

## Factors affecting bioleaching kinetics of sulfide ores using acidophilic micro-organisms

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**Recovery of metal values from sulfide ores by use of acidophilic microorganisms is gaining importance. A number of commercial/pilot plants are setup to find out the techno-economic feasibility of the overall process. The main drawback in the process is the slow kinetics of dissolution of metal values from the sulfide ores. To make the technology more attractive the kinetics should be improved considerably. There are various factors which determine the overall kinetics such as bacterial activity and concentration, iron and sulfur oxidation, oxygen consumption, reactor design and nature of ore. A brief review has been made dealing with the above parameters.**

**Keywords:** acidophilic, strain, oxidation, kinetics.

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### Introduction

The advancement of science has lead to the development of various new technologies that can meet the demand of ever-increasing population so that the mass at large would lead a comfortable life. Biotechnology is one of the recently developed frontier technologies and has been well exploited commercially, specially in the areas of pharmaceuticals, chemicals, food and enzyme production. The recovery of metal values from ores and minerals is also a part of vast area of biotechnology.

The popularity of biometallurgy is increasing due to two factors, mainly depletion of high grade ores and stricter enforcement of anti-pollution laws. The biometallurgy has several advantages compared to the conventional techniques, such as:

- a) Ecofriendly process
- b) Low capital investment
- c) Low manpower and less requirement of costly sophisticated controls.

Biometallurgy has a good potential for solving various metallurgical problems such as recovery of metal values from ores and minerals popularly known as bio-leaching, biodressing, biosorption and desulphurization of coal. Bioleaching technique has been widely used to recover metal values from ores. In this process the acidophilic microorganisms such as *Thiobacillus*, *Leptospirillum*, *Sulfobacillus*, *Acidianus* and *Sulfolobus* are generally used (Jordan *et al.* 1994, Bailey & Hansford 1993, Ahonen & Tuovinen 1994, Brierly 1993.) Recent trends also show that this technology can be applied to enhance the recovery of precious metals like gold and silver from refractory sulfide ores (Wang & Guan 1994, Miller & Hansford 1992, Jordan *et al.* 1996) as an alternative to pressure oxidation and roasting and a number of plants have been commissioned to recover gold in commercial scale (Hanzhao & Yongduo 1995, Dew *et al.* 1993). Apart from this, preliminary studies have also been carried out to recover metal values like zinc, cobalt, lead etc. from their respective sulfide ores (Donaldson *et al.* 1992, Holmes & Debus 1991).

Although the process is widely studied to recover the metal values from different ores but so far commercial application is only limited to low grade

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ores. The primary reason for low commercial importance of this technique is the slow bioleaching kinetics. The present review discusses the important parameters determining the bioleaching kinetics.

## Microorganisms

The acidophilic microorganisms that take part in dissolution of metals from the sulfide ores are autotrophic in nature. They can grow in inorganic medium having low pH values. They can tolerate high metal ion concentrations. The two main functions of this type of bacteria are oxidation of Fe(II) to Fe(III) and S to H<sub>2</sub>SO<sub>4</sub>.

These two reactions mainly control the reaction kinetics. To understand the reaction kinetics, it is mandatory to know about the characterization of the micro-organisms and conditions favorable to carry out the oxidation process.

The acidophilic microorganisms that actively take part in oxidizing Fe(II) to Fe(III) and S to H<sub>2</sub>SO<sub>4</sub> are *Thiobacillus*, *Sulfolobus*, *Acidianus* and *Leptospirillum*. *Thiobacillus* species are rod-shaped, gram-negative, non-spore forming and mesophilic except the thermophilic *Thiobacilli*, which can grow at a higher temperature (Buchanan & Gibbons 1974). *Sulfolobus* species are gram-positive, rod shaped with rounded or tapered ends and can grow at higher temperature. *Acidianus* species are spherical with tetrahedron, pyramid disc or saucer shaped lobes (Karavaiko 1988). *Leptospirillum* species are spiral shaped, non spore forming and gram negative (Blake *et al.* 1993).

Depending on their tolerance to temperature the acidophilic micro-organisms are categorized into three sub-groups such as mesophiles, moderate thermoacidophiles and extreme thermoacidophiles.

### Mesophiles

Mesophiles are those micro-organisms which grow at a prevailing room temperature, i.e. 28–37°C. Among the mesophiles, the most popular and widely used strain is *Thiobacillus ferrooxidans* (Sugio & Akhter 1996, Kai *et al.* 1989, Das & Mishra 1996, Bhattacharya *et al.* 1990). Although many strains of *Thiobacillus ferrooxidans* have been isolated from different sources, most of the strains showed the following optimum growth conditions, i.e. pH 1.5–2.5 and a temperature range of 28–37°C (Ahonen & Tuovinen 1989). *T. ferrooxidans* being a lithotroph, derives energy for its growth by oxidizing Fe(II) to Fe(III) and sulfur, sulfide and different oxyanion of

sulfur to sulfate. The assimilation of carbon dioxide by the microorganism is through Calvin Benson cycle catalysed by the ribulose-biphosphate carboxylase enzyme. The nitrogen requirement is met through ammonium compounds present in the medium. There is a poor resemblance of DNA homology between different strains of *Thiobacillus ferrooxidans*.

*Leptospirillum ferrooxidans* is an acidophilic microorganism like *Thiobacillus ferrooxidans*. The major drawback with *Leptospirillum ferrooxidans* is, it cannot oxidise sulphur to sulphate (Helle & Onken 1988). Therefore in order to oxidise Fe(II) and S it is essential to use a mixture of *Leptospirillum ferrooxidans* and *Thiobacillus ferrooxidans*.

*Thiobacillus thiooxidans* has the same morphological characteristics as that of *Thiobacillus ferrooxidans*. The main difference between the two microorganisms is that the former cannot oxidize Fe(II). The bacterium gets its energy by oxidizing S and soluble sulfur compounds to sulfate (Tuovinen *et al.* 1991, Suzuki *et al.* 1994).

### Moderate thermophiles

Moderate thermophiles are able to grow at a temperature of around 50°C. There are a number of thermophilic strains isolated from different geothermal environments and mine sites (Tuovinen *et al.* 1991). An important moderate thermophile is *Sulfolobus thermosulfidooxidans* which has the ability to oxidize sulfur and iron. (Lizama & Suzuki 1989, Morris & Barr 1985).

