

The bioleaching of sulphide minerals with emphasis on copper sulphides — A review

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Abstract

This review outlines current research in heap bioleaching, particularly in respect of the bioleaching of chalcopyrite, assesses the status of the bioprocessing of copper sulphides and evaluates promising developments.

The bioleaching of sulphide minerals is reviewed with emphasis on the contribution from the microbial community, especially attachment and biofilm formation, bioleaching mechanisms and the potential benefits to be gained by a greater understanding of the molecular genetics of the relevant microbial strains.

The leaching and bioleaching of copper sulphides is examined. The main focus is on heap bioleaching of whole ores, and the development of models to describe heap and dump processes that can be applied in the design phase as well as to optimise metal extraction. The characteristics of chalcopyrite leaching are discussed in respect of those conditions and controls that might be needed to make a heap bioleach commercially productive.

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1. Introduction

World copper production has increased steadily in the period 1984–2005, from 9 Mt to 16 Mt per annum, and is predicted by the Australian Bureau of Agricultural and Resource Economics (ABARE) to reach close to 18 Mt in 2006. More than 20% of that copper is now produced via hydrometallurgy. An indirect indicator of the notable increase in hydrometallurgical copper production over recent years is the increased overall capacity of solvent extraction-electrowinning (SX-EW) plants producing cathode copper. Copper production from SX-EW rose from 0.8 to 2.0 Mt in the period 1993 to 1997 (Arbiter and Fletcher, 1994; Readett and Townson, 1997). In

2001, the combined copper production of Chile and the USA using SX-EW was about 2.1 Mt (Bartos, 2002) with additional production of about 0.16 Mt from other countries.

While world demand for copper is growing, the minerals industry is increasingly faced with the need to process low grade ores, overburden and waste from current mining operations. The economic extraction of copper from low-grade ores requires low-cost processing methods such as in situ, dump and heap leaching. Bacterially-assisted heap leaching of low-grade copper sulphides is a developing technology that has been applied successfully to the extraction of copper from secondary sulphide minerals such as chalcocite at a number of operations worldwide. However, heap bioleaching of the refractory primary copper sulphide,

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chalcopyrite, has yet to be implemented at commercial scale. Traditionally, the advantages of bacterial leaching technology are compatible with these requirements:

- Moderate capital investment with low operating costs,
- Appropriate recovery of metals from low-grade ores and waste materials,
- Basic equipment and simple operating procedures.

The most successful copper heap leaching operations have been those processing copper oxides and secondary copper sulphides (Table 1). Chalcocite (Cu_2S) is the main copper sulphide mineral mined at bioleaching operations. Some of the chalcocite heap operations began as oxide (chemical) leach operations and were converted to bioleach (oxidative) operations by heap aeration and/or inoculation, when the oxidised ore was depleted. However, even if bacterial activity is not facilitated, microbial assisted air

oxidation of iron(II) and sulphur will contribute to copper extraction if sulphide minerals are present in a heap.

Millions of tonnes of low-grade ore and copper-rich tailings await the development of an efficient and economic bioleach process for chalcopyrite (CuFeS_2). The bioleaching of chalcopyrite in situ and in dumps is a practical option only because the low and slow recoveries are countered by the low processing costs (Schnell, 1997). Not surprisingly, the bioleaching of chalcopyrite, both the most abundant and the most refractory copper sulphide, is a key industry target. However most of the technological developments have taken place with the bioleaching of chalcocite and other less refractory sulphide minerals.

Straits Resources operated a test heap with chalcopyrite ore in parallel with their copper oxide/chalcocite heap leach operation at Girilambone with promising results (D. Readett, personal communication). Titan Resources operated a trial mixed nickel sulphide and chalcopyrite heap at their Radio Hill deposit with some success

Table 1
Heap bioleaching of copper ores (historical and current)

Region/mine	Operation reserves (t)	Ore processed (t/day)	Cu production (t/year)
Lo Aguirre, Chile 1980–1996	Heap bioleach 12×10^6 at 1.5% Cu	Oxides/chalcocite 16×10^3	$14\text{--}15 \times 10^3$
Cerro Colorado, Chile 1993–	Heap bioleach 80×10^6 at 1.4% Cu	Chalcocite, covellite 16×10^3	100×10^3
Ivan Zar, Chile 1994–	Heap bioleach 5×10^6 at 2.5% Cu	Oxides/sulphides 1.5×10^3	12×10^3
Quebrada Blanca, Chile 1994–	Heap/dump bioleach 85×10^6 at 1.4% Cu 45×10^6 at 0.5% Cu	Chalcocite 17.3×10^3	75×10^3
Punta del Cobre, Chile 1994–	Heap (bio)leach 10×10^6 at 1.7% Cu	Oxides/sulphides –	$7\text{--}8 \times 10^3$
Andacollo, Chile 1996–	Heap/dump bioleach 32×10^6 at 0.58% Cu	Chalcocite 15×10^3	21×10^3
Dos Amigos, Chile 1996–	Heap bioleach 2.5%	Chalcocite 3×10^3	–
Zaldivar, Chile 1998–	Heap/dump bioleach 120×10^6 at 1.4% Cu 115×10^6 at 0.4% Cu	Chalcocite 20×10^3	150×10^3
Lomas Bayas, Chile 1998–	Heap/dump 41×10^6 at 0.4% Cu	Oxides/sulphides 36×10^3	60×10^3
Cerro Verde, Peru 1977–	Heap bioleach — at 0.7% Cu	Oxide/sulphide 32×10^3	54.2×10^3
Escondida, Chile	Heap bioleach 1.5×10^9 at 0.3–0.7%	Oxides, sulphides	200×10^3
Lince II, Chile, 1991–	Heap leach 1.8% Cu	Oxides, sulphides	27×10^3
Toquepala, Peru	Heap leach	Oxides, sulphides	40×10^3
Morenci, Arizona 2001–	Mine for leach 3450×10^6 0.28% Cu	Chalcocite, pyrite 75×10^3	380×10^3
Equatorial Tonopah, Nevada, 2000–2001	Heap bioleach 0.31% Cu	25×10^3	25×10^3
Gunpowder Mammoth Mine, Australia, 1991–	In situ (bio)leach 1.2×10^6 at ~1.8% Cu	chalcocite and bornite –	33×10^3
Girilambone, Australia 1993–2003	Heap bioleach — at 2.4% Cu	Chalcocite/chalcopyrite 2×10^3	14×10^3
Nifty Copper, Australia, 1998–	Heap bioleach — at 1.2%	Oxides/chalcocite 5×10^3	16×10^3
Whim Creek and Mons Cupri, Australia 2006–	Heap bioleach 900×10^3 at 1.1% Cu 6×10^6 at 0.8% Cu	Oxides/sulphides	17×10^3
Mt Leyshon, Australia 1992–1997	Heap bioleach — 0.15%	Chalcocite 1.3×10^3	750
S&K Copper, Monywa, Myanmar, 1999–	Heap bioleach 126×10^6 at 0.5% Cu	Chalcocite 18×10^3	40×10^3
Phoenix deposit, Cyprus, 1996–	Heap (bio)leach 9.1×10^6 at 0.78% Cu 5.9×10^6 at 0.31% Cu	Oxide/sulphide –	8×10^3
Jinchuan Copper, China 2006–	240×10^6 at 0.63% Cu	Chalcocite, covellite, enargite	10×10^3

(Hunter, 2002a). Currently, Mintek, with the National Iranian Copper Industries Company (NICICO), are undertaking a large-scale pilot test of Mintek's heap bioleaching technology for Darehzar chalcopyrite ore at the Sarcheshmeh Copper Complex in southern Iran (van Staden et al., 2005).

Clearly, there remain impediments to the acceptance and implementation of bioleaching for the processing of chalcopyrite, not necessarily restricted to the biological aspects. According to Holmes and Debus (1991), biological processing would need to have >20% advantage over conventional processing to interest the mining industry. Poulter et al. (1999) concluded that reluctance to embrace the technology is "partly a reflection of the relatively refractory nature of chalcopyrite, partly the inherent process and economic advantage of modern smelting technologies, and partly the real and perceived technical risks associated with the introduction of a novel processing technology".

The purposes of this review are: (i) to outline the focus of current research, particularly in respect of the bioleaching of chalcopyrite; (ii) to assess the status of the bioprocessing of copper sulphides and, (iii) to evaluate promising developments. The theory and practice of heap, dump and in situ leaching are not described in any detail because these topics have been covered in several substantial reviews and numerous more specific publications (e.g., Murr, 1980; Rossi, 1990; Bartlett, 1998).

2. The bioleaching of sulphide minerals

The main research topics in the bioleaching literature are (i) fundamentals and modeling of bioleaching chemistry (and to a small extent, mineralogy), (ii) microbiology, (iii) bioleaching of selected sulphide minerals, commonly associated with specific ores or mines, and (iv) the engineering aspects of heap and dump leaching. The fundamental chemical and microbiological studies are usually generic, rather than copper-oriented, and conducted on sulphide concentrates rather than ores. The engineering aspects are outside the scope of this review.

The bioleaching of sulphide minerals occurs in an acidic medium that often contains a considerable concentration of iron(III). While the emphasis here is on bioleaching, acid leaching and galvanic interactions between sulphide minerals and iron(III) also contribute to the overall efficiency of the leach.

2.1. Leaching chemistry

The extraction of selected metals from mineral sulphides such as chalcopyrite can be an oxidative process

in which ferric ions are the oxidant and the sulphide component of the mineral is oxidized to elemental sulphur Eq. (1).



The reaction is known to be sensitive to redox potential; and surprisingly, higher dissolution rates have often been measured at lower potentials, in the range 0.45–0.65 V SHE (Peters, 1976; Third et al., 2000; Hiroyoshi et al., 1997; 2001). In addition, it has been shown that a parallel, non-oxidative dissolution process that is also sensitive to potential, contributes to the kinetics of chalcopyrite leaching in sulphuric acid solutions Eq. (2) (Nicol and Lazaro, 2003; Lazaro and Nicol, 2003).



The main disadvantage of (bio)leaching of sulphides is that the process is perceived to be slow relative to pyrometallurgical process routes and other high-intensity hydrometallurgical processes. However, the kinetics of mineral dissolution may change when two minerals are in electrical contact with each other, as is often the case in mineralized ores. For example, in isolation, the dissolution rate of pyrite is faster than that of sphalerite, galena or chalcopyrite, but when pyrite is in intimate contact with one of these minerals, the situation is reversed (Ramachandra Rao and Finch, 1988; Das et al., 1999b; Sui et al., 1995). This response is due to galvanic interaction and could be exploited to enhance leaching rates for base metal sulphides of interest.

In the case of chalcopyrite, both sulphur- and iron-containing reaction products have been invoked as the cause of slow dissolution. The insoluble reaction products formed on the chalcopyrite surface during leaching and bioleaching have been examined using X-ray photoelectron spectroscopy (XPS), a surface sensitive analytical technique. Four sulphur-containing species were detected on the leached chalcopyrite surface, namely a sulphide phase (unreacted chalcopyrite), elemental sulphur Eq. (1), a basic ferric sulphate phase akin to jarosite, and a disulphide phase (Fig. 1) (Klauber, 2003; Klauber et al., 2001; Parker et al., 2004). Similar speciation was found for abiotic chemical leaching and bioleaching under both aerobic and anaerobic conditions, suggesting that there was a common leaching mechanism.

On the basis of the sulphur speciation, a mechanism for the ferric ion oxidation of chalcopyrite has been proposed. A key feature of the mechanism is the oxidation of the disulphide phase, which forms rapidly on freshly fractured chalcopyrite and persists on leached

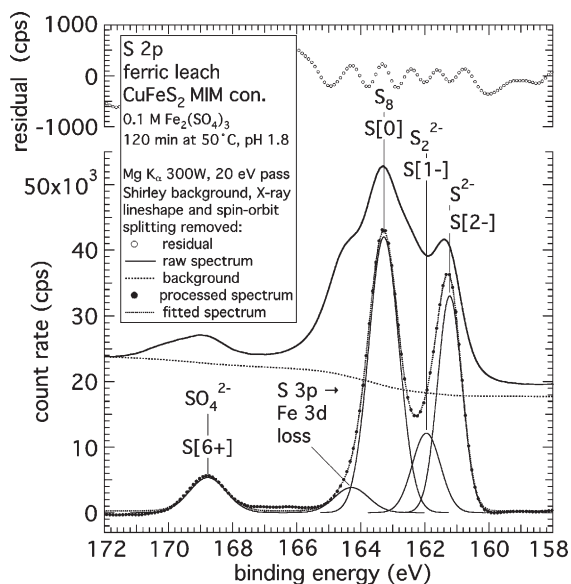


Fig. 1. Sulphur speciation of a chalcopyrite concentrate after leaching with ferric sulphate solution and surface analysis using X-ray photoelectron spectroscopy (Klauber et al., 2001, redrawn).

surfaces. Oxidation of the disulphide phase directly produces thiosulphate which is then oxidized to sulphate, generating the basic ferric sulphate that then acts as a template for jarosite formation. The jarosite layer then builds to the point that it hinders further chalcopyrite oxidation (Klauber, 2003).

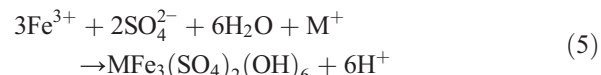
The chemistry of microbial oxidation of sulphides is closely related to that of ferric ion oxidation in acidic solutions Eq. (1). The microorganisms play a catalytic role in oxidising ferrous ion to ferric ion, thus regenerating the oxidant Eq. (3). They also oxidize sulphur to sulphate, generating acid Eq. (4).



The costs associated with the maintenance of sulphide bioprocessing microorganisms are minimal because many gain energy from the redox reactions Eqs. (3) and (4), utilize carbon dioxide from the air as C source and obtain their phosphorus, nitrogen, potassium and micronutrients, etc.), from the bioleach environment.

Because the microorganisms are particularly efficient at oxidising ferrous ions to ferric ions, the bioleaching conditions typically exhibit a relatively high redox potential around 0.65–0.70 V SHE, which is less conducive to chalcopyrite dissolution. One consequence of the high solution potential is that ferric ion readily precipitates as a

basic sulphate, like jarosite Eq. (5) in an environment containing monovalent alkali cations and sulphate ions.



where $\text{M} = \text{K}^{+}, \text{Na}^{+}$ or NH_4^{+}

Basic iron sulphate precipitates have been implicated in hindering the complete dissolution of chalcopyrite during bioleaching (Stott et al., 2001; Petersen et al., 2001). Kinnunen et al. (2003) proposed leaching at pH 1 to hinder/delay the formation of jarosite precipitates, and used a pH 1-adapted, immobilized biomass of *Leptospirillum*-like organisms to regenerate the ferric ion oxidant. More recently, Tshilombo et al. (2002) showed that the “passivation” of chalcopyrite during bacterial leaching could be countered by controlling the thermal (45–65 °C) and electrochemical (0.45–0.65 V SCE) conditions. The authors noted that the “passive layers” formed at 25 °C strongly inhibit ferric ion reduction on polarized chalcopyrite surfaces.

2.2. Mineralogy

The impact of mineralogy on leaching and bioleaching should not be ignored. Extensive mineralogical analysis of ore types around a deposit is required in developing a flow sheet. In heap leaching, acid consumption by gangue minerals is a key parameter and therefore sulphuric acid is usually a major processing cost. Maintenance of the pH in the preferred range between 1 and 2 for microbial iron and sulphur oxidation is also important for ferric ion and acid regeneration by the microbial population.

Surprisingly little quantitative research on the correlation between ore mineralogy, reaction chemistry and leach residue mineralogy has been reported in the public domain. The application of quantitative mineralogical analysis of feed and leach residues proved valuable in understanding autoclave chemistry during the high pressure acid leaching of nickel laterites (Whittington et al., 2003a,b) and is being refined to describe copper sulphide concentrates as part of a systematic leaching study (Tiller-Jeffery et al., 2004). A combination of X-ray fluorescence and X-ray diffraction analysis of leach residues was combined with elemental composition to understand the leaching of gangue minerals in mild sulphuric acid column leaching experiments; mineral characterisation by microscopy together with clay phase identification using X-ray diffraction was correlated with acid leaching performance (Helle and Kelm, 2005; Helle et al., 2005; Kelm and Helle, 2005). When the automated SEM techniques (Gottlieb et al., 2000; Gu, 2003) are

combined with quantitative analysis of X-ray diffraction data, SEM-microprobe data and elemental analysis of ores and residues, they can provide new insights into leach chemistry and reaction mechanisms for difficult to process ores.

2.3. Microbiology and leaching

Sulphide ore dumps and heaps, with their varying mineralogical compositions and different climatic environments represent extremely complex microbiological habitats. Yet only a modest number of iron- and sulphur-oxidising bacteria (Table 2) have been isolated from mineral sulphide ores, characterised physiologically and phylogenetically, and deposited in data banks (Hallberg and Johnson, 2003 and references therein). These extreme acidophiles that grow optimally at $\text{pH} < 3$, must comprise only a small selection of those that have mineral processing capabilities.

The limited number of bacteria that have been discovered is partly a consequence of the selective methods by which bacteria are enriched and isolated. The perceived importance of *Acidithiobacillus ferrooxidans* in bioleaching is a case in point. For many years, *A. ferrooxidans* was thought to be the dominant bacterial strain in bioreactors operated at temperatures lower than $40\text{ }^{\circ}\text{C}$ because, when cultures were grown on soluble iron media in batch tests, *A. ferrooxidans* outgrew *Leptospirillum ferriphilum* (previously thought to be *L. ferrooxidans*). It has since been shown that the reverse is true in bioreactors. This is because high iron(III) concentrations inhibit *A. ferrooxidans* growth, whereas *L. ferriphilum* is relatively unaffected by high ferric ion concentrations and is also more tolerant of both higher temperatures and lower pH (Norris et al., 1988; Hansford, 1997; Schrenk et al., 1998; Rawlings et al., 1999). The dominance of *L. ferrooxidans* and *Acidithiobacillus thiooxidans* in highly acidic (pH 0.7) copper heap leach environments has been reported (Vasquez and Espejo, 1997).

