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New Substrates

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The Role of Thiobacillus ferrooxidans in Hydrometallurgical Processes

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The present article illustrates the increased interest which is manifested in the microorganisms, *Thiobacillus ferrooxidans*, involved in the biohydrometallurgical extraction processes. The wide varieties of problems currently studied are very important in order to gain a better understanding about the factors which are governing the growth of microorganisms, and as a consequence, the metal dissolution phenomena. In several mining sites, the microbiological leaching techniques are currently practiced at industrial-scale, especially for recovery of copper and uranium from low-grade materials. However, an accurate assessment of further potential possibilities for the application of

microorganisms in leaching metal sulfides requires a more fundamental knowledge about the interactions of the physical and chemical factors with the growth of *T. ferrooxidans* in pure and mixed cultures including heterotrophic and thermophilic cohabitants. Altogether, the future industrial exploitation of these microbiological leaching techniques are very attractive in many countries of the world.

1. Introduction

In spite of the fact that bacterial oxidation of sulfide minerals has been occurring for centuries, microbiological leaching is only a recent development. The microorganisms, *Thiobacillus ferrooxidans,* responsible for this oxidation were first isolated in 1947 from the acid mine drainage of bituminous coal mines [1]. The presence of copper in mine drainage waters was observed by the Phoenicians, Romans, Arabs and Spaniards. The earliest leaching of copper from copper sulfide-bearing materials was recorded in 1970 at Rio Tinto in Spain [2]. However, the presence of bacteria in leach waters of the Rio Tinto mines was not confirmed until 1963 [3]. Dump leaching techniques were practised in the United States of America, Peru, Canada, Africa and in many other locations without knowing about the contribution of the microorganisms in these processes.
The earliest report on microbiological leaching of metal sulfides was published in 1922, using some non identified autotrophic bacteria [4, 5] and suggesting that the biological treatment might be an economical way for the extraction of metals from low-grade sulfide-bearing ores. This idea was neglected for the next twenty five years until the discovery and characterization of the chemolithotrophic *T. ferrooxidans* [1, 9–11]. These bacteria can tolerate exceptionally high metal and hydrogen ion concentrations, for example, 120 g/l of zinc [12], 72 g/l of nickel [13], 30 g/l of cobalt [13], 12 g/l U_3O_8 [14], 55 g/l of copper [15], 160 g/l of iron (II) [16] and an acid medium of pH 1.0 to 5.0 [17]. These facts are of considerable economic significance from metallurgical point of view and because, unlike many other fermentations, the bacterial leaching does not require an expensive sterilisation of the medium prior to inoculation. *T. ferrooxidans* are virtually ubiquitous. They can be found everywhere in nature, wherever an acidic environment is maintained in the presence of sulfide minerals [6–8].
The chemolithotrophic microorganisms [18] have the ability to utilize energy released from the metabolic oxidation of inorganic substrates [19; 20] such as reduced-valence inorganic sulfur compounds [21–23] and ferrous ion [24]. The chemical energy is converted by oxidative phosphorylation to ATP [25]. This is universally recognized to be the form of metabolic energy which can be utilized by the cell [26] for transportation work (substrate and nutrients into the cell and product out of the cell), mechanical work (muscle work for vibration and locomotion) and biosynthesis work (synthesis of cellular material). In this process the ATP is hydrolyzed to ADP and inorganic phosphate. These latter two species will be recombined into high energy carrier ATP in the followed-up respiration. The carbon metabolism by the chemolithotrophic bacteria may be either autotrophic, or facultative which represents a nutritional mode between the autotrophic and heterotrophic metabolism. The autotrophic capabilities of bacteria

were established in 1887 [27] and can be defined as the ability to grow on strictly inorganic substrates providing energy for growth and carbon dioxide as the main source of carbon for the biosynthesis of cell materials [28, 30, 31].

The discovery of *T. ferrooxidans*, opened up an area of research which has had and will continue to have considerable economic significance. It represents a potential solution to the problem faced in many countries where continuing depletion of high-grade ore deposits has created a need to develop effective methods for recovering metals from low-grade sulfide ores.

