Journal of Industrial Microbiology & Biotechnology (1998) 20, 268–274

http://www.stockton-press.co.uk/jim

Industrial practice and the biology of leaching of metals from ores
The 1997 Pan Labs Lecture

DE Rawlings

Department of Microbiology, University of Cape Town, Rondebosch, 7700, South Africa

Biomining processes have been used successfully on a commercial scale for the recovery of metals, the most important of which are copper, uranium and gold. These processes are based on the activity of chemoautolithotrophic bacteria which are able to use either iron or sulfur as their energy source and which grow in highly acid conditions. In general, low-rate dump and heap leaching processes are used for copper recovery while the biooxidation of difficult-to-treat gold-bearing arsenopyrite ores is carried out commercially in highly aerated stirred tank reactors. Because of the high levels of bacterial activity required, limitations in the growth rate of the microorganisms which were not apparent in low-rate processes have become an important factor. A key to the commercialization of the gold-bearing arsenopyrite biooxidation process was the development of a rapidly-growing, arsenic-resistant bacterial consortium. The empirical technique of mutation and selection in a continuous-flow system was used to improve the ability of the bacteria to decompose the ore. This approach resulted in a dramatic initial enhancement in growth rate but a plateau in improvement of performance has been reached. Further advances will require a more direct approach based on an understanding of the underlying physiological mechanisms and an application of the tools of molecular biology. Considerable advances have been made in our understanding of the molecular biology of *Thiobacillus ferrooxidans*. However much less is known about the other biomining bacteria. Recent studies using 16S rRNA analysis techniques have indicated that *T. ferrooxidans* may play a smaller role in continuous flow stirred tank biomining processes than was previously thought.

Keywords: biomining; bioleaching; biooxidation; ore decomposition; *Thiobacillus; Leptospirillum*

Introduction

Research on biomining and the microbes involved in biomining is a subject in which I have been involved for the past 19 years. During that time the use of microbes in the recovery of metals from ores has greatly expanded and one of the aims of this report is to convince the reader that biomining has become a very significant part of industrial microbiology. Large scale copper bioleaching has been taking place for centuries using what may be considered to be a low technology process, the irrigation of dumps [4]. Since the early 1960s, the copper recovery process was made more efficient by the construction and irrigation of specially designed heaps [4,46]. Also in the 1960s, it was discovered that uranium could be recovered by bioleaching, and industrial scale uranium bioleaching was carried out by spraying stope walls with acid mine drainage and the *in situ* irrigation of fractured underground ore deposits [29]. Then in the 1980s, a process for the biooxidation of gold-bearing arsenopyrite ores in highly aerated stirred tank reactors was developed by Gencor, South Africa (now renamed Billiton) [27,43]. With that process, biomining became a significant part of the fermentation industry. An illustration of this is that the biooxidation plant built at the Ashanti gold-fields in Ghana [10] is currently (with the possible exception of some sewage treatment facilities), the largest fermentation plant in the world. Today at least six commercial stirred-tank gold-ore biooxidation plants operate in five countries using highly controlled fermentation technology. Biomining has come of age as an important part of industrial microbiology.

An aspect of biomining that has interested me is the fascinating physiology of the microorganisms involved [23,28]. The most important microbes are characterized by their ability to grow chemolithotrophically using ferrous iron or reduced sulfur compounds as an energy source. Microorganisms with this ability are found in a large number of environments ranging from the mesophilic to the hyperthermophilic and in water which varies from neutral pH to highly acidic [31,32]. All current industrial bioleaching or biooxidation processes are carried out at below 50°C, using a consortium of mesophilic or moderately thermophilic iron and sulfur-oxidizing bacteria, at a pH of 1.6 or less. With the exception of a single biooxidation plant [30], currently operating commercial bioleaching plants operate at 40°C or below.

Biomining bacteria

Mesophiles

The most important 'players' in biomining operations that take place at less than 40°C are the bacteria, *Thiobacillus ferrooxidans*, *Leptospirillum ferrooxidans* (Figure 1) and *Thiobacillus thiooxidans*. These Gram-negative bacteria have a physiology which is ideally suited for growth in

Correspondence to DER at his address after 1 July 1998: Department of Microbiology, University of Stellenbosch, Stellenbosch, 7600, South Africa
Received 20 November 1997; accepted 2 March 1998

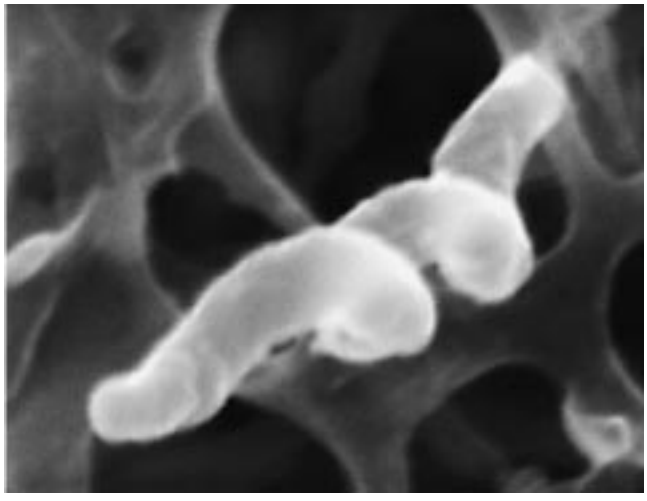

Figure 1 A scanning electron microscope illustrating the typical spiral shape of a strain of a *Leptospirillum* from the biooxidation tanks of the Fairview mine (magnification approx. 20000×).