Moderate thermophiles and hyperthermophiles also have a role to play in making bioleaching more efficient, because they permit the use of higher temperatures, which in turn result in faster reaction rates. At intermediate temperatures ($40\text{--}45\text{ }^{\circ}\text{C}$), *Acidithiobacillus caldus* is most likely the dominant sulphur oxidizer in bioreactors treating arsenopyrite or copper concentrates (Rawlings et al., 1999; Okibe et al., 2003). At temperatures greater than $60\text{ }^{\circ}\text{C}$ *Sulfolobus metallicus* and *Metallosphaera* spp are thought to be the most important bioleaching strains (Hallberg and Johnson, 2001).

While *A. ferrooxidans* was the first iron-oxidising acidophile to be isolated from acidic bioleaching

environments (Colmer et al., 1950), and has since been subjected to the most intensive characterisation, it is not necessarily the most important. Microbial consortia responsible for the solubilisation of metals from sulphide minerals are expected to be complex mixes of autotrophic and heterotrophic bacteria (Tuovinen et al., 1991). *A. ferrooxidans* and *L. ferrooxidans* share their environment with other acidophiles that have a similar physiology and can compete for the available nutrient and energy sources.

With new molecular microbiological methods of enumeration and identification of organisms, it is now possible to follow changes in microbial consortia as a function of time or location. This is a valuable tool in describing biodiversity and/or understanding bioleaching processes (Gonzalez-Toril et al., 2003; Okibe et al., 2003). Microbiological surveys of specific environments, such as hydrothermal sites (e.g. Atkinson et al., 2000; Burton and Norris, 2000; Simmons and Norris, 2000; Plumb et al., 2002), mine sites (Robertson et al., 2002; Keeling et al., 2004; Demergasso et al., 2005) or acidic mine drainage systems (e.g., Hallberg and Johnson, 2003; Dopson et al., 2004) are yielding more robust data on biodiversity. A group of acidophiles, possibly overlooked because they grow in a mid pH range (pH 3–6) in an acid mine drainage system, are of particular interest because of their role in promoting the oxidation and precipitation of iron (Hallberg and Johnson, 2003). Acidophilic heterotrophs are being discovered and characterised with increasing frequency (Hallberg and Johnson, 2001). The new methods also facilitate elucidation of the nature of microbial interactions in enhancing bioleaching (Johnson et al., 2001; Okibe and Johnson, 2004), a research topic worthy of more focused attention.

In the context of bioleaching, most microbiological research is conducted utilising known iron- and sulphur-oxidising bacteria, either as single strains or in mixed cultures. However, in many instances, bacteria indigenous to the ore are not excluded and may thus contribute to bioleaching. Indeed, it is often noted that indigenous bacteria, being acclimatised to high levels of selected metals in their environment, are more effective as bioleaching catalysts. The acclimatisation of bacteria to a particular mineral system by subjecting them to progressively greater amounts of the major elements present is common practice in test work. In addition, there have been many fundamental studies on the tolerance of single strains and cultures to base metal ions (e.g., Das et al., 1997; Dopson et al., 2003). In the same way, microbial growth can be promoted at heap operations by the addition of nutrients to leach solutions and by creating conditions that result in increased iron concentrations (Readett et al., 2003).

Table 2
Iron and sulphur oxidising acidophiles

Organism	Reported growth substrates	Characteristics
<i>Acidianus ambivalens</i>	S oxidation and reduction	Hyperthermophiles
<i>Acidianus brierleyi</i>	Sulphides	pH opt 1.5–2.5
<i>Acidianus infernus</i>	Poor, if any, Fe oxidation	
“ <i>Acidianus tengchongensis</i> ”		
<i>Acidimicrobium ferrooxidans</i>	Mixotroph Fe oxidation and reduction Sulphides (poor)	Moderate thermophile pH opt 2
<i>Acidiphilium</i> spp	Obligate heterotrophs	Mesophiles
<i>Acidiphilium</i> SJH	S oxidation Fe(III) reduction	pH opt ~2–3
<i>Acidiphilium acidophilum</i>	Facultative autotroph S oxidation Fe(III) reduction	Mesophile pH opt ~2–3
<i>Acidithiobacillus albertensis</i>	Autotrophs	Mesophiles
<i>Acidithiobacillus ferrooxidans</i>	S oxidation, sulphides	pH range 2–4
<i>Acidithiobacillus thiooxidans</i>	(<i>Ad</i> , facultative autotroph; <i>At</i> , mixotroph, Fe(III) reduction as a facultative anaerobe)	
<i>Acidithiobacillus caldus</i>	Mixotroph 3S oxidation, sulphides	Moderate thermophile pH opt 2–2.5
<i>Acidolobus aceticus</i>	Heterotroph S reduction to H ₂ S	Hyperthermophile pH opt 3.8
<i>Alicyclobacillus</i> spp	S oxidation, sulphides	Mesophiles — moderate thermophiles
“ <i>Alicyclobacillus disulfidooxidans</i> ”	(<i>Ad</i> , facultative autotroph; <i>At</i> , mixotroph, Fe(III) reduction)	pH 1.5–2.5
“ <i>Alicyclobacillus tolerans</i> ”		
“ <i>Ferrimicrobium acidiphilium</i> ”	Heterotroph Fe(II) oxidation, sulphides Fe(III) reduction	Mesophile pH opt 1.7–1.8
<i>Ferroglobus placidus</i>	Fe oxidation	Thermophile pH neutral
“ <i>Ferroplasma acidarmanus</i> ”	Possibly autotroph	Moderate thermophiles
“ <i>Ferroplasma cyprexacervatum</i> ”	Iron oxidation	pH range <1–2
<i>Ferroplasma acidophilum</i>	Pyrite oxidation poor	
<i>Ferroplasma</i> MT17		
<i>Hydrogenobaculum acidophilus</i>	S, H oxidation to produce sulphuric acid	Thermophile pH opt 3–4
<i>Leptospirillum ferriphilum</i>	Fe oxidation	Mesophiles, some thermo-tolerant strains
<i>Leptospirillum thermoferrooxidans</i>	Pyrite	pH range 1.6–1.9
<i>Leptospirillum ferrooxidans</i>	Fe oxidation, pyrite	Mesophile pH opt 1.5–1.7
<i>Metallosphaera sedula</i>	S oxidation	Thermophiles
<i>Metallosphaera prunae</i>	Sulphides	pH 1–4
“ <i>Metallosphaera hakonensis</i> ”		
<i>Sulfobacillus acidophilus</i>	Fe(II) oxidation; Fe(III) reduction, Sulphides	Moderate thermophiles
<i>Sulfobacillus thermosulfidooxidans</i>	S oxidation	pH 1–2.5
<i>Sulfolobus metallicus</i>	Strict chemolithoautotroph	Hyperthermophiles
“ <i>Sulfolobus rivotincti</i> ”	S oxidation, sulphides	Various pH in range 1–4.5
<i>Sulfolobus shibatae</i>		
“ <i>Sulfolobus tokodaii</i> ”		
<i>Sulfolobus yangmingensis</i>		
“ <i>Sulfolobus</i> ” JP2 and JP3		
<i>Sulfolobus acidocaldarius</i>	Heterotrophs	Hyperthermophiles
<i>Sulfolobus solfataricus</i>	Not S oxidation	pH 2–4.5
<i>Sulfurococcus yellowstonensis</i>	S and Fe oxidation	Hyperthermophile
<i>Thiobacillus prosperus</i>	S and Fe oxidation sulphides	Mesophile, halophile pH opt 2
<i>Thiomonas cuprina</i>	S oxidation, sulphides	Mesophile pH opt 3–4

Related to this is the issue of acclimatisation to the high total dissolved solids (TDS) content of solutions that develop during prolonged leaching, due to the dissolution of the gangue minerals. Shiers et al. (2005) used a quantitative batch culture method to investigate adaptation or habituation of a mixed culture of acidophiles to growth media containing increased concentrations of sodium sulphate or sodium chloride. Their results indicated relatively rapid adaptation to sodium sulphate at levels in excess of those normally found in process water. However, concentrations of only 7 g/L sodium chloride inhibited cell replication by more than 50% and no significant culture adaptation occurred during prolonged exposure. The search for acidophilic halophiles that do function well in high TDS water has not been particularly successful. Most iron oxidising halophiles prefer a higher pH range (e.g. Holden et al., 2001) which would promote greater precipitation of ferric compounds, thus diminishing the ferric ion concentration available to oxidise the sulphide mineral. Acidophiles extracted from samples (pH 2–3.5, temperatures 35–75 °C) taken close to vents of Vulcano (Aeolian Islands) exhibited salt tolerance but grew better in the absence of salt or at salinities lower than seawater (Simmons and Norris, 2000).

Recently, the possibility of utilising mineral specific (in this case chalcopyrite-specific) bacteria has been raised (Williams et al., 1999). One [mixed] culture, in particular, apparently had a high affinity for oxidising chalcopyrite ores. The bacteria were active over a wide range of temperatures (45–60 °C) and solution pH values (0.8–2.2) that might be encountered within a heap. Evidence of the chalcopyrite specificity was obtained by leaching a chalcopyrite/pentlandite and a chalcopyrite/pyrite concentrate. Normally, in both cases, the oxidation of chalcopyrite would be slow and incomplete, in comparison with that of the other sulphide. However, in agitated, aerated tank tests, chalcopyrite was leached to completion in 13–14 days. The rate of nickel dissolution was slow, and that of pyrite almost non-existent, until most of the chalcopyrite had been oxidised. The chalcopyrite-specific culture was tested on 11 chalcopyrite ores or concentrates of diverse origins with a range of mineral phases, sulphur and copper contents. Copper extractions of 95–99% were achieved consistently in 8–36 days. While the redox potentials of leach solutions were not reported by Williams et al. (1999), it was noted more recently (Hunter, 2002b,c) that the proprietary culture contained sulfur-oxidising microorganisms but not iron-oxidising organisms. It may be deduced, therefore, that the reported enhanced bioleaching of chalcopyrite was a consequence of low solution potentials (e.g. Hiroyoshi et al., 1997; Third et al., 2000) rather than chalcopyrite-specific microorganisms.

2.3.1. Microbial attachment and biofilm formation

It is well known that bacteria attach to substrates and form biofilms that have well-developed community structures, with mechanisms for the delivery of nutrients and the disposal of waste products. There are many observations that surfaces are the main sites of microbial activity in natural environments. van Loosdrecht et al. (1990) reviewed the many influences of interfaces on microbial activity. Those aspects relevant to bioleaching environments have been drawn out by Crundwell (1997) and expanded to include more recent studies in bioleaching systems.

In the past, attachment of bacteria to sulphide surfaces and the enhanced rates of dissolution of the sulphides were, together, used as evidence that bioleaching proceeded, in part, via a direct mechanism (enzymatic attack). The search for evidence to support this hypothesis has resulted in innovative studies on the role of attachment and biofilm formation in bioleaching.

It has been reported that microorganism–mineral interactions result in changes in the surface chemistry of the microorganisms.

- The surface charge on cells grown in media with soluble iron (Fe^{2+}) was different to that on cells grown on a solid substrate (sulphur, pyrite). The altered cell surface charge was attributed to higher protein content in the latter (Sharma et al., 2003).
- The absence of a lag phase when microorganisms are grown on a solid substrate (sulphur), compared with soluble sulphur (tetrathionate), was attributed to the presence of a proteinaceous cell-surface appendage that assisted adhesion (Devasia et al., 1996). Adhesion is thought to be promoted both by lipopolysaccharides as well as cell surface proteins (Amaro et al., 1993; Arredondo et al., 1994).
- Organic compounds with sulpho-hydryl groups (e.g., cysteine) might react with the sulphide surface with the subsequent release of iron and sulphur species. Bacteria could take advantage of the biochemical corrosion process by uptake and oxidation of the released species (Rojas-Chapana and Tributsch, 2000; Tributsch and Rojas-Chapana, 2000).

Many studies have been focused on the role of the extra-cellular polymeric substances (EPS) which are thought to mediate attachment.

- Cells with the EPS layer removed could not attach to covellite until the EPS layer was regenerated (Pogliani and Donati, 1999).
- Bacterial cells adapted the chemical composition of their exo-polymers to the substrate (Gehrke et al., 2001).

- The EPS might facilitate the concentration of Fe^{3+} by complexation through uronic acids or other residues at the mineral surface, resulting in an enhanced oxidative attack on the sulphide (Kinzler et al., 2003).
- Fe^{3+} reduction by attached bacteria could be a relevant process involving the EPS layer in the bioleaching of sulphides in aerobic conditions (Pronk et al., 1992). The behaviour of attached bacteria is very dependent on the $\text{Fe}^{3+}/\text{Fe}^{2+}$ ratio in the EPS layer, which is, in turn, very dependent upon the redox potential in solution and the concentration of soluble iron (Hansford and Vargas, 2001).

There is also evidence that bacterial attachment is mineral and site specific, and that it results in changed properties of the mineral surface. It is reported that:

- Attachment was rapid and most leaching bacteria grew attached to mineral sulphide surfaces (Gonzalez et al., 1999; Lizama et al., 2003). More than 80% of an inoculum was fixed throughout the process in inoculated bioreactors not limited by surface area (DiSpirito et al., 1983; Monroy et al., 1994).
- Bacteria attached to small pyrite and chalcocopyrite inclusions in low grade ore, rather than to siliceous phases (Murr and Berry, 1976). *Acidithiobacillus ferrooxidans* attached selectively to iron containing sulphides (Ohmura et al., 1993), and cell adsorption density was related to mineral phase (Devasia et al., 1993; Das et al., 1999a; Santhiya et al., 2001).
- Bacterial hydrophobicity increased as pH decreased; the degree of adhesion per unit area was greater to sulphide surfaces (hydrophobic) than to quartz surfaces (hydrophilic) (Solari et al., 1992). Sulphides can be separated from quartz through selective flocculation/dispersion following bio-treatment (Natarajan and Das, 2003; Chandraprabha et al., 2004a,b). Rojas-Chapana et al. (1998) proposed a chemo-tactic bacterial response to pyrite and to sulphur globules, which might have evolved as an adaptive advantage.
- Bacteria might be influenced by crystallographic orientation when “selecting” a site for attachment (Berry and Murr, 1977; Ndlovu and Monhemius, 2005). The degree of crystallization of synthetic pyrite films might influence bacterial behaviour (Sanhueza et al., 1999).
- Shape rather than size of a micro-topographic feature could be the key to attachment of *A. caldus* grown on pyrite. Edwards and Rutenberg (2001) concluded that small local surface alterations due to bacterial metabolism could strongly affect local adhesion parameters and account for bacterial adhesion on mineral surfaces.

Attachment may also be influenced by solution chemistry parameters. For example:

- Ferrous ion inhibited the attachment of *A. ferrooxidans* to pyrite and chalcocopyrite while ferric ion was much less inhibiting (Ohmura et al., 1993).
- Bacteria (*A. ferrooxidans*) attached to jarosite precipitates, actively oxidised ferrous ion (Pogliani and Donati, 2000).

Microbial attachment and biofilm formation may provide a mechanism through which the microorganism can locate itself near an energy source. For example, in the case where only a low concentration of ferrous ions is present in a bioleaching solution, the most reliable source of further ferrous ions will be the sulphide surface e.g. Eq. (1).

- Bagdigian and Myerson (1986) demonstrated that *A. ferrooxidans* preferentially attached at dislocations and grain boundaries on pyrite surfaces; proximity to such dislocations and boundaries might confer an advantage. Andrews (1988) suggested that sulphur atoms could diffuse along dislocations in pyrite in coal. Escobar et al. (2004) concluded that sulfur oxidation by *S. metallicus* grown on chalcocopyrite (70 °C, pH 1.8) was initiated by attached microorganisms after a significant lag period, during which sulphur built up on the mineral surface. They estimated that about 68% of the cell population was attached to chalcocopyrite particles and that attachment occurred within 100 h of inoculation.

2.3.2. Bioleaching mechanisms

The traditional hypothesis that bacteria oxidise sulphides by either a direct mechanism or an indirect mechanism (Silverman and Ehrlich, 1964), has evolved into a complex chemical/electrochemical/biochemical description of the interactions of bacteria with sulphide minerals.

Tributsch (2001) proposed that the term “contact” leaching be used in place of “direct” leaching because it described the association of bacteria with a surface rather than the means of attack. This approach has been refined further and summarized by Crundwell (2003), who described three mechanisms by which microorganisms (specifically *A. ferrooxidans*) might interact with a sulphide mineral, as follows:

- Bacteria oxidize ferrous ions to ferric ions in the bulk solution, and the ferric ions oxidize the sulphide phase — the *indirect mechanism*.
- Bacteria attached to the mineral surface oxidize ferrous ions to ferric ions within a biofilm

comprised of bacteria and exo-polymeric material, and the ferric ions generated within this layer oxidize the sulphide phase — the *indirect contact mechanism*.

- iii. Bacteria attached to the mineral surface oxidize the sulphide phase by biological means directly, without any requirement for ferric or ferrous ions — the *direct contact mechanism*.

Importantly, Crundwell noted that the distinguishing feature of both the direct and indirect mechanisms of sulphide oxidation was the necessity for ferric ions to participate in the mineral dissolution. It is a separate issue as to whether or not bacterial attachment (*contact*) in itself contributes to enhanced bioleaching by either the direct or indirect mechanism (Fig. 2). Crundwell's terminology has been adopted in the summary that follows.

- There is, to date, no evidence that *direct contact* leaching occurs, in which the bacteria break the metal–sulphide bond of a mineral.
- Attached microorganisms may cause an increased *indirect* rate of sulphide dissolution above the chemical rate of dissolution when the dissolution of the sulphide is hindered by the formation of a porous

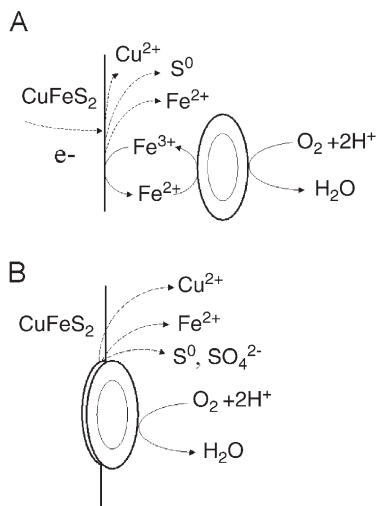


Fig. 2. Microbial roles in bioleaching of sulphide minerals. Indirect mechanism (A): bacteria oxidise soluble iron(II) to iron(III) and sulphur to sulphate. Ferric ions oxidise the sulphide minerals in an acidic environment. Indirect contact mechanism (B): bacterial attachment is important physiologically, but ferric ions oxidise the sulphide minerals. The specifics of bacterial (electro)chemical interactions with mineral surfaces and/or their direct contact (enzymatic) contribution to sulphide dissolution are unknown.

sulphur product layer, which is removed by the bacteria.