The microbiological leaching of metal sulfides can be defined as a biochemical oxidation process catalyzed by living organisms. However, only the insoluble sulfides are of commercial consequence. This process can be represented by the following simplified equation:

$$MS + 2\,O_2 \xrightarrow{\text{microorganisms}} MSO_4, \qquad (1)$$

where M is a bivalent metal. When the oxidation product is insoluble, as it is the case, i.e., for the lead sulfide leaching, this fact can be used for selective leaching purpose [30] to separate the insoluble from the solubilized metals.

The microbiological leaching processes involve complex interactions between the microorganisms, substrates and the nutrient concentrations, which are not yet completely understood. Altogether, a more economic use of these leaching processes require a better understanding of the various factors influencing bacterial growth and as a consequence, the microbiological metal dissolution processes.

2. Microbiological Background

a) Morphology of *T. ferrooxidans*

T. ferrooxidans possesses the following morphological characteristics: it is a motile, flagellated [32–34], non spore forming, Gram-negative, rod shaped (0.1 by 1.5 μm) bacterium occurring single or occasionally in pairs [9, 10]. The growing cell goes through lag, log, stationary and death phases. When cell growth reaches about the double of a single cell size, it divides by binary fission. In the death cell, the mechanisms which regulate the permeability of the cell wall and cytoplasmic membrane do not function and the cell is plasmolyzed and broken down under the influence of the acid medium [35].

b) Physiology of *T. ferrooxidans*

The microorganisms, *T. ferrooxidans*, derives the necessary energy for its life processes from oxidation of ferrous ion and of reduced-valence inorganic sulfur compounds and utilizes carbon dioxide for growth [33]. It is morphologically and, in some aspects, physiologically similar to *T. thiooxidans*, which is often present in acid mine drainage [36, 37]. The fundamental difference between the two species is generally recognized

to be the inability of *T. thiooxidans* to oxidize ferrous iron and insoluble metal sulfides [9, 10, 36].

Other bacteria have been identified from acid mine waters, oxidizing ferrous iron but not elemental sulfur or thiosulfate. It was considered to be a new genus and assigned the name of *Ferrobacillus ferrooxidans* [38, 39]. Similarly, the name *Ferrobacillus sulfooxidans* was assigned to a microorganism which utilized ferrous iron and elemental sulfur but not thiosulfate [40]. Subsequent investigations [41–45] indicated that the microorganisms (*T. ferrooxidans, F. ferrooxidans* and *F. sulfooxidans*) were identical and should be called *T. ferrooxidans.* All these organisms were capable of oxidizing elemental sulfur and thiosulfate in addition to ferrous ion [44]. The earlier, apparent fragmentation of the nomenclature and classification for this single species resulted from the use of different techniques in studying it.

A new approach in the naming and classifying of bacteria is to reflect the manner in which present organisms are related by virtue of descent [53]. The increasing knowledge of comparative cytology and biochemistry led to the characterization of bacteria and blue-green algae as being *procaryotic* cells [54], possessing a simpler and an evolutionary more primitive structure than do all other cells (eucaryotic, i.e., possessing true mitotically dividing nuclei). The latest edition (8th) of the Bergey's Manual carries now the GC content of the DNA of each described nomenclatural type of organisms. The studies of DNA base composition appear to be the most beneficial when the cultures analysed are characterized by other biochemical means [55–57].

