

## REVIEW ARTICLE

# Reasons why '*Leptospirillum*'-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores

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## Background

A variety of chemoautolithotrophic bacteria which are capable of oxidizing iron- or sulphur-containing minerals may be readily isolated from acidic mine drainage or places where an ore body is naturally exposed to water and the atmosphere. The mineral-oxidizing bacteria found in these 'natural', ambient temperature conditions are ubiquitous and the most commonly encountered have been characterized as *Thiobacillus ferrooxidans*, '*Leptospirillum ferrooxidans*', *Thiobacillus thiooxidans* (Kelly & Harrison, 1989) and more recently *Thiobacillus caldus* (Hallberg & Lindström, 1994). *T. ferrooxidans* (an iron and sulphur oxidizer) and '*L. ferrooxidans*' (an iron oxidizer) are capable of oxidizing an ore such as pyrite when growing in pure culture. *T. thiooxidans* and *T. caldus* (a more thermotolerant bacterium with a growth optimum at 45 °C) are both sulphur oxidizers and are not able to oxidize pyrite alone but grow on the sulphur released after the iron has been oxidized. Also found in similar environments are a variety of acidophilic heterotrophs or facultative heterotrophs such as those of the genera *Acidiphilium*, *Acidocella* and '*Ferromicrobium*' (Johnson & Roberto, 1997). Oxidation of ore by consortia of bacteria generally takes place at a higher rate than with pure cultures.

For many years *T. ferrooxidans* was considered to be the most important micro-organism in commercial bioleaching and biooxidation plants that operate at 40 °C or less (Lundgren & Silver, 1980; Brierley, 1982). However, recent findings based primarily on the use of PCR to amplify and characterize the 16S rRNA genes of

the bacteria present have suggested that this bacterium may be less important in many commercial bioleaching/biooxidation processes than was once thought. The reasons why commercial processes may be dominated by '*L. ferrooxidans*' in combination with *T. thiooxidans* or *T. caldus* is apparent by an examination of ore dissolution kinetics and mineral surface electrochemistry.

## Molecular ecology of biooxidation/bioleaching operations

Investigation into the presence of the obligately acidophilic chemoautolithotrophs has presented a number of difficulties. These bacteria are difficult to cultivate on solid media as they are very sensitive to organic matter including the small quantities of sugar present as impurities in polysaccharide-based gelling agents such as agar or agarose (Tuovinen *et al.*, 1971). Even if highly purified agars are used, the bacteria grow poorly, probably because some of the sugar molecules in the gelling agent are subject to acid hydrolysis and the released sugars inhibit cell growth. A number of alternative gelling agents have met with partial success but most of these are difficult to work with. The most successful approach has been the use of a double-layer plate technique, where freshly grown acidophilic heterotrophs (which are frequently found growing in close association with the autotrophic iron and sulphur oxidizers) are mixed into an inorganic pour plate medium (Johnson & McGinness, 1992). After the first layer has set, a second layer of inorganic medium but without the heterotrophs is poured on top. The starving

heterotrophs adsorb any free sugars and metabolic waste products allowing for good growth of the obligate chemoautolithotrophs. However, even a good isolation medium does not solve all the problems. Many of the bacteria in mineral environments grow in biofilms which adhere strongly to the surface of the particulate matter or grow deep within the pores which form during ore decomposition. Furthermore there is always the possibility that some of these fastidious bacteria may be unculturable on any laboratory media.

A breakthrough in investigating the ecology of bio-mining processes was achieved by the application of the now widely used techniques of PCR amplification of 16S rRNA genes from total DNA extracted from environmental samples. PCR-based methods for detecting bacteria do have their drawbacks in that not all bacteria may lyse equally well, attached bacteria may be difficult to free from solid material and the technique is almost certainly not a quantitative estimate of bacterial numbers. Nevertheless, PCR-based methods have allowed an analysis of the composition of the bacteria in heaps and stirred tank reactors in a manner that does not require growth of the bacteria in a laboratory.

### Percolation and steady-state continuous-flow tank systems

There are two main types of commercial biomining processes. One type involves the percolation of leaching solutions through crushed ore or concentrates that have been stacked in columns, heaps or dumps (Brierley, 1982; Schnell, 1997). The second type employs continuously operating highly aerated stirred tank reactors (Rawlings & Silver, 1995; Dew *et al.*, 1997). The types of bacteria that occur in both types of process have been examined.

#### Dominant bacteria in stacked column or heap-type reactors

Using PCR-based technology to measure species-dependent 16S and 23S rDNA intergenic spacing, Espejo and co-workers (Pizarro *et al.*, 1996) were not able to detect *T. ferrooxidans* in a Chilean copper heap leaching environment operating at low ferrous iron concentration. '*L. ferrooxidans*' and *T. thiooxidans* dominated the population. However, when samples were grown on plates *T. ferrooxidans* was detected, indicating that low numbers of *T. ferrooxidans* were present and that there was a strong selection for these bacteria on plating. *T. ferrooxidans* was detected when ferrous iron was added during heap leaching, although the addition of up to 5 g ferrous iron l<sup>-1</sup> did not change the recovery of copper by more than 5%. These workers concluded that '*L. ferrooxidans*' and *T. thiooxidans* were responsible for bioleaching and were more important than previously recognized by plate cultivation analysis. In a similar study, organisms capable of leaching copper ore in conditions of high acidity (pH 0.7) had 16S–23S rRNA spacers of a size consistent with those of '*L. ferrooxi-*

*dans*' and *T. thiooxidans* but no spacers of a size corresponding to *T. ferrooxidans* were present (Vásquez & Espejo, 1997). Iron oxidation under conditions of high acidity was therefore attributed to the leptospirilli.