- Attached microorganisms may also cause an increased rate of sulphide dissolution above the chemical rate of dissolution in those cases where the dissolution of the mineral increases with decreased acidity; the corrosion or mixed potential also decreases in the presence of the bacteria, relative to an abiotic system. Mineral dissolution is achieved *indirectly* by ferric ion oxidation.

In both *indirect* leaching and *indirect contact* leaching, microorganisms catalyse the oxidation of ferrous ions to ferric ions (Crundwell, 2003). The oxidation of a sulphide mineral by ferric ions may proceed via a thiosulphate intermediate or a polysulphide intermediate depending upon its solubility in acid (Schippers and Sand, 1999).

- Acid-insoluble sulphides (e.g. pyrite, molybdenite, tungstenite) are oxidised to metal ions and sulphate via a thiosulphate intermediate. Bacteria catalyse the thiosulphate oxidation. For the case of pyrite, the thiosulphate is oxidised rapidly to sulphate by ferric ions and, therefore, may not be available as a substrate for the microorganisms (Hansford and Vargas, 2001).
- Acid soluble sulphides (e.g. pyrrhotite, sphalerite, chalcopyrite Eq. (2) dissolve to form metal ions and H_2S . In this way, the metal–sulphide bond in the mineral lattice is broken prior to sulphur oxidation. Bacteria catalyse the soluble sulphide–polysulphide oxidation to sulphate, and generate acid. The mechanism of acid dissolution of chalcopyrite and the aqueous sulphide oxidation to polysulphide have been described (Lazaro and Nicol, 2003; Nicol and Lazaro, 2003; Stuedel, 1996).

Crundwell and colleagues (Crundwell, 1999a,b; Fowler et al., 1999, 2001; Fowler and Crundwell, 1998, 1999; Holmes et al., 1999; Holmes and Crundwell, 2000) used a novel apparatus to determine the rates of biotic and abiotic leaching of both pyrite and sphalerite under strictly controlled solution conditions, specifically total soluble iron and controlled potential. They concluded that the increased biotic rate of pyrite dissolution was the result of attached bacteria consuming protons near the mineral surface while oxidising ferrous ions Eq. (2). In contrast, analysis of the data obtained for the leaching of sphalerite at low potential (high ferrous ion concentration) indicated that the rate limiting step was the diffusion of the ferrous ions away from the sulphide surface through the sulphur product layer that forms on the mineral surface in abiotic leaching but is

subsequently consumed in biotic leaching Eq. (3). In bioleaching, bacteria clean the mineral surface of sulphur, allowing the mineral to be more rapidly oxidized by ferric ions and ferrous ions to escape the surface into the bulk solution phase. In both cases, while bacterial attachment results in enhanced leaching, mineral dissolution is the result of ferric ion attack on the sulphide particles.

In their study of the bioleaching of a sphalerite concentrate, Rodriguez et al. (2003a,b) concluded that there are two steps to sphalerite dissolution. In the first step, the rapid attachment of microorganisms to active pyrite surfaces lead to the oxidation of the pyrite and concomitant bio-generation of ferric ions and protons. This was the key to enhanced sphalerite leaching. In the second step, the key contributions in sphalerite dissolution were the continued regeneration of ferric ions by planktonic bacteria and the oxidation of the elemental sulphur reaction product. These authors termed this *cooperative bioleaching* and suggested that there is a direct relationship between the magnitude of cell attachment in the first step of the process; and the dissolution rate in the second step.

2.3.3. Molecular chemistry and genetics

Acclimation of bacteria to different conditions, such as increased tolerance to high metal levels, is a simple means of genetic improvement. The technique is dependent upon the small number of errors in the DNA sequence that are made during chromosomal replication. Most errors are harmful or neutral but some may be advantageous. Thus, when a selective pressure is applied to a population, those bacteria that acquire an advantageous mutation will outperform the rest and dominate the population. For example, by growing bacteria in a continuous flow-reactor under conditions of increasing flow rate, fast growing bacteria will be enriched while slow growing bacteria will be washed out.

The advantage of “mutation and selection” is that it can be applied in the laboratory without requiring specialised knowledge of bacterial physiology and biochemistry. The disadvantage is that it is a slow process and yields little information about the bacterial population itself. Nevertheless, *A. ferrooxidans* leach rates have been improved ten-fold, making possible the commercialisation of bio-oxidation (van Aswegen et al., 1988).

Studies on the genome and genetic engineering of mineral processing bacteria are in their infancy and mainly relate to *A. ferrooxidans* (Barreto et al., 2003; Holmes et al., 2001; Leduc and Ferroni, 1994; Quatrini et al., 2004; Rawlings, 1999, 2001; Rawlings and Kusano, 1994; Valdes et al., 2004) This is not surprising. *Acidithiobacillus ferrooxidans* is the best characterised

and fastest growing of the iron- and sulphur-oxidisers and, fortuitously, the structures of many of its genes have been found to resemble those of *Escherichia coli* (a well-characterised bacterium of physiological importance). Genomes of other acidophiles such as *Picrophilus torridus* (Futterer et al., 2004), *Ferroplasma acidarmanus* (Tyson et al., 2004) and *Sulfolobus acidocaldarius* (Chen et al., 2005) have been sequenced more recently.

A number of studies using *A. caldus* (de Groot et al., 2003 and references therein) or *L. ferrooxidans* (Coram and Rawlings, 2002; Coram et al., 2005) have been reported. Recent progress on the molecular genetics of *Sulfolobus* spp has also been reviewed (Zillig et al., 1998; Ciaramella et al., 2002). The role of *Ferroplasma acidarmanus* in bioleaching is as yet unknown but this strain is dominant in mine water at pH < 1.5 and thus implicated in acid rock drainage (Barreto et al., 2003; Holmes et al., 2001).

Currently, the genetic engineering of mineral processing bacteria tends not to have a high priority among mineral bioleaching researchers. There are a number of reasons for this:

- i there is a great deal of uncertainty about regulatory issues concerning the release of genetically engineered strains into the environment;
- ii there is some doubt that engineered strains would be sufficiently robust to survive and compete effectively in the complex environment of a mineral processing operation;
- iii before strain improvement by genetic engineering can be contemplated, it will be necessary to establish those attributes that provide the organisms with their competitive edge and, from that, determine which genes to target;
- iv there is the generally held view that indigenous bacteria growing in a particular environment are likely to be those best adapted to that environment.

Natural, but sometimes extreme environments, have been the source of all bacterial strains used in mineral processing thus far (Rawlings, 1997; Norris, 1997; Hallberg and Johnson, 2001).

3. Copper sulphide bioleaching

The majority of sulphide bioleaching research studies have been carried out with one of two main goals. The first has been to assess and/or compare the capability of bacterial strains to oxidise different minerals. Related to these studies, are those concerned with quantifying bacterial tolerance to particular leaching environments

(e.g. Franzmann et al., 2005) and/or with adapting bacteria to changed conditions. Increasing bacterial tolerance to high concentrations of heavy metals is a topic that has also received close attention (e.g. Das et al., 1997). The mechanisms of metal resistance in acidophilic microorganisms have been reviewed by Dopson et al. (2003).

The second goal has been to test the possibility of utilising bioleaching/bio-oxidation to process different ores and concentrates, particularly those associated with a particular ore body or mine. While the results of such studies are seldom directly comparable, because most ores contain a variety of copper minerals, some generalisations can be made.

In those locations where leaching is a secondary operation with the purpose of processing tailings, low-grade ore or material unsuited to concentration, then the preferred approach for processing, based on the number of operations are ranked:

- Minerals: copper oxides > secondary sulphides > chalcopyrite
- Methods: dumps > heaps > in situ

The efficiency of leaching depends strongly upon the minerals that make up the ore (Table 3). For example, whereas tenorite [CuO], cuprite [Cu₂O], malachite [Cu₂(CO₃)(OH)₂] and chrysocolla [CuSiO₃·2H₂O] might require only hours of leaching, chalcocite [Cu₂S] and covellite [CuS] require months of heap leaching and chalcopyrite would require years of dump leaching. Chalcopyrite leaches at about one fifth the rate of chalcocite (Rear et al., 1994). While grinding of the ore to a smaller particle size very often increases copper recovery in a given time (Rhodes et al., 1998), the benefit conferred by the treatment will be offset by increased acid and energy consumption. In addition, fine particles in a heap can decrease the permeability of the heap to both air and solution.

The bioprocessing of whole ores presents different challenges to those experienced in stirred tank technologies. For example, during the dump and heap leaching of whole ores, leaching progress can be monitored by chemical and physical means, but only minimal control can be exercised over the conditions within the ore in situ and the reactions occurring there. The most widely practiced example is the heap leaching of chalcocite ores (Table 1).

3.1. Whole ore heap (and dump) bioleaching

Thin-layer leaching exemplifies heap leaching in that it is the foundation of so many of the current heap leach

operations. Originally thin layer leaching was developed to process copper oxides, but it was subsequently modified to accommodate an increased contribution from sulphide minerals in the ore (Montealegre et al., 1995). The key features are (i) curing, in which concentrated acid is added to the crushed ore and reacts with acid soluble and gangue minerals, and (ii) consolidation of fines with larger particles in a rotating drum, which ensures even acidification and wetting of the ore prior to stacking. In those cases where organic free raffinate is used for curing/acidification, bacteria may also be distributed through the ore before it is stacked. The ore is stacked in carefully designed heaps so as to create a bed that exhibits good permeability for both solutions and gases. The bed is irrigated from sprinklers or drippers arranged in a grid across the top of the heap. Typically, a short-duration acid leach (18 days) recovers 80% of copper from oxides, and between 30 and 40% of copper from bornite and chalcocite. The transition from a heap leach to a bioleach is accomplished by constructing heaps of the acid-leached, crushed ore (100% less than 6 mm particle size) stacked to 5–6 m high. Solution pH is controlled to ~1.8 to optimize bacterial activity. Leach effluents typically contains 2–3 g/L total soluble iron, 20–30 g/L sulphate and ~10⁶ bacterial cells/mL. Temperatures inside the heap are in the range 12–27 °C. Leach duration is markedly longer than that required for the oxides, and typically ranges from 150 to 210 days for 75–80% recovery of copper from bornite and chalcocite.

The success of many recent bioleaching operations, mainly chalcocite (Table 1) is testament to this robust technology, which has been successfully modified to accommodate ore characteristics peculiar to different mineral deposits. Some of those modifications have been directed towards optimizing heaps as bioreactors to increase copper production (Fig. 3).

Sulphur- and iron-oxidising microorganisms colonise sulphide ores naturally, and are well known contributors to the process of acid rock drainage (Colmer et al., 1950; Temple and Colmer, 1952). It is therefore surprising that forced aeration of heaps to optimize bacterial activity by meeting their physiological requirements for O₂ and CO₂ was only implemented in the mid 1990s, concurrently in Chile and Australia (Readett and Sylwestrzak, 2002). In Chile, the calculated required amount of air was delivered to the heap, whereas in Australia excess air was applied by means of low-pressure blowers. The significantly improved recovery of copper from an aerated chalcocite test heap at Girilambone, NSW prompted the strategy of re-mining and aeration of under-performing heaps (Lancaster and Walsh, 1997). This was accomplished by using an

Table 3
Dissolution and oxidation reactions of some copper-containing minerals in heaps and dumps

Mineral	Leaching and oxidation reactions	Duration
Atacamite	$\text{Cu}_2\text{Cl}(\text{OH})_3 + 3\text{H}^+ \rightarrow 2\text{Cu}^{2+} + \text{Cl}^- + 3\text{H}_2\text{O}$	Hours to days
Chrysocolla	$\text{CuSiO}_3 \cdot 2\text{H}_2\text{O} + 2\text{H}^+ \rightarrow \text{Cu}^{2+} + \text{SiO}_2 \cdot 3\text{H}_2\text{O}$	
Neotocite	$(\text{Cu}, \text{Mn})_2\text{H}_2\text{Si}_2\text{O}_5(\text{OH})_4 \cdot n\text{H}_2\text{O} + 4\text{H}^+ \rightarrow \text{Cu}^{2+} + \text{Mn}^{2+} + 4\text{SiO}_2 + 6 + n\text{H}_2\text{O}$	
Tenorite	$\text{CuO} + \text{H}_2\text{SO}_4 \rightarrow \text{CuSO}_4 + \text{H}_2\text{O}$	
Malachite	$\text{Cu}_2(\text{CO}_3)(\text{OH})_2 + 2\text{H}_2\text{SO}_4 \rightarrow 2\text{CuSO}_4 + \text{CO}_2 + 3\text{H}_2\text{O}$	
Azurite	$\text{Cu}_3(\text{CO}_3)_2(\text{OH})_2 + 3\text{H}_2\text{SO}_4 \rightarrow 3\text{CuSO}_4 + 2\text{CO}_2 + 4\text{H}_2\text{O}$	
Brochantite	$\text{Cu}_4(\text{SO}_4)(\text{OH})_6 + 6\text{H}^+ \rightarrow \text{CuSO}_4 + 3\text{Cu}^{2+} + 6\text{H}_2\text{O}$	
Native copper	$\text{Cu} + 1/2\text{O}_2 + \text{H}_2\text{SO}_4 \rightarrow \text{CuSO}_4 + \text{H}_2\text{O}$	Days to months
Cuprite	$\text{Cu}_2\text{O} + 1/2\text{O}_2 + 2\text{H}_2\text{SO}_4 \rightarrow 2\text{CuSO}_4 + 2\text{H}_2\text{O}$	
Chalcocite	$\text{Cu}_2\text{S} + 1/2\text{O}_2 + \text{H}_2\text{SO}_4 \rightarrow \text{CuS}^* + \text{CuSO}_4 + \text{H}_2\text{O}$ $\text{Cu}_2\text{S} + \text{Fe}_2(\text{SO}_4)_3 \rightarrow \text{CuS}^* + \text{CuSO}_4 + 2\text{FeSO}_4$ * CuS is a reaction product rather than the mineral covellite	
Bornite	$\text{Cu}_5\text{FeS}_4 + 2\text{Fe}_2(\text{SO}_4)_3 \rightarrow 2\text{CuS} + \text{CuFeS}_2 + 2\text{CuSO}_4 + 4\text{FeSO}_4$	Months to years
Covellite	$\text{CuS} + 2\text{O}_2 \rightarrow \text{CuSO}_4$ $\text{CuS} + \text{Fe}_2(\text{SO}_4)_3 \rightarrow \text{CuSO}_4 + 2\text{FeSO}_4 + \text{S}^0$	
Enargite	$\text{Cu}_3\text{AsS}_4 + 41/2\text{Fe}_2(\text{SO}_4)_3 + 2\text{H}_2\text{O} \rightarrow 3\text{CuSO}_4 + 9\text{FeSO}_4 + 4\text{S}^0 + \text{HAsO}_2 + 11/2\text{H}_2\text{SO}_4$	
Chalcopyrite	$\text{CuFeS}_2 + \text{O}_2 + 2\text{H}_2\text{SO}_4 \rightarrow \text{CuSO}_4 + \text{FeSO}_4 + 2\text{S}^0 + 2\text{H}_2\text{O}$	Years

excavator to turn the heap whilst aeration piping was placed in the slot where the ore was turned. The heaps were brought back on line with drippers and air was introduced to the heap from both sides via a vent bag. The additional copper extracted in the short term by this strategy amply compensated the cost of re-mining.

When heaps are stacked and irrigated, there can be a lag time before bacterial growth and metabolism contributes

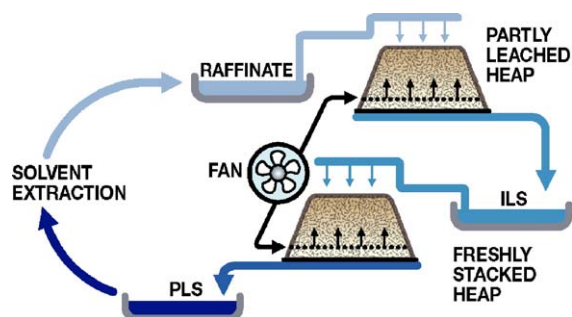


Fig. 3. Simplified heap leach schematic showing solution management and heap aeration to promote bioleaching of sulphide minerals. Solution management is aimed at minimizing water use and maximizing PLS to the SX circuit.

to sulphide oxidation. Lag times can be shortened by utilising heap recycle solutions (e.g. raffinate from SX) that contain indigenous microbial populations already acclimated to the leaching conditions, for the acid conditioning step, thus ensuring that an active bacterial population is distributed throughout the ore. Heap irrigation utilizing similarly populated recycle solutions maintains the population for the duration of leaching and serves to inoculate subsequent heaps. The patented BIOPRO™ process, which involves heap inoculation during acid conditioning and agglomeration, (Brierley, 1997) and has been implemented for the biooxidation of refractory gold ores prior to gold extraction, claims rights over an inoculation strategy that has been widely practiced because water recycle is cost effective, if not critical, in heap leaching operations.

Dump leaching and in situ leaching can be thought of as sub-sets of heap leaching in that solutions percolate through a bed of ore and copper is recovered from the leachate. Typically, dump leaching, with minimal ore preparation, is used to extract copper from low-grade (“run-of-mine”) ore (0.1–0.5% Cu). Dump leaching is relatively inefficient because large sized particles

present small surface areas to lixivants whilst small particles block solution flows and impede aeration. Furthermore, haulage vehicles and time cause the ore to compact, making it even less permeable. Modern dump practices that minimise compaction and maximise aeration and solution permeability, can improve copper recoveries. Dump material may be pre-conditioned with high acid levels, but the value of this strategy depends upon the mineralisation and on the acid consumption of gangue minerals. Variable irrigation programs may be employed as the permeability of the dump decreases with age. The enormous tonnages and the fact that the cost of removal is already borne by the mine make it economical to scavenge copper from low-grade (0.1–0.5% Cu) dumps (Schnell, 1997). Studies on dump biodiversity and biodynamics are sparse (e.g. Edwards et al., 1999) but it can be assumed that bacteria can readily colonise exposed mineral sulphide surfaces and engage in iron and sulphur oxidation.