The DNA base composition of ferrous iron grown *T. ferrooxidans,* which belongs to the procaryote group of microorganisms, has been found to be in a narrow range of 56.0–57.0% GC [33]. However, recent studies [58, 59] on ferrous iron, chalcopyrite and lead sulfide grown *T. ferrooxidans* indicated 56.0, 60.1 and 54.4% GC respectively. The relatively important variations obtained for *T. ferrooxidans* grown on different substrate seemed to be not attributable to the analytical methods (melting temperature, cesium chloride density gradient centrifugation and ultraviolet absorbancy ratios) because of the good reproducibility of the results (51.0, 51.5, and 51.8% GC respectively) obtained for *E. coli* reference DNA. These variations cannot be explained by the adaption of *T. ferrooxidans* to the specific substrate. This problem is very complex and considerable caution must be exercised in extrapolating laboratory observations to microorganisms in their natural habitat [60]. Under natural conditions pure culture do not occur, growth rates are very slow by laboratory standards. In the development of heavy metal resistance in various organisms, it is difficult to decide whether adaptation, mutation, cohabitation or a combination of these is involved. However, the possibility exists that in these studies a kind of selection of microorganisms took place as suggested by other investigators [61–63] who isolated new species of bacteria from cultures of *T. ferrooxidans* by changing environmental conditions and substrates. Data are available indicating that in the relatively strong sulfuric acid media [64] other (mixotrophic) microorganisms (algae, molds, protozoa and bacteria) than *T. ferrooxidans* can develop simultaneously. This view is supported by recent studies on microbial mutualism in ore leaching [65, 66], i.e., use of an aerobic, nitrogen fixing *Beijerinckia lacticogenes* in presence of *T. ferrooxidans.* Further, it has been pointed out [67], that considerable caution must be exercised when GC-data are compared from different laboratories.

c) Chemical Composition and Structure of *T. ferrooxidans*

The first report [46] on cell composition of *T. ferrooxidans* indicated that it contained approximately 20% protein which consisted of 13 amino acids, and two B-vitamins: riboflavin and thiamine. These results have been refined [47] and the following cell composition has been obtained: 44% protein, 26% lipid, 15% carbohydrate, 10% ash, and at least, the two B-vitamins mentioned before.

The cell structure of *T. ferrooxidans* has been found to be similar to that of other Gram-negative bacteria [47, 48]. The cell envelope, which is semi-permeable to nutrient [49, 50], appears to be composed of three osmophilic and three osmophobic layers, the total thickness measures 125 to 215 Å [51, 52]. These layers are composed of lipoprotein, lipopolysaccharide, globular protein and peptidoglycan passing from outer to inner layers [51, 68]. The lipopolysaccharide layer consists of heptose, glucose, galactose, mannose and 2-keto-3-deoxyoctulosonate. Iron, mostly in the ferric form is associated with the lipopolysaccharide, suggesting it might serve as the initial binding site for the substrate. The peptidoglycan layer consists of glutaminic acid, α-ϵ-diaminopimelic acid, alamine, glucosamine and muramic acid [69]. These two layers are of similar composition as those of *E. coli* strains [70–72]. Phospholipids and neutral lipids are also related to the cell envelope structure [73]. The cytoplasma of *T. ferrooxidans* contains ribosomes, nuclear materials and cell inclusions [52, 68, 69].

At the present time, the relation between the structure and the function of cell envelope of *T. ferrooxidans* remains to be obscure. However, a better understanding of these problems would be very important from the point of view of evolution of chemolithotrophs and elucidation of oxidation pathways of insoluble inorganic sulfur substrates.

d) Mechanisms of Bacterial Action

The fact that *T. ferrooxidans* is capable of oxidizing ferrous ion and the reduced-valence forms of inorganic sulfur compounds, is an indication that it should have an enzymatic system similar to those of the iron and sulfur oxidizing bacteria. With regard to the mechanisms of metabolism of these substrates, there exist still different opinions over the basic concepts of oxidation pathways. There is agreement however, that the solid substrates must be rendered soluble before the bacterial oxidation could take place [17, 74] and the same time, nutrients have to be available in the environment of the contacted mineral surface.

d. 1) Oxidation of Inorganic Sulfur Compounds.

The metabolism of inorganic sulfur compounds has been studied extensively [20, 21, 75, 76, 77, 80, 81]. The inorganic sulfide to sulfate oxidation can be represented by a simplified schema as follows:

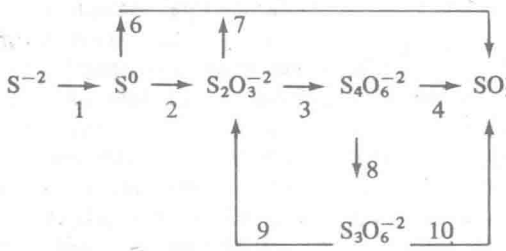
$$S^{-2} \rightarrow S^{+6} + 8\,e^{-}. \qquad (2)$$