The bioleaching kinetics by moderate thermophiles is more than mesophiles as the leaching experiments are carried out at higher temperature. The moderate thermophiles are usually available inside the core of the dump as the core temperature is 10–15°C higher than the ambient.

### Extreme thermophiles

The extreme thermophiles are those which can grow actively even at a temperature as high as 80°C. The most important extreme thermophiles belong to the genus *Sulfolobus*. A number of *Sulfolobus* species have been isolated such as *S. acidocaldarius*, *S. solfatarius*, *S. brierleyi*, *S. ambioalvus*. They show the following properties such as :

- (i) anaerobic growth coupled with reduction of elemental sulfur.
- (ii) aerobic growth coupled with oxidation of sulfur.
- (iii) optimum growth temperature of 65–70°C.
- (iv) able to oxidize both Fe(II) to Fe(III) and sulfur to sulfate.

Since the extreme thermophiles can grow at higher temperature, so oxidation kinetics is more compared to mesophiles and moderate thermophiles (Lindstorm and Gunneriusson 1990).

The bioleaching kinetics in presence of extreme thermophiles is higher than that of mesophiles and moderate thermophiles. The bioleaching dissolution reaction is exothermic therefore the temperature increases during the reaction. So if extreme thermophiles are used then heat exchanger may not be required to control the leaching temperature.

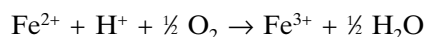
## Mechanisms of bacterial oxidation

As discussed above, the primary functions that are carried out by the micro-organism during bioleaching of sulfide ores are :

1. Oxidation of Fe(II) and S
2. Oxidation of metal sulfides to metal sulfates

### *Oxidation of Fe(II) and S*

The oxidation of Fe(II) can be presented as:



Cytochrome a and c along with the co-enzyme Q actively participates during Fe(II) oxidation (Sato *et al.* 1989). Co-enzyme Q acts as an intermediary electron carrier between Fe(II)-SO<sub>4</sub> organic complex associated with the cellular envelope (Nunzi & Bruschi 1993). Iron-cytochrome-c-oxidoreductase has been isolated, purified and characterized. The enzyme containing one atom of Fe(III) binds one atom of Fe(II), which reduces the enzyme bound iron and is then released. Then one molecule of oxidized cytochrome-c is bound by the enzyme, whose ferrous iron reduces the Fe(II) of the cytochrome-a. The iron of the enzyme is thus oxidized and the reduced cytochrome-c is released (Blake & Guinness 1993).

The iron oxidation by acidophilic microorganism is an important reaction in determining the dissolution kinetics. The Fe(III) reacts with metal sulfide (MS) to oxidize the same to MSO<sub>4</sub> and the former reduces to Fe(II). The Fe(II) is again re-oxidized to Fe(III) which reacts with MS. Therefore the strain having higher Fe oxidation rate would favor MS dissolution rate. Elemental sulfur is present in the form of a circular 8-atom molecule. The sulfhydryl of the cell reacts with the sulfur to form an organic polysulfide. The sulfur oxidising enzyme then oxidise the terminal sulfur atom to SO<sub>4</sub> via intermediate SO<sub>3</sub> (Blake & Shute 1994). Sulfite thus formed is oxidised to sulfate

by two principal methods such as via adenosine phosphosulfate or by cytochrome-c mediated oxidation (Silverman 1967, Srihari *et al.* 1992).

The above conversion of SO<sub>3</sub><sup>2-</sup> to SO<sub>4</sub><sup>2-</sup> essentially follows 3-steps, such as, in the 1st step, APS is formed by the APS reductase followed by the action of ADP sulfurylase, the sulfate moiety of APS, is replaced by phosphoric acid to form ADP and SO<sub>4</sub><sup>2-</sup> and in the final step, in the presence of adenylate kinase, the phosphoric acid group from one ADP molecule is transferred to another thus forming AMP and ATP. The iron and sulfur oxidation mechanism is being reviewed by several authors (Kuenen *et al.* 1993, Wiertz 1993).

The bacterial S oxidation rate is vital in determining the MS dissolution rate in two ways, firstly the S produced during MS dissolution acts as a product layer and forms an impervious layer over the reactant thus retarding the reaction kinetics and secondly the in-situ production of H<sub>2</sub>SO<sub>4</sub> which provides the necessary acidic environment required for the growth of acidophilic microorganisms.

### *Oxidation of metal sulfide (MS) to metal sulfate (MSO<sub>4</sub>)*

Conversion of metal sulfides to metal sulfates is possible either through indirect or direct mechanisms. In the indirect mechanism Fe(III) oxidised by the microorganism reacts with metal sulfide to form metal sulfate and in the process Fe(III) is reduced to Fe(II). The Fe(II) is further reoxidised to Fe(III) which again reacts with metal sulfide. But in the direct mechanism, the microorganism itself reacts with metal sulfide thus converting it to metal sulfate. In the direct mechanism initially a thin film is formed (Levia & Tributsch 1988) between the outer membrane of the attached bacterial cell and the surface of the sulfate mineral, followed by corrosion within the interfacial film. The mechanism of bacterial adhesion may be due to electrostatic, hydrophobic or other short or long range forces. The acidophilic microorganisms like *Thiobacillus* oxidise Fe(II) to Fe(III) in liquid medium, in which these are grown in absence of solid substrates. But if the bacteria are forced to utilize the mineral substrates to derive energy for their metabolism then it becomes necessary for the bacteria to secrete surface active reagents for the development of pilli that helps the same for attachment to the mineral surface (Natarajan 1995). It was reported that the development of flagella in *Thiobacillus ferrooxidans* depends on the culture conditions (Ohmura *et al.* 1996, Seeger & Jerez 1993).

So the necessary condition for direct mechanism is the attachment of bacteria with the sulfide minerals (Pogliani *et al.* 1990, Boon 1996). Espijo and Ruiz in 1987 attempted to determine the concentration of attached cell by radio-tracer technique. Several authors reported (Ohmura *et al.* 1994) that most of the cells are attached to the sulfide matrix. Yunker and Radovich in 1885 and 1986, concluded that the pyrite oxidation rate was dependent on the adsorption of *Thiobacillus ferrooxidans* to the pyrite surface. Konishi and Asai in 1993, postulated a direct bacterial action at mineral surfaces by means of a langmuir adsorption isotherm.