There is only limited evidence that bacteria play a role in the in situ processing of undisturbed copper ores. For example, solutions circulating through a chalcocite/chalcopyrite/pyrite ore at La Hermosa (part of the Andacollo mining district) were found to contain 10^6 cells/mL (Concha et al., 1991). *Leptospirillum ferrooxidans* is thought to play a role in the leaching of a chalcocite and bornite ore body below an old open-pit mine, relatively near the surface (Concha et al., 1991). However, while solutions injected into deep in situ ore bodies may contain bacteria, it is unlikely that the bacteria will be particularly active at depth under conditions of oxygen limitation. That is not to say that an in situ leach of the future cannot be engineered to meet bacterial physiological requirements.

3.2. Modelling applied to heaps or dumps

Sulphide heaps are complex but poorly understood bioreactors. To a degree, the lack of understanding has arisen because many investigators have focused on specific sub processes in isolation, such as the chemistry, the microbiology or the hydrodynamics, and have failed to account for the interactions between those processes. In order to realize fully the potential of heap bioleaching, a holistic model is required that accounts for as many as possible of the complex micro- and macro-scale processes and their interactions.

There is currently a considerable effort being made to develop predictive models of a generic nature to facilitate heap design and control. Much of the early research has been summarized by Ritchie (1997). Recent studies in respect of both heap leaching and acid rock drainage

(Casas et al., 1998; Bouffard and Dixon, 2001; Lefebvre and colleagues, 2001a,b) are testament to the growing interest in models as tools to improve heap design and management.

A critical assessment of the significant, public-domain, heap leach models has been undertaken by Dixon (2003). The review cites 47 references describing developments over the last 30 years. Fifteen models including heap and dump leaching (five models), heap and dump bioleaching (eight models) and acid rock drainage (two models) are compared in terms of the extent to which each represents the most important macro-scale transport, chemical and kinetic, and biological phenomena involved in heap leaching. Dixon uses an extensive matrix of heap sub-processes in his collation. Table 4 presents a simplified matrix summarising the capabilities of the heap bioleaching models reviewed by Dixon (2003), augmented with some more recent work. As was the case in Dixon's review, distinction is made between those phenomena that are accounted for explicitly, and are well documented, and those that are accounted for only implicitly or in a cursory fashion or are poorly documented.

Ritchie and colleagues (Pantelis et al., 2002 and references therein) describe the development of a model initially focused on dump leaching and sulphidic mine waste stockpiles. In respect of heap leach phenomena, there seem to be limitations in respect of boundary phenomena, reaction networks, gangue mineral reactions and interactions, and microbial growth and oxidation, as is also the case for the copper dump model (Casas et al., 1998).

The point of departure for the development of the HeapSim[©] model (Dixon and Petersen, 2003, 2004) has been a systematic conceptual and mathematical description of many of the processes relevant in heap leaching drawn together within a comprehensive simulation engine. A relatively large set of parameters are required by the model. Many can be taken from the literature but some must be measured, usually through targeted laboratory-scale column tests. The dual experimental and modelling approach has been applied in a number of heap bioleach contexts, including high-grade zinc sulphide (marmatite) ores, low grade chalcocite ores and supported copper concentrates. HeapSim[©] is currently being revised and refined with the focus on the heap bioleaching of chalcopyrite.

The key differentiator of the multi-dimensional models – Phelps Dodge (PD) copper stockpile model (Bennett et al., 2003a,b) and CSIRO Heap model (Leahy et al., 2005, submitted for publication) – is the ability to simulate spatial structures in heaps. The PD model can simulate different conditions within individual lifts at various locations within the canyon

topography. The CSIRO model has been used to investigate the local structure related to individual air spargers and acid drips. These two models also have the capability of simulating two phases (gas and liquid) simultaneously, conferring greater predictive model power.

Bennett et al. (2003a,b, 2006) described the design and development of a comprehensive model for a copper stockpile leach process based on the computational fluid dynamic software framework PHYSICA+. The authors report that the model accounts for transport phenomena through the stockpile, reaction kinetics for the important mineral species and bacterial affects on the leach reactions — plus heat, energy and acid balances for the overall leach process. However, in comparison with others (Table 4), boundary phenomena, gangue mineral reactions and interactions, and microbial growth and oxidation are either dealt with in cursory fashion or poorly documented.

The CSIRO model is built on the computational fluid dynamics CFX platform (Leahy et al., 2005 and references therein). Like HeapSim©, it incorporates a mathematical description of many of the important heap phenomena. It is perhaps less robust in accounting for boundary phenomena and, like all its predecessors, thus far fails to address gangue mineral reactions and interactions explicitly.

4. Chalcopyrite bioleaching

Not surprisingly nearly all the research on chalcopyrite leaching and bioleaching has been undertaken with concentrates and, therefore, not all of the examples given below are directly relevant to the heap leaching of low grade ores. Nevertheless, it is worth briefly summarizing some of the peculiarities of chalcopyrite leaching as a means of determining what controls may be desirable in a heap leach environment. Some of the detail is specific to agitated tank leaching processes, for example BioCOP™ (Clark et al., 2005) or the BacTech-Mintek process (ANON, 2001). The key points that do carry across to heaps are:

- Chalcopyrite (or the target sulphide) surface must be exposed to the leach solution, particularly the oxidant (iron(III)). Thus appropriate ore preparation and iron chemistry is important.
- Microbial catalysts are needed to regenerate the oxidant and acid and to enhance leaching rates. Thus conditions conducive to growth are important.
- Chalcopyrite leaches more rapidly at higher temperatures.
- Chalcopyrite may leach more rapidly at lower redox potentials.

- Silver catalyses chalcopyrite dissolution, but is not an economic additive. No other effective catalyst has been identified.
- Chloride enhances chalcopyrite leaching.

4.1. Fine grinding

The slow and incomplete extraction of copper from chalcopyrite concentrates and ores can be overcome when the ore/concentrate is finely ground ($p_{80} < 15 \mu\text{m}$) (Rhodes et al., 1998; van Staden, 1998). Small volume particles combined with a high surface area promoted rapid copper extraction. By the time the “inhibiting layer” had developed to the point of hindering further reaction, copper extraction was all but complete. However, care must be taken not to damage cells physically during agitation (e.g., Deveci, 2002, 2004).

Moderate leach temperatures and fine grinding, made economic by a very favourable power cost, combined to make the recent pilot trial at Mt Lyell a success (Rhodes et al., 1998). This process utilised moderate thermophiles (45–47 °C) and pH controlled between pH 0.8–2.0; the residence time was about 5–6 days for 96% copper extraction. When operating in the thermophilic temperature range, fine grinding may not be necessary. Greater than 95% copper extraction from a chalcopyrite/pyrite concentrate was achieved in a three-stage pilot plant (Gericke and Pinches, 1999; Gericke et al., 2001). The key parameters were delivery of sufficient oxygen and carbon dioxide for bacterial growth and oxidation, and accommodation of an increased microbial sensitivity to solids concentration and particle size distribution. The process has been operated at pilot and demonstration scales with a view to commercialization (Rhodes et al., 1998; ANON, 2005).

4.2. Heap inoculation

Copper bioleaching operations typically rely upon natural colonisation by indigenous microbial strains well-acclimatised to the ore. However, with the continued push to achieve the desired metal extractions in ever shorter times, the need arises to not only promote microbial colonisation throughout the heap efficiently and in a short time period but also to maintain a population appropriate to the conditions that become established in the heap as a function of time. Further, for chalcopyrite, heaps may need to be operated at increased temperature to achieve the necessary copper extraction rate, in which case inoculation with a consortium of microorganisms that grow well at different temperatures would be required to overcome the delay in natural colonisation. In the case of a

Table 4
Comparison of factors considered in various bioleach models in terms of heap leach phenomena

Model	A	B	C	D	E	F	G	H	I
<i>Hydrology and solute transport</i>									
Heap wetting			●	●	●	●	●	●	●
Advection			●	●		●	●	●	●
Diffusion							●	●	●
Multidimensional solute transport				●				●	●
<i>Gas transport</i>									
Advection (forced aeration)	●	●	●	●	●	●	●	●	●
Convection (natural aeration)	●	●	●	●	●			○	●
Diffusion		●	●	●	●			●	●
<i>Heat transport</i>									
Advection and conduction	●	●	●	●	●		●	●	●
In situ evaporation/condensation	○		○	●	●			●	●
<i>Boundary phenomena</i>									
Non-uniform solution application							●	○	○
Surface evap'n, radiation, convection							●	○	○
Heap base evaporative cooling	●				○		●	○	○
<i>Operator-induced phenomena</i>									
Solution stacking/change			○				●		
Rest/rinse cycles			○		○		○		
<i>Leaching kinetics</i>									
Leaching reaction kinetics	○			●		○	●	●	●
Pore diffusion kinetics	●	●		●		●	●	●	●
Liquid–solid mass transfer				●		●	○	●	●
<i>Reaction networks</i>									
Leaching in series			○	○	○	●	●	●	●
Homogeneous reaction kinetics						●	●	●	●
Gas–liquid mass transfer						●	●	●	●
Multiple minerals leaching in parallel	○				○	○	●	●	●
<i>Component sources/sinks</i>									
Gangue interactions	○		○				●		
Precipitation/adsorption/solution	○		○				○		
Solution speciation			○				○		
<i>Non-linear effects</i>									
Changes in pellet porosity/liberation						●			
<i>Microbial growth and oxidation</i>									
Microbial speciation by T and function							●	○	●
Growth and oxidation kinetics						●	●	○	●
Temperature factors	○	○	○	●	●		●	○	●
Substrate limitations/inhibitory factors			○	●	○	●	●	○	●
Cell yield						●	●	○	●
Maintenance/decay/death							●	○	●
<i>Microbial transport</i>									
Microbial diffusion/mobility						○	●		●
Microbial attachment/adsorption						●	●		●
Inoculation (continuous, periodic)							●		●

Adapted from Dixon (2003) and augmented.

chalcocite (minor chalcopyrite) test heap that was not specifically inoculated, cell numbers indicative of a functioning population, including moderate thermophiles, were only observed at about three months (Readett et al., 2003). In the same test heap, thermophiles were not detected, even though the heap temperatures rose and remained quite high for a significant period.

Examination of the literature shows that a number of heap inoculation methods have been proposed, including:

- Heap irrigation from ponds in which microbial growth has been enhanced through the addition of nutrients (P, K, N) to the iron-rich solution. Ponds are sometimes covered and aerated.
- Formation of agglomerates from ore particles using an inoculum containing iron and sulphur oxidising organisms and then stacking the ore (and variations) (Brierley and Hill, 1993).
- Inoculating a biological contactor with iron oxidising strains and feeding the ferric-rich stream (containing some microorganisms washed out) to the heap (Hunter, 2001).
- Creating a culture of microorganisms on part of the ore and mixing this ore with the bulk ore being stacked — a means of distributing a viable culture already attached to the ore throughout the heap (King, 2001). In an apparently similar invention, the microorganisms are first adapted to an ore and then scaled up to the required volume for heap inoculation (Hunter and Williams, 2002).
- Inoculating with a tailored bacterial culture that grows particularly well on a specific mineral (e.g. chalcopyrite) and feeding the culture from a pond to the heap (Hunter, 2002c). A similar application could be heap inoculation with tailored microbial cultures that grow well under the targeted heap conditions such as above ambient temperatures, increased acidity, or the presence of organic compounds originating from the ore or the downstream processing.
- Microbial inoculation with strains that have been rendered temporarily non-adhesive so that they permeate to greater depths in the heap when delivered via irrigation to the heap surface (StickiBugs™) (Gericke et al., 2005).
- Delivery of ultra-small microorganisms via a gaseous suspension into the heap using the aeration lines (du Plessis, 2003).

4.3. Heap leach at above ambient temperatures

It is well known that the bioleaching of chalcopyrite proceeds more rapidly if the temperature is raised above

about 55 °C (Fig. 4). Greater copper extraction is achieved before leaching rates are slowed by the formation of inhibitory layers on the mineral surface.

The exploitation of thermophiles in the bioleaching of base metal concentrates represents a breakthrough development for the bioleaching of chalcopyrite. In batch bioleaching tests using thermophiles and temperatures 68 and 78 °C, a relatively-long residence time of 30 days yielded 95–97% copper extraction from chalcopyrite (Dew et al., 1999). Process improvements have been achieved by utilising oxygen-enriched air to sparge the bioreactors, thus overcoming oxygen limitation due to the low solubility of oxygen in aqueous solutions at high temperatures. The BioCOP™ process continues to be improved and tested with a view to commercialization, for example the Alliance Copper demonstration plant (Clark et al., 2005).

There is no doubt that heaps of quite low grade sulfidic material can be self-sustaining at above ambient temperatures, but this may not be achieved in practice. Norton and Crundwell (2004) cite insufficient and/or inefficient aeration and the implementation of constant irrigation rates, carried over from heap leaching of oxides, as key factors why heaps seldom heat to the temperatures required to recover copper from chalcopyrite. A number of methods have been described in respect of generating and then maintaining heat in a heap.

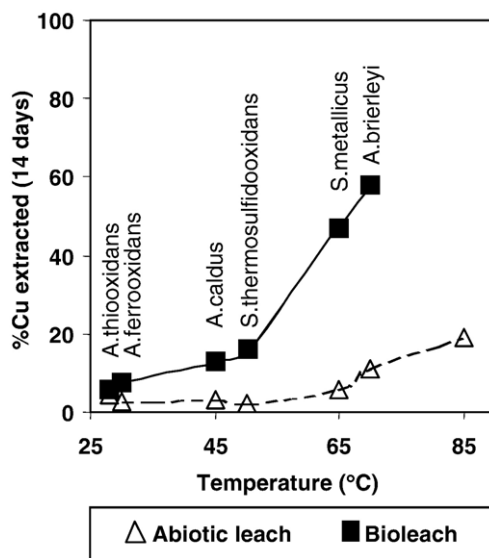


Fig. 4. Thermophilic sulphur- and iron-oxidising microorganisms are required in order to take advantage of increased chalcopyrite leaching rates. Tests used a chalcopyrite concentrate of composition 64% chalcopyrite, 6.6% pyrite, 3.3% pyrrhotite and 25% quartz, mean particle size 27 μm , P_{80} -66 μm and surface area 0.36 m^2/g (5-point BET analysis).

- Acid conditioning of ore using hot water to add heat to the heap and promote more rapid oxidation (Schnell, 1997).
- Heating the heap (Kohr et al., 2000).
- Increasing the heat-generating capacity of the heap with, for example, additional sulfides (Kohr et al., 2002).
- The use of covers to limit evaporation and/or insulate the heap (Schnell, 1997).
- Heat transfer to heap irrigation solution via a flexible, heat-absorbing distribution mat with parallel spaced emitter tubes (solar heating) (Lane, 2000).
- Heat transfer to heap leach solution from another process (Batty and Norton, 2003; Schnell, 1997).
- Aeration with humidity and temperature control to maintain heap environment suitable for thermophiles (Winby and Miller, 2000).
- Managed aeration and irrigation to maximise heat conservation in the heap (Norton and Crundwell, 2004).
- Addition of carbon (carbonate, carbon dioxide, organic carbon) to the heap to enhance microbial growth and activity at temperatures above 60 °C (du Plessis and de Kock, 2005).

Combinations of these management and control measures should deliver and maintain heap temperatures in a range that favours the necessary microbial oxidation and promotes more rapid release of copper from chalcopyrite.

4.4. Silver catalysis

The acceleration of chalcopyrite dissolution by the presence of some catalytic ions, particularly silver, is well known (Hu et al., 2002). Silver ions react with the chalcopyrite surface to form Ag_2S which modifies the anodic dissolution of chalcopyrite and inhibits passivation. The problem has been to develop a means of recovering the silver added to a chalcopyrite dissolution process from the solids with which the silver reports. For example, silver is readily locked up as a silver jarosite and does not report to the solution. The IBES and BRISA two-stage processes for the treatment of a chalcopyrite concentrate (Romero et al., 2003; Carranza et al., 2004) involve the optimisation of both the biooxidation of ferrous ions and the chemical oxidation of the sulphides in separate reactors — the latter at higher temperatures conducive to chalcopyrite leaching. Silver, added as a catalyst to the chemical leach reactor, significantly enhanced copper extraction from the chalcopyrite. Silver ion toxicity to the microbial population was avoided by

the use of a two-stage process. The efficient recovery of added silver, by brine treatment of the leach residues to solubilise silver as a chloro-complex, is an important part of the process economics. No doubt, this would also require transformation of jarosite into other iron oxides.

Canfell et al. (1998) propose a process for the bio-leaching of chalcopyrite ores that comprises treating the ore with silver during acid conditioning to distribute the silver through the ore, and bioleaching the ore to oxidise ferrous to ferric ion.

4.5. Controlled Eh

The effect of redox potential on the leaching of chalcopyrite has been investigated extensively. Recently Hiroyoshi et al. (1997, 2000, 2001) reported that the dissolution rate of chalcopyrite is higher at redox potentials of leach solutions below a critical value. They have since proposed a practical method to control the redox potential during the leaching of low grade copper ore (Okamoto et al., 2003).

Earlier, Bruynesteyn et al. (1986) had taken advantage of the increased reaction rates afforded by a silver catalysed bioleach of chalcopyrite. They achieved further enhancement of the copper extraction by controlling the oxidation potential of the slurry between 0.54 and 0.66 V SHE with the addition of thiosulphate and soluble copper added as copper sulphate. The process was applied to the leaching of a chalcopyrite concentrate as a means of oxidising sulphide stoichiometrically to elemental sulphur at ambient temperature and pressure (Lawrence et al., 1985). While this particular process was not developed to commercial scale, redox control is an inherent part of some more modern processes (e.g. Dixon and Tshilombo, 2005), and can be achieved by prescribing the total iron concentration in bioleach solutions and/or controlling the ferric/ferrous ion ratio or potential. The two-stage bioprocesses such as IBES (Carranza et al., 1997) are particularly amenable to this kind of control. Redox-controlled bioleaching of chalcopyrite, either as concentrate in agitated tank reactors or as ore in heaps, is a key claim of recent patents (van der Merwe et al., 1998; Gericke and Pinches, 1999; Pinches et al., 2001).