In this reaction 8 electrons are removed from the substrate and will be carried out through a series of intermediate products: S_x^{-2}, SO^{-2}, SO_2^{-2}, $S_2O_2^{-2}$, $S_2O_4^{-2}$, $S_xO_6^{-2}$, SO_3^{-2}, and so on. However, many of these products are highly unstable [78, 92] and probably

could not exist under physiological conditions. It has been proposed [79] that the first intermediate of sulfur oxidation is sulfite:

$$S^0 + O_2 + H_2O \xrightarrow{\text{bacteria}} H_2SO_3 \tag{3}$$

which is probably the key reaction in the pathways of the oxidation of sulfides, polysulfides or polythionates. On the basis of the available data [22, 82–85] the following scheme can be suggested for sulfides oxidation by *T. ferrooxidans:*



(4)

Reactions 1–4 are catalyzed by the sulfur-oxidizing enzyme [89, 83, 90, 91] where sulfite is the product of the reaction. Sulfite is oxidized to sulfate [85] by sulfite-oxidase (reaction 5) with the formation of ATP [88]. Thiosulfate is formed [84] by oxidation of elemental sulfur (reaction 7). It can be cleaved [82] by rhodanese (reaction 8) to sulfite and elemental sulfur. The thiosulfate is oxidized to tetrathionate (reaction 3) by the thiosulfate–oxidizing enzyme [84]. The reactions 8–10 may exist if *T. ferrooxidans* species behave like other Thiobacilli [86, 87].

d. 2) Oxidation of Ferrous Ion

The oxidation of ferrous ion has been studied by many investigators [34, 93–99] using *T. ferrooxidans:*

$$Fe^{+2} \rightarrow Fe^{+3} + e^- . \tag{5}$$

A mechanism of iron oxidation is proposed [24] in which the complexing of ferrous ion with molecular oxygen precedes the electron transport involving sulfate [100–102] and phosphatidylserine [73, 103] in the oxidation. The isolation of cytochrome *c* and cytochrome *a* from *T. ferrooxidans* [104] allowed the postulation of iron oxidation through the cytochrome system with oxygen as the final electron acceptor [96, 104–106]:

$$\left.\begin{matrix} Fe^{+2} \\ Fe^{+3} \end{matrix}\right) \quad \left(\begin{matrix} \text{oxidized} \\ \text{cytochrome } c \end{matrix}\right) \quad \left(\begin{matrix} \text{reduced} \\ \text{cytochrome } c \end{matrix}\right) \quad \left(\begin{matrix} \text{reduced} \\ \text{cytochrome } a \end{matrix}\right) \quad \left(\begin{matrix} \text{oxidized} \\ \text{cytochrome } a \end{matrix}\right) \quad \left(\begin{matrix} \frac{1}{2}\,O_2 + H_2 \\ H_2O \end{matrix}\right) \tag{6}$$

It has been reported that the cytochrome *c* reductase is associated with DNA [107] and the nucleic acid involved was RNA [108]. Further, the iron-cytochrome *c* reductase was

inhibited by copper, nickel, chromium and mercury ions [107, 109]. The resting cell suspensions consume oxygen at high rate [93, 94, 96], however the highest rate is reported to be 21 000 μl O_2/mg cell N/h [95]. The mean generation time on ferrous ion was reported to be 7.3 h [41].

e) Kinetics of Microbial Growth

Studies of microbial growth involve periodic observations of bacterial growth, substrate utilization and product formation. Kinetic analysis makes the connection between metabolism and growth of the living organisms [26]. This fundamental information allows one to make maximum advantage of bacterial conversion, and also, suggests techniques which could lead to an increase in the yield of the desired product [123]. However, the biokinetic modeling is especially difficult because of the many different metabolic pathways and side reactions involved which are important to the life of bacterial cell. Major complications in modeling come from the fact that many of the reaction mechanisms of the cell's metabolisms are not completely understood. Factors influencing bacterial growth are numerous, and the biological knowledge and mathematical tools necessary for the formulation and study of a completely general model do not exist [110].

The kinetic character of individual growth processes differs widely. However, certain characteristics typical for a group permit their classification in three different ways: phenomenological [11, 112], thermodynamic [113, 114] and kinetic [115].