There are certain controversies regarding the direct mechanism, as the observation of pits (Normal & Snijman 1987, Zuo-Mei *et al.* 1984) does not conclusively prove the dissolution of metal sulfides through bacterial oxidation considering several other factors, such as, biooxidation mechanism, cyclic voltammetry analysis, amount of biomass attached on the mineral surface and bacterial kinetics using selective inhibitors (Chia *et al.* 1989; Hazen *et al.* 1986; Hazen *et al.* 1987; Boogerd *et al.* 1991; Verbaran & Huberts 1988; Boon 1996).

## Factors affecting the bioleaching kinetics

There are various factors by which the oxidation reactions of acidophilic microorganisms are affected, such as :

1. Activity of microorganisms
2. Bacterial concentration
3. pH and Fe(III) concentration
4. Supply of oxygen
5. Product layer
6. Galvanic interaction

### Activity of microorganisms

The activities of *Thiobacillus* and other acidophiles are indirectly measured in terms of iron and sulfur oxidation rate. The Fe and S oxidation rate differs from strain to strain (Mason *et al.* 1987, Bhattacharya *et al.* 1992). The difference in oxidation rate is due to many factors, such as change of morphology (Silverman and Roghoff 1961), difference in the chemical composition of lipopolysaccharide which is the main component of the cell-envelope (Vestal *et al.* 1973, Hirt & Vestal 1975), nutrient metabolism (Ralph 1985), tolerance towards organic materials (Wichlacz 1986) and DNA

base ratio (Rawlings *et al.* 1986). High strain microbes can be isolated from different mine sources or by UV irradiation or genetic manipulation, the activities can be increased manifold. (Summers *et al.* 1986, Torma 1988). The adaptation technique is another way of increasing the bacterial activity (Babij & Madgwick 1983). Most of the metal ions are toxic to the micro-organisms, therefore the adaptation technique helps the micro-organism to survive and grow in alien conditions. *Thiobacilli* and other acidophilic micro-organisms have the capability to grow in the presence of various kinds of metal ions after adaptation. The adaptation also helps to reduce the lag period, thus enhancing the overall leaching kinetics (Attia 1993).

### Bacterial concentration

Bacterial concentration in the solution is one of the controlling factors in determining the oxidation kinetics for sulfide minerals both in direct as well as indirect mechanism. In a continuous stirred tank reactor, the maximum concentration of microbial population varied between  $10^3$  to  $10^9$  cells/ml (Karavaiko 1988). Hence to increase the kinetics, the biopopulation has to be increased. The bacterial concentration in the lixiviant can be increased by harvesting the biomass from the bacterial solution and then inoculating the same in lesser volume of the solution. The harvesting can be done either by centrifugation or membrane filtration, but both the processes are rather costly and moreover it requires complicated instrumentation. The other way of increasing the microbial population is by the use of a biofilm type of reactor known as bacterial film oxidation (BACFOX) (Murayama *et al.* 1987, Gzrishin & Tuovinen 1988, Nikolov *et al.* 1988, Grishin *et al.* 1991). *Thiobacillus* and other acidophilic micro-organisms have special affinity towards jarosite, i.e. the microbial population gets concentrated on jarosite. Therefore, in a reactor if a thin film of jarosite can be developed under suitable conditions, then the acidophilic microorganisms would get necessary site for surplus growth. Pesic *et al.* in 1993, proposed a mechanism for jarosite formation and growth of microbial population over the same (Pesic & Kim 1993). The surface area of the reactor for jarosite precipitation can be increased by many ways such as by packing the reactor with corrugated sheet, tubes sulfide ores etc. The enhanced oxygen requirement by the bacterium can be supplied by aeration, either through biological contactor or distributor. Iron oxidation rate by the acidophilic microorganisms in this type of reactor

was reported (Murayama *et al.* 1987, Grishin & Tuovinen 1988, Nikolov *et al.* 1988, Grishin *et al.* 1991) to be high. The iron oxidation rate can reach a figure as high as 5–6 g/l.h. depending on the reactor design and the air utilization efficiency (Grishin *et al.* 1991). Apart from higher oxidation rate the other advantages of using this type of reactor are:

- a. precipitation of excess iron compounds so that the quantum of precipitate during leaching can be minimized.
- b. the reactor can also be utilized as an iron precipitator during further processing of leach liquor to recover the metal values (Toro *et al.* 1986, Kar *et al.* 1991).

The other alternative to increase the bacterial population in a solution is to apply an electrical potential. The bacterial population directly depends on the amount of Fe(II) oxidized. In a rough estimate, to produce 1 g of biomass it requires to oxidize 100 g of Fe(II). Therefore to produce 1 g of biomass a very high amount of FeSO<sub>4</sub> is required. To reduce the FeSO<sub>4</sub> requirement the bacterial oxidation reaction can be carried out under applied potential. In this system, anode and cathode chambers are separated by a membrane which allows the mobility of the electron. In the cathode chamber the Fe(II) which is oxidised by bacteria is then reduced back to Fe(II) by application of potential. Therefore in the cathode cell there would be perennial source of Fe(II) without external addition. In an ideal condition if the bacterial iron oxidation rate is equal to iron reduction rate by applied potential then it would give a lixiviant containing high bacterial concentration as well as high oxidation potential which are both favorable for sulfide dissolution kinetics (Natarajan 1992).

#### pH and Fe(III) concentration

pH of the growth medium significantly affects the growth and activity of acidophilic microorganisms. Amaro *et al.* in 1991 reported that *Thiobacillus ferrooxidans* responds to external pH changes by regulating the synthesis of several of its cellular components. According to Apel and Dugan in 1978, *Thiobacillus ferrooxidans* takes up H<sup>+</sup> from its external environment during the oxidation of Fe(II) to Fe(III). Hence H<sup>+</sup> is considered to be an essential nutrient for the bacterium. In general, the acidophilic bacteria, *Thiobacillus ferrooxidans*, are unable to initiate growth on Fe(II) at a pH greater than 3.0. The growth of the bacteria is usually initiated at a very low pH range and as the growth

continues the pH of the medium also increases without affecting the bacterial activity (Apel & Dugan 1978). Several authors have reported that the bacterial activity is enhanced many folds by adaptation of the bacterial strain to the growth medium maintained at a suitable pH (Elzeky & Altia 1995, Menon & Dave 1995, Barr & Jordan 1992).