In an independent study carried out in Australia, a PCR-based technique was used to analyse the microbial population in a silver-catalysed column for the leaching of chalcopyrite ore at 37 °C (De Wulf-Durand *et al.*, 1997). '*L. ferrooxidans*' was readily detected, but no 16S rRNA corresponding to *T. ferrooxidans* or *T. thiooxidans* was apparent.

#### Bacteria in biooxidation continuous-flow steady-state tank reactors

Researchers in Australia have used PCR-based technology to investigate the bacteria present in laboratory-scale batch and continuous-flow bioreactors treating a mixed zinc–lead ore at 35–40 °C and at an acidity of pH 1–2 (Goebel & Stackebrandt, 1994). Bacteria identified as *Acidiphilium cryptum*, '*L. ferrooxidans*', *T. thiooxidans* and *T. ferrooxidans* were isolated from batch cultures. However, in a continuous-flow bioreactor at steady-state conditions, only '*L. ferrooxidans*' and bacteria belonging to *T. thiooxidans* physiological group II (now known to be *T. caldus*) were detected. A related study was carried out on commercial-scale, continuous-flow, high-rate biooxidation tanks used to pretreat gold-bearing arsenopyrite concentrates of the type used at the Fairview mine (Barberton, South Africa) and which operate at 40 °C and pH 1.6. Restriction enzyme patterns of 16S rDNA that was amplified from known cultures of *T. ferrooxidans*, *T. thiooxidans* and '*Leptospirillum*' were compared with those from total DNA isolated from biooxidation tanks. A restriction pattern corresponding to *T. ferrooxidans* was undetectable and the population was reported to be dominated by '*Leptospirillum*' and *T. thiooxidans* (Rawlings, 1995). Subsequent studies have shown that the restriction enzyme patterns of *T. thiooxidans* and *T. caldus* are very similar and that the bacteria were almost certainly *T. caldus* (M. N. Gardner & D. E. Rawlings, unpublished).

An examination of the bacteria present in commercial biooxidation tanks using an immunofluorescent antibody microscope count detection technique differed from the PCR-based work in that *T. ferrooxidans* cells were detected in most samples. *T. ferrooxidans* cells were, however, in the minority. The proportions of bacterial types in continuous-flow biooxidation tanks from Sao Bento (Brazil) and Fairview (South Africa) were 48–57% '*L. ferrooxidans*', 26–34% *T. thiooxidans* and 10–17% *T. ferrooxidans*. The proportions varied within the ranges given depending on whether the sample had been treated with Triton X to release attached bacteria and from which tank in the series of biooxidation tanks the sample was derived (Dew *et al.*, 1997). Using the immunofluorescent technique, a slightly different distribution of bacteria was found in pilot-scale bioreactors treating a nickel pentlandite-pyrrhotite ore.

**Table 1.** List of symbols

Symbol	Definition (units)
$B$	Kinetic constant in pyrite oxidation (dimensionless)
$c_x$	Concentration of bacteria (mol l <sup>-1</sup> )
$E_h$	Redox potential – standard hydrogen electrode (mV)
$F$	Faraday constant (C mol <sup>-1</sup> )
$[Fe^{2+}]$	Concentration of Fe <sup>2+</sup> (mol l <sup>-1</sup> )
$[Fe^{3+}]$	Concentration of Fe <sup>3+</sup> (mol l <sup>-1</sup> )
$[FeS_2]$	Concentration of FeS <sub>2</sub> (mol l <sup>-1</sup> )
$K$	Kinetic constant (dimensionless)
$n$	Number of electrons involved in reaction
$q_{Fe^{2+}}$	Bacterial specific ferrous iron oxidation rate [mol Fe <sup>2+</sup> (mol C) <sup>-1</sup> h <sup>-1</sup> ]
$q_{Fe^{2+}}^{max}$	Maximum bacterial specific ferrous iron oxidation rate [mol Fe <sup>2+</sup> (mol C) <sup>-1</sup> h <sup>-1</sup> ]
$R$	Universal gas constant (kJ mol <sup>-1</sup> K <sup>-1</sup> )
$r_{Fe^{2+}}$	Ferrous iron production rate (mol Fe <sup>2+</sup> l <sup>-1</sup> h <sup>-1</sup> )
$r_{Fe^{2+}}^{bact}$	Bacterial ferrous iron production rate (mol Fe <sup>2+</sup> l <sup>-1</sup> h <sup>-1</sup> )
$r_{Fe^{2+}}^{chem}$	Chemical ferrous iron production rate (mol Fe <sup>2+</sup> l <sup>-1</sup> h <sup>-1</sup> )
$T$	Temperature (K)
$\alpha$	Specific surface area of pyrite (m <sup>2</sup> mol <sup>-1</sup> )
$\xi_{Fe^{2+}}$	Area specific ferrous iron production rate (mol Fe <sup>2+</sup> m <sup>-2</sup> h <sup>-1</sup> )
$\xi_{Fe^{2+}}^{max}$	Maximum area specific ferrous iron utilization rate (mol Fe <sup>2+</sup> m <sup>-2</sup> h <sup>-1</sup> )

In this case numbers of *T. ferrooxidans* were higher although they never exceeded about 33% of the total population (Lawson, 1997).

