Extremophiles (2000) 4:71–76

MINI-REVIEW

Paul R. Norris · Nicolas P. Burton · Nathalie A.M. Foulis

Acidophiles in bioreactor mineral processing

Received: July 11, 1999 / Accepted: December 27, 1999

Abstract Mineral processing in bioreactors has become established in several countries during the past decade with industrial application of iron- and sulfur-oxidizing bacteria to release occluded gold from mineral sulfides. Cobalt extraction in bioreactors has also been commercialized, and development of high-temperature biooxidation of copper sulfides has reached pilot-plant scale. A variety of potentially useful mineral sulfide-oxidizing thermophiles have been recognized, but the most active strains have not been fully characterized. Progress continues to be made in identifying key organisms in mineral sulfide oxidation and in identifying factors that influence their activities and their relative numbers in mixed cultures, where elements of competition and mutually beneficial interactions have been observed. A summary of mineral-processing bioreactors and cultures used in industry is presented, followed by discussion of recent microbiological and process developments with thermoacidophiles that could expand the range of mineral sulfides processed commercially using microorganisms.

Key words Mineral-processing bioreactors · Thermoacidophilic Archaea

Introduction

The development of industrial mineral processing in bioreactors has utilized mineral sulfide-oxidizing acidophiles (Rawlings 1997). Many of the described species have been found in both natural and industrial environments, for example, in proximity to sulfurous geothermal springs and at mine sites that have acidic drainage. The heterogeneous conditions in such environments with gradients of temperature and acidity support a wide diversity of acidophiles. The most useful mixed cultures or individual strains can be selected for their oxidative capacities and their adaptability with regard to industrial mineral-processing conditions.

Communicated by K. Horikoshi

P.R. Norris (✉) · N.P. Burton · N.A.M. Foulis
Department of Biological Sciences, University of Warwick, Coventry CV4 7AL, UK
Tel. +44 24 76 523733; Fax +44 24 76 523701
e-mail: P.R.Norris@warwick.ac.uk

Commercial bioreactors

The successful operation of mineral-processing bioreactors at the Fairview mine in South Africa led to commissioning of several similar plants in the early 1990s (Table 1). In these reactors, gold has been liberated from sulfides (arsenopyrite, pyrite, and pyrrhotite) before its downstream recovery by cyanidation. Several modules of reactors in series at the largest plant have treated approximately 1000 tonnes of refractory gold concentrates per day (Table 1). Commercial extraction of less valuable metals has begun with bacterial oxidation of cobaltiferous pyrite in the largest reactors in use for mineral bioprocessing with tank volumes of 1350 m^3 (d'Hugues et al. 1999; Briggs and Millard 1997). The development of bioreactor processes for nickel and copper extraction from mineral sulfide flotation concentrates has reached pilot-plant scale (Dew et al. 1999). Most industrial plants for biooxidation of gold-bearing concentrates have been operated at about 40°C with mixed cultures of mesophiles and, in some cases, thermotolerant organisms. Moderately thermophilic bacteria were utilized at the Youanmi plant at about 50°C (Table 1) (Miller 1997). The potential of thermophilic Archaea in copper extraction has attracted particular interest following recognition that the most efficient oxidation of mineral sulfides that are most recalcitrant to dissolution can be achieved using organisms which tolerate the most extreme conditions (Brierley and Brierley 1986; Norris 1990).

Table 1. Mineral-processing bioreactors operating in the early 1990s

Location	Operating period	Tonnes per day	Total capacity (m^3)
Fairview, South Africa	1986–1991	10	
	1991–	40	900
São Bento, Brazil	1990–1995	150	
	1995–	110	1160
Harbour Lights, Australia	1991–1993	40	
Wiluna, Australia	1993–1996	115	
	1996–	152	4240
Youanmi, Australia	1994–1998	120	~3000
Obuasi, Ghana	1994–1995	720	
	1995–	1152	21360

Sources: D. Dew, Billiton Process Research (personal communication); Dew et al. (1997)