A low Fe(III) concentration enhances the oxygen uptake by the acidophilic microorganism. At a higher concentration, Fe(III) competitively inhibited ferrous iron oxidation by *Thiobacillus ferrooxidans* (Nyavor *et al.* 1996). Curtchet *et al.* 1992 reported that in the absence of other sources of energy the presence of soluble Fe(III) inhibits the growth of *Thiobacillus ferrooxidans*.

#### Supply of oxygen

Bacteria need oxygen during oxidation of Fe(II) and sulfur. The solubility of oxygen in water at 35°C is 8 g/m<sup>3</sup> and it decreases with increase of ionic concentration in the solution (Karavaiko 1988). As per the stoichiometry, iron oxidation reaction by the bacterium requires 0.07 g of oxygen per gram of Fe(II) oxidized and this amount cannot be available from the solution considering the oxygen solubility, therefore, the extra oxygen has to be provided externally. The acidophilic micro-organisms are obligate aerobes and hence, low concentration of oxygen would impose constraints on the rate of oxidation by the same. It was reported by Liu *et al.* in 1987, that more than 0.2 g/dm<sup>3</sup> dissolved oxygen concentration in the solution is sufficient for the bacterium to retain the metabolic activities.

The copper solubilization rate in a dump or heap leaching operation greatly depends on the availability of oxygen. In an ideal situation the dump is constructed in such a way so that the air flow through the face of the dump and convects horizontally before the same ascends vertically. The main driving force of air inside the dump is the difference of air density. The differences of air density is directly related to the temperature distribution inside the dump. Due to the exothermic nature of sulfide dissolution, a temperature gradient is observed inside the dump. The air convection also depends on dump height, irrigation rate and dump permeability. Usually the air convection is good if the dump height is between 15–30 m and beyond this the air convection would be centered in and around the face. In a larger dump the higher solution flow rate favors a good air distribution inside the dump as the core temperature of the dump is higher and higher flow rate would bring down the

temperature where as a smaller dump requires a lower solution flow rate to attain good air convection. Apart from natural air convection, the availability of oxygen in a dump leaching operation can be increased by forced aeration. The oxygen deficiency in the dump usually occurs due to compaction of the ore and under these circumstances, the leaching rate can be improved by injecting air either through bore holes or tunnels (Hiskey & Bhapu 1987). A number of mathematical models have been developed regarding the air distribution inside the dump considering various leaching parameters such as temperature, permeability, flow rate, ore minerals (Pantelis & Ritchie 1991, 1992).

Bacterial leaching of sulfide ores has previously been limited to only dump or heap leaching operation, but due to possible utilization of acidophilic microorganism to treat the refractory ore containing precious metals in commercial scale, much interest is given to carry out the bioleaching studies in stirred reactors (Gormely 1992). In a stirred tank, the reactor design determines the overall oxygen mass transfer. The biological dissolution of sulfide ore is specific to the particular mineral/bacterial system. Therefore after evaluating the overall kinetics in terms of metal dissolution, iron as well as sulfur oxidation, the total demand of oxygen in the system can be evaluated. In order to utilize the oxygen to the maximum extent there should be proper design of the reactor, impeller, oxygen distribution as well as bubble size (Gormely 1990, Oolman *et al.* 1990, Boogerd *et al.* 1990, Fraser 1993, Hoffman *et al.* 1993). There are a number of proprietary technologies such as BIOX, Bac Tech and MINBAC process to treat the ore in a stirred reactor (Nicholsol *et al.* 1994, Brierley & Brans 1994, Torma 1991).

#### Product layer

The dissolution of sulfide ore through direct as well as indirect mechanism can be explained through shrinking core model (Sohn & Wadsworth 1979). In the shrinking core model, it is assumed that the substrate volume goes on decreasing with leaching. In this mechanism the following steps are involved:

- The attacking species have to diffuse through the thin liquid film as well as the product layer to reach the substrate or reacting species.
- The metal ions from the substrate have to diffuse from the product layer as well as the thin liquid film.
- If the product layer is impervious then there would be gradual reduction of fluxes of the reactants and products and as the quantum of

product layer increases the reaction rate falls off. On the contrary, if the product layer is porous or creates no additional diffusion barrier, then the reaction rate is independent of the product layer thickness. Therefore in shrinking core model, the product layer plays an important role in determining the rate kinetics.

In bioleaching process, there are three main product layers such as gypsum, precipitated iron compounds and sulfur. The ore contains various acid consuming gangue minerals such as carbonates and silicates and during leaching, the gangue minerals are neutralized by the acid forming gypsum. For better bacterial activity a part of the acid consuming minerals have to be neutralized as the acidophilic microorganism can grow in acidic medium. The iron chemistry is rather complicated and iron can be precipitated as hydroxide, goethite, jarosite or hematite. The formation of precipitated iron compounds depends on pH, Eh and concentration of Fe(III). Therefore, by carrying out the leaching experiment under control conditions, the extent of precipitation can be minimized (Canterford *et al.* 1985). Sulfur is formed during the dissolution of metal sulfides and forms a dense, tenacious, protective and insulating layer over the sulfide matrix. The layer limits the transport of species to and from the reaction site thereby limiting the dissolution rate of sulfide minerals. There are a number of reports (Ballester *et al.* 1992, Sukla *et al.* 1990, Ahonen & Tuovinen 1990) where the nature of product layer is changed from impervious to pervious layer by using catalyst like Ag ion and therefore the same would not hamper the reaction kinetics. The Ag ion reacts with the MS forming  $MSO_4$  and  $Ag_2S$ . The  $Ag_2S$  then reacts with  $Fe_2(SO_4)_3$  to form Ag ion and the cycle continues. The electrochemical reaction of  $Ag^+/Ag$  changes the nature of sulfur product layer from impervious to porous layer. Before using the Ag ion, the acidophilic microorganisms should be adapted to grow in presence of Ag as the same is extremely toxic (Tuovinen *et al.* 1985).