4.6. Iron chemistry

The acidic dissolution of sulphide minerals requires an oxidant that, in heap leaching, is typically ferric ion Eq. (1). Once a sufficient iron concentration has built up in the leachate (Readett et al., 2003), the natural bacterial population regenerates the ferric ions reduced by reaction

with the sulphide, thus sustaining dissolution over long periods of time. At the same time, the acidic dissolution of gangue minerals will result in increased solution pH within the heap. Under the conditions of high pH and high ferric ion concentrations, iron hydroxy compounds, most commonly jarosites $[\text{MFe}_3(\text{SO}_4)_2(\text{OH})_6]$, will precipitate Eq. (5). The immediate consequence is to diminish the concentration of ferric ions available for further sulphide oxidation, but this is unlikely to be a limiting factor for a low-grade sulphide ore. A second consequence is that iron precipitation could contribute to the production of fines within the heap, possibly impacting heap permeability. Anecdotal evidence suggests that the contribution from iron precipitation is a minor contributor. However, iron precipitation preferentially on the target sulphide surfaces has the potential to impact significantly on leaching rates.

The results of a systematic study of the acidic oxidative dissolution of chalcopyrite surfaces indicated that, while a rapidly formed copper-deficient sulphide or disulphide played a key role in the leaching mechanism, subsequent reactions of a likely thiosulphate intermediate and ferric ion occurred in the immediate vicinity of the chalcopyrite surface, leading to the formation of a ferric sulphate phase (Parker et al., 2004). This sulphate is thought to be the precursor for the eventual mass deposition of jarosite on the sulphide surface. The precipitate forms a coherent layer that adheres strongly to the chalcopyrite surface and cannot be removed completely by bio-reduction (Fig. 5). It constitutes a physical barrier that prevents microbial access and slows the diffusion of ferric ions to the sulphide surface and of reaction products away from the sulphide surface (Boon and Heijnen, 1993; Stott et al., 2000).

Kohr et al. (1997), recognising the importance of iron control, proposed a method of precipitating some iron from bioleach solutions prior to recycle to the heap. Marsden et al. (2002) described a method of “seeded precipitation” for iron control. However, such reactions are slow and difficult to control.

4.7. Chloride effect

The leaching of chalcopyrite in chloride media has been reviewed and advanced by Dutrizac (1990, 1992). Early processes were focused on the leaching of concentrates under aggressive conditions, for example the CLEAR process and the INTEC process (Lunt et al., 1997). In general, leaching rates are more rapid in chloride media than in sulphate media. In their work on the dissolution of chalcopyrite under dump leaching conditions, Dutrizac and MacDonald (1971) investigated conditions under which modest salt addition (6 g/L)

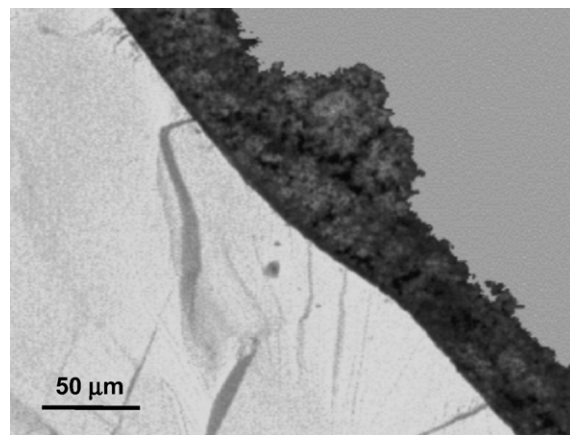


Fig. 5. Massive chalcopyrite cleaved after 24 h exposure to ferric sulphate solution shows a thick, coherent layer of jarosite closely adhered to the exposed surface (reprinted with permission from Stott et al., 2000).

would accelerate leaching in low-grade ores. The ores they tested gave variable responses to leaching, with or without added sodium chloride. They concluded that some ores did respond to chloride addition, but that a significant acceleration was only achieved at temperatures above 50 °C. At lower temperatures (25 °C) the presence of chloride might inhibit chalcopyrite dissolution. Kinnunen and Puhakka (2004) reported similar results; the presence of 0.25 g/L of Cl^- concentrate enhanced copper yield at temperatures between 67 and 90 °C but decreased copper yield at 50 °C.

Lu et al. (2000a,b) focused their electrochemical studies on the leaching of chalcopyrite in mixed sulphate–chloride media and found that the presence of chloride enhanced chalcopyrite oxidation. Examination of leached chalcopyrite surfaces showed that the sulphur formed in the presence of chloride was crystalline and porous, while that deposited in the absence of chloride formed an amorphous or cryptocrystalline film of sulphur (Fig. 6). Given that ground- or bore-water often contains significant concentrations of chloride, and that some copper ores contain atacamite, it is possible that the chloride effect could be exploited in a heap leach situation, if the microbial catalysts continued to grow well.

The impact of chloride on the growth of a number of bioleaching strains has been reported, for example, *A. ferrooxidans* (Alexander et al., 1987; Lawson et al., 1995), *L. ferriphilum* (Kinnunen and Puhakka, 2004), *S. metallicus* (Huber and Stetter, 1991), *S. rivotincti* (Gomez et al., 1999) and a mixed mesophilic culture (Shiers et al., 2005). In summary, the results indicated that the presence of chloride is detrimental to microbial growth, but that the

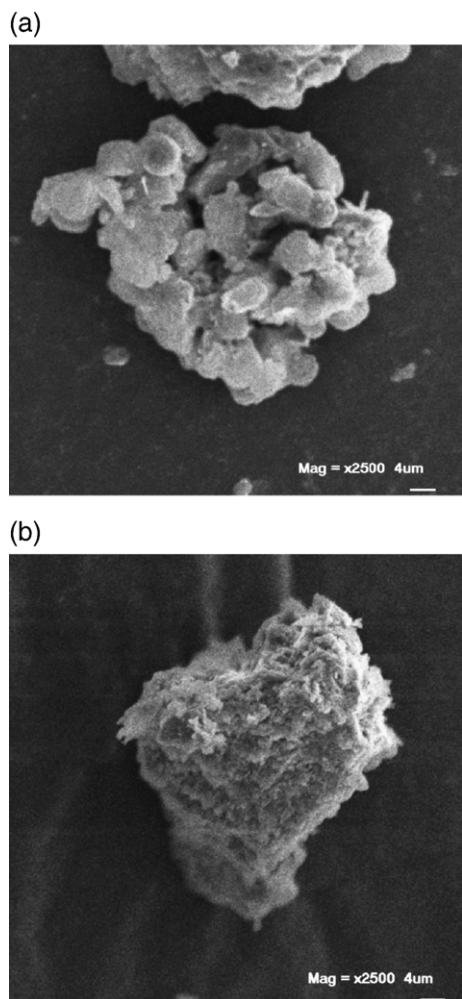


Fig. 6. SEM micrographs showing a residues particle leached (a) in the presence of NaCl and (b) in its absence. The concentrate in these experiments had a d_{50} of 15.1 μm (Lu et al., 2000b, reprinted with permission).

degree of inhibition varies between strains. In addition, Shiers et al. (2005) showed that concentrations of 7 g/L NaCl reduced cell replication by 50% and that no significant culture adaptation or habituation occurred with prolonged exposure to that concentration.

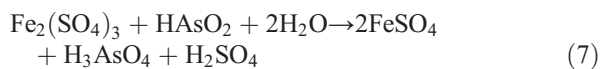
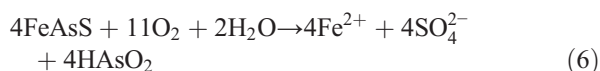
The question remains as to whether the benefits of chloride enhanced chalcopyrite dissolution outweigh the inhibition of the microbial population in a heap of low-grade sulphide ore. The downstream impact of a mixed sulphate–chloride solution on solvent extraction must also be considered.

5. Enargite bioleaching

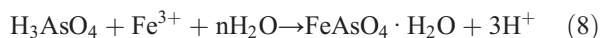
Enargite is a copper arsenic sulphide (Cu_3AsS_4) that occurs frequently with chalcocite. It does not easily

separate from the other sulphide minerals during flotation concentration, and thus the resulting high-arsenic concentrates cannot be roasted. Heap leaching of enargite-rich copper ores may be a process option.

Arsenic toxicity to bioleaching microorganisms is well documented, often in respect of the biooxidation of pyrite–arsenopyrite refractory gold concentrates (e.g., Breed et al., 1996). Barrett et al. (1989) reported that an arsenic-acclimated culture of moderate thermophiles grew vigorously at up to 15 g/L of arsenic in a biooxidation process but that if As(III) production Eq. (6) exceeded As(III) oxidation to As(V) by ferric ions Eq. (7), then microbial growth rates diminished. In addition they noted that the onset of biotoxicity could be sudden and was accompanied by an increase in pH of the medium. Arsenic in either oxidation state is toxic to bacteria, but As(III) is more toxic than As(V) (Silver et al., 1981). The mechanisms of toxicity are not the same, as evidenced by measurements of the maximum oxygen utilization rate, and of soluble arsenic concentrations and solution speciation; relative levels of toxicity may be influenced by the energy source (Breed et al., 1996).



In a biooxidation reactor, arsenic(III) oxidation by ferric ions occurs in the presence of excess ferric ions. Under such conditions, the precipitation of ferric arsenate is favoured Eq. (8), thus lowering soluble arsenic levels to below the bacterial toxicity threshold (Escobar et al., 1997a; Wiertz et al., 1997). The same condition of excess ferric ions and consequent “control” of soluble As(III) may also prevail in a heap leach system.



Enargite is known to be refractory, but data on its leaching/bioleaching are sparse (Dutrizac and MacDonald, 1972; Ehrlich, 1964; Hao et al., 1972; Wiertz et al., 1997; 1998; Escobar et al., 1997a,b). Only 24% copper recovery was achieved during the bioleaching of an enargite concentrate using mesophilic organisms. Some improvement was achieved when the air used to sparge the reactors was supplemented with carbon dioxide (Acevedo et al., 1997, 1998). Biooxidation tests in a continuous reactor showed that bacterial attack was directed towards the pyrite in an enargite rich concentrate (Canales et al., 2002). Neither enargite nor luzonite (its dimorph) were oxidized

during the bioleaching at 25 °C and 50 °C of a copper concentrate that also contained chalcocite, covellite and pyrite (Olson and Clark, 2001).

In contrast, Inoue et al. (2001) reported that silver catalysis of a mesophilic bioleach of a concentrate containing chalcopyrite, tennantite and enargite and pyrite resulted in preferential biooxidation of the copper minerals compared with pyrite. Pyrite was oxidized preferentially in the absence of silver, leading the authors to propose that silver-catalysed biooxidation could be the key to bioleaching enargite. Dew et al. (1999) noted that enargite dissolution required a high redox potential. They achieved this by pre-leaching the mixed sulphide concentrate with ferric sulphate to remove easily oxidised minerals such as chalcocite before inoculating with bacteria. Redox potentials remained high during the subsequent bioleach stage, promoting the dissolution of enargite, but not of chalcopyrite.

6. Future directions

The last two decades have witnessed exponential growth in the technological advancement and commercialisation of heap bioleaching of copper ores, particularly acid soluble and secondary sulphides such as chalcocite.

Heap (bio)leaching of copper ores is a stand alone technology that is

- robust and proven under different climatic conditions for oxides and secondary sulphides;
- flexible — heap engineering and management can accommodate site peculiarities in remote localities; suited to small deposits;
- simple — a technology that can be communicated to non-scientific personnel;
- low cost — stacking, irrigation, aeration, solution collection are all basic;

The technology has also benefited from concomitant improvements in SX-EW for the production of LME grade cathode copper from sulphate solutions and in the control of solvent extraction reagent losses.

However, heap leaching technology has yet to be demonstrated on a large scale for the bioleaching of chalcopyrite ore. In heap and dump leaching of run-of-mine chalcopyrite ores, relatively rapid leaching rates decrease as a function of time and overall copper recoveries seldom exceed 50%. Nevertheless, there are processing and economic reasons why the development of whole ore bioleaching is the preferred option for low-grade chalcopyrite ores.

6.1. Microbiology

Among the many groups researching biodiversity, a few are focused on the discovery and characterization of acidophiles across a range of temperatures. The knowledge gained by identifying and characterising more bioleaching strains can only enhance our understanding of bioleaching and acid mine drainage processes. The symbiotic relationships between mining microbial strains in mixed culture are not well understood and should be a continued focus for researchers.

In heaps, the reduction in microbial growth rates in high ionic strength process water might become the rate-controlling step for the bioleaching of copper sulphide. Acidophiles that oxidize iron and sulphur in high ionic strength solutions should be targeted. Further quantitative data on microbial growth rates obtained from systematic targeted experimental programs under varied but controlled conditions, relevant to heap leaching, would be valuable for understanding heap behaviour and for model development.

The variety of organisms that abound in acidic environments, many of which are associated with sulphide mineralization, necessitates the use of novel enrichment techniques and modern molecular methods of characterization that are beyond the scope of most minerals industry in-house research laboratories. It may be argued that the microbial capability to enhance sulphide dissolution can be assessed without knowing what organisms are present in a mixed culture and without determining their roles in bioleaching. However, the information that is arising from this research, and indeed from the related molecular genetics research, will undoubtedly benefit the improvement of mineral processing technologies in the longer term. Continued research on the biodiversity of sulphidic environments and on microbial characterization and growth kinetics should be supported.

The ability to quantify microbial population composition and strain-specific activities and to monitor changes in populations and their activity as a result of changes in different environments would confer two benefits. First, knowledge of the changes in populations that occur as a response to changes in the microbial environment would facilitate the design and operation of bioreactors that optimize the desired activity. For example, it may be beneficial to promote sulphur oxidation but reduce iron oxidation in a bioreactor to maximize the extraction of a selected metal. Secondly, by monitoring changes in the microbial mix that is found in leachates, for example from a heap operation, it should be possible to infer the prevailing conditions in the heap — having identified and quantified those leach parameters that

impact on microbial population dynamics. If they were not as required, measures could then be taken to restore the appropriate conditions.

There are a variety of microbiological and biochemical methods that can be used to identify and quantify microbial groups or strains in environmental samples, for example 16S rRNA gene sequencing, analysis of cell membrane phospholipids, fluorescent in situ hybridisation, and denaturing gradient gel electrophoresis. However, most were developed for use in “cleaner” systems and problems arise in their application to acidic, mineral leaching environments. Currently, no one method gives reliable results for all microorganisms of interest. The development of relatively rapid, robust methods for the identification and quantification of microbial strains, both attached and planktonic, in bioreactor samples would greatly assist research into microbial roles in mineral bioprocesses (e.g., Lehman et al., 2001; Johnson et al., 2005; Coram-Uliana et al., 2005).

Finally, with the need to operate heaps at above ambient temperatures to increase leaching chemistry rates, there is a need to improve methods of generating inocula to suit the selected leach conditions and to deliver them effectively to the heap. This is particularly important in the heap temperature range. Inocula could also be tailored to specific ores or minerals.

6.2. Leaching chemistry and mineralogy

Even at high temperatures, faster leaching rates would be beneficial to processing. Ferrous/ferric ion ratios in solution impact on dissolution rates and total soluble iron concentrations increase to a point where massive precipitation of iron oxides, hydroxides and basic sulphates can occur. Iron-rich reaction products report with the leach residues and must be disposed of in an environmentally responsible manner. Focused research on the iron chemistry of bioleaching as part of an optimization study should be well rewarded.

The mineralogy of ores and concentrates impacts directly on the efficiency of leaching and bioleaching, and on the nature of reaction products. Yet, to date, there has been no (published) systematic quantitative study of mineralogical effects in respect of leach chemistry or dissolution rates. With the development of automated scanning electron microscopic techniques and robust quantitative analyses of XRD data, the opportunity exists to quantify the effects of specific sulphide, oxide and gangue minerals on leaching efficiency under controlled but varied conditions. The results of such studies would be relevant to the development and optimization of both agitated tank and heap leach processes.

6.3. Modelling

The refractory character of chalcopyrite makes it certain that an efficient heap leach with the desired extraction rates and copper recovery will require greater management and control than has so far been necessary in oxide and chalcocite heap leaching. Improved models that are equipped to describe the impact of selected changed parameters on sulphide heap productivity will assist in understanding and optimizing these very complex bioreactors.

Key to models:	E Chile/Sweden copper dump
A Kennecott copper dump	F University of Chile
B ANSTO FIDHELM	G UBC HeapSim
C ANSTO SULFIDOX	H PD copper stockpile
D ANSTO Conceptual	I CSIRO heap

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References

- Acevedo, F., Gentina, J.C., Algegre, C., Arevalo, P., 1997. Biooxidation of a gold-bearing enargite concentrate. Proc. IBS '97 — Biomine '97 (Sydney). Australian Mineral Foundation, Glenside, pp. M3.2.1–M3.2.9.
- Acevedo, F., Gentina, J.C., Garcia, N., 1998. CO₂ supply in the biooxidation of an enargite–pyrite gold concentrate. *Biotechnology Letters* 20, 257–259.
- Alexander, B., Leach, S., Ingledew, W.J., 1987. The relationship between chemiosmotic parameters and sensitivity to anions and organic acids in the acidophile *Thiobacillus ferrooxidans*. *Journal of General Microbiology* 133, 1171–1179.
- Amaro, A.M., Seeger, M., Arredondo, R., Moreno, M., Jerez, C.A., 1993. The growth conditions affect *Thiobacillus ferrooxidans* attachment to solids. In: Torma, A.E., Apel, M.L., Brierley, C.L. (Eds.), *Biohydrometallurgical Technologies II*. TMS, Warrendale, pp. 577–585.
- Andrews, G.F., 1988. The selective adsorption of *Thiobacilli* to dislocation sites on pyrite surfaces. *Biotechnology and Bioengineering* 31, 378–381.
- ANON, 2001. Penoles bio-leach demonstration plant. Bateman press release.
- ANON, 2005. Bioleaching produces first cathodes. *Engineering News* 12 January, at www.engineeringnews.co.za/eng/features/environment/?show=13436.
- Arbiter, N., Fletcher, A.W., 1994. Copper hydrometallurgy — evolution and milestones. *Mining Engineering* 118–123 (February).