The hypothesis that the enzyme (E) and the substrate (S) form a complex (ES) in enzyme catalyzed product (P) formation was originally derived in 1913 [116, 121]:

$$E + S \underset{k_2}{\overset{k_1}{\rightleftarrows}} \{ES\} \overset{k_3}{\rightarrow} E + P. \tag{7}$$

In batch systems where the substrate concentration is a limiting factor the growth rate (V) may be expressed [117] by:

$$V = V_m \, S/(K + S), \tag{8}$$

where V_m is the maximum growth rate and K constant.

A plot of $1/V$ versus $1/S$ gives a linear relationship [118] where the intercept is $1/V_m$ and the slope K/V_m. There exist many alternative forms [119, 120, 122] of the linearized Eq. (8).

When sulfide minerals are used as substrate, S can be represented by the specific or total surface area [124]. With increasing fineness of the particles, and with all other nutrients in excess, the metal extraction rate, which is proportional to the growth rate [125], increases towards a limit.

A more general form for bacterial growth limited by a single nutrient is given by the next equation [126]:

$$V = V_m \, (1 - \exp (1 - S/K)). \tag{9}$$

When S/K is small the Eq. (9) becomes approximately equal to Eq. (8), which is known to be the hyperbolic rate equation relating the effect of a single limiting nutrient on the specific growth rate. However, in the most important processes this condition is not maintained and more than one substrate is used. For those cases Eq. (8) has to be modified. For example for the two-substrate reaction [127] it can be written:

$$V = V_m S_1 S_2/(1 + K_1 S_1)(1 + K_2 S_2). \tag{10}$$

Similarly an equation has been developed for ternary complex formation [128] in which, ordered sequence of substrate addition is supposed.

Despite the fact that most workers [117, 126, 129–132, 162] have regarded the specific growth rate of a population as a single function of the concentration of the limiting substrate, others were able to show that it is also a function of the population density [133, 134] and the mass transfer or the assimilation processes [135]. These authors [133–135] also claim general applicability of their models to both batch and continuous systems.

For a single stage continuous culture [182] the rate of bacterial growth, dX/dt, and substrate utilization, dS/dt, can be defined as follows:

$$\frac{dX}{dt} = (\mu - D) X \tag{11}$$

and

$$\frac{dS}{dt} = (S_0 - S) - D\frac{\mu X}{Y}. \tag{12}$$

Under steady state conditions the specific growth rate, μ, is equal to the dilution rate, D, which is the reciprocal of the mean holding time. Hence the bacterial output and the substrate utilization can be given by:

$$X = Y(S_0 - S) = Y\left(S_0 - K\frac{D}{\mu_m - D}\right) \tag{13}$$

since

$$S = K\frac{D}{\mu_m - D}. \tag{14}$$

Further, the efficiency (η) of substrate utilization can be expressed by:

$$\eta = \frac{S_0 - S}{S_0} = \frac{S_0 - K\dfrac{D}{\mu_m - D}}{S_0}. \tag{15}$$

Equation (15) shows that the maximum substrate utilization could be obtained at low dilution rates, whereas the maximum bacterial growth respectively substrate consumption, will be achieved at high dilution rates.

It has been shown [183] that for the oxidation of one mole of the limiting substrate,

B moles of oxygen are required. The rate of oxygen uptake, dC/dt, may be expressed by:

$$-\frac{dC}{dt} = -B\frac{dS}{dt} \tag{16}$$

It follows, therefore, that the rate of oxygen uptake is directly proportional to the growth rate.

$$-\frac{dC}{dt} = \frac{B}{Y}\frac{dX}{dt} = \frac{B}{Y}\mu X. \tag{17}$$

For steady-state condition $\mu = D$ and

$$Y = X/(S_0 - S). \tag{18}$$

Incorporating these terms we get

$$-\frac{dC}{dt} = B\mu(S_0 - S) \cong BDS_0. \tag{19}$$

S may be neglected since it is usually small as compared to S_0. The oxygen demand of a bacterial culture in a continuous system is directly proportional to the dilution rate and the concentration of substrate in the inlet medium.