The reason why *T. ferrooxidans* is not the dominant bacterium in most commercial bioleaching operations may be understood by an examination of the kinetics of the bioleaching/biooxidation of a model ore such as pyrite.

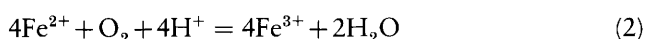
## Kinetics of biooxidation

### A two-subprocess mechanism for biooxidation kinetics

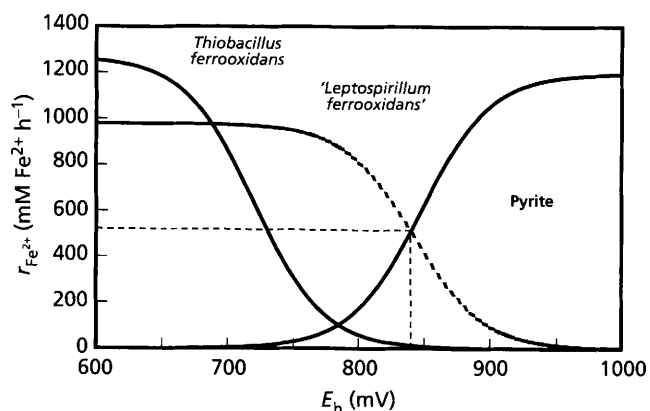
Boon *et al.* (1995) have proposed that the bioleaching of sulphide minerals involves two subprocesses. These are the ferric leaching of pyrite to form ferrous iron and sulphate in solution



and the bacterial oxidation of ferrous iron to the ferric form



The two subprocesses are balanced when the rate of



**Fig. 1.** Rates of ferrous iron production from the chemical ferric leaching of 64  $\mu$ m pyrite and the rates of ferrous iron consumption by bacterial oxidation to the ferric form by '*L. ferrooxidans*' and *T. ferrooxidans*, respectively. The curves are calculated using the kinetic constants for bacterial ferrous iron oxidation reported by Boon (1996) and van Scherpenzeel (1997) and the kinetic constants for ferric leaching of pyrite reported by May *et al.* (1997). Pyrite concentration was calculated as mol (l culture volume)<sup>-1</sup> even though not all of the pyrite will be soluble under all conditions. The concentration of pyrite was assumed to be 0.1 mM and the bacterial concentration 0.012 mM carbon.

ferrous/ferric iron turnover between them is balanced (see Table 1 for list of symbols).

$$r_{Fe^{2+}}^{chem} = -r_{Fe^{2+}}^{bact} \quad (3)$$

The ferric leach kinetics have been described by

$$\xi_{Fe^{2+}} = \frac{-r_{Fe^{2+}}}{\alpha[FeS_2]} = \frac{\xi_{Fe^{2+}}^{max}}{1 + B \frac{[Fe^{2+}]}{[Fe^{3+}]}} \quad (4)$$

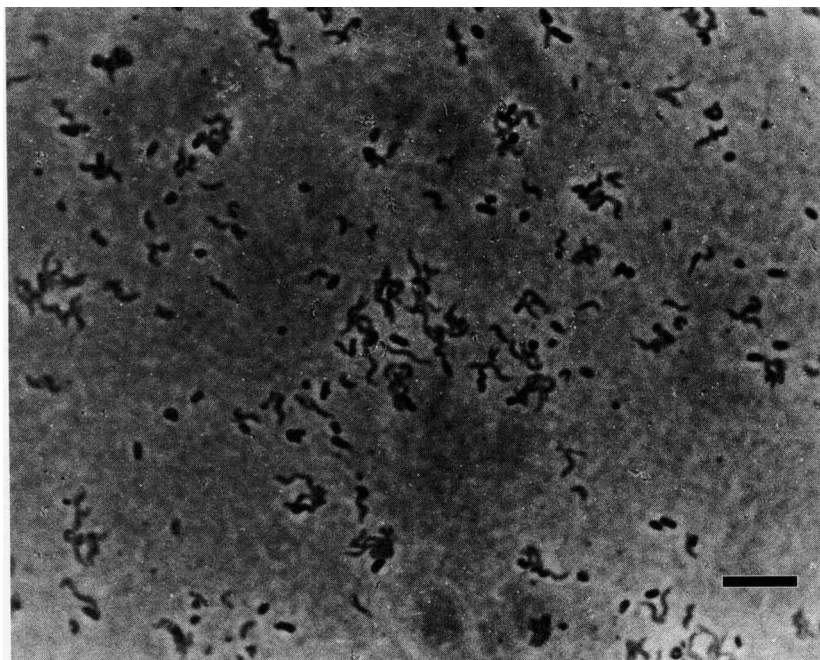
and the kinetics of the bacterial oxidation of ferrous iron by

$$q_{Fe^{2+}} = \frac{-r_{Fe^{2+}}}{c_x} = \frac{q_{Fe^{2+}}^{max}}{1 + K \frac{[Fe^{3+}]}{[Fe^{2+}]}} \quad (5)$$

In both of these the ferric/ferrous iron ratio can be expressed using the Nernst equation

$$E_h = E^0 + \frac{RT}{zF} \ln \left( \frac{[Fe^{3+}]}{[Fe^{2+}]} \right) \quad (6)$$