an inorganic mining environment [4,28,41]. They are all obligately chemoautolithotrophic bacteria which obtain their energy through the oxidation of ferrous to ferric iron (*T. ferrooxidans* and *L. ferrooxidans*) or reduced inorganic sulfur compounds to sulfate (*T. ferrooxidans* and *T. thiooxidans*) [22]. The production of sulfate results in the accumulation of sulfuric acid so that the pH of their environment is typically pH 1.5–2.0 (or lower) and all of the important biomining bacteria are obligately acidophilic. A typical source of energy is the oxidation of a mineral such as pyrite.

$$4FeS_2 + 15O_2 + 2H_2O \rightarrow 2Fe_2(SO_4)_3 + 2H_2SO_4$$

Biomining bacteria are considered to be aerobic, although in the absence of oxygen, *T. ferrooxidans* is able to grow on reduced inorganic sulfur using ferric iron as an alternate electron acceptor [47]. *T. ferrooxidans* has been shown to fix atmospheric nitrogen and all *T. ferrooxidans* and *L. ferrooxidans* tested have *nif* genes [34,39]. Their unique physiology means that biomining bacteria are able to grow in what would appear to be very nutrient-poor solutions. Aeration of a suitable ore in water is usually sufficient to satisfy their essential growth requirements. Air provides the carbon (CO_2), nitrogen (N_2) and electron acceptor (O_2) source, ore is the source of energy and water the growth medium. Essential trace elements are provided as impurities in the ore or water. The organisms are probably not capable of fixing nitrogen when growing in an aerobic environment so that when growing in highly aerated stirred tank reactors, small amounts of inexpensive fertilizer-grade ammonium sulfate and potassium phosphate are added to ensure that sufficient nutrients are present. Biomining bacteria have other advantages. They tolerate moderate to high levels of many metal ions [48] and because few other organisms are capable of growth in an inorganic low pH environment, it is unnecessary to ensure the sterility of industrial processes which employ these bacteria.

Moderate thermophiles

A number of bacteria are capable of oxidizing iron or sulfur compounds at temperatures of between 45–55°C. These include the Gram-negative bacterium, *Thiobacillus caldus* [17] which is capable of oxidizing sulfur optimally at 45°C. *T. caldus* strains rapidly oxidize sulfur within the temperature range 35–50°C and occur in greater numbers in biooxidation plants than *T. thiooxidans* (Gardner and Rawlings, unpublished). *T. caldus* appears to be the moderately thermophilic equivalent of *T. thiooxidans*. A comparison of 16S rRNA sequences from *T. caldus* and *T. thiooxidans* indicates that the two bacteria are phylogenetically closely related [17]. In contrast to the mesophilic iron-oxidizing bacteria, the iron-oxidizing moderate thermophiles are more commonly Gram-positive spore-forming bacteria belonging to the genus *Sulfobacillus* [32]. *Sulfobacillus thermosulfidooxidans* has been reported to be the most efficient iron-oxidizing moderate thermophile and its growth and ability to oxidize iron is stimulated in the presence of yeast extract and carbon dioxide-enriched air [32]. *Acidimicrobium ferrooxidans* is a moderate thermophile which grows well even in the absence of added carbon dioxide, but its ability to oxidize iron is less than *Sulfobacillus thermosulfidooxidans* even when grown in carbon dioxide-enriched air. Mixed cultures of both bacteria are efficient oxidizers of iron in the absence of added carbon dioxide [32]. It has been suggested that the reason for this is that *Acidimicrobium* is more efficient at fixing carbon dioxide than *Sulfobacillus thermosulfidooxidans* and when grown in mixed culture, *Acidimicrobium* secretes small amounts of organic nutrients which are used by the *Sulfobacillus*. This enables the *Sulfobacillus* to oxidize iron rapidly.

Current industrial processes

Although several minerals are amenable to commercial scale bioleaching/biooxidation, currently only two metals are recovered using this technology; these are copper and gold. In the 1980s substantial amounts of uranium were mined using *in situ* bioleaching technology [29]. During 1988 approximately 300 tons of uranium with a value of over US$ 25 million were recovered from a single mine (Dennison mine, Lake Elliot district, Canada). However, with the reduction in demand for uranium in more recent years, this mine has stopped production.