- Arredondo, R., Garcia, A., Jerez, C.A., 1994. Partial removal of lipopolysaccharide from *Thiobacillus ferrooxidans* affects its adhesion to solids. *Applied and Environmental Microbiology* 60, 2846–2851.
- Atkinson, T., Cairns, S., Cowan, D.A., Danson, M.J., Hough, D.W., Johnson, B., Norris, P.R., Raven, N., Robinson, C., Robson, R., Sharp, R.J., 2000. A microbiological survey of Montserrat Island hydrothermal biotopes. *Extremophiles* 4, 305–313.
- Bagdigian, R.M., Myerson, A.S., 1986. The adsorption of *Thiobacillus ferrooxidans* on coal surfaces. *Biotechnology and Bioengineering* 28, 467–479.
- Barreto, M., Quatrini, R., Bueno, S., Arriagada, C., Valdes, J., Silver, S., Jedlicki, E., Holmes, D.S., 2003. Aspects of the predicted physiology of *Acidithiobacillus ferrooxidans* deduced from an analysis of its partial genome sequence. *Hydrometallurgy* 71, 97–105.
- Barrett, J., Ewart, D.K., Hughes, M.N., Nobar, A.M., Poole, R.K., 1989. The oxidation of arsenic and arsenopyrite: the toxicity of As (III) to a moderately thermophilic mixed culture. In: Salley, J., McReady, R.G.L., Wichlaz, P.L. (Eds.), *Biohydrometallurgy-89* (Jackson Hole). Department of Supplies and Services, Canadian Government, Ottawa, pp. 49–57.
- Bartlett, R.W., 1998. *Solution Mining: Leaching and Fluid Recovery of Materials*. Second expanded and updated edition. Gordon and Breach Science Publishers, Australia.
- Bartos, P.J., 2002. SX-EW copper and the technology cycle. *Resources Policy* 28, 85–94.
- Batty, J. de K., Norton, A., 2003. Heat transfer in heap leaching of sulphide ores. U. S. Patent Application 20,030,167,879, September 11.
- Bennett, C.R., Cross, M., Croft, T.N., Uhrig, J.L., Green, C.R., Gebhardt, J.E., 2003a. A comprehensive copper stockpile leach model: background and model formulation. In: Young, C.A., Alfantazi, A.M., Anderson, C.G., Dreisinger, D.B., Harris, B., James, A. (Eds.), *Hydrometallurgy 2003. Volume 1: Leaching and Solution Purification*. TMS, Warrendale, pp. 315–328.
- Bennett, C.R., Cross, M., Uhrig, J.L., Green, C.R., Gebhardt, J.E., 2003b. A comprehensive copper stockpile leach model: background and parameter sensitivity. In: Riveros, P.A., Dixon, D., Dreisinger, D.B., Menacho, J. (Eds.), *Copper-Cobre 2003 Volume VI — Hydrometallurgy of Copper (Book 2) Modeling, Impurity Control and Solvent Extraction*. Canadian Institute of Mining, Metallurgy and Petroleum, Montreal, pp. 563–579.
- Bennett, C.R., McBride, D., Cross, M., Gebhardt, J.E., Taylor, D.A., 2006. Simulation technology to support base metal ore heap leaching. *Mineral Processing and Extractive Metallurgy* 115, 41–48.
- Berry, V.K., Murr, L.E., 1977. An SEM study of bacterial catalysis and its effect on surface reactions at sulfide phases in the leaching of low-grade copper ore. *Scanning Electron Microscopy* 10, 137–146.
- Boon, M., Heijnen, J.J., 1993. Mechanisms and rate limiting steps in bioleaching of sphalerite, chalcopyrite and pyrite with *Thiobacillus ferrooxidans*. In: Torma, A.E., Wey, J.E., Lakshmanan, V.L. (Eds.), *Biohydrometallurgical Technologies*. TMS, Warrendale, pp. 217–235.
- Bouffard, S.C., Dixon, D.G., 2001. Investigative study into the hydrodynamics of heap leaching processes. *Metallurgical and Materials Transactions B* 32, 763–776.
- Breed, A.W., Glatz, A., Hansford, G.S., Harrison, S.T.L., 1996. The effect of As(III) and As(V) on the batch bioleaching of a pyrite–arsenopyrite concentrate. *Minerals Engineering* 9, 1235–1252.
- Brierley, J.A., 1997. Heap leaching of gold-bearing deposits: theory and operational description. In: Rawlings, D.E. (Ed.), *Biomining: Theory, Microbes and Industrial Processes*. Springer, Berlin, pp. 103–115.
- Brierley, J.A. and Hill, D.L., 1993. Biooxidation process for recovery of gold from heaps of low-grade sulfidic and carbonaceous sulfidic ore materials. U. S. Patent 5,246,486. September 21.
- Bruynesteyn, A., Hackl, R.P., Lawrence, R.W. and Vizsolyi, A.I. 1986, *Biological-acid leach process*. U. S. Patent No. 4,571, 387, February 18.
- Burton, N.P., Norris, P.R., 2000. Microbiology of acidic, geothermal springs of Montserrat: environmental rDNA analysis. *Extremophiles* 4, 315–320.
- Canales, C., Acevedo, F., Gentina, J.C., 2002. Laboratory-scale continuous bio-oxidation of a gold concentrate of high pyrite and enargite content. *Process Biochemistry* 37, 1051–1055.
- Canfield, A.J., Winborne, A., Greenfield, F., Winborne, D.A. and Greenfield, P.F., 1998. Process for bioleaching chalcopyrite ore comprises treating ore with silver and bioleaching ore body to oxidize ferrous ion. Australian Patent AU 9878560-A; AU 749366-B.
- Carranza, F., Palencia, I., Romero, R., 1997. Silver catalyzed IBES process: application to a Spanish copper–zinc sulphide concentrate. *Hydrometallurgy* 44, 29–42.
- Carranza, F., Iglesias, N., Mazuelos, A., Palencia, I., Romero, R., 2004. Treatment of copper concentrates containing chalcopyrite and non-ferrous sulphides by the BRISA process. *Hydrometallurgy* 71, 413–420.
- Casas, J.M., Martinez, J., Moreno, L., Vargas, T., 1998. Bioleaching model of a copper-sulfide ore bed in heap and dump configurations. *Metallurgical and Materials Transactions B* 29, 899–909.
- Chandraprabha, M.N., Natarajan, K.A., Modak, J.M., 2004a. Selective separation of pyrite and chalcopyrite by biomodulation. *Colloids and Surfaces B: Biointerfaces* 37, 93–100.
- Chandraprabha, M.N., Natarajan, K.A., Somasundaran, P., 2004b. Selective separation of arsenopyrite from pyrite by biomodulation in the presence of *Acidithiobacillus ferrooxidans*. *Journal of Colloid and Interface Science* 276, 323–332.
- Chen, L.M., Brugger, K., Skovgaard, M., Redder, P., She, Q.X., Torarinsson, E., Greve, B., Awayez, M., Zibat, A., Klenk, H.P., Garrett, R.A., 2005. The genome of *Sulfolobus acidocaldarius*, a model organism of the Crenarchaeota. *Journal of Bacteriology* 187, 4992–4999.
- Ciaramella, M., Pisani, F.M., Rossi, M., 2002. Molecular biology of extremophiles: recent progress on the hyperthermophilic archaeon *Sulfolobus*. *Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology* 81, 85–97.
- Clark, M.E., Batty, J., van Buuren, C., Dew, D., Eamonn, M., 2005. *Biotechnology in Minerals Processing: Technological Breakthroughs Creating Value*. In: Harrison, S.T.L., Rawlings, D.E., Petersen, J. (Eds.), *Proc. 16th Intl. Biohydrometallurgy Symposium (Cape Town)*. IBS, Cape Town, pp. xvii–xxiv.
- Colmer, A.R., Temple, K.L., Hinkle, M.E., 1950. An iron-oxidizing bacterium from the acid mine drainage of some bituminous coal mines. *Journal of Bacteriology* 59, 317–328.
- Concha, A., Oyarzun, R., Lunar, R., Sierra, J., 1991. Over a century of bioleaching copper sulphides at Andacollo. *Mining Magazine* 165, 325–328.
- Coram, N.J., Rawlings, D.E., 2002. Molecular relationship between two groups of the genus *Leptospirillum* and the finding that *Leptospirillum ferriphilum* sp., nov., dominates South African commercial biooxidation tanks that operate at 40 °C. *Applied and Environmental Microbiology* 68, 838–845.
- Coram, N.J., van Zyl, L.J., Rawlings, D.E., 2005. Occurrence of plasmids in the *Leptospirilli* and a comparison of two plasmids from the biomining bacterium *Leptospirillum ferrooxidans* ATCC49879. In: Harrison, S.T.L., Rawlings, D.E., Petersen, J.

- (Eds.), Proc. 16th Intl. Biohydrometallurgy Symposium (Cape Town). IBS, Cape Town, pp. 797–804.
- Coram-Uliana, N.J., van Hille, R.P., Kohr, W.J., Harrison, S.T.L., 2005. Development of a method to assay the microbial populations in heap bioleaching operations. In: Harrison, S.T.L., Rawlings, D.E., Petersen, J. (Eds.), Proc. 16th Intl. Biohydrometallurgy Symposium (Cape Town). IBS, Cape Town, pp. 647–656.
- Crundwell, F.K., 1997. Physical chemistry of bacterial leaching. In: Rawlings, D.E. (Ed.), *Biomining: Theory, Microbes and Industrial Processes*. Springer Verlag, Berlin, pp. 177–200.
- Crundwell, F.K., 1999a. Mechanism of pyrite dissolution in the presence of *Thiobacillus ferrooxidans*. *Applied and Environmental Microbiology* 65, 2987–2993.
- Crundwell, F.K., 1999b. Leaching of zinc sulfide by *Thiobacillus ferrooxidans*: bacterial oxidation of the sulfur product layer increases the rate of zinc sulfide dissolution at high concentrations of ferrous ions. *Applied and Environmental Microbiology* 65, 5285–5292.
- Crundwell, F.K., 2003. How do bacteria interact with minerals? *Hydrometallurgy* 71, 75–81.
- Das, A., Modak, J.M., Natarajan, K.A., 1997. Studies on multi-metal ion tolerance of *Thiobacillus ferrooxidans*. *Minerals Engineering* 10, 743–749.
- Das, A., Rao, K.H., Sharma, P., Natarajan, K.A., Forssberg, K.S.E., 1999a. Surface chemical and adsorption studies using *Thiobacillus ferrooxidans* with reference to bacterial adhesion to sulfide minerals. In: Amils, R., Ballester, A. (Eds.), Proc. Intl. Biohydrometallurgy Symposium (Madrid, Spain) Part A. Elsevier, Amsterdam, pp. 697–707.
- Das, T., Ayyappan, S., Chaudhury, G.R., 1999b. Factors affecting bioleaching kinetics of sulfide ores using acidophilic microorganisms. *BioMetals* 12, 1–10.
- de Groot, P., Deane, S.M., Rawlings, D.E., 2003. A transposon-located arsenic resistance mechanism from a strain of *Acidithiobacillus caldus* isolated from commercial arsenopyrite biooxidation tanks. *Hydrometallurgy* 71, 115–123.
- Demergasso, C.S., Galleguillos, P.A., Escudero G., L.V., Zepeda A., V.J., Castillo, D., Casamayor, E.O., 2005. Molecular characterization of microbial populations in a low-grade copper ore bioleaching test heap. *Hydrometallurgy* 80, 241–253.
- Devasia, P., Natarajan, K.A., Sathyanaraya, D.N., Ramananda Rao, G., 1993. Surface chemistry of *Thiobacillus ferrooxidans* relevant to adhesion on mineral surfaces. *Applied and Environmental Microbiology* 59, 4051–4055.
- Devasia, P., Natarajan, K.A., Rao, G.R., 1996. Role of bacterial growth conditions and adhesion in the bioleaching of chalcopyrite by *Thiobacillus ferrooxidans*. *Minerals and Metallurgical Processing* 13 (2), 82–86.
- Deveci, H., 2002. Effect of solids on viability of acidophilic bacteria. *Minerals Engineering* 15, 1181–1189.
- Deveci, H., 2004. Effect of particle size and shape of solids on the viability of acidophilic bacteria during mixing in stirred tank reactors. *Hydrometallurgy* 71, 385–396.
- Dew, D.W., van Buuren, C., McEwan, K., Bowker, C., 1999. Bioleaching of base metal sulphide concentrates: a comparison of mesophilic and thermophilic bacterial cultures. In: Amils, R., Ballester, A. (Eds.), Proc. Intl. Biohydrometallurgy Symposium (Madrid, Spain) Part A. Elsevier, Amsterdam, pp. 229–238.
- DiSpirito, A.A., Dugan, P.R., Tuovinen, O.H., 1983. Sorption of *Thiobacillus ferrooxidans* to particulate matter. *Biotechnology and Bioengineering* 25, 1163–1168.
- Dixon, D.G., 2003. Heap leach modelling — the current state of the art. In: Young, C.A., Alfantazi, A.M., Anderson, C.G., Dreisinger, D.B., Harris, B., James, A. (Eds.), *Hydrometallurgy 2003: Volume 1: Leaching and Solution Purification*. TMS, Warrendale, pp. 289–314.
- Dixon, D.G., Petersen, J., 2003. Comprehensive modelling study of chalcocite column and heap bioleaching. In: Riveros, P.A., Dixon, D., Dreisinger, D.B., Menacho, J. (Eds.), *Copper-Cobre 2003 (Santiago) Volume VI — Hydrometallurgy of Copper (Book 2) Modeling, Impurity Control and Solvent Extraction*. Canadian Institute of Mining, Metallurgy and Petroleum, Montreal, pp. 493–516.
- Dixon, D.G., Petersen, J., 2004. Modelling the dynamics of heap bioleaching for process improvement and innovation. *Hydro-Sulfides 2004: Intl. Colloquium on Hydrometallurgical Processing of Copper Sulfides (Santiago)*. University of Chile, Santiago, pp. 13–45.
- Dixon, D.G. and Tshilombo, A.F., 2005. Leaching process for copper concentrates. U.S. Patent Application 2005/0269208 A1, 8 December: WO20051188994-A1.
- Dopson, M., Baker-Austin, C., Koppineedi, P.R., Bond, P.L., 2003. Growth in sulfidic mineral environments: metal resistance mechanisms in acidophilic micro-organisms. *Microbiology* 149, 1959–1970.
- Dopson, M., Baker-Austin, C., Hind, A., Bowman, J.P., Bond, P.L., 2004. Characterization of ferroplasma isolates and *Ferroplasma acidarmanus* sp. nov., extreme acidophiles from acid mine drainage and industrial bioleaching environments. *Applied and Environmental Microbiology* 70, 2079–2088.
- du Plessis, C., 2003. Delivery system for heap bioleaching. World Patent WO 2,003,068,999 21 August.
- du Plessis, C.A., de Kock, S.H., 2005. Heap bioleaching process. World Patent WO 2,005,073,414. 11 August.
- Dutrizac, J.E., 1990. Elemental sulphur formation during the ferric chloride leaching of chalcopyrite. *Hydrometallurgy* 23, 153–176.
- Dutrizac, J.E., 1992. The dissolution of chalcopyrite in ferric sulfate and ferric chloride media. *Metallurgical Transactions. B, Process Metallurgy* 12B, 371–378.
- Dutrizac, J.E., MacDonald, R.J.C., 1971. The effect of sodium chloride on the dissolution of chalcopyrite under simulated dump leaching conditions. *Metallurgical Transactions* 2, 2310–2312.
- Dutrizac, J.E., MacDonald, R.J.C., 1972. The kinetics of dissolution of enargite in acidified ferric sulphate solutions. *Canadian Metallurgical Quarterly* 11, 469–476.
- Edwards, K.J., Rutenberg, A.D., 2001. Microbial response to surface microtopography: the role of metabolism in localized mineral dissolution. *Chemical Geology* 180, 19–32.
- Edwards, K.J., Goebel, B.M., Rodgers, T.M., Schrenk, M.O., Gihring, T.M., Cardona, M.M., Hu, B., McGuire, M.M., Hamers, R.J., Pace, N.R., Banfield, J.F., 1999. Geomicrobiology of pyrite (FeS₂) dissolution: case study at Iron Mountain, California. *Geomicrobiology Journal* 16, 155–179.
- Ehrlich, H.L., 1964. Bacterial oxidation of arsenopyrite and enargite. *Economic Geology* 59, 1306–1312.
- Escobar, B., Wiertz, J., de los Angeles Ruiz, M., 1997a. The effect of silver in the leaching and bioleaching of enargite. Proc. IBS '97 — Biomine '97. Australian Mineral Foundation, Glenside, pp. PM18.1–PM18.2.
- Escobar, B., Huenupi, E., Wiertz, J.V., 1997b. Chemical and biological leaching of enargite. *Biotechnology Letters* 19, 719–722.
- Escobar, B., Hevia, M.J., Vargas, T., 2004. Evaluating the growth of free and attached cells during the bioleaching of chalcopyrite with *Sulfolobus metallicus*. In: Tzesos, M., Hatzikioseyan, A., Remoundaki, E. (Eds.), *Biohydrometallurgy: A Sustainable Technology in*