In studies at 30 °C and pH = 1.6 (Boon, 1996) the following can be calculated:  $\xi_{Fe^{2+}}^{max} = 4.80 \times 10^{-5}$  (kmol Fe<sup>2+</sup>) (m<sup>2</sup> FeS<sub>2</sub>)<sup>-1</sup> h<sup>-1</sup> and  $B = 2500$  for the bioleaching of pyrite and  $q_{Fe^{2+}}^{max} = 8.8$  (mol Fe) (mol C)<sup>-1</sup> h<sup>-1</sup> and  $K = 0.05$  for the oxidation of ferrous iron by *T. ferrooxidans* (Hansford, 1997). van Scherpenzeel (1997) found  $q_{Fe^{2+}}^{max} = 6.8$  (mol Fe) (mol C)<sup>-1</sup> h<sup>-1</sup> and  $K = 0.0005$  for the oxidation of ferrous iron by '*L. ferrooxidans*' at



**Fig. 2.** Light micrograph of the bacteria present after repeated successive batch growth on pyrite of a culture taken from an exploration adit in the Gamsberg in Namaqualand, South Africa (Boon *et al.*, 1995). This shows the predominance of 'Leptospirillum'-like bacteria. Bar, 10  $\mu\text{m}$ .

the same temperature and pH (Hansford, 1997). '*L. ferrooxidans*' has a lower maximum specific utilization rate than *T. ferrooxidans* at low redox potential but can sustain higher activity up to higher redox potentials. These findings are in agreement with the report by Norris *et al.* (1988) that '*L. ferrooxidans*' has a higher affinity for ferrous iron ( $K_m = 0.25 \text{ mM Fe}^{2+}$ ) than *T. ferrooxidans* ( $K_m = 1.34 \text{ mM Fe}^{2+}$ ). It has also been shown by Norris *et al.* (1988) that '*L. ferrooxidans*' is less inhibited by ferric iron ( $K_i = 42 \text{ mM Fe}^{3+}$ ) than *T. ferrooxidans* ( $K_i = 3.1 \text{ mM Fe}^{3+}$ ).

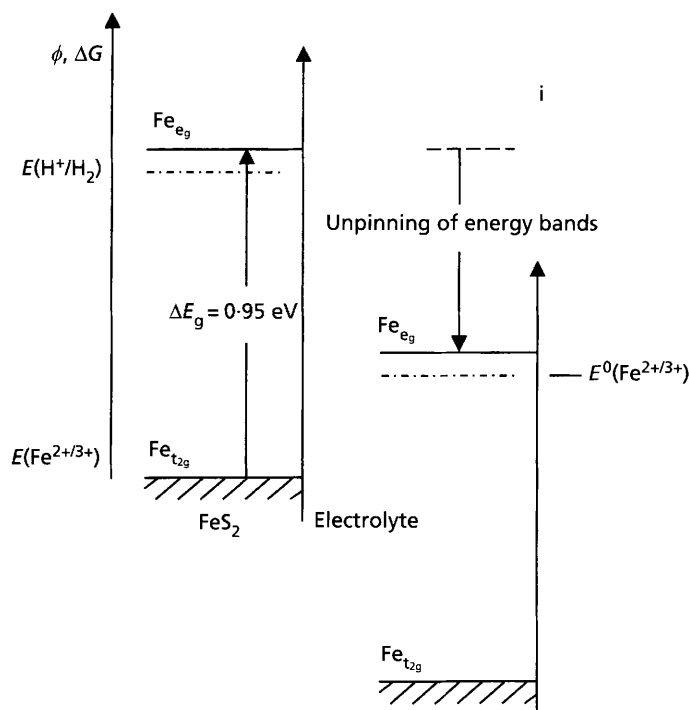
The effect of the differences in affinity for ferrous iron and the inhibition by ferric iron can be seen in Fig. 1, where the rate of ferrous iron produced by the ferric leaching of pyrite and the rate of consumption during bacterial oxidation by *T. ferrooxidans* and '*L. ferrooxidans*' are shown. It can be seen that the ferric leach curve and bacterial oxidation curve for '*L. ferrooxidans*' intersect at a high redox potential (about 840 mV) where there is a very low activity for *T. ferrooxidans*. The intersection point of the two curves defines the conditions where the rate of iron turnover between the mineral and the bacteria will be balanced and also provides an explanation for why '*L. ferrooxidans*' will out-compete *T. ferrooxidans* in the bioleaching of pyrite.

This predicted dominance of '*L. ferrooxidans*' has been confirmed in fed-batch bioleaching studies of pyrite using an enrichment culture as shown in Fig. 2 (Boon *et al.*, 1995). Similarly, Norris (1983) has shown that pyrite dissolution with '*Leptospirillum*'-like bacteria was more extensive than with *T. ferrooxidans* and that pyrite enrichment subcultures were frequently dominated by '*L. ferrooxidans*'. In a related observation the accelerated bioleaching of pyrite by '*L. ferrooxidans*'

over *T. ferrooxidans* was reported by Helle & Onken (1988).