Some kind of inhibitory products of metabolism are always formed and accumulating during the growth processes. These will compete with the substrate for the active sites of the enzyme and could result in a diminution of product formation and the number of viable microorganisms. Several models are proposed [136, 137] to describe the relationship between the growth rate and the concentration of the toxic substances. Also, equations have been derived to fit the sigmoid type growth curves by exponential expressions [138–140] or by logistic exponential forms [141–154]. The product formation in product limited cultures [155] can be described by the following equation:

$$\frac{1}{X}\frac{dP}{dt} = a\frac{1}{X}\frac{dX}{dt} + b, \tag{20}$$

where X is cell number and a and b are constants. The first term on the right hand side is an expression for growth associated product formation and the second term for non-growth associated product formation. Based on Eq. (20), a plot of the specific rate of product formation, $\frac{1}{X}\frac{dP}{dt}$, versus the specific growth rate, $\frac{1}{X}\frac{dX}{dt}$, should give a straight line, where b would be the intercept and a the slope of the regression line.

Further models have been developed describing simultaneous effect of product and substrate [156, 157], cell age distribution [158–161] and statistics of cell division [163–171] on the growth or product formation rate. Growth curves have been approximated and described by mathematical expressions involving the cell age and size distribution [172–174] the cell composition (ribosome, protein and nucleic acid) [175–178] and allosteric enzyme actions [179–181].

As shown, the current literature contains many examples of mathematical models. These can be applied in description of many of the microbiological phenomena or they may be used to derive new expressions. However, in the choice of a model which quantitatively describes the biological phenomena, one should be certain that it has generality and predictive ability [184]. This mathematical model should further depend on what is already known about the system and on what type of results are expected.

3. Factors Influencing Bacterial Activity

The metabolic activity of *T. ferrooxidans* growing on mineral sulfide is largely influenced by environmental factors. In this inorganic system, the available energy from the substrate is only in the form of electrons.
A maximum rate of metal extraction can be achieved when the environment is maintained at the optimum leaching conditions. The problem is in turn an engineering one. It consists of transporting the limiting nutrient and substrate materials into the medium with a rate required for maximum bacterial growth.

a) Effect of Environment

The limits of bacterial activity of the natural environment have been studied [182, 185, 186, 188, 189] in terms of the pH and the oxidation reduction potential (*Eh*). This latter is defined by:

$$Eh = E^0 + \frac{RT}{nF} \log K, \tag{21}$$

where K is the reaction equilibrium constant. The *Eh*-scale extends from +850 to –450 mV, while the pH ranges from 1.0 to 10.2. However, these values do not represent the extreme limits [187], life still exists outside of them. A more sensitive indication of the effect of the environment on the activities of microorganisms is given by:

$$r\mathrm{H}_2 = -\log[a_{\mathrm{H}_2}] \tag{22}$$

where a_{H_2} is the activity of molecular hydrogen, which can be defined from the next equation:

$$\mathrm{H}_2 \rightleftarrows 2\,\mathrm{H}^+ + 2\,e^-. \tag{23}$$

Through application of Eq. (21) on Eq. (23) a relation between *Eh* and $r\mathrm{H}_2$ can be derived:

$$r\mathrm{H}_2 = \frac{Eh}{0.029} + 2\ \mathrm{pH\ at\ 25\ °C}. \tag{24}$$

The rH_2 values may range from 0 to 41.7 and are directly related to Gibb's free energy change:

$$\Delta G = -nF\,\Delta Eh. \tag{25}$$

Further, ΔG is related to the equilibrium constant representing the metabolic conversion of substrate:

$$\Delta G = -RT \log K. \tag{26}$$

The ΔG-values correspond to the maximum amount of energy which is available for the microorganisms in form of ATP, to do work. However, no fondamental studies exist concerning the relation between *Eh*, rH_2, *K*, and ΔG for *T. ferrooxidans*. Such studies should be undertaken to gain a better understanding on the energetic phenomena associated with the microbiological leaching of metal sulfides.

b) Effect of pH

The biological oxidation of ferrous ion and metal sulfides involves movements of hydrogen ions as well as of electrons. Therefore, the pH has a definite effect on their metabolism.