- Evolution (Athens, 2003). National Technical University of Athens, Athens, pp. 1091–1097.
- Fowler, T.A., Crundwell, F.K., 1998. The leaching of sphalerite by *Thiobacillus ferrooxidans*: experiments at controlled redox potentials indicate that there is no direct mechanism of bacterial action. *Applied and Environmental Microbiology* 64, 3570–3575.
- Fowler, T.A., Crundwell, F.K., 1999. Leaching of zinc sulfide by *Thiobacillus ferrooxidans*: bacterial oxidation of the sulfur product layer increases the rate of zinc sulfide dissolution at high concentrations of ferrous ions. *Applied and Environmental Microbiology* 65, 5285–5292.
- Fowler, T.A., Holmes, P.R., Crundwell, F.K., 1999. The mechanism of bacterial leaching of pyrite by *Thiobacillus ferrooxidans*. *Applied and Environmental Microbiology* 65, 2987–2993.
- Fowler, T.A., Holmes, P.R., Crundwell, F.K., 2001. On the kinetics and mechanism of the dissolution of pyrite in the presence of *Thiobacillus ferrooxidans*. *Hydrometallurgy* 59, 257–270.
- Franzmann, P.D., Haddad, C.M., Hawkes, R.B., Robertson, W.J., Plumb, J.J., 2005. Effects of temperature on the rates of iron and sulfur oxidation by selected bioleaching Bacteria and Archaea: application of the Ratkowsky equation. *Minerals Engineering* 18, 1304–1314.
- Futterer, O., Angelov, A., Liesegang, H., Gottschalk, G., Schleper, C., Schepers, B., Dock, C., Antranikian, G., Liebl, W., 2004. Genome sequence of *Picrophilus torridus* and its implications for life around pH 0. *Proceedings of the National Academy of Sciences of the United States of America* 101, 9091–9096.
- Gehrke, T., Hallman, R., Kinzler, K., Sand, W., 2001. The EPS of *Acidithiobacillus ferrooxidans* — a model for structure–function relationships of attached bacteria and their physiology. *Water Science and Technology* 43, 159–167.
- Gericke, M., Pinches, A., 1999. Bioleaching of copper sulphide concentrate using extreme thermophilic bacteria. *Minerals Engineering* 12, 893–904.
- Gericke, M., Pinches, A., van Rooyen, J.V., 2001. Bioleaching of a chalcopryrite concentrate using an extremely thermophilic culture. *International Journal of Mineral Processing* 62, 243–255.
- Gericke, M., Muller, H.H., Neale, J.W., Norton, A.E., Crundwell, F.K., 2005. Inoculation of heap leaching operations. In: Harrison, S.T.L., Rawlings, D.E., Petersen, J. (Eds.), *Proc. Intl. Biohydrometallurgy Symposium* (Cape Town). IBS, Cape Town, pp. 255–264.
- Gomez, E., Ballester, A., Gonzalez, F., Blazquez, M.L., 1999. Leaching capacity of a new, extremely thermophilic microorganisms, *Sulfolobus rivotincti*. *Hydrometallurgy* 52, 349–366.
- Gonzalez, R., Gentina, J.C., Acevedo, F., 1999. Attachment behaviour of *Thiobacillus ferrooxidans* cells to refractory gold concentrate particles. *Biotechnology Letters* 21, 715–718.
- Gonzalez-Toril, E., Llobet-Brossa, E., Casamayor, E.O., Amann, R., Amils, R., 2003. Microbial ecology of an extreme acidic environment, the Tinto River. *Applied and Environmental Microbiology* 69, 4853–4865.
- Gottlieb, P., Wilkie, G., Sutherland, D., Ho-Tun, E., Suthers, S., Perera, K., Jenkins, B., Spencer, S., Butcher, A., Rayner, J., 2000. Using quantitative electron microscopy for process mineralogy applications. *Journal of Minerals, Metals and Materials Society* 52 (4), 24–25.
- Gu, Y., 2003. Automated scanning electron microscope based mineral liberation analysis. *Journal of Minerals and Materials Characterization & Engineering* 2, 33–41.
- Hallberg, K.B., Johnson, D.B., 2001. Biodiversity of acidophilic prokaryotes. *Advances in Applied Microbiology* 49, 37–84.
- Hallberg, K.B., Johnson, D.B., 2003. Novel acidophiles isolated from moderately acidic mine drainage waters. *Hydrometallurgy* 71, 139–148.
- Hansford, G.S., 1997. Recent developments in modeling the kinetics of bioleaching. In: Rawlings, D.E. (Ed.), *Biomining: Theory, Microbes and Industrial Processes*. Springer Verlag, Berlin, pp. 153–175.
- Hansford, G.S., Vargas, T., 2001. Chemical and electrochemical basis of bioleaching processes. *Hydrometallurgy* 59, 135–145.
- Hao, P.L.C., Chang, W.S., Wang, N.N., 1972. Microbioleaching of copper from a low-grade enargite. In: Terui, G. (Ed.), *Proc. IV IFS: Fermentation Technology Today* (Kyoto, Japan). Osaka Society of Fermentation, Osaka, pp. 509–512.
- Helle, S., Kelm, U., 2005. Experimental leaching of atacamite, chrysocolla and malachite: relationship between copper retention and cation exchange capacity. *Hydrometallurgy* 78, 180–186.
- Helle, S., Kelm, U., Barrientos, A., Rivas, P., Reghezza, A., 2005. Improvement of mineralogical and chemical characterization to predict the acid leaching of geometallurgical units from Mina Sur, Chuquicamata, Chile. *Minerals Engineering* 18, 1334–1336.
- Hiroyoshi, N., Hirota, M., Hirajima, T., Tsunekawa, M., 1997. A case of ferrous sulfate addition enhancing chalcopryrite leaching. *Hydrometallurgy* 47, 37–45.
- Hiroyoshi, N., Miki, H., Hirajima, T., Tsunekawa, M., 2000. A model for ferrous-promoted chalcopryrite leaching. *Hydrometallurgy* 57, 31–38.
- Hiroyoshi, N., Miki, H., Hirajima, T., Tsunekawa, M., 2001. Enhancement of chalcopryrite leaching by ferrous ions in acidic ferric sulfate solutions. *Hydrometallurgy* 60, 185–197.
- Holden, P.J., Foster, L.J., Neilan, B.A., Vu, Q.M., 2001. Characterisation of novel salt tolerant iron-oxidising bacteria. In: Ciminelli, V. S.T., Garcia, O. (Eds.), *Biohydrometallurgy: Fundamentals, Technology and Sustainable Development Part A*. Elsevier, Amsterdam, pp. 283–290.
- Holmes, P.R., Crundwell, F.K., 2000. The kinetics of the oxidation of pyrite by ferric ions and dissolved oxygen: an electrochemical study. *Geochimica et Cosmochimica Acta* 64, 263–274.
- Holmes, D.S., Debus, K.A., 1991. Biological opportunities for metal recovery. In: Malik, K.A., Naqvi, S.H.M., Aleem, M.I.H. (Eds.), *Proc. Intl. Symposium on Biotechnology for Energy* (Faisalabad, Pakistan, 1989). NIAB/NIBGE, Faisalabad, pp. 341–358.
- Holmes, P.R., Fowler, T.A., Crundwell, F.K., 1999. The mechanism of the bacterial leaching of pyrite: an electrochemical study. *Journal of the Electrochemical Society* 146, 2906–2912.
- Holmes, D.S., Barreto, M., Valdes, J., Dominguez, C., Arriagada, C., Silver, S., Bueno, S., Jedlicki, E., 2001. Whole genome sequence of *Acidithiobacillus ferrooxidans*: metabolic reconstruction, heavy metal resistance and other characteristics. In: Ciminelli, V.S.T., Garcia, O. (Eds.), *Biohydrometallurgy: Fundamentals, Technology and Sustainable Development Part A*. Elsevier, Amsterdam, pp. 237–251.
- Hu, Y., Qiu, G., Wang, J., Wang, D., 2002. The effect of silver-bearing catalysts on bioleaching of chalcopryrite. *Hydrometallurgy* 64, 81–88.
- Huber, G., Stetter, K.O., 1991. *Sulfolobus metallicus*, sp. nov., a novel strictly chemolithoautotrophic thermophilic archaeal species of metal-mobilizers. *Systematic and Applied Microbiology* 14, 349–366.
- Hunter, C.J., 2001. A bacterially assisted heap leach, World Patent WO 0144,519, 21 June.
- Hunter, C., 2002a. Bioheap leaching of a primary nickel–copper sulphide ore. ALTA Nickel/Cobalt 2002 (Perth, WA). ALTA, Melbourne. 11 pp.

- Hunter, C.J., 2002b. A method for the bacterially assisted heap leaching of chalcopyrite, European Patent EP 1,366,202, 3 December.
- Hunter C.J., 2002c. A method for the bacterially assisted heap leaching of chalcopyrite. World Patent 2002 70757 (A1) (US 2004 091984).
- Hunter C.J., Williams, T.L., 2002. Adaptation of bacteria for leaching, World Patent WO 02,066,689, 29 August.
- Inoue, C., Suto, K., Terashima, M., Takeshima, T., Chida, T., 2001. Bioleaching of an arsenic bearing copper concentrate. In: Ciminelli, V.S.T., Garcia, O. (Eds.), *Biohydrometallurgy: Fundamentals, Technology and Sustainable Development*, Part A. Elsevier, Amsterdam, pp. 573–579.
- Johnson, D.B., Bacelar-Nicolau, P., Okibe, N., Yahya, A., Hallberg, K.B., 2001. Role of pure and mixed cultures of Gram-positive eubacteria in mineral leaching. In: Ciminelli, V.S.T., Garcia, O. (Eds.), *Proc. Intl. Biohydrometallurgy Symposium (Ouro Preto)* Part A. Elsevier, Amsterdam, pp. 461–470.
- Johnson, D.B., Okibe, N., Hallberg, K.B., 2005. Differentiation and identification of iron-oxidizing acidophilic bacteria using cultivation techniques and amplified ribosomal DNA restriction enzyme analysis (ARDREA). *Journal of Microbiological Methods* 60, 299–313.
- Keeling, S.E., Davies, K.L., Johnson, J.A., Watling, H.R., 2004. Isolation and phenotypic characterization of Australian mining microbes from a spent heap. *Proc. BacMin Conference (Bendigo)*. AusIMM, Melbourne, pp. 35–39.
- Kelm, U., Helle, S., 2005. Acid leaching of malachite in synthetic mixtures of clay and zeolite-rich gangue. An experimental approach to improve the understanding of problems in heap leaching operations. *Applied Clay Science* 29, 187–198.
- King, J.A., 2001. Method for initiating heap bioleaching of sulfidic ores. U.S. Patent 6,207,443, March 27.
- Kinnunen, P.H.-M., Puhakka, J.A., 2004. Chloride-promoted leaching of chalcopyrite concentrate by biologically-produced ferric ion. *Journal of Chemical Technology* 79, 830–834.
- Kinnunen, P.H.-M., Robertson, W.J., Plumb, J.J., Gibson, J.A.E., Nichols, P.D., Franzmann, P.D., Puhakka, J.A., 2003. The isolation and use of iron-oxidizing, moderately thermophilic acidophiles from the Collie coal mine for the generation of ferric iron leaching solution. *Applied Microbiology and Biotechnology* 60, 748–753.
- Kinzler, K., Gehrke, T., Telegdi, J., Sand, W., 2003. Bioleaching — a result of interfacial processes caused by extracellular polymeric substances (EPS). *Hydrometallurgy* 71, 83–88.
- Klauber, C., 2003. Fracture induced reconstruction of a chalcopyrite CuFeS_2 surface. *Surface and Interface Analysis* 35, 415–428.
- Klauber, C., Parker, A., van Bronswijk, W., Watling, H., 2001. Sulphur speciation of leached chalcopyrite surfaces as determined by X-ray photoelectron spectroscopy. *International Journal of Mineral Processing* 62, 65–94.
- Kohr, W.J., Johansson, C., Shield, J. and Sharder, V., 1997. Method for improving the heap biooxidation rate of refractory sulfide ore particles that are biooxidized using recycled bioleachate solution. U. S. Patent 5,688,304. November 18.
- Kohr, W.J., Shrader, V. and Johansson, C., 2000. High temperature heap bioleaching process. U. S. Patent 6,110,253, August 29.
- Kohr, W.J., Shrader, V. and Johansson, C., 2002. High temperature heap bioleaching process. U. S. Patent Application 20,020,194,962. December 26.
- Lancaster, T., Walsh, D., 1997. The development of the aeration of copper sulphide ore at Girilambone. *Proc. IBS—Biomine'97*. AMF, Glenside South Australia, pp. M5.4.1–M5.4.10.
- Lane, R.P., 2000. Method and apparatus for solar heating and distributing a mining leach solution. U. S. Patent 6,149,711, November 21.
- Lawrence, R.W., Vizsolyi, A., Vos, R.J., 1985. The silver catalyzed bioleach process for copper concentrate. In: Clum, J.A., Haas, L.A. (Eds.), *Microbiological Effects on Metallurgical Processes* (New York). TMS, Warrendale, pp. 65–82.
- Lawson, E.N., Nicholas, C.J., Pellat, H., 1995. The toxic effects of chloride ions on *Thiobacillus ferrooxidans*. In: Vargats, T., Jerez, C.A., Wiertz, J.V., Toledo, H. (Eds.), *Biohydrometallurgy Processing*. University of Chile, Santiago, pp. 165–174.
- Lazaro, I., Nicol, M.J., 2003. The mechanism of the dissolution and passivation of chalcopyrite: an electrochemical study. In: Young, C.A., Alfantazi, A.M., Anderson, C.G., Dreisinger, D.B., Harris, B., James, A. (Eds.), *Hydrometallurgy 2003*. TMS, Warrendale, pp. 405–417.
- Leahy, M.J., Davidson, M.R., Schwarz, M.P., 2005. A model for heap bioleaching of chalcocite with heat balance: bacterial temperature dependence. *Minerals Engineering* 18, 1239–1252.
- Leahy, M.J., Davidson, M.R., Schwarz, M.P., submitted for publication. A model for heap bioleaching of chalcocite with heat balance: mesophiles and moderate thermophiles. *Hydrometallurgy*.
- Leduc, L.G., Ferroni, G.D., 1994. The chemolithotrophic bacterium *Thiobacillus ferrooxidans*. *FEMS Microbiology Reviews* 14, 103–120.
- Lefebvre, R., Hockley, D., Smolensky, J., Gelinas, P., 2001a. Multiphase transfer processes in waste rock piles producing acid mine drainage. 1. Conceptual model and system characterization. *Journal of Contaminant Hydrology* 52, 137–164.
- Lefebvre, R., Hockley, D., Smolensky, J., Lamontagne, A., 2001b. Multiphase transfer processes in waste rock piles producing acid mine drainage. 2. Applications of numerical solution. *Journal of Contaminant Hydrology* 52, 165–186.
- Lehman, R.M., Colwell, F.S., Bala, G.A., 2001. Attached and unattached microbial communities in a simulated basalt aquifer under fracture- and porous-flow conditions. *Applied and Environmental Microbiology* 67, 2799–2809.
- Lizama, H.M., Fairweather, M.J., Dai, Z., Allegretto, T.D., 2003. How does bioleaching start? *Hydrometallurgy* 69, 109–116.
- Lu, Z.Y., Jeffrey, M.I., Lawson, F., 2000a. An electrochemical study of the effect of chloride ions on the dissolution of chalcopyrite in acidic solutions. *Hydrometallurgy* 56, 145–155.
- Lu, Z.Y., Jeffrey, M.I., Lawson, F., 2000b. The effect of chloride ions on the dissolution of chalcopyrite in acidic solutions. *Hydrometallurgy* 56, 189–202.
- Lunt, D., Poulter, S. and Canterford, J., 1997. *Hydrometallurgical treatment of copper concentrates: What are the real process options?* Unpublished paper presented at ALTA Copper 1997: (Brisbane, Qld.), ALTA Metallurgical Services, Melbourne, 24 pp.
- Marsden, J.O., Brewer, R.E., Robertson, J.M., Baughman, D.R., Thompson, P., Hazen, W., Schmidt, R., 2002. Method for improving metals recovery using high temperature leaching. U.S. Patent 6,451,088, 17 September.
- Monroy, M.G., Dziurla, M.A., Lam, B.-T., Bethelin, J., Marion, P.A., 1994. Laboratory study on the behaviour of *Thiobacillus ferrooxidans* during pyrite bioleaching in percolation columns. In: Galindo, E., Ramirez, O.T. (Eds.), *Advances in Bioprocess Engineering*. Kluwer, Dordrecht, pp. 509–517.
- Montealegre, R., Bustos, S., Rauld, J., Ruiz, P., Arriagada, F., Rojas, J., Jo, M., Neuberger, H., Yanez, H., Araya, C., Espejo, R., D'Amico, J., Reyes, R., 1995. Copper sulphide hydrometallurgy and the thin layer bacterial leaching technology of Sociedad Minera Pudahuel. In: Cooper, W.C., Dreisinger, D.B., Dutrizac, J.E., Hein, H., Ugarte, G. (Eds.), *Copper '95–Cobre '95: Proc. Intl. Conference (Santiago, Chile) Volume III — Electrorefining and Hydrometallurgy of Copper*. TMS, Warrendale, pp. 781–793.