### Potential-dependent interfacial chemistry of pyrite and availability of chemical energy for bacteria

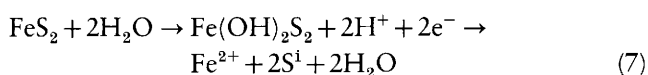
The state of interfacial chemistry of pyrite, which serves as the source of energy for bacteria, should be re-examined in the light of recent photoelectron spectroscopic (XPS) and electrochemical studies (Ennaoui *et al.*, 1993). It is now well-established that natural pyrite is a predominantly n-type semiconducting material with an energy gap of  $\Delta E_g = 0.95 \text{ eV}$  between the valence and conduction bands. Important for the reactivity with electrolytes is the fact that conduction and valence bands are derived from iron d-states ( $e_g$  and  $t_{2g}$ , respectively) (Fig. 3). XPS spectra have shown that sulphur states only begin to occur at 1 eV below the  $t_{2g}$  valence band in the energy scheme. This observation determines the interfacial reactivity of pyrite and has two important consequences. Firstly, the extraction of electrons from pyrite, e.g. by  $\text{Fe}^{3+}$ , does not directly weaken existing chemical bonds (the electrons come from non-bonding d-states). Secondly, the presence of extracted electrons (positive holes, equivalent to a positive electrode potential) induces interfacial pyrite iron to engage in coordination reactions with water species. The consequence is a significant double-layer charging of the interface dependent on the electrochemical potential applied to pyrite by an external potential or by an added redox electrolyte ( $\text{Fe}^{2+}/\text{Fe}^{3+}$ ). In the energy scheme of Fig. 3 this means that with increasingly positive potential the energy bands of pyrite move down towards more positive potentials (unpinning of energy bands). Addition of an  $\text{Fe}^{2+}/\text{Fe}^{3+}$  solution,



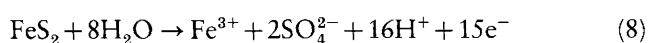
**Fig. 3.** Energy scheme of pyrite and the downshift (unpinning of energy bands). This occurs with increasingly positive potential.

which applies a redox potential of  $E^0 = +0.67$  V (standard hydrogen electrode, SHE) to the pyrite, may shift its energy band position down by as much as half a volt (compared to a salt solution of the same pH without the iron redox system), thereby charging its interfacial capacitance of iron-based surface states. Due to this shift of electron levels in the pyrite correspondingly less free energy of electrons is available for the bacteria at positive potentials.

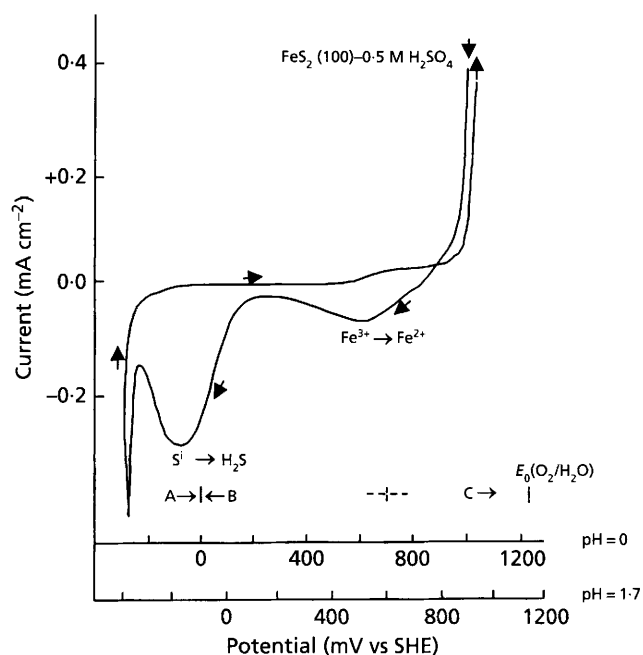
If electrons extracted from pyrite by  $\text{Fe}^{3+}$  do not disrupt chemical bonds directly, how does  $\text{Fe}^{3+}$  induce pyrite corrosion? Electrochemical studies of pyrite in organic electrolyte (propylene carbonate) with gradual addition of water (Abd El-Halim *et al.*, 1995) have shown that corrosion occurs only in the presence of water molecules and at sufficiently positive potentials [thermodynamically starting at  $E^0 = 0.389$  V and  $E^0 = 0.546$  V (SHE) but shifted towards positive potentials because of the required breaking up of the pyrite interface]. Two reactions with water can be recognized: one leading to an interfacial sulphide-hydroxide complex, which liberates  $\text{Fe}^{2+}$  and creates surface-bound sulphur states  $\text{S}^1$  [sulphur species which still maintain chemical bonds with the pyrite lattice, and which can be reduced to  $\text{H}_2\text{S}$  negative of  $E^0 = 0.142$  V (SHE)]



and another leading to  $\text{Fe}^{3+}$  and sulphate



Here, the unstable oxidized iron complex chemically reacts with sulphur atoms and transfers hydroxide species initiating the oxidation of pyrite sulphur to



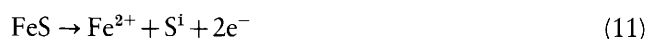
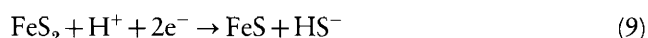
**Fig. 4.** Dynamic current-voltage curve ( $10 \text{ mV s}^{-1}$ ) of pyrite showing reduction of  $\text{Fe}^{3+}$  and of interfacially bound  $\text{S}^1$  generated during the anodic corrosive reaction. The situation when the leaching solution of pH 1.7 is determining the pyrite electrochemical potential is also shown.

sulphate. The surface-bound sulphur species formed during reaction 7 and  $\text{Fe}^{3+}$  formed during reaction 8 can again be reduced to  $\text{H}_2\text{S}$  and  $\text{Fe}^{2+}$ , respectively, during the back sweep towards negative potentials, which confirms their presence (Fig. 4). The reduction peaks

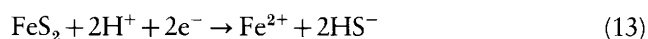
become bigger with increasingly positive polarization of the electrode, until saturation is reached. This causes corrosion.