Copper leaching

In terms of tonnage, copper is the most important metal to be recovered by biomining [5]. Bioleaching of copper involves the conversion of water-insoluble copper sulfides to water-soluble copper sulfates. Copper-containing minerals such as chalcocite (Cu_2S) or covellite (CuS) are crushed, acidified with sulfuric acid and agglomerated in rotating drums to bind fine material to coarser particles [46]. The agglomerate is stacked in heaps onto lined pads on which aeration piping may be placed. The stacked heaps are irrigated with an iron-containing solution (usually recycled spent leach liquor) through a second system of pipes laid on or just below the heap surface (Figure 2). Aeration may be enhanced by forcing air through the heap

Figure 2 Irrigation of heaps of copper ore at a mine near Santiago, Chile. The crushed ore is stacked on pads and sprayed with a raffinate leaching solution (8 g sulfuric acid plus 0.3–0.5 g residual copper per L) through a system of pipes and nozzles placed on top of the ore. (By kind permission of EM Domic, Sociedad Minera Pudahuel Ltd, Santiago, Chile.)

from the bottom using low pressure fans. The solution percolates through the heap and bacteria growing on the surface of the ore and in solution catalyze the release of copper. Small amounts of inorganic nutrients in the form of fertilizer grade ammonium sulfate and potassium phosphate may aid microbial growth and copper dissolution. The ferric iron generated by the bacteria plays an important role in the production of copper sulfate.

$$FeSO_4 + O_2 + H_2SO_4 \xrightarrow{\text{bacteria}} 2\ Fe_2(SO_4)_3 + H_2O$$

$$Cu_2S + 1/2\ O_2 + H_2SO_4 \xrightarrow{\text{bacteria}} CuS + CuSO_4 + H_2O$$

$$CuS + 2\ O_2 \xrightarrow{\text{bacteria}} CuSO_4$$

$$Cu_2S + 2\ Fe_2(SO_4)_3 \longrightarrow 2\ CuSO_4 + 4\ FeSO_4 + S$$

$$CuS + Fe_2(SO_4)_3 \longrightarrow CuSO_4 + 2\ FeSO_4 + S$$

The pregnant leach solution containing 1.5–6 g L^{-1} soluble copper and up to 20 g L^{-1} iron is collected and sent to a recovery plant. The most common methods for copper recovery are by precipitation using iron filings (cementation), electrowinning or solvent extraction followed by electrowinning. The latter procedure produces the highest grade of copper. The most important copper-producing country in the world is Chile and the largest heap bioleaching operations are located in that country. Copper mines at Quebrada Blanca and Cerro Colorado use bioleaching to produce 75 000 and 60 000 metric tons of copper per annum respectively [5].

Gold biooxidation

The potential of microbes to assist in the extraction of gold from some recalcitrant ores was first realized in the early 1980s. Much of the credit for this belongs to the late Eric Livesey-Goldblatt [27], who at the time was director of the Gencor Process Research Laboratory in Krugersdorp, South Africa. Gold is usually extracted from ores using cyanide. Recalcitrant ores are those in which gold is encased in a matrix of arsenopyrite/pyrite, so that even after fine milling, the gold cannot be efficiently recovered. Pretreatment of the ore is required to open up the molecular structure of the ore which permits cyanide to make contact with and extract the gold. Since the quantities of ore to be treated are huge and most of the gold is present in a small pyrite/arsenopyrite fraction, the ore is crushed and a gold-bearing concentrate is prepared by flotation. Prior to 1986, concentrate pretreatment processes were physico-chemical. For example, the concentrate was roasted at 700°C in the presence of oxygen or digested with acid under pressure in an oxygen-enriched atmosphere (autoclaved). In contrast, biomining bacteria decompose ores and concentrates at atmospheric pressure and at temperatures which are closer to ambient.

$$2\ FeAsS\ (\text{arsenopyrite}) + 7\ O_2 + H_2SO_4 + 2\ H_2O \xrightarrow{\text{bacteria}} 2\ H_3AsO_4 + Fe_2(SO_4)_3$$

Without pretreatment, only 30–50% of the gold is recovered depending on the concentrate, while after biooxidation more than 95% of the gold is recoverable. Since gold-bearing concentrates are valuable substrates relative to copper, it is economically viable to carry out the pretreatment biooxidation processes in efficient, aerated, temperature- and pH-controlled vat-type fermenters. Unlike the bioleaching of copper ores, where the copper is solubilized in the biological process, the gold is not solubilized by microbial action but a separate chemical process is required. Since gold is not leached from the ores the term 'biooxidation' is used to describe the treatment of gold ores as opposed to the term 'bioleaching'. Bioleaching strictly applies only to processes in which uranium and base metals such as copper, nickel, zinc or lead are solubilized as a result of microbial activity.

Development of the Biox process: Early experiments on gold-biooxidation were carried out in a series of three or four continuous-flow, aerated, stirred tank reactors [44]. As these reactors are expensive to construct and operate, the rate of concentrate decomposition has an important effect on the economics of the process. The initial processes were very slow because unadapted cultures of biooxidation bacteria were sensitive to the arsenic released from the arsenopyrite. Concentrate was decomposed until the build-up of arsenic inhibited further microbial activity. Arsenic toxicity was reduced by the introduction of an arsenic precipitation step between each of the aerated reactors. This process was unworkable and uneconomical. A retention time of over 12 days was required for sufficient biooxidation to allow more than 95% gold recovery. However, a continuous flow process provides strong selection for arsenic tolerance because slow growing cells were preferentially washed out and the faster growing cells retained. Over a period of 2 years the arsenic resistance of the cultures increased from less than 1 g to over 13 g total arsenic L^{-1}. Largely because of this increase in arsenic resistance, the retention time of concentrate in the reactors was