Bioreactor microflora

The mesophilic *Thiobacillus ferrooxidans* was the focus of early studies of iron- and sulfur-oxidizing bacteria that could be applied to mineral processing. However, another iron-oxidizing acidophile, *Leptospirillum ferrooxidans*, has been increasingly studied since its capacity to grow more successfully than *T. ferrooxidans* in mixed cultures on pyrite concentrates was observed (Norris and Kelly 1982; Helle and Onken 1988). Phylogenetic inference from 16*S* rRNA sequence data groups *L. ferrooxidans* with *Nitrospira* species and not among the Protobacteria with *T. ferrooxidans* (B.M. Goebel, personal communication). Competition between these iron-oxidizing bacteria has been reviewed (Norris 1990; Rawlings et al. 1999a); factors cited in favor of *L. ferrooxidans* include its greater tolerance of acidity, greater resistance to inhibition by ferric iron, and greater thermotolerance. *Leptospirillum ferrooxidans* outnumbers *T. ferrooxidans* in the bioreactors at the Fairview mine (see Table 1) (Lawson 1997). The relative numbers and distribution of these bacteria in mine drainage (Walton and Johnson 1992; Schrenk et al. 1998) and in an in situ bioleaching operation (Hallman et al. 1992) have also been related to localized ferrous/ferric iron ratios, acidity, and temperature. Although individual bioreactors represent a relatively homogeneous environment compared to these sites, the operation of reactors in series with progressive oxidation of substrate ensures variable concentrations of iron and acid and consequently different ratios of acidophiles in primary or secondary tanks (Lawson 1997). *Thiobacillus thiooxidans*, which is incapable of pyrite degradation in pure culture, can utilize the sulfide moiety of the mineral when it is released by the action of iron-oxidizing *L. ferrooxidans* (Norris 1983). *Thiobacillus thiooxidans* initially shared attention with *T. ferrooxidans* in the study of sulfur oxidation in bioleaching of metals, but just as *L. ferrooxidans* has become recognized alongside *T. ferrooxidans* for iron oxidation, *Thiobacillus caldus* (Hallberg and Lindström 1994) rather than *T. thiooxidans* has become recognized as the significant sulfur-oxidizing bacterium in mineral-processing bioreactors that are operated at about 40°C (Rawlings et al. 1999b). The dominance of *T. caldus* reflects its thermotolerance with a growth rate that exceeds that of *T. thiooxidans* at temperatures above about 30°C (Norris et al. 1986).

The species of moderately thermophilic bacteria that were used in commercial bioreactors at about 50°C (Miller 1997) were not defined. *Sulfobacillus* species are the most studied moderately thermophilic, mineral sulfide-oxidizing acidophiles (Golovacheva and Karavaiko 1979; Norris et al. 1996). However, analysis of cultures from laboratory reactors processing mineral sulfides at about 50°C have indicated that *Acidimicrobium ferrooxidans* (Clark and Norris 1996) can outnumber *Sulfobacillus* species, while *T. caldus* is the dominant organism overall (A. Cleaver and P. Norris, unpublished data; noted in Dew et al. 1999).

The potential of thermophilic mineral sulfide-oxidizing Archaea for biomining was evident enough to be reviewed in 1986 (Brierley and Brierley), but pilot-scale development work has only recently made progress toward their industrial application in bioreactors. The principal target of high-temperature biooxidation of mineral sulfides is the dissolution of chalcopyrite to release copper. Comparison of chalcopyrite oxidation by mesophiles, moderate thermophiles, and thermoacidophilic Archaea has shown the efficiency of extraction to increase roughly in proportion to temperature up to about 80°C (Norris 1990; Norris and Owen 1993). Comparison of mineral sulfide oxidation by different *Sulfolobus*-like Archaea has considered their relative capacities for mineral oxidation (Norris and Owen 1992, 1993). *Sulfolobus metallicus* (optimum activity at about 70°C, and referred to as *Sulfolobus* strain BC in early studies) and *Metallosphaera sedula* showed greater capacity for mineral dissolution than *Acidianus brierleyi*, but the most rapid oxidation of pyrite and most efficient extraction of copper from chalcopyrite were obtained with an uncharacterized mixed culture of *Sulfolobus*-like organisms capable of optimum activity at 80°–85°C. The nature of uncharacterized cultures that grow optimally at about 80°C and their capacity for copper extraction in continuous bioreactors is considered further.

Diversity of high-temperature strains in pyrite enrichment cultures

It has so far been difficult to grow some of the autotrophic, iron-oxidizing thermoacidophilic Archaea on solid media, which has hindered their purification and characterization. The mineral oxidation capacities of cultures at the high temperatures required for efficient extraction of copper from chalcopyrite have mostly been assessed with mixed enrichment cultures.

The establishment of pyrite enrichment cultures at about 80°C has been used to preclude survival of *Sulfolobus metallicus*, which has dominated enrichment cultures established at about 70°C with samples from various natural and industrial acidic environments (unpublished observations). Initial evidence for diversity within mineral-oxidizing