Since no chemical energy for bacteria can be obtained from the final chemical products of reaction 8, only the products of reaction 7 and maybe some intermediate sulphur species of reaction 8 (though with reduced free energy, because of the downshift of energy states; Fig. 3), will be relevant for bacterial activity. However, the surplus electrons made available through reaction 8 can serve for reduction of  $\text{Fe}^{3+}$  ions; in other words, the electrons extracted from pyrite by  $\text{Fe}^{3+}$  will generate the corrosion reaction 8. It has to be concluded that  $\text{Fe}^{3+}$ , used for mineral leaching, only acts indirectly by generating a sufficiently positive potential to induce reactions 7 and 8 with water species. Ion-scattering experiments have confirmed that water species react with the iron centre in the  $\text{FeS}_2$  surface. Some chemical surface bonds could, however, be broken by electron extraction from surface states, the electronic nature of which deviates from the pyrite bulk structure and which may develop polar bonding between iron and sulphur (FeS-type surface states).

On the basis of these observations the availability of chemical energy from pyrite for the bacteria will depend on the electrochemical potential of the leaching solution. At strongly reducing conditions (negative of the hydrogen potential, section A in Fig. 4) the proton concentration will control pyrite dissolution according to the reactions ( $\text{S}^{\text{i}}$  = sulphur still chemically bound to the surface)



with the global reaction



which determines the pH-dependent solubility product of the pyrite.  $\text{Fe}^{2+}$ ,  $\text{S}^{\text{i}}$  and  $\text{H}_2\text{S}$  are available as chemical energy carriers, so that *T. ferrooxidans*, *T. thiooxidans*, *T. caldus* and '*L. ferrooxidans*' should find a suitable energy-providing environment. However, the rate of corrosion will be determined by the rate of reaction 13.

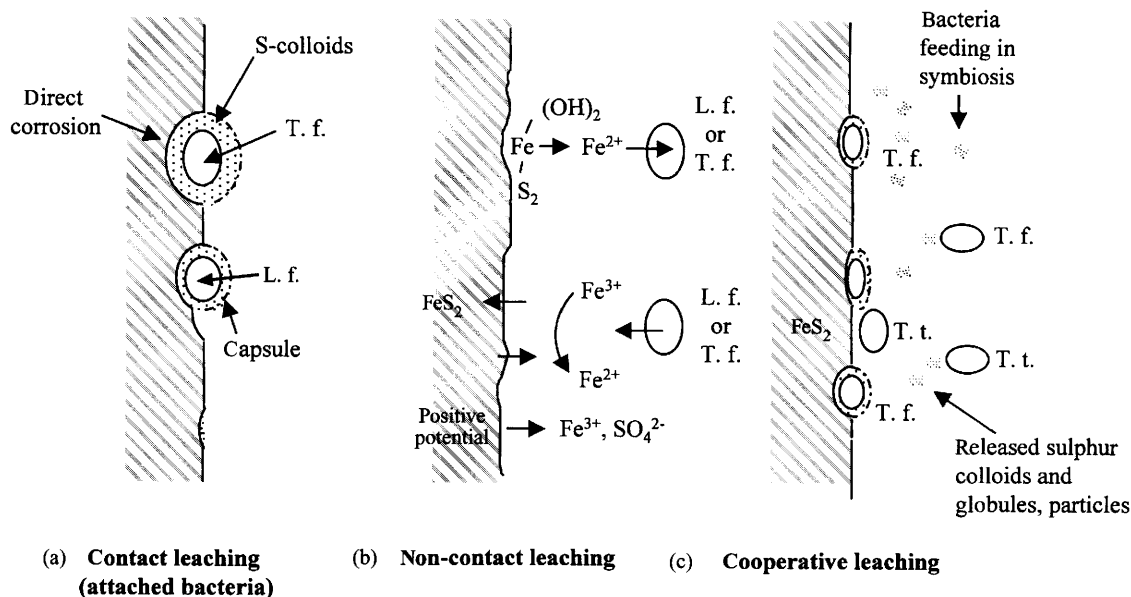
At potentials between 0 V and up to approximately 600–700 mV (SHE), considering the effective overpotential for reactions 7 and 8 (region B in Fig. 4), pyrite corrosion is not possible electrochemically. It occurs only if the bacterium itself is able to disrupt the pyrite structure or is supported by a chemical reaction, which is able to disrupt chemical bonds in the interface (e.g. thiols reacting with interfacial sulphur forming polysulphides, complexing agents strongly reacting with pyrite-iron). The capacity to interact with the pyrite surface has been demonstrated with *T. ferrooxidans*, which dissolves pyrite using an organic capsule as a reaction medium. The capsule also serves to absorb

pyrite sulphur in the form of nanodimensioned sulphur colloids (Rojas *et al.*, 1995). Under suitable conditions, controllable by a detergent (e.g. Tween 80), the bacterium generates corrosion pits under the contact zone with a corrosion rate of up to  $2 \text{ nm h}^{-1}$ .