reduced to 7 days. During 1986 the first full-scale continuous biooxidation plant designed to treat 10 tons of gold-bearing arsenopyrite concentrate per day was built at the Fairview mine (Figure 3) in South Africa [50]. By 1989, the growth rate of the bacteria had improved still further so that the retention time had been reduced to 3.5 days. At the same time the solid concentration in the liquor was increased from 10 to 18% so that the same equipment could be used to treat almost four times the amount of concentrate per day as initially. This process which had been developed by Gencor SA [10,50] was registered as the Biox process. The Biox process has proved to be highly robust and since 1990, plants using Biox technology were commissioned at Sao Bento, Brazil (150 tons day^{-1}); Harbour Lights (40 tons day^{-1}) and Wiluna (115 tons day^{-1}) both in Western Australia and Sansu, Ghana (1000 tons day^{-1}). The latter plant consists of 24 1000 m^3 aeration tanks and is the largest fermentation plant in the world. A typical Biox plant operates at 40°C and a considerable amount of energy for cooling is required to maintain the process at this temperature.

The BacTech process: An alternative to the Biox process was developed by BacTech (Australia). In 1994 a plant using this BACOX technology was constructed to treat 120 tons of gold-bearing concentrate per day at the Yuoanmi mine, Western Australia [30]. The BacTech and Biox processes use similar highly aerated stirred tank reactors with the major difference being that the BacTech process is operated at close to 50°C. As a result biooxidation of the concentrate is carried out by moderately thermophilic bacteria, although the exact composition of the bacterial population is unclear [14]. Like the Biox process, the BACOX process has been reported to be highly robust and has the advantage that less cooling is required. A disadvantage is that the solubility of oxygen and carbon dioxide is lower at the higher temperatures.

Use of heaps to treat gold-bearing ores: A process in which a gold-bearing concentrate is treated in a heap rather than a tank reactor has recently been reported. In this process (developed by Geobiotics, Hayward, CA, USA), the concentrate is agglomerated onto a support rock which is then stacked on a pad, inoculated with microorganisms and irrigated [52]. Air is blown through the stack and after a period the partly oxidized material is removed for gold recovery.

Figure 3 A photograph of the first production-scale biooxidation plant for the treatment of gold-bearing arsenopyrite ore. The plant was commissioned at the Fairview mine, Barberton, South Africa and was originally designed to treat 30% of the mine's production. In 1991 this demonstration plant was extended to treat the full production of approximately 40 tons of concentrate per day. (By kind permission of Gencor Ltd, Johannesburg, South Africa.)

Advantages and disadvantages of biooxidation: A major advantage of biooxidation is that relatively little of the ore needs to be decomposed to allow near complete gold recovery. The gold particles create a weakness in the pyrite/arsenopyrite crystal lattice and biooxidation takes place preferentially in these areas of weakness [9]. Capital costs for biooxidation have been reported to be about 2-fold lower than roasting or pressure oxidation and operating costs are also lower [10]. Waste disposal following biooxidation is comparatively easy. The pH of the ferric arsenate effluents is raised to above pH 5 with lime. This precipitates arsenic as $FeAsO_4$ which is almost insoluble (<0.2 mg L^{-1} arsenic) and may be disposed of to a tailings dam [10]. Biooxidation of gold-bearing ores has proved to be a remarkably reliable process. Early concerns about the possibility of phage infection and process instability were unfounded. A major drawback of the biooxidation compared with the physico-chemical pretreatment processes is that in many cases the consumption of cyanide in the subsequent gold-recovery process is considerably higher following biooxidation.

Development of genetic systems for biomining bacteria

There are several challenges in the development of genetic systems for the highly acidophilic, obligately autotrophic bacteria involved in biomining. These are suitable cloning vectors, selectable markers and methods for getting DNA into the cells.

Plasmids: Because of their unique physiology and specialized ecological niche, there was a concern that these bacteria may have been genetically isolated from the more commonly studied bacteria for which cloning vectors are available. As a result, replicons from plasmids considered to be broad-host-range might not have been functional in these bacteria. Early studies concentrated on the use of indigenous plasmids (which are widespread in the thiobacilli) as cloning vectors. Several *T. ferrooxidans* plasmids have been cloned and some completely or partially sequenced (see Reference 42 for a review). In general, *T. ferrooxidans* plasmids have replicons that are closely related to those of more commonly studied bacteria [12] and the broad-host-range plasmid replicons that have been tested in *T. ferrooxidans* and *T. thiooxidans* have been functional [19,36]. Mobilization functions of *T. ferrooxidans* plasmids are likewise related to those of plasmids of hospital bacteria isolates [13,42]. There are no reports on the frequency of plasmid occurrence in the leptospirilli and no broad-host-range plasmids have been tested in these bacteria.