#### Galvanic interaction

The biokinetics of pyritic dissolution is faster compared to either sphalerite, galena or chalcopyrite. But when pyrite is in intimate contact with any of the above sulfide mineral then the rate of dissolution is just reversed, i.e. kinetics of dissolution of pyrite is least. The retardation of kinetics is due to galvanic interaction. The sulfide minerals are regarded as semiconductors and therefore can act as a carrier for electrons. Due to the electronic nature

of the sulfide minerals, the sulfide dissolution reaction can be considered as two half cells such as cathodic and anodic. Each sulfide mineral has its own rest potential depending on various factors such as crystallographic structure, solution composition, ionic concentration and nature of micro-organism (Natarajan 1992, Palencia *et al.* 1991). If pyrite is in intimate contact with sphalerite then pyrite having higher rest potential will act as a cathode and sphalerite as anode. Therefore sphalerite would be anodically dissolved whereas pyrite would be cathodically protected. Therefore in galvanic interaction the dissolution reaction of those sulfide minerals which acts as a cathode would be enhanced whereas the dissolution of anodic sulfide minerals would be minimized. So the galvanic interactions not only increase the dissolution reaction but preferentially leach a particular mineral. The galvanic effect depends on several factors (Natarajan 1988, Rao & Finch 1988, Jyothi *et al.* 1989) such as:

- a. rest potential of sulfide minerals
- b. nature and duration of contact
- c. presence of oxygen
- d. nature of electrolyte such as pH, conductivity and presence of other redox species.

## Conclusion

Biorecovery process is a widely accepted commercial technology to recover metal values from low/off grade sulfide ores which cannot be exploited commercially by usual techniques. So far the application of biorecovery technique to recover metal values from high grade ore concentrate has very limited application due to slow kinetics. With the possible use of this technique to enhance the recovery of precious metals from refractory ores, much emphasis is now being given to modifying the kinetics. In-depth knowledge regarding the reaction mechanisms including the characterization of microorganism and the enzyme system is required in order to enhance the kinetics by finding out the rate determining steps.

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## References

- Ahonen L, Tuovinen OH. 1989 Microbial oxidation of Fe(II) at low temperature, *Appl Environ Biotechnol* **55**, 312–316.
- Ahonen L, Tuovinen OH. 1994 Solid phase alteration and iron transformation in column biorecovery of a complex sulfide ore, *ACS Symp Ser* **550** (Env. Geochemistry of sulfide oxidation) 79–89.
- Ahonen L, Tuovinen OH. 1990 Silver catalysis of the bacterial leaching of chalcopyrite containing ore materials in column reactors. *Min Eng* **3**, 437–445.
- Amaro AM, Chamorro D, Seeger M, Arredondo R, Peirano I, Jerez C A. 1991 Effect of external pH perturbations on in vivo protein synthesis by the acidophilic bacterium *Thiobacillus ferrooxidans*. *J Bacteriol*, **173**(2), 910–915.
- Apel AW, Dugan RP. 1978 Hydrogen ion utilization by iron grown *Thiobacillus ferrooxidans*: *Metallurgical Applications of Bacterial Leaching and Related Microbiological Phenomena*. Eds. Murr EL, Torma AE, Brierley AJ, Academic Press, New York, San Francisco, London, 45–58.
- Attia YA, Eleky M, Ismail M 1993 *Int J Min Proc.* **37**, 61
- Babji T, Madgwick JC. 1993 High yield bacterial leaching of copper concentrate, *Proc Aust Inst Min Met* **287**, 61–64.
- Bailey AD, Hansford GS. 1993, Factors affecting bio-oxidation of sulfide minerals at high concentrations of solids. *Biotech Bioeng* **42**, 1164–1174
- Ballester A, Gonzalez F, Blazquez ML, Gomez C, Mier JL. 1992 The use of catalytic ions in biorecovery. *Hydromet* **29**, 145–160.
- Barr DW, Jordan MA, Norris PR, Phillips CV. 1992 An investigation into bacterial cell, Fe(II), pH and Eh interactions during thermophilic leaching of copper concentrate, *Miner Eng* **5**, 557–567.
- Bhattacharya D, Hsieh M, Francis H, Kermode RI, Khalid AM, Aleem HMI. 1990 Biological desulfurization of coal by mesophilic and thermophilic microorganisms, *Res Cons Recycl* **3**, 81–96.
- Bhattacharya P, Sarkar P, Mukherjee RN. 1990 Copper leaching from low grade ore, *Enzyme-Microb Technol*, **12**, **11**, 873–76
- Bhattacharya S, Das A, Chakrabarti BK, Banerjee PC 1992 A comparative study of characteristic properties of *Thiobacillus ferrooxidans* strains, *Folia Microbiol* **37**, 169–175.
- Blake RC, Guinness S Mc. 1993 Electron -transfer proteins of bacteria that respire on iron, *Biohydrometall. Technol. Proc Int Biohydrometall Symp*, **2**, 615–628.
- Blake RC, Shute EA. 1994 Respiratory enzymes of *T. ferrooxidans*. Kinetic properties of an acid-stable Iron-Rusticyanin Oxido-reductase, *Biochemistry*, **33**(31), 9220–9228,
- Blake RC, Shute EA, Greenwood MM, Spencer GH, Ingledew WJ. 1993 *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* and *Metallosphaera sedula*