When the electrochemical potential is shifted to quite positive potentials by the presence of sufficient  $\text{Fe}^{3+}$  (potential region C in Fig. 4), then electrochemical pyrite corrosion proceeds, whereby  $\text{Fe}^{2+}$  and  $\text{S}^{\text{i}}$  are made available according to reaction 7, and electrons for  $\text{Fe}^{3+}$  reduction from reaction 8. In addition sulphur species, during their oxidation to sulphate (reaction 8), may be partially recoverable by bacteria on the pyrite interface and provide chemical energy. Here '*L. ferrooxidans*' may favourably exist by the oxidation of  $\text{Fe}^{2+}$  and *T. caldus* by the oxidation of surface-bound  $\text{S}^{\text{i}}$ . The electrochemical energy available from  $\text{Fe}^{2+}$  at such positive potentials may already be too low for *T. ferrooxidans*.

The downward shift of the energy bands of pyrite (Fig. 3) and the corresponding decrease in the free energy of electrons by approximately 0.5 V decreases also the electrochemical energy available from surface-bound sulphur species from reaction 7 and from possible intermediates of reaction 8. The availability of chemical energy therefore significantly decreases towards positive potentials, favouring '*Leptospirillum*', which can harness energy from  $\text{Fe}^{2+}$  at surprisingly positive potentials. Corrosion reaction 8, which dissipates free energy of pyrite into heat, will in addition be increasingly dominant with the corrosion rate increasing with increasingly positive potential. No energy can be gained beyond  $E^0$  ( $\text{O}_2/\text{H}_2\text{O}$ ) = 1.23 V (for pH = 0). The shift of potentials in a leaching solution of pH = 1.7 has been indicated in Fig. 4 by a second axis. Electrons from ferrous iron are required to react with the protons which enter the cell via the ATP synthase and serve to maintain the cytoplasm of the cells at a pH close to neutral. However, free energy to drive the transfer of  $\text{Fe}^{2+}$  to oxygen will stop being available for bacterial growth at approximately  $E^0 = 1 \text{ V}$  (SHE) and 0.9 V for pH = 1.7.

In many bioleaching operations typically little  $\text{Fe}^{3+}$  will be present at the beginning. The starting electrochemical potentials will be in region B (Fig. 4). *T. ferrooxidans* will therefore be initially favoured, because it can rapidly attack  $\text{FeS}_2$ . This situation may also correspond to natural leaching conditions, where the leaching solution is not circulated but constantly renewed by the existing water percolation. This would explain why *T. ferrooxidans* is frequently isolated from many sulphide mines, giving the impression that this bacterium is dominating leaching of sulphides. However, in commercial bioleaching installations, the leaching solution is circulated or steady-state conditions allow  $\text{Fe}^{3+}$  ions to accumulate. Therefore, a more positive potential region C (Fig. 4) will be reached, where '*Leptospirillum*' can thrive and sulphur-oxidizing bacteria (e.g. *T. caldus*) can coexist on surface-bound sulphur species  $\text{S}^{\text{i}}$ . With increasingly positive redox potential *T. ferrooxidans*



**Fig. 5.** Patterns of direct and indirect interaction of bacteria with pyrite: (a) contact leaching by *T. ferrooxidans* (T.f.) or '*L. ferrooxidans*' (L.f.); (b) non-contact leaching by inducing electrochemical corrosion through  $\text{Fe}^{2+}$  oxidation (generation of positive redox potential); (c) co-operative leaching in which soluble energy carriers are generated by *T. ferrooxidans* for use by bacteria in solution such as *T. thiooxidans* (T.t.) or *T. ferrooxidans*.

will cease to find adequate chemical energy, since also  $\text{Fe}^{2+}$  generated from  $\text{Fe}^{3+}$  by the electrons from the corrosion reaction 8 will be available at a redox potential that is too positive.

This simplified scenario of the energy availability from pyrite for bacteria is complicated by additional circumstances. Recent studies of spherical sulphur and  $\text{FeS}_2$  particles have shown that when bacteria such as *T. thiooxidans* and *T. ferrooxidans* are feeding interfacially they do so in an apparently wasteful way by releasing unconsumed chemical energy (sulphur colloids, substrate fragments) into the surrounding medium (Rojas-Chapana *et al.*, 1998). Therefore, chemical energy becomes available to bacteria not directly interacting with the solid energy source. For pyrite this means that in the presence of *T. ferrooxidans* or *T. thiooxidans* which are attached to the surface, unattached *T. thiooxidans* and *T. caldus* may develop in symbiosis. Also, non-adhering *T. ferrooxidans* may take advantage of chemical energy released by surface-attached bacteria in a kind of symbiotic leaching. '*L. ferrooxidans*' is also known to attach to the surface of pyrite (Schippers *et al.*, 1996), but whether a similar release of colloidal sulphur takes place during iron oxidation by '*Leptospirillum*' is not known. Possible patterns of the bacterial leaching of pyrite are presented in Fig. 5.