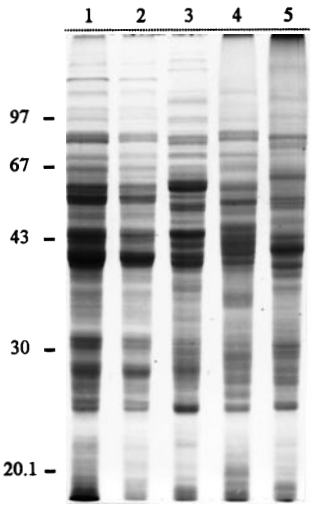

Fig. 1. SDS-PAGE of whole-cell proteins of *Sulfolobus*-like organisms grown on pyrite at 78°C. Enrichment cultures were established with samples from Iceland (*lane 1*), Montserrat (*lane 2*), United States (*lane 3*), and Java (*lane 4*). A pure culture (strain J1; *lane 5*) was isolated from a Javan enrichment culture

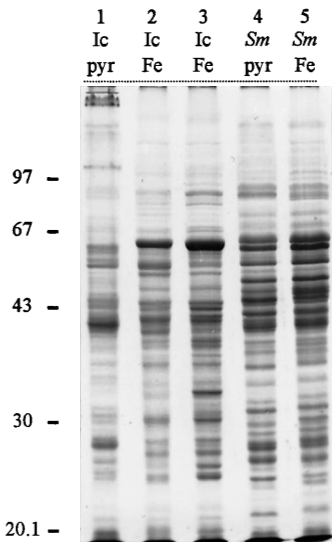

Fig. 2. SDS-PAGE of whole-cell proteins of *Sulfolobus*-like organisms. Enrichment cultures of a sample from Iceland (*Ic*) were grown at 78°–80°C on pyrite (*pyr*, *lane 1*) and on ferrous iron (*Fe*) in the absence (*lane 2*) and presence (*lane 3*) of yeast extract. *Sulfolobus metallicus* (*Sm*) was grown on pyrite (*lane 4*) or ferrous iron (*lane 5*) at 70°C

enrichment cultures at 80°C has come from comparative SDS-PAGE of whole-cell proteins (Fig. 1). Some of the water–sediment samples from geothermal sites that were used to establish these enrichment cultures samples were not collected by the authors and there are no details of the precise sample sites, but these were geographically widespread (hot springs at Krísuvík, Iceland; in Yellowstone National Park, United States; two different hot springs at Bandung, Java; and from Galway's and Upper Gages Soufrieres, Montserrat). Some enrichment cultures (e.g., cultures from Iceland and Montserrat) gave different electrophoretic protein profiles soon after the cultures were established but showed almost identical patterns after serial subculture under the same conditions for several years (see Fig. 1, lanes 1 and 2). In contrast, protein profiles of cultures originating from Yellowstone National Park and Java have remained relatively stable for several years and are different from each other and the Iceland/Montserrat enrichment culture profiles (Fig. 1), which has indicated that a variety of organisms are active in mineral sulfide oxidation at high temperature.

The diversity of strains within particular enrichment cultures as opposed to between them is not yet clear, but at least three species have been observed in one culture (data not shown). The mixed nature of some of the cultures has been indicated by comparative PAGE of cell extracts after growth with different substrates. For example, serial culture of the enrichment from Iceland in medium that contained ferrous iron instead of pyrite as the substrate resulted in many differences in the whole-cell protein profiles (Fig. 2, lanes 1 and 2), indicating selection of a strain that was probably a minor component of the pyrite enrichment culture. The profiles of major cell proteins of pure cultures of these thermoacidophiles do not change greatly with this substrate change, as illustrated with *S. metallicus* grown on pyrite or ferrous iron (Fig. 2, lanes 4 and 5). Further examples of diversity among these thermoacidophiles have come from isolation in pure culture from a Javan enrichment culture of an iron-oxidizing strain with a protein profile distinct from those of enrichment cultures from the same area (see Fig. 1, lanes 4 and 5) and from sequencing of ribosomal RNA genes from environmental samples (see following).

Phylogeny of mineral-oxidizing thermoacidophilic Archaea

In the absence of distinctive phenotypic characteristics that might indicate its closest relatives, the Javan isolate obtained in pure culture as noted earlier has been provisionally designated as a species of *Metallosphaera* to reflect its phylogenetic proximity to the other species of this genus (Fig. 3). The lack of definitive physiological or biochemical characteristics that might allow the grouping of previously undescribed isolates within particular subgroups of the *Sulfolobales* (Fuchs et al. 1996) has been reflected in the appearance of *Sulfolobus* species on various branches of the thermoacidophile evolutionary tree (Fig. 3). The current appreciation of the diversity of *Sulfolobus*-like organisms is based largely on the study of strains that have been readily isolated in the laboratory. The finding of novel sequences of *Sulfolobus*-like rRNA genes that were most readily cloned from DNA extracted directly from Montserrat hot spring samples (Burton and Norris, unpublished work; clones MS1 and MS2, Fig. 3) might indicate that some strains make the transition to the laboratory less readily than others or that their optimum growth conditions have not yet been replicated. The relative phylogenetic