- application in metal leaching: a review, *FEMS-Microbiol Rev* **11**, 1–3, 9–18.
- Boogerd FC, Bos P, Kuenen JG, Heijnen JJ, Van der Lans RGJM. 1990 Oxygen and carbon dioxide mass transfer and the aerobic, autotrophic cultivation of moderate and extreme thermophiles: A case study related to the microbial desulfurization of coal. *Biotech Bioeng* **35**, 1111–1119.
- Boogerd SC, Beemd CVD, Stoelwinder T, Bos P, Kuenen JG. 1991 Relative contribution of biological and chemical reaction to the overall rate of pyrite oxidation at temperatures between 30° and 70°. *Biotech Bioeng*, **38**, 109–115
- Boon M. 1996 Theoretical and experimental methods in the modeling of Biooxidation kinetics of sulfide minerals, Ph.D. Thesis (Delft University).
- Brierley CL. 1993 Practical role of thermophilic bacteria in bioleaching and biooxidation. BIOMINE-93.
- Brierley CL, Brans R. 1994 Selection of Bactech's thermophilic bio-oxidation process for Youanmi Mine, Biomine-94.
- Buchanan RE, Gibbons RE. 1974 Bergey's Manual Of Determinative Bacteriology, Baltimore .
- Canterford JH, Davey PT, Tsambourakis T. 1985 Gangue mineral dissolution and jarosite formation in copper solution mining, *Hydromet.* **13**, 327- 343.
- Chia LM, Choi WK, Ghay R, Torma AE. 1989 Electrochemical aspects of pyrite oxidation by *Thiobacillus ferrooxidans* during leaching of a Canadian uranium ore. *Biohydrometallurgy Proc Int Symp Edts Salley J, McCready RGL, Wichlacz PL*, 35–47.
- Curutechet G, Pogliani C, Donati E, Tedesco P. 1992 Effect of Fe(III) and its hydrolysis products (jarosite) on *Thiobacillus ferrooxidans* growth and on bacterial leaching. *Biotechnol Lett* **14**, 329–334.
- Das A, Mishra AK. 1996 Anaerobic and aerobic pyrrhotite, copper concentrate and pyrite oxidation and solubilization using sulfur (sulfide) dependent ferric ion oxidoreductase, application in leaching, *Appl Microbial Biotechnol.* **45**, 3, 377–82.
- Dew DW, Miller DM, Van Aswegen PC 1993 GEMIN's commercialization of the bacterial oxidation process for the treatment of refractory gold concentrates, in Randol Gold Forum, Beaver Creek 93, Randol International Ltd., Golden, Co., 229–237.
- Donaldson J, Grimes S, Tadesse B. 1992 (July) Cobalt transport in the environment-II-Bioleaching of gold. *Cobalt News* 12–13.
- Elzeky M, Attia YA 1995, Effect of bacterial adaptation on kinetics and mechanisms of bioleaching ferrous sulfides. *Chem Eng J*, **56**(2), B115-B125.
- Espejo RT, Ruiz P. 1987 Growth of free and attached *Thiobacillus ferrooxidans* in ore suspension, *Biotech Bioeng* **30**, 586–592.
- Fraser GM. 1993 Mixing and oxygen transfer in mineral bioleaching, Biomine' 93, 16.1–16.11.
- Gormely L. 1990 Mathematical modeling of oxygen transfer in a stirred tank bioreactor, *Adv. Gold and Silver Processing*, Nevada, USA, 217–223.
- Gormely L. 1992 Mechanical agitation and aeration in hydrometallurgical reactors. *Hydromet* **29**, 217–230.
- Grishin SI, Kachelkin AV, Adamov EV, Gusakov SE, Larin VK, Pendrov ED. 1991 Intensification of ferrous iron oxidation process using fixed film biomass (*Thiobacillus ferrooxidans*), *17th Int Mineral Proces Congr.* **5**, 91–104.
- Grishin SI, Tuovinen OH. 1988 Fast kinetics of Fe(II) oxidation in packed bed reactors. *Appl Environ Microbiol* **54**, 3093–3110.
- Hanzhao L, Yongduo J. 1995 The search for a process to recover Au from sub microscopic ores rich in As and C, *IX IMP*, **4**, 9.
- Hazen W, Bijleveld W, Grotenhns JTC, Kakes E, Kuenen JG. 1986 Kinetics and energetics of reduced sulfur oxidation by chemostat cultures of *Thiobacillus ferrooxidans*. *Antonie V. Leeuwenhoek*, **52**, 507–518.
- Hazen W, Schmedding DJ, Goddijin O, Bos P, Kuenen JG. 1987 The importance of the sulfur oxidizing capacity of *Thiobacillus ferrooxidans* during during leaching of pyrite. *Proc. 4th Europ. Congr. Biotech.* Edts. Neysel OM, Meervander RR, *Lujben KChAM*, **3**, 497–499.
- Helle U, Onken U. 1988 Continuous bacterial leaching of a pyrite concentrate by *Leptospirillum* like bacteria, *Appl. Microbiol Biotechnol* **28**, 553- 558.
- Hirt WE, Vestal JR. 1975 Physical and chemical studies of *Thiobacillus ferrooxidans*, lipopolysaccharides, *J. Bacteriol*, **123**, 642–650.
- Hiskey JB, Phule PP, Pritzker M D 1987 Studies on the effect of addition of silver ions on direct oxidation of pyrite. *116th AIME Annu. Meet*, Denver.
- Hiskey JB, Bhapu R. 1987 Role of oxygen in dump leaching, *Proc Int Symp Impact Oxygen Production Non-ferrous Metall Proces*, Edts Kachaniwsky G, Newman CJ, 165–183.
- Hoffman W, Batterham R, Conochie D. 1993 Design of a reactor bioleach process for refractory gold treatment, *FEMS Microbiol Rev* **11**, 221–230.
- Holmes DS, Debus KA. 1991 Opportunities for biological metal recovery. *Mineral Bioprocessing Edts. Smith RW, Misra M, TMS*, 53–78.
- Jordan MA, Barr DW, Phillips CV. 1993 Iron and sulfur speciation and cell surface hydrophobicity during bacterial oxidation of a complex copper concentrate. *Miner Eng.* **6**(8–10), 1001–1011.
- Jordan MA, McGinness S, Phillips CV. 1996 Acidophilic bacteria – their potential mining and environmental applications. *Minerals Engineering*, **9**(2), 169–181.
- Jyothi N, Sudha KN, Natarajan KA. 1989 Electrochemical aspects of selectivity bioleaching of sphalerite and chalcopyrite from mixed sulfides. *Int J Min Proc* **27**, 189–203.
- Kai M, Yano T, Fukumori Y, Yammanaka T. 1989 Cytochrome Oxidase of an acidophilic iron oxidizing bacterium, *Thiobacillus ferrooxidans* functions at pH 3.5, *Biochem Biophys Res Commun.* **160**, 839–843.
- Kar RN, Roy Chaudhury G, Sukla LB, Das RP. 1991 Kinetics of iron oxidation as well as precipitation by *Thiobacillus ferrooxidans* in presence and absence of various metal ions. *Erzmetal* **44**, 212–215.