Heterologous gene expression and selectable markers: It was not initially known whether genes

from other bacteria would be expressed in the autotrophic thiobacilli or which genetic markers would be suitable. Phylogenetic studies based on 16S rRNA [26], RecA [21], and ATP synthase sequence data [7] have indicated that *T. ferrooxidans* is closely related to bacteria like *E. coli*, more so than might be expected given the differences in their physiology. Gene expression studies of *T. ferrooxidans* genes cloned in *E. coli* have confirmed that the majority of housekeeping genes from *T. ferrooxidans* that have been tested complement corresponding *E. coli* mutants (see Reference 42 for a review). In one study the transcription start of the cloned *T. ferrooxidans* thioredoxin (*trxA*) gene in *E. coli* was found to be identical to the start of the same gene in *T. ferrooxidans* [38]. No complementation or chromosomal gene expression studies have been reported with either *T. thiooxidans* or members of the genus *Leptospirillum*.

There are strong indications that expression of heterologous genes in *T. ferrooxidans* and *T. thiooxidans* should not be difficult. In spite of this, finding suitable selectable markers for the genetic manipulation of *T. ferrooxidans* and *T. thiooxidans* has been problematic. One reason for this would appear to be that the low pH produced (when growing on sulfur media) and presence of metal ions (when growing on iron media) result in the inactivation of most antibiotics. Another is that the bacteria are slow growing and the antibiotic becomes inactive during the 10–14 day incubation period before colonies appear. Kanamycin is the only selectable marker that has been successfully used on sulfur [19,36] and mercury resistance has been used on iron media [24]. However, selection for mercury resistance was not completely effective and only about 50% of the colonies that grew on mercury plates possessed the gene for the selected marker. Presumably the long incubation period allowed for the non-biological reduction in mercury toxicity. Clearly, the choice of selectable markers is less than satisfactory. No selectable markers for use with the leptospirilli have been identified.

Gene delivery systems: Gene delivery systems have been reported for only two of the bacteria involved in bioleaching, *T. ferrooxidans* and *T. thiooxidans*. After many unsuccessful attempts to transform *T. ferrooxidans* by conjugation either directly from *E. coli* or via a less acidophilic facultatively heterotrophic *Thiobacillus* intermediate, the first report of success in transformation of the bacterium was by electroporation [24]. More than 30 isolates of *T. ferrooxidans* were tested, only one of which was successfully transformed at a very low frequency using a mercury resistance marker. After many years work in several laboratories the transformation of both *T. ferrooxidans* [36] and *T. thiooxidans* [19] by conjugation directly from *E. coli* at a low frequency was eventually achieved. IncP or IncQ plasmids were used with kanamycin as the selective marker on solid media with a rather high initial pH of 4.6–4.8. The high plate pH was almost certainly the secret of success as the mating frequency from *E. coli* and the stability of kanamycin decrease rapidly with pH. No experiments on the transformation of leptospirilli have been reported. Since leptospirilli will only grow on iron media at a very low pH, conjugation directly from *E. coli* is unlikely to be successful and electroporation is the most promising option.

Molecular studies of unique aspects of the physiology of biomining bacteria

An investigation into the molecular genetics and biochemistry of the systems associated with the ability of biomining bacteria to oxidize iron and sulfur in a highly acidic environment has yielded interesting findings [1–3,8,53]. These are also the properties of the bacteria that make them valuable from a commercial viewpoint. Since DNA probes for these genes or defined mutants lacking them were unavailable, these studies have been highly challenging. In spite of this, a considerable amount of success has been achieved in an investigation of the iron oxidation system of *T. ferrooxidans*. Most if not all of the components comprising the iron oxidation electron transport chain have been identified although the exact role of each component remains to be settled [53]. For example, the extensively studied small copper protein rusticyanin is considered to form part of the electron transport chain but recently it was reported that the aporusticyanin acts as specific receptor which stimulates the adhesion of the bacterium to pyrite [35]. Several of the genes involved in iron oxidation have been cloned and sequenced [1,25,42,53]. A particularly interesting finding was that the ability to oxidize iron may have evolved independently on more than one occasion [2,3] since there seems to be very little in common between the components of the iron oxidation systems of *T. ferrooxidans*, *L. ferrooxidans* and some of the moderately thermophilic iron-oxidizing bacteria.

Organisms in Biox tanks and heap reactors

For many years *T. ferrooxidans* was thought to dominate the microbial population in stirred tank and heap reactors that operate at 40°C or less. However, several recent findings have led researchers to re-evaluate the role of *T. ferrooxidans* in bioleaching/biooxidation processes. Because of their sensitivity to organic matter including the free sugars present in or released from agar [49], considerable difficulties have been experienced in growing bacteria on plates. Some success has been achieved using heterotrophic acidophilic bacteria (which are frequently found growing in close association with the autotrophic iron and sulfur oxidizers) to mop up free sugars [20]. A breakthrough in investigating the ecology of biomining processes was achieved by the application of the now widely used techniques of polymerase chain reaction (PCR) amplification of 16S rRNA genes from total DNA extracted from environmental samples. This has allowed an analysis of the composition of the bacteria in stirred tank reactors in a manner that does not require growth of the bacteria in a laboratory [15,40,51]. Sequences of 16S rRNAs are available for most of the bacteria commonly found in biomining environments [26]. Using this technology, several workers have reported that *T. ferrooxidans* occurs in very low numbers in stirred tank reactors and that the population of bacteria is dominated by *Leptospirillum*, *T. thiooxidans* [15,16,40] or *T. caldus* (unpublished). Using similar techniques to measure species-dependent 16S and 23S rDNA intergenic spacing, the near absence of *T. ferrooxidans* and dominance of *Lep-*

tospirillum and *T. thiooxidans* has also been reported in copper heap leaching environments, operating in conditions of high acidity (pH 0.7) [51]. *T. ferrooxidans* has however been reported to be dominant during the heap leaching of copper ores to which ferrous iron had been added [37] and is frequently the dominant bacterium in the drainage from mining wastes [45].

