is sufficiently conserved in all species. Hence sequence homologies are evident even between organisms that have immeasurably low overall deoxyribonucleic acid (DNA) homology. Mixed or contaminated cultures are readily evident by detection of more than one type of 5S rRNA. The rRNA sequence, therefore, provides incisive criteria for the classification of new strains. It is understood that phylogenetic framework will offer a more directed approach toward the comparative biochemistry of these organisms. Since there is presently a great deal of interest in 'engineering' these economically important bacteria, knowledge of their natural evolutionary relationships should be of use in constructing fruitful *recombinants*.

Thus, DNA and rRNA analyses are being used to classify strains into different genomic groups within the species. This will provide useful selection of genotypes and phenotypes best suited for particular application. Twenty-three strains of *T. ferrooxidans* were examined by Harrison (1982), which were genomically diverse.

A strategy for obtaining recombinants by genetic engineering is presented here.

- (i) The chromosomal and plasmid DNA from *T. ferrooxidans* are isolated by standard procedures.
- (ii) Both the chromosomal and plasmid DNA are subjected to restriction digestion with the help of restriction enzymes to cut the DNA into fragments.
- (iii) With advanced molecular biology techniques the gene of interest is selected and isolated. (Explanation of the procedures is beyond the scope of this review.)
- (iv) Once the gene of interest is isolated, it is incorporated into an *Escherichia coli* plasmid. This plasmid is a small circular DNA.
- (v) The circular plasmid DNA is cut with a restriction enzyme to form a linear strand.
- (vi) The gene of interest is then added to the linear strand of DNA. The gene then gets attached to the linear plasmid to form a recombinant molecule comprising part of the plasmid DNA, hence the term *recombinant DNA*.
- (vii) The recombinant DNA molecule is then sealed to reform a circle with an enzyme called ligase.
- (viii) The recombinant plasmid is then put back into *E. coli*. This process is called *transformation*.
- (ix) The *E. coli* cell is then allowed to divide many times in the culture media, producing a large number of colonies, each identical to the starting bacterium. Since the recombinant plasmid is also present in all the progeny bacteria, the inserted gene of interest is said to have been *cloned*.
- (x) After cloning, the recombinant plasmids are then isolated from the host *E. coli*, giving amplified copies of a single gene of interest. The characteristics of this gene are then expressed.

A schematic representation of this method is depicted in figure 9.

7. Summary

Biological processing of minerals and metals is a rapidly growing, diverse field whose importance will become considerable in the near future. The use of genetic engineering

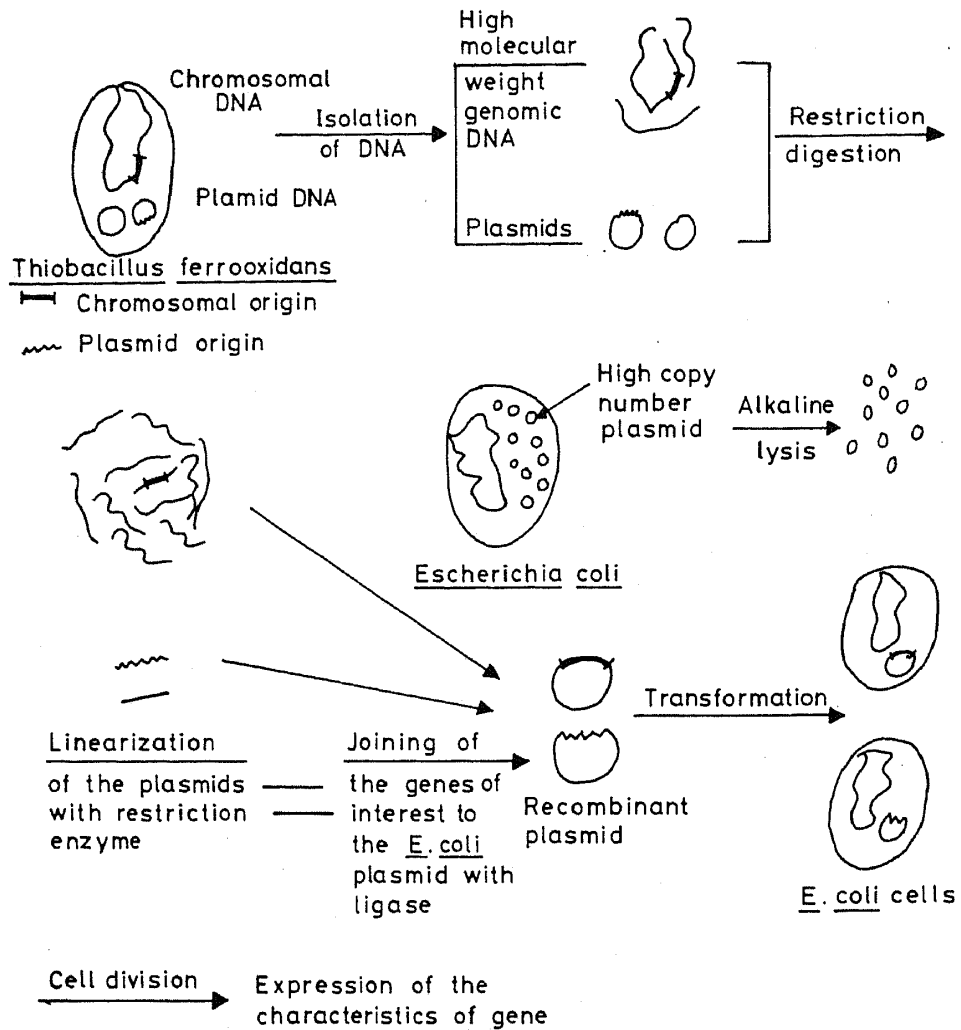


Figure 9. A strategy for obtaining recombinants by genetic engineering.

techniques will allow organisms to be precisely tailored to their applications and thus improve their effectiveness.

Further development of bioleaching technology is aimed at extending the range of metals and ores that may be processed. New organisms, especially thermophilic bacteria, may accelerate the rates of metal extraction. There is a need for more fundamental research to determine the factors which limit the rate of microbial ore leaching, the characteristics of the ore bioleaching environment, the interactions of microorganisms and their effect on ore leaching.

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