- Karavaiko, GI 1988 Microorganisms & their significance for biotechnology of metals: Biogeotechnology of Metals. Manual. Edts. Karavaiko GI, Rossi G, Agate AD, Groudev SN, Avakyan ZA, Centre for International Projects GKNT, Moscow.
- Karavaiko GI Golovacheva RS, Pivovarova TA, Tzaplina IA, Vartanjan NS. 1988. Thermophilic bacteria of the genus *Sulfobacillus*. *Biohydrometallurgy: International Symp 1987*, Edts Norris PR, Kelly DP, 29–41.
- Konishi I, Asai S. 1993 A biochemical engineering approach to the of pyrite in the batch continuous tank reactors. In: *Biohydrometallurgical Technologies Proc Int Symp*. Wyoming, (Eds) Torma AE, Wey JE, Lakshmanan VI. 1, 259–268.
- Kuenen JG, Pronk JT, Hazen W, Meulenber R, Bas P. 1993 Areview of bioenergetics and enzymology of sulfur compound oxidation by acidophilic *Thiobacilli* *Biohydromet. Technol Proc Int Biohydrometall Symp 2*, 287–94.
- Lindstrom EB, Gunneriusson L. 1990 Thermophilic bioleaching of arsenopyrite using *Sulfolobus* and semi continuous laboratory procedure, *J Ind Microbiol 5*, 375–382.
- Liu MS, Branion RMR, Duncan DW. 1987 Oxygen transfer in *Thiobacillus* cultures. *Biohydrometall Proc Int Symp* Edts. Norris PR, Kelly DP 375–384.
- Lizama HM, Suzuki I. 1989 Bacterial leaching of sulfide ore by *Thiobacillus ferrooxidans* and *Thiobacillus thiooxidans*, Part II, Column leaching studies. *Hydromet.*, **22**(3), 301–310.
- Lizama HM, Suzuki I. 1988 Bacterial leaching of a sulfide ore by *Thiobacillus ferrooxidans*: Shake flask studies. *Biotech. & Bioeng.* **32**, 110–116.
- Mason J, Kelly DP, Wood AP. 1987, Chemolithotrophic and autotrophic growth of *Thiobacillus thiooxidans* and some *Thiobacilli* on thiosulphate and polythionates and a reassessment of growth yields of *Thiobacillus thiooxidans* in chemostat culture, *J Gen Microbiol 133*, 1249–1256.
- Menon AG, Dave SR. 1995, Growth behavior of various *Thiobacillus ferrooxidans* isolates on different substrates. *Trans Ind Inst Met 48*(2), 135–138.
- Miller DM, Hansford GS. 1992 Batch biooxidation of a gold-bearing pyrite arsenopyrite concentrate, *Miner Eng 5*, 613–629.
- Morris PR, Barr DW. 1985 Growth and iron oxidation of acidophilic moderate thermophiles, *FEMS Microbiol Lett 28*, 221.
- Murayama T, Konna Y, Sakata T, Imaizumi T. 1987 Application of immobilised *Thiobacillus ferrooxidans* for large scale treatment of acid mine drainage. *Methods Enzymol 136*, 530–540.
- Natarajan KA. 1992 Application of applied potential and growth of *Thiobacillus ferrooxidans*, *Biotech Bioeng 39*, 907–913.
- Natarajan KA. 1988 Electrochemical aspects of multisulfide minerals. *Miner Metall Process*, **5**, 61–65.
- Natarajan KA. 1992 Electrobioleaching of base metal sulfides, *Met Trans 23B*, 5–11.
- Natarajan KA. 1995 Biomining Beneficiation, Mineral processing, Recent Advances and Future trends. Edts. Mehrotra SP, Shekhar R (Allied Publisher), 489
- Nicholson HM, Smith GR, Stewart RJ, Kock FW, Marais HJ. 1994 Design and commissioning of Ashati's Sansu BIOX plant , Biomine-94.
- Nikolov LN, Valkova-Vulchanova M, Mekhochev D. 1988 Oxidation of high ferrous iron oxidation concentrations by chemolithotrophic *Thiobacillus ferrooxidans* in packed bed bio-reactors. *J Bacteriol 7*, 87–94.
- Normal PF, Snijman CP. 1987 The biological and chemical leaching of an auriferous pyrite/arsenopyrite flotation concentrate: a microscopic examination. *Geomicrobiol J 6*, 1–10.
- Nunzi F, Bruschi M. 1993 Iron oxidn. by T. ferrooxidans structure, function, relationships of a blue copper protein rusticyanin, *Biohydrometall Technol Proc Int Biohydrometall. Symp.*, **2**, 603–613.
- Nyavor K, Egiebor NO, Fedorak PM. 1996, the effect of ferric ion on the rate of ferrous oxidation by *Thiobacillus ferrooxidans*. *Appl. Microbial. Biotechnol.* **45**(5), 688–691.
- Ohmura N, Tsugita K, Koizumi J, Saika H. 1996 *J Bacteriol.* **178**, 5776.
- Ohmura N, Kitamura K, Saiki H. 1993 Selective adhesion of *Thiobacillus ferrooxidans* to pyrite. *Appl. Environ. Microbiol.* **59**(12), 4044–50.
- Oolman T, Nagpal S, Dahlstrom DA. 1990 Oxygen and carbon dioxide consumption with steady state continuous flow bioleaching. *Adv Gold and Silver Processing*, Nevada, USA, 237–245.
- Palencia I, Wan RY, Miller JD. 1991 The electrochemical behaviour of a semiconducting natural pyrite in the presence of bacteria. *Met Trans 22B*, 765–774.
- Pantelis G, Ritchie AIM. 1992 Rate -limiting factors in dump leaching of pyrite ores, *Appl Math Modelling 16*, 553–560.
- Pantelis G, Ritchie AIM. 1991 Macroscopic transport mechanism as a rate limiting factor in dump leaching of pyrite ores. *Appl Math Modelling 15*, 136–143
- Pesic B, Oliver DJ, Wichlacz P. 1989 An electrochemical method of measuring the oxidation rate of Fe(II) to Fe(III) with oxygen in the presence of *Thiobacillus ferrooxidans*. *Biotech Bioeng 33*, 428–439.
- Pesic B, Kim I. 1993 Electrochemistry of *Thiobacillus ferrooxidans* interactions with pyrite. *Metallurgical Transactions 24B*, 717–727.
- Pogliani C, Curutchet G, Donati E, Tedesco PH. 1990 A need for direct contact with particle surfaces in bacterial oxidation of covellite in absence of chemical lixiviant, *Biotechnol Lett 12*, 515–518.
- Pott BM, Olson G, Larson L, Karlsson HT, Holst O. 1995 Consecutive desulphurization of coal with *Thiobacillus ferrooxidans* and *Acidianus brierleyi*, *Res Env Biotech 1*, 21–32.
- Ralph BJ. 1985 Biotechnology applied to raw mineral processing, Comprehensive Biotechnology, Vol. IV, Edt. Moo-Young M, Pergamon.
- Rao SR, Finch JA. 1988 Galvanic interaction studies on sulfide minerals, *Can Metll Q 27*, 253–259.