An important question is why researchers believed that *T. ferrooxidans* was the dominant bacterium in stirred tank reactors for so long. Part of the answer is that when samples from biooxidation tanks were grown on soluble iron media in batch culture, *T. ferrooxidans* outgrew its iron-oxidizing competitors and dominated the population. This is because in batch culture *T. ferrooxidans* has a faster growth rate on iron than does *Leptospirillum* [18]. However, the ability of *T. ferrooxidans* to oxidize ferrous iron is severely inhibited by the presence of ferric iron whereas the iron-oxidizing ability of *Leptospirillum* is relatively unaffected [33]. In continuous flow stirred tank reactors, ferric iron is always present at high levels relative to ferrous iron and growth of *T. ferrooxidans* is inhibited. However when samples from biooxidation tanks are placed in iron media and grown in batch culture, there is no ferric iron present at the start of growth and the few *T. ferrooxidans* cells present are able to outcompete the leptospirilli and dominate the population. Other reasons for the dominance of *Leptospirillum* is that these bacteria are somewhat more tolerant of temperatures as high as 40°C and pH values of 1.4–1.6 than *T. ferrooxidans* [33].

The future

Biomining is set to expand into the recovery of other base metal sulfides such as those of nickel, cobalt and zinc. The Billiton Company (UK) has registered a process for the recovery of nickel from metal sulfide concentrates called BioNIC [11] and a cobalt recovery plant is due to be commissioned by the Kasese Cobalt Company in Uganda in 1999 [6]. Both of these processes use stirred tank bioreactors and huge reactors of 1380 m^3 have been designed for the Kasese site. There is much potential for the recovery of copper from ores such as chalcopyrite which it is uneconomic to treat biologically at present. Bioleaching is a combination of chemical solubilization and microbial activity to regenerate the chemicals. The solubilization of copper from chalcopyrite is temperature-dependent and intensive research is currently being carried out to develop processes which operate at above 65°C. Such processes would require the use of hyperthermophilic iron and sulfur-oxidizers. Biomining has clearly become a significant part of industrial microbiology and its application is likely to further increase still further during the coming century.

References

1 Appia-Ayme C, A Bengrine, M Chippaux and V Bonnefoy. 1997. C4-type cytochrome *c*552 encoding gene from *Thiobacillus ferrooxidans*: cloning, characterization and expression in *Escherichia coli*. In: IBS Biomine '97, pp P2.1–10, Australian Mineral Foundation, Glenside, Australia.

2 Blake RC and S McGinness. 1993. Electron-transfer proteins of bacteria that respire on iron. In: Biohydrometallurgical Technologies, Vol II (Torma AE, ML Apel and CL Brierley, eds), pp 616–628, TMS Press, Warrendale, Pennsylvania.

3 Blake RC, EA Shute, J Waskovsky and AP Harrison. 1992. Respiratory components in acidophilic bacteria that respire on iron. Geomicrobiol J 10: 173–192.

4 Brierley CL. 1982. Microbiological mining. Scient Am 247: 42–51.

5 Brierley CL. 1997. Mining biotechnology: research to commercial development and beyond. In: Biomining: Theory, Microbes and Industrial Processes (Rawlings DE, ed), pp 3–17, RG Landes and Springer-Verlag, Berlin.

6 Briggs A and M Millard. 1997. Cobalt recovery using bacterial leaching at the Kasese project, Uganda. In: IBS Biomine '97, pp M2.4.1–12, Australian Mineral Foundation, Glenside, Australia.

7 Brown LD and DE Rawlings. 1993. A comparison of the structure of the H^+-translocating ATP synthase from *Thiobacillus ferrooxidans* with those of other organisms. In: Biohydrometallurgical Technologies, Vol II (Torma AE, ML Apel, CL Brierley, eds), pp 519–528, TMS Press, Warrendale, Pennsylvania.

8 Cavazza C and M Bruschi. 1995. Iron oxidation by *Thiobacillus ferrooxidans:* characterization of two electron transfer proteins. In: Biohydrometallurgical Processing, Vol II (Jerez CA, T Vargas, H Toledo and JV Wiertz, eds), pp 97–107, University of Chile Press, Santiago.

9 Claasen R, CT Logan and CP Snyman. 1993. Bio-oxidation of refractory gold-bearing arsenopyritic ores. In: Biohydrometallurgical Technologies, Vol I (Torma AE, JE Wey and VI Lakshmanan, eds), pp 479–488, TMS Press, Warrendale, Pennsylvania.