An additional factor to be considered for understanding the kinetics of pyrite leaching is the amount of energy to be gained from the chemical species released from pyrite. Approximately  $200 \text{ kcal mol}^{-1}$  ( $840 \text{ kJ mol}^{-1}$ ) can be obtained from pyrite oxidation to iron sulphate, but only  $7\text{--}9 \text{ kcal mol}^{-1}$  ( $29.4\text{--}37.8 \text{ kJ mol}^{-1}$ ), depending

on the pH, from oxidation of  $\text{Fe}^{2+}$  to  $\text{Fe}^{3+}$ . Much more energy can be gained from oxidation of sulphur compared to oxidation of  $\text{Fe}^{2+}$ . Iron-oxidizing bacteria may have evolved to optimize electron extraction from pyrite to obtain  $\text{Fe}^{2+}$  (corrosion reaction 8). This explains the adaptation of '*Leptospirillum*' to very positive redox potentials. The sulphate produced is soluble and does not block the pyrite interface. On the other hand, bacteria utilizing sulphide sulphur may have evolved to dissolve the insoluble sulphide at a high rate, which is limited by the disruption of chemical bonds. This would explain the direct interaction with pyrite, which gives access to the chemical energy of the sulphur, and the 'wasteful' handling of sulphur which is released into the medium where it can be consumed by non-adhering cells. In this way, the overall leaching rate is high. *T. ferrooxidans* and '*Leptospirillum*' can not find a common optimum for their energy harvest. Industrial operations which allow  $\text{Fe}^{3+}$  to accumulate apparently favour '*L. ferrooxidans*'.

### Effects of temperature and pH

The major reason for the dominance of '*Leptospirillum*' over *T. ferrooxidans* in industrial processes is almost certainly the ferric/ferrous ratio (redox potential). However, there may be other reasons which contribute to the dominance of '*Leptospirillum*'. The optimum pH for the growth of *T. ferrooxidans* is within the range pH 1.8–2.5. In contrast, '*L. ferrooxidans*' is more acid resistant than *T. ferrooxidans* and will grow at a pH of 1.2 (Norris, 1983). With regard to temperature, *T. ferrooxidans* is considered to be more tolerant of low

temperatures and less tolerant of high temperatures than is '*L. ferrooxidans*'. Some strains of *T. ferrooxidans* are able to oxidize pyrite at temperatures as low as 10 °C (Norris, 1990) but 30–35 °C is considered to be optimal. '*Leptospirillum*'-like bacteria have been reported to have an upper limit of around 45 °C (Norris *et al.*, 1986) with a lower limit of about 20 °C (Sand *et al.*, 1993). The majority of continuous-flow biooxidation processes which are used to treat gold-bearing arsenopyrite ores or concentrates operate at 40 °C and pH 1.6 (Dew *et al.*, 1997). This is a little below the optimum pH range and a little above the optimum temperature range for *T. ferrooxidans* but well within the optimum range of most '*Leptospirillum*' isolates. The preference of *T. ferrooxidans* for growth at higher pH values is probably not the main reason for the dominance of '*Leptospirillum*' since there is a report of the adaptation of the bacterium to pH 1.5 after selection in continuous culture (Vian *et al.*, 1986).

## Conclusions

The main factor in determining which bacteria are likely to dominate commercial bioleaching or biooxidation processes is the ferric to ferrous iron ratio (redox potential). The redox potential will be affected by whether treatment of an ore is carried out using a batch culture or continuous culture type of process. Under batch culture conditions the ferric to ferrous iron concentration (redox potential) is low at the start of oxidation, but high once all the ferrous iron has been oxidized to ferric. When grown in batch culture in a liquid ferrous iron or pyrite medium, *T. ferrooxidans* will initially outgrow its iron-oxidizing competitors and dominate the population. This is largely because during the initial stages of batch culture the redox potential is low and *T. ferrooxidans* has a faster growth rate than '*Leptospirillum*' (Fig. 1, below 690 mV). Under such conditions *T. ferrooxidans* is able to build up large numbers of cells before conditions become more favourable for '*L. ferrooxidans*'. However, because the leptospirilli have a greater affinity for ferrous iron and are less sensitive to inhibition by ferric iron on prolonged aeration '*L. ferrooxidans*' is likely to dominate (Norris *et al.*, 1988). Single pass percolation-type reactors may be viewed as plug-flow systems where the amount of dissolved metals (and the ferric/ferrous iron ratio) is lower near the top of the reactor than near the bottom. Provided no recycling of leach liquor takes place such a system would be analogous to a batch culture system and *T. ferrooxidans* is likely to be the dominant iron-oxidizing bacterium.

In percolation systems where the leach liquor is collected and recycled, differences between the metal ion concentrations within the reactor will be reduced and the redox potential will be higher throughout. In continuously operating stirred tank reactors, a steady state is reached at which the redox potential within each tank remains approximately constant and high. Under such conditions the ability of *T. ferrooxidans* to oxidize ferrous iron is severely inhibited by the presence of ferric iron

whereas the iron-oxidizing ability of '*Leptospirillum*' is much less affected (Fig. 1, above 700 mV and Fig. 4, section C). Because of its ability to oxidize pyritic ores better at high redox potentials '*L. ferrooxidans*' will be the dominant iron oxidizer. In the case of non-pyrite minerals whose ferric leach curve is shifted to the left (Fig. 1) such that the intersection with the bacterial ferrous oxidation curve occurs at lower redox potentials (or Fig. 4, section B), it is possible that *T. ferrooxidans* could be the predominant species.

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