- Murr, L.E., 1980. Theory and practice of copper sulphide leaching in dumps and in-situ. *Minerals Science and Engineering* 12, 121–189.
- Murr, L.E., Berry, V.K., 1976. Direct observations of selective attachment of bacteria on low grade sulfide ores and other mineral surfaces. *Hydrometallurgy* 2, 11–24.
- Natarajan, K.A., Das, A., 2003. Surface chemical studies on '*Acidithiobacillus*' group of bacteria with reference to mineral flocculation. *International Journal of Mineral Processing* 72, 189–198.
- Ndlovu, S., Monhemius, A.J., 2005. The influence of crystal orientation on the bacterial dissolution of pyrite. *Hydrometallurgy* 78, 187–197.
- Nicol, M.J., Lazaro, I., 2003. The role of non-oxidative processes in the leaching of chalcopyrite. In: Riveros, P.A., Dixon, D., Dreisinger, D.B., Menacho, J. (Eds.), *Copper 2003 — Cobre 2003* (Santiago) Volume VI (Book 1). Canadian Institute of Mining, Metallurgy and Petroleum, Montreal, pp. 367–381.
- Norris, P.R., 1997. Thermophiles and bioleaching. In: Rawlings, D.E. (Ed.), *Biomining: Theory, Microbes and Industrial Processes*. Springer Verlag, Berlin, pp. 247–258.
- Norris, P.R., Barr, D.W., Hinson, D., 1988. Iron and mineral oxidation by acidophilic bacteria: affinities for iron and attachment to pyrite. In: Norris, P.R., Kelly, D.P. (Eds.), *Biohydrometallurgy: Proc. Intl. Symposium* (Warwick, UK, 1987). Science and Technology Letters, Kew, pp. 43–59.
- Norton, A.E., Crundwell, F.K., 2004. The HotHeap™ process for the heap leaching of chalcopyrite ores. SAIMM Colloquium: Innovations in Leaching Technologies (Saxonwold, RSA). SAIMM, Johannesburg. 24 pp.
- Ohmura, N., Kitamura, K., Saiki, H., 1993. Selective adhesion of *Thiobacillus ferrooxidans* to pyrite. *Applied and Environmental Microbiology* 59, 4044–4050.
- Okamoto, H., Nakayama, R., Tsunekawa, M., Hiroyoshi, N., 2003. Improvement of chalcopyrite leaching in acidic sulfate solutions by redox potential control. In: Riveros, P.A., Dixon, D., Dreisinger, D.B., Menacho, J. (Eds.), *Copper-Cobre 2003* (Santiago) Volume VI — *Hydrometallurgy of Copper* (Book 1) Leaching and Process Development. Canadian Institute of Mining, Metallurgy and Petroleum, Montreal, pp. 67–81.
- Okibe, N., Johnson, D.B., 2004. Biooxidation of pyrite by defined mixed cultures of moderately thermophilic acidophiles in pH-controlled bioreactors: significance of microbial interactions. *Biotechnology and Bioengineering* 8, 574–583.
- Okibe, N., Gericke, M., Hallberg, K.B., Johnson, D.B., 2003. Enumeration and characterization of acidophilic microorganisms isolated from a pilot plant stirred tank bioleaching operation. *Applied and Environmental Microbiology* 69, 1936–1943.
- Olson, G.J., Clark, T.R., 2001. Bioleaching of a mixed copper sulfide ore containing enargite and luzonite. In: Ciminelli, V.S.T., Garcia, O. (Eds.), *Biohydrometallurgy: Fundamentals, Technology and Sustainable Development*, Part A. Elsevier, Amsterdam, pp. 543–551.
- Pantelis, G., Ritchie, A.I.M., Stepanyants, Y.A., 2002. A conceptual model for the description of oxidation and transport processes in sulphidic waste rock dumps. *Applied Mathematical Modelling* 26, 751–770.
- Parker, A., Klauber, C., Stott, M., Watling, H., van Bronswijk, W., 2004. An X-ray photoelectron spectroscopy study of the mechanism of microbially assisted dissolution of chalcopyrite. In: Tzesos, M., Hatzikioseyan, A., Remoundaki, E. (Eds.), *Biohydrometallurgy: A Sustainable Technology in Evolution* (Athens, 2003). National Technical University of Athens, Athens, pp. 1011–1022.
- Peters, E., 1976. Direct leaching of sulfides: chemistry and applications. *Metallurgical Transactions B* 505–517.
- Petersen, J., Dixon, D.G., Timmins, M., Ruitenbergh, R., 2001. Batch reactor studies of the leaching of a pyrite/chalcopyrite concentrate using thermophilic bacteria. In: Ciminelli, V.S.T., Garcia, O. (Eds.), *Biohydrometallurgy: Fundamentals, Technology and Sustainable Development*, Part A. Elsevier, Amsterdam, pp. 525–533.
- Pinches, A., Myburgh, P.J., van der Merwe, C., 2001. Process for the rapid leaching of chalcopyrite in the absence of catalysts. U.S. Patent 6,277,341.
- Plumb, J.J., Gibbs, B., Stott, M.B., Robertson, W.J., Gibson, J.A.E., Nichols, P.D., Watling, H.R., Franzmann, P.D., 2002. Enrichment and characterisation of thermophilic acidophiles for the bioleaching of mineral sulphides. *Minerals Engineering* 15, 787–794.
- Pogliani, C., Donati, E., 1999. The role of copolymers in the bioleaching of a non-ferrous metal sulphide. *Journal of Industrial Microbiology and Biotechnology* 22, 88–92.
- Pogliani, C., Donati, E., 2000. Immobilisation of *Thiobacillus ferrooxidans*: importance of jarosite precipitation. *Process Biochemistry* 35, 997–1004.
- Poulter, S., Canterford, J., Lunt, D., 1999. Biotechnology for sulphide minerals. A comparison with other leaching processes. *Biomine '99—WAMMO '99* (Perth, WA). Australian Mineral Foundation, Glenside, pp. 1–14.
- Pronk, J.T., De Bruyn, J.C., Kuenen, J.G., 1992. Anaerobic growth of *Thiobacillus ferrooxidans*. *Applied Environmental Microbiology* 58, 2227–2230.
- Quatrini, R., Veloso, F., Jedlicki, E., Holmes, D.S., 2004. A model for iron uptake in *Acidithiobacillus ferrooxidans* based upon genome analysis. In: Tzesos, M., Hatzikioseyan, A., Remoundaki, E. (Eds.), *Biohydrometallurgy: A Sustainable Technology in Evolution* (Athens, 2003). National Technical University of Athens, Athens, pp. 989–995.
- Ramachandra Rao, S., Finch, J.A., 1988. Galvanic interaction studies on sulphide minerals. *Canadian Metallurgical Quarterly* 27, 253–259.
- Rawlings, D.E., 1997. Mesophilic, autotrophic bioleaching bacteria: description, physiology and role. In: Rawlings, D.E. (Ed.), *Biomining: Theory, Microbes and Industrial Processes*. Springer Verlag, Berlin, pp. 229–245.
- Rawlings, D.E., 1999. The molecular genetics of mesophilic, acidophilic, chemolithotrophic, iron- or sulfur-oxidizing microorganisms. In: Amils, R., Ballester, A. (Eds.), *Proc. Intl. Biohydrometallurgy Symposium* (Madrid, Spain), Part B. Elsevier, Amsterdam, pp. 3–20.
- Rawlings, D.E., 2001. The molecular genetics of *Thiobacillus ferrooxidans* and other mesophilic, acidophilic, chemolithotrophic, iron- or sulfur-oxidizing bacteria. *Hydrometallurgy* 59, 187–201.
- Rawlings, D.E., Kusano, T., 1994. Molecular genetics of *Thiobacillus ferrooxidans*. *Microbiological Reviews* 58, 39–55.
- Rawlings, D.E., Tributsch, H., Hansford, G.S., 1999. Reasons why '*Leptospirillum*'-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidising bacteria in many commercial processes for the biooxidation of pyrite and related ores. *Microbiology* 145, 5–13.
- Readett, D., Sylwestrzak, L., 2002. Bioleaching at Nifty Copper Operation. *Aus. I.M.M. Bulletin* 12–18 Nov.–Dec.
- Readett, D., Townson, B., 1997. Practical aspects of copper solvent extraction from acidic leach liquors. Unpublished paper presented at ALTA Copper 1997: Copper Hydrometallurgy Forum (Brisbane, Qld.), ALTA Metallurgical Services, Melbourne, 37 pp.
- Readett, D., Sylwestrzak, L., Franzmann, P.D., Plumb, J.J., Robertson, W.R., Gibson, J.A.E., Watling, H., 2003. The life cycle of a chalcocite heap bioleach system. In: Young, C.A., Alfantazi, A.M., Anderson, C.G., Dreisinger, D.B., Harris, B., James, A. (Eds.),

- Hydrometallurgy 2003, Volume 1: Leaching and Solution Purification. TMS, Warrendale, pp. 365–374.
- Rear, B., Taylor, A., Busby, P., 1994. Chalcocite ore leaching at Girilambone Copper Mine. Biomine '94: Applications of Biotechnology to the Minerals Industry (Perth, WA). Australian Mineral Foundation, Glenside, pp. 6.0–6.25.
- Rhodes, M., Deeplaul, V., van Staden, P.J., 1998. Bacterial oxidation of Mt Lyell concentrates, Unpublished paper presented at ALTA Copper 1998: (Brisbane, Qld.), ALTA Metallurgical Services, Melbourne, 24 pp.
- Ritchie, A.I.M., 1997. Optimization of biooxidation heaps. In: Rawlings, D.E. (Ed.), Biomining: Theory, Microbes and Industrial Processes. Springer Verlag, Berlin, pp. 201–226.
- Robertson, W.J., Kinnunen, P.H.-M., Plumb, J.J., Franzmann, P.D., Puhakka, J.A., Gibson, J.A.E., Nichols, P.D., 2002. Moderately thermophilic iron oxidising bacteria isolated from a pyritic coal deposit showing spontaneous combustion. Minerals Engineering 15, 815–822.
- Rodriguez, Y., Ballester, A., Blazquez, M.L., Gonzalez, F., Munoz, J.A., 2003a. New information on the pyrite bioleaching mechanism at low and high temperature. Hydrometallurgy 71, 37–46.
- Rodriguez, Y., Ballester, A., Blazquez, M.L., Gonzalez, F., Munoz, J.A., 2003b. Study of bacterial attachment during the bioleaching of pyrite, chalcopyrite and sphalerite. Geomicrobiology Journal 20, 131–141.
- Rojas-Chapana, J.A., Tributsch, H., 2000. Bio-leaching of pyrite accelerated by cysteine. Process Biochemistry 35, 815–824.
- Rojas-Chapana, J.A., Bärtels, C.C., Pohlmann, L., Tributsch, H., 1998. Co-operative leaching and chemotaxis of *Thiobacilli* studied with spherical sulphur/sulphide substrates. Process Biochemistry 33, 239–248.
- Romero, R., Mazuelos, A., Palencia, I., Carranza, F., 2003. Copper recovery from chalcopyrite concentrates by the BRISA process. Hydrometallurgy 70, 205–215.
- Rossi, G., 1990. Biohydrometallurgy. McGraw Hill, Hamburg, pp. 331–492.
- Sanhueza, A., Ferrer, I.J., Vargas, T., Amils, R., Sanchez, C., 1999. Attachment of *Thiobacillus ferrooxidans* on synthetic pyrite of varying structural and electronic properties. Hydrometallurgy 51, 115–129.
- Santhiya, D., Subramanian, S., Natarajan, K.A., 2001. Role of microorganisms in surface chemical changes on sphalerite and galena. In: Ciminelli, V.S.T., Garcia, O. (Eds.), Proc. Intl. Biohydrometallurgy Symposium, Part A. Elsevier, Amsterdam, pp. 13–22.
- Schippers, A., Sand, W., 1999. Bacterial leaching of metal sulfides proceeds by two indirect mechanisms via thiosulfate or via polysulfides and sulfur. Applied and Environmental Microbiology 65, 319–321.
- Schnell, H., 1997. Bioleaching of Copper. In: Rawlings, D.E. (Ed.), Biomining: Theory, Microbes and Industrial Processes. Springer Verlag, Berlin, pp. 21–43.
- Schrenk, M.O., Edwards, K.J., Goodman, R.M., Hamers, R.J., Banfield, J.F., 1998. Distribution of *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*: implications for generation of acid mine drainage. Science 279, 1519–1522.
- Sharma, P.K., Das, A., Hanumantha Rao, K., Forssberg, K.S.E., 2003. Surface characterization of *Acidithiobacillus ferrooxidans* cells grown under different conditions. Hydrometallurgy 71, 285–292.
- Shiers, D.W., Blight, K.R., Ralph, D.E., 2005. Sodium sulphate and sodium chloride effects on batch culture of iron oxidising bacteria. Hydrometallurgy 80, 75–82.
- Silver, S., Budd, K., Leahy, K.M., Shaw, W.V., Hammond, D., Novick, R.P., Willsky, G.R., Malamy, M.H., Rosenberr, H., 1981. Inducible plasmid-determined resistance to arsenate, arsenite and antimony (III) in *Escherichia coli* and *Staphylococcus aureus*. Journal of Bacteriology 146, 983.
- Silverman, M.P., Ehrlich, H.L., 1964. Microbial formation and degradation of minerals. Advances in Applied Microbiology 6, 153–206.
- Simmons, S., Norris, P.R., 2000. Acidophiles of saline water at thermal vents of Vulcano, Italy. Extremophiles 4, 201–207.
- Solari, J.A., Huerta, G., Escobar, B., Vargas, T., Badilla-Ohlbaum, R., Rubio, J., 1992. Interfacial phenomena affecting the adhesion of *Thiobacillus ferrooxidans* to sulphide mineral surfaces. Colloids and Surfaces 69, 159–166.
- Studel, R., 1996. Mechanism for the formation of elemental sulfur from aqueous sulfide in chemical and microbiological desulfurization processes. Industrial & Engineering Chemistry Research 35, 1417–1423.
- Stott, M., Watling, H., Franzmann, P., Sutton, D., 2000. The role of iron hydroxyprecipitates in the passivation of chalcopyrite. Minerals Engineering 13, 1117–1127.
- Stott, M.B., Watling, H.R., Franzmann, P.D., Sutton, D.C., 2001. The effect of solution chemistry on jarosite deposition during the leaching of chalcopyrite by the thermophilic archaeon *Sulfolobus metallicus*. In: Ciminelli, V.S.T., Garcia, O. (Eds.), Proc Intl. biohydrometallurgy Symposium (Ouro Preto) Part A. Elsevier, Amsterdam, pp. 207–215.
- Sui, C.C., Brienne, S.H.R., Ramachandra Rao, S., Xu, Z., Finch, J.A., 1995. Metal ion production and transfer between sulphide minerals. Minerals Engineering 8, 1523–1539.
- Temple, K.L., Colmer, A.R., 1952. The autotrophic oxidation of iron by a new bacterium *Thiobacillus ferrooxidans*. Journal of Bacteriology 62, 605–611.
- Third, K.A., Cord-Ruwisch, R., Watling, H.R., 2000. The role of iron-oxidizing bacteria in stimulation or inhibition of chalcopyrite bioleaching. Hydrometallurgy 57, 225–233.
- Tiller-Jeffery, R.E., Watling, H.R., Browner, R.E., Whittington, B.I., 2004. Microbial versus chemical leaching of chalcopyrite concentrate — a quantitative mineralogical investigation. Proc. Bac-Min Conference (Bendigo). Aus. I.M.M., Melbourne, pp. 57–62.
- Tributsch, H., 2001. Direct versus indirect bioleaching. Hydrometallurgy 59, 177–185.
- Tributsch, H., Rojas-Chapana, J.A., 2000. Metal sulfide semiconductor electrochemical mechanisms induced by bacterial activity. Electrochimica Acta 45, 4705–4716.
- Tshilombo, A.F., Petersen, J., Dixon, D.G., 2002. The influence of applied potentials and temperature on the electrochemical response of chalcopyrite during bacterial leaching. Minerals Engineering 15, 809–813.
- Tuovinen, O.H., Kelley, B.C., Groudev, S.N., 1991. Mixed cultures in biological leaching processes and mineral biotechnology. In: Zeikus, J.G., Johnson, E.A. (Eds.), Mixed Cultures in Biotechnology. McGraw Hill, New York, pp. 373–427.
- Tyson, G.W., Chapman, J., Hugenholtz, P., Allen, E.E., Ram, R.J., Richardson, P.M., Solovyev, V.V., Rubin, E.M., Rokhsar, D.S., Banfield, J.F., 2004. Community structure and metabolism through reconstruction of microbial genomes from the environment. Nature 428, 37–43 (March).
- Valdes, J., Jedlicki, E., Holmes, D.S., 2004. Sulfur assimilation in *Acidithiobacillus ferrooxidans*. In: Tzesos, M., Hatzikioseyan, A., Remoundaki, E. (Eds.), Biohydrometallurgy: A Sustainable Technology in Evolution (Athens, 2003). National Technical University of Athens, Athens, pp. 1187–1194.
- van Aswegen, P.C., Haines, A.K., Marais, H.J., 1988. Design and operation of a commercial bacterial oxidation plant at Fairview. Randol Gold Forum '88 (Perth, WA). Randol International Ltd., Golden, Colorado, pp. 144–147.
- van der Merwe, C., Pinches, A. and Myburgh, P.J., 1998. A process for the leaching of chalcopyrite. World Patent No. WO 9,839,491 (September 11, also published as AU 2224697).

- van Staden, P.J., 1998. The Mintek/Bactech copper bioleach process. Unpublished paper presented at ALTA Copper in press: Copper Sulphides Symposium (Brisbane, Qld.), ALTA Metallurgical Services, Melbourne, 12 pp.
- van Staden, P.J., Shaisaee, B., Yazdani, M., 2005. A collaborative plan towards the heap bioleaching of low grade chalcopyritic ore from a new Iranian mine. In: Harrison, S.T.L., Rawlings, D.E., Petersen, J. (Eds.), Proc. 16th Intl. Biohydrometallurgical Symposium (Cape Town). IBS, Cape Town, pp. 115–123.
- van Loosdrecht, M.C.M., Lyklema, J., Norde, W., Zehnder, A.J.B., 1990. Influence of interfaces on microbial activity. *Microbiological Reviews* 54, 75–87.
- Vasquez, M., Espejo, R.T., 1997. Chemolithotrophic bacteria in copper ores leached at high sulfuric acid concentration. *Applied and Environmental Microbiology* 63, 332–334.
- Whittington, B., McDonald, R., Johnson, J., Muir, D., 2003a. Pressure acid leaching of Bulong nickel laterite ore, Part I: Effect of water quality. *Hydrometallurgy* 70, 31–46.
- Whittington, B., Johnson, J., McDonald, R., Muir, D., 2003b. Pressure acid leaching of Bulong nickel laterite ore, Part II: Effect of ore type. *Hydrometallurgy* 70, 47–61.
- Wiertz, J., Espinoza, S., Mendoza, C., Casas, J.M., Escobar, B., 1997. Bacterial leaching study and process development for enargite bearing ores and concentrates. IBS '97 — Biomine '97 (Sydney, NSW). Australian Mineral Foundation, Glenside, pp. PM17.1–PM17.2.
- Wiertz, J., Espinoza, S., Mendoza, C., Ruiz, M.A., Huenupi, E., Casas, J.M., Escobar, B., 1998. Preliminary study of (bio)leaching process for enargite bearing ores and concentrates. In: Sanchez, M.A., Vergara, F., Castro, S.H. (Eds.), *Environment and Innovation in Mining and Mineral Technology: Proc. IV Int. Conf. On Clean Technologies for the Mining Industry, Volume 1* (Santiago, Chile). Univ. of Concepcion, Concepcion, Chile, pp. 141–153.
- Williams, T., Hunter, C. and Arnall, B., 1999. Pacific Ore Technology's bacterial leaching of chalcopyrite. Unpublished paper presented at ALTA Copper 1999: Copper Sulphides Symposium and Copper Hydrometallurgy Forum (Gold Coast, Qld.), ALTA Metallurgical Services, Melbourne, 11 pp.
- Winby, R., Miller, P.C., 2000. An improved method for heap leaching of chalcopyrite, World Patent 0071,763 A, A1 30 November; Australian Patent 43863-A.
- Zillig, W., Arnold, H.P., Holz, I., Prangishvili, D., Schweier, A., Stedman, K., She, Q., Phan, H., Garrett, R., Kristjansson, J.K., 1998. Genetic elements in the extremely thermophilic archaeon *Sulfolobus*. *Extremophiles* 2, 131–140.