The influence of pH on the activity of *T. ferrooxidans* has been studied by the majority of investigators [17, 190] in the range of 1 to 5. Optimum pH-values were reported to lie between 2.3 and 2.5 for chalcopyrite [3, 15, 191], zinc sulfide [192], chalcocite [193, 195], covellite [193] and ferrous ion [194]. These values are derived in terms of shortest lag time, fastest rates of substrat oxidation or highest yield of metal extraction. The acidophilic character of *T. ferrooxidans* is shared by a number of microorganisms: *T. thiooxidans* [37], algae, molds, protozoa [64], yeast [196] and so on. However, no data are available to indicate the interactions which may exist between these microorganisms and *T. ferrooxidans* during the leaching of the sulfide-bearing ores.

c) Effect of Temperature

The temperature range in which microbiological leaching of metal sulfides by *T. ferrooxidans* functions best, is limited. There are two competing factors to be considered in this process: the usual rise in the reaction rate with an increase in temperature (activation) and at the same time, an increase in the rate of thermal death of the microorganisms (inactivation) due to denaturation of the proteins [197, 198]. This denaturation reaction leads to a loss in the biological activity and as the temperature increases, the denaturation becomes much faster than the sulfide oxidation until the thermal death of the microorganisms occurs.

The optimum temperature has been found to be in the range of 25 to 45 °C [199] for the different strains of *T. ferrooxidans*. For ferrous iron oxidation optimum values reported are 28 °C [200], 32 °C [40], and 35 °C [194, 201]. For metal sulfide leaching the optimum temperature is 35 °C [3, 192, 193, 194, 202]. The biological oxidation ceases at around 55 °C and at higher temperatures only chemical oxidation occurs

[202]. No minimum temperature has been established for growth of *T. ferrooxidans,* but is is generally accepted that bacterial activity stops at the freezing point of the culture media. On the basis of its temperature optimum, 35 °C, this bacterium can be classified as a mesophilic organism, despite its soil origin. Soil organisms are generally psychrophilic.

However, the temperature range for growth and reproduction of different microorganisms extends from −18° to 104 °C [187]. These limits exceed those defining the stability field of pure water under one atmosphere, but they do not exceed the stability field of water in the liquid state when it is impure and under variable pressure. According to relatively recent studies [284–286, 290], the microbiological leaching of metal sulfides can be realized in the temperature range of 50° to 80 °C by thermophilic acidophilic thiobacteria [287–289]. These microorganisms are present in nature and are associated with the oxidation of reduced valence inorganic sulfur compounds. These bacteria present new possibilities for leaching, since the increase in the temperature may result in an increase in the rate of metal extraction. Further studies are needed to understand this phenomenon.

A quantitative treatment of the effect of temperature can be given by the temperature coefficient [203]:

$$Q_{10} = \left(\frac{k_1}{k_2}\right)^{\frac{10}{T_2 - T_1}}. \tag{27}$$

The Q_{10}-value for many chemical reaction is about 2; that is, the reaction rate doubles approximately for each 10 °C rise in the temperature. The respective Q_{10}-values for growth of *T. ferrooxidans* on different substrates are given in Table 1. The dependence

Table 1. Comparison of Q_{10}- and $E_{a,i}$-values for oxidation of different substrates by *Thiobacillus ferrooxidans*

Substrate	Temperature range °C	Q_{10}	$E_{a,i}$ kcal/mole	References
ZnS	25–30	2.0	−12.0	192
Cu_2S	25–30	2.4	−16.3	193
CuS	25–30	1.9	−11.7	193
$FeSO_4$	23–32	1.8	−13.9	99
$FeSO_4$	40–45	–	53.3	99
CuS	40–45	–	55.5	193
Cu_2S	40–45	–	61.5	193

of reaction rate constant on temperature is merely an extension of the kinetic theory of matter. As the thermal energy increases, thereby increasing the electron spin, the concentration of molecules with this higher level of energy rises. That means, there are more molecules with an energy of activation [204], which can be expressed mathematically as follows:

$$E_a = -\frac{R \, d\ln(k)}{d(1/T)}. \tag{28}$$