- Rawlings DE, Pretrins IM, Woods DR. 1986 Expression of *Thiobacillus ferrooxidans*, plasmid, functions and the development of genetic system for the *Thiobacilli*. *Biotechnol Bioeng Symp.* **16**, 281–287.
- Rodriguez-Levia M, Tributsch H. 1988 Morphology of bacterial leaching patterns by *Thiobacillus ferrooxidans* on synthetic pyrite. *ArchMicrobiol.* **149**, 401–405.
- Roy P, Misra AK. 1981 Factors affecting oxidation of pyrite by *Thiobacillus ferrooxidans*, *Ind J of Exptl Biol* **19**, 728–732.
- Sato A, Fukumori Y, Yano T, Kai M, Yamanka T. 1989 *Thiobacillus ferrooxidans*. cytochrome-c, purification and some of its molecular features. *Biochim Biophys Acta.* **976**, 129–134.
- Seeger M, Jerez CA. 1993, Phosphate-starvation induced changes in *Thiobacillus ferrooxidans*. *FEMS Microbial Lett.* **108**, 35.
- Silverman MP1967 Mechanism of bacterial pyrite oxidation. *J Bacteriol.* **94**, 1046
- Sohn HY, Wadsworth ME. 1979 In Rate processes of extractive metallurgy, Edts. Sohn H Y, Wadsworth M E, Plenum.
- Srihari B, Modak SR, Kumar JM, Gandhi KS 1992 Dissolution of sulfur by *Thiobacillus ferrooxidans*: Substrate for unattached cells. *Biotechnol Bioeng.* **41**, 612
- Sugio T, Akhter F. 1996 *Thiobacillus* mediated leaching and the effect of hydrogen sulfide ferric ion oxidoreductase activity, *J Ferment Bioeng.* **8**, 4, 346–50.
- Sukla LB, Roy Chaudhury G, Das RP. 1990 Effect of silver ion on kinetics of biochemical leaching of chalcopyrite concentrate. *Trans Inst Min Met* **90**, C43–C46.
- Summers AO, Roy P, Davidson MS, 1986 Current techniques for the genetic manipulation of bacteria and their application to the study of sulfur-based autotrophy in *Thiobacillus*. *Biotechnol Bioeng Symp.* **16**, 267–279.
- Suzuki H, Tanaka T, Tano T, Sugio T. 1993 Existence of sulfide-binding protien in iron-oxidizing bacteria, *Biohydrometall Technol Proc Int. of Biohydrometall Symp.* **2**, 423–31
- Torma AE, 1988 Use of Biotechnology in Mining and Metallurgy, *Biotech Adv.* **6**,1–8.
- Torma AE. 1991 In: New Trends in Biohydrometallurgy, *Proc Min Bioproc.* California, 43–55.
- Toro L, Paponetti B, Cantalini C. 1986 Precipitate formation in the oxidation of Ferrous ion in the presence of *Thiobacillus ferrooxidans*. *Hydromet.* **20**,1–9.
- Tuovinen OH, Kelly BC, Groudev SN. 1991 *Mixed-culture in biological leaching processes and mineral biotechnology, Mixed cultures in Biotechnology*, Eds. Zeikus, J. G., Johnson, E. A.; McGraw Hills, New York.
- Tuovinen OH, Puhakka J, Hiltunen P, Dolan KM. 1985 Silver toxicity to Fe(II) iron and pyrite oxidation and its alleviation by yeast extract in cultures of *Thiobacillus ferrooxidans*. *Biotech Lett.* **7**, 389–394.
- Verbaan B, Huberts R. 1988 An electrochemical study of bacterial leaching of synthetic Ni<sub>3</sub>S<sub>2</sub>. *Int J Min Proc* **24**, 185–202.
- Vestal JR, Lundgren DG, Milner KG. 1973 Toxic and Immunological differences among lipopolysaccharides from *Thiobacillus ferrooxidans*, grown autotrophically and heterotrophically, *Can. J.Microbiol.* **19**, 1335–1339.
- Wang E, Guan G. 1992 Bacterial leaching of high-arsenic gold ores, *Dongbei Gongxueyuan Xuebao*, **13**(6), 527–32.
- Wichlacz PC, Unz RF, Langworthy TA, 1986 *Acidiphilum angustum*, *A. facilis* and *A. rubrum* : acidophillic heterotrophic bacteria isolated from acidic coal mine drainage, *Int. J. Syst. Bacteriol*, **36**, 197–201.
- Wiertz JV. 1993 Ferrous and sulfur oxidation by *Thiobacillus ferrooxidans*, *Biohydrometall Technol Proc Int. Biohydrometall. Symp.* **2**, 463–71.
- Yunker SB, Radovich JM, 1986 Enhancement of growth and ferrous iron oxidation rates of *Thiobacillus ferrooxidans* by electrochemical reduction of ferric iron, *Biotech Bioeng.* **28**, 1867–1875.
- Yunkur, SB, Radovich, JM. 1985 Enhanced growth of *Thiobacillus ferrooxidans* in an electrolytic bioreactor, *Biotechnol Bioeng Symp Ser.* **15**, 307–319.
- Zuo-Mei J, Warren GW, Henein, H. 1984 Reaction kinetics of ferric chloride leaching of sphalerite – an experimental study. *Metall Trans. B.* **15B**, 5–12.