10 Dew DW, EN Lawson and JL Broadhurst. 1997. The Biox process for biooxidation of gold-bearing concentrates. In: Biomining: Theory, Microbes and Industrial Processes (Rawlings DE, ed), pp 45–80, RG Landes and Springer-Verlag, Berlin.

11 Dew DW and DM Miller. 1997. The BioNIC process: bioleaching of mineral sulphide concentrates for recovery of nickel. In: IBS Biomine '97, pp 7.1.1–9, Australian Mineral Foundation, Glenside, Australia.

12 Dorrington RA and DE Rawlings. 1990. Characterization of the minimum replicon of the broad-host-range plasmid pTF-FC2 and similarity between pTF-FC2 and the IncQ plasmids. J Bacteriol 172: 5697–5705.

13 Drolet M, P Zanga and PCK Lau. 1990. The mobilization and origin of transfer regions of a *Thiobacillus ferrooxidans* plasmid: relatedness to plasmids RSF1010 and pSC101. Mol Microbiol 4: 1381–1391.

14 Franzmann PD and TL Williams. 1997. Biomass and microbial community composition in an arsenopyrite oxidation bioreactor determined by phospholipid-derived fatty acid methyl ester composition and content. In: IBS Biomine '97, pp QP1.1–10, Australian Mineral Foundation, Glenside, Australia.

15 Goebel BM and E Stackebrandt. 1994. Cultural and phylogenetic analysis of mixed microbial populations found in natural and commercial bioleaching environments. Appl Environ Microbiol 60: 1614–1621.

16 Goebel BM and E Stackebrandt. 1995. Molecular analysis of the microbial biodiversity in a natural acidic environment. In: Biohydrometallurgical Processing, Vol II (Jerez CA, T Vargas, H Toledo and JV Wiertz, eds), pp 43–51, University of Chile Press, Santiago.

17 Hallberg KB and EB Lindström. 1994. Characterization of *Thiobacillus caldus* sp nov, a moderately thermophilic acidophile. Microbiology 140: 3451–3456.

18 Hansford GS. 1997. Recent developments in modelling the kinetics of bioleaching. In: Biomining: Theory, Microbes and Industrial Processes (Rawlings DE, ed), pp 153–175, RG Landes and Springer-Verlag, Berlin.

19 Jin SM, WM Yan and ZN Wang. 1992. Transfer of IncP plasmids to extremely acidophilic *Thiobacillus thiooxidans*. Appl Environ Microbiol 58: 429–430.

20 Johnson DB and S McGinness. 1991. A highly efficient and universal solid medium for growing mesophilic and moderately thermophilic, iron-oxidizing, acidophilic bacteria. J Microbiol Meth 13: 113–122.

21 Karlin S, GM Weinstock and V Brendel. 1995. Bacterial classifications derived from RecA protein sequence comparisons. J Bacteriol 177: 6881–6893.

22 Kelly DP and AP Harrison. 1989. Genus *Thiobacillus* Beijerinck, Vol 3. In: Bergey's Manual of Systematic Bacteriology (Staley JT, MP Bryant, N Pfennig and JG Holt, eds), pp 1842–1858, The Williams & Wilkins Co, Baltimore.

23 Kelly DP, PR Norris and CL Brierly. 1979. Microbiological methods for the extraction and recovery of metals. In: Microbial Technology: Current State and Future Prospects (Bull AT, DG Ellwood and C Ratledge, eds), pp 263–308, Cambridge University Press, Cambridge.
24 Kusano T, K Sugawara, C Inoue, T Takeshima, M Numata and T Shiratori. 1992. Electrotransformation of *Thiobacillus ferrooxidans* with plasmids containing a *mer* determinant as the selective marker by electroporation. J Bacteriol 174: 6617–6623.
25 Kusano T, T Takeshima, K Sugawara, C Inoue, T Shiratori, T Yano, Y Fukumori and T Yamanaka. 1992. Molecular cloning of the gene encoding *Thiobacillus ferrooxidans* Fe(II) oxidase. J Biol Chem 267: 11242–11247.
26 Lane DJ, AP Harrison Jr, D Stahl, B Pace, SJ Giovannoni, GJ Olsen and NR Pace. 1992. Evolutionary relationships among sulfur- and iron-oxidizing eubacteria. J Bacteriol 174: 269–278.
27 Livesey-Goldblatt E, P Norman and DR Livesey-Goldblatt. 1983. Gold recovery from arsenopyrite/pyrite ore by bacterial leaching and cyanidation. In: Recent Progress in Biohydrometallurgy (Rossi G and Torma AE, eds), pp 627–641, Azzociazione Mineraria Sarda, Iglesias, Sardinia.
28 Lundgren DG and M Silver. 1980. Ore leaching by bacteria. Annu Rev Microbiol 34: 263–283.
29 McCready RGL and WD Gould. 1990. Bioleaching of uranium. In: Microbial Mineral Recovery (Erlich HL and CL Brierley, eds), pp 107–126, McGraw-Hill, New York.
30 Miller PC. 1997. The design and operating practice of a bacterial oxidation plant using moderate thermophiles (the BacTech process). In: Biomining: Theory, Microbes and Industrial Processes (Rawlings DE, ed), pp 81–102, RG Landes and Springer-Verlag, Berlin.
31 Norris PR. 1990. Acidophilic bacteria and their activity in mineral sulfide oxidation. In: Microbial Mineral Recovery (Erlich HL and CL Brierley, eds), pp 3–27, McGraw-Hill, New York.
32 Norris PR. 1997. Thermophiles and bioleaching. In: Biomining: Theory, Microbes and Industrial Processes (Rawlings DE, ed), pp 247–258, RG Landes and Springer-Verlag, Berlin.
33 Norris PR, DW Barr and D Hinson. 1988. Iron and mineral oxidation by acidophilic bacteria: affinities for iron and attachment to pyrite. In: Biohydrometallurgy (Norris PR and DP Kelly, eds), pp 43–59, Science and Technology Letters, Kew, Surrey.
34 Norris PR, JC Murrell and D Hinson. 1995. The potential for diazotrophy in iron- and sulfur-oxidizing acidophilic bacteria. Arch Microbiol 164: 294–300.
35 Ohmura N and R Blake II. 1997. Aporusticyanin mediates the adhesion of *Thiobacillus ferrooxidans* to pyrite. In: IBS Biomine '97, pp PB.1–10, Australian Mineral Foundation, Glenside, Australia.
36 Peng J-B, W-M Yan and X-Z Bao. 1994. Plasmid and transposon transfer to *Thiobacillus ferrooxidans*. J Bacteriol 176: 2892–2897.
37 Pizarro J, E Jedlicki, O Orellana, J Romero and RT Espejo. 1996. Bacterial populations in samples of bioleached copper ore as revealed by analysis of DNA obtained before and after cultivation. Appl Environ Microbiol 62: 1323–1328.
38 Powles RE, SM Deane and DE Rawlings. 1995. Molecular genetic analysis of a thioredoxin isolated from *Thiobacillus ferrooxidans*. Microbiology 141: 2175–2181.
39 Pretorius I-M, DE Rawlings and DR Woods. 1986. Identification and cloning of *Thiobacillus ferrooxidans* structural *nif* genes in *Escherichia coli*. Gene 45: 59–65.
40 Rawlings DE. 1995. Restriction enzyme analysis of 16S rRNA genes for the rapid identification of *Thiobacillus ferrooxidans*, *Thiobacillus thiooxidans* and *Leptospirillum ferrooxidans* strains in leaching environments. In: Biohydrometallurgical Processing, Vol II (Jerez CA, T Vargas, H Toledo and JV Wiertz, eds), pp 9–17, University of Chile Press, Santiago.
41 Rawlings DE. 1997. Mesophilic, autotrophic, bioleaching bacteria: description, physiology and role. In: Biomining: Theory, Microbes and Industrial Processes (Rawlings DE, ed), pp 229–245, RG Landes and Springer-Verlag, Berlin.
42 Rawlings DE and T Kusano. 1994. Molecular genetics of *Thiobacillus ferrooxidans*. Microbiol Rev 58: 39–55.
43 Rawlings DE and S Silver. 1995. Mining with microbes. Bio/Technology 13: 773–778.
44 Rawlings DE and DR Woods. 1995. Development of improved biomining bacteria. In: Bioextraction and Biodeterioration of Metals (Gaylarde CG and HA Videla, eds), pp 63–84, Cambridge University Press, Cambridge.
45 Schippers A, R Hallmann, S Wentzien and W Sand. 1995. Microbial diversity in uranium waste heaps. Appl Environ Microbiol 61: 2930–2935.
46 Schnell HA. 1997. Bioleaching of copper. In: Biomining: Theory, Microbes and Industrial Processes (Rawlings DE, ed), pp 21–43, RG Landes and Springer-Verlag, Berlin.
47 Sugio T, C Domatsu, O Munakata, T Tano and K Imai. 1985. Role of a ferric ion-reducing system in sulfur oxidation of *Thiobacillus ferrooxidans*. Appl Environ Microbiol 49: 1401–1406.
48 Touvinen OH, SI Niemelä and HG Gyllenberg. 1971. Tolerance of *Thiobacillus ferrooxidans* to some metals. Antonie van Leeuwenhoek 37: 489–496.
49 Touvinen OH, SI Niemelä and HG Gyllenberg. 1971. Effect of mineral nutrients and organic substances on the development of *Thiobacillus ferrooxidans*. Biotech Bioeng 13: 517–527.
50 van Aswegen PC, MW Godfrey, DM Miller and AK Haines. 1991. Developments and innovations in bacterial oxidation of refractory ores. Min Metallurg Proc 8: 188–192.
51 Vásquez M and RT Espejo. 1997. Chemolithotrophic bacteria in copper ores leached at high sulfuric acid concentration. Appl Environ Microbiol 63: 332–334.
52 Whitlock JL. 1997. Biooxidation of refractory gold ore, the Geobiotics process. In: Biomining: Theory, Microbes and Industrial Processes (Rawlings DE, ed), pp 117–127, RG Landes and Springer-Verlag, Berlin.
53 Yamanaka T and Y Fukumori. 1995. Molecular aspects of the electron transfer system which participates in the oxidation of ferrous iron by *Thiobacillus ferrooxidans*. FEMS Microbiol Rev 17: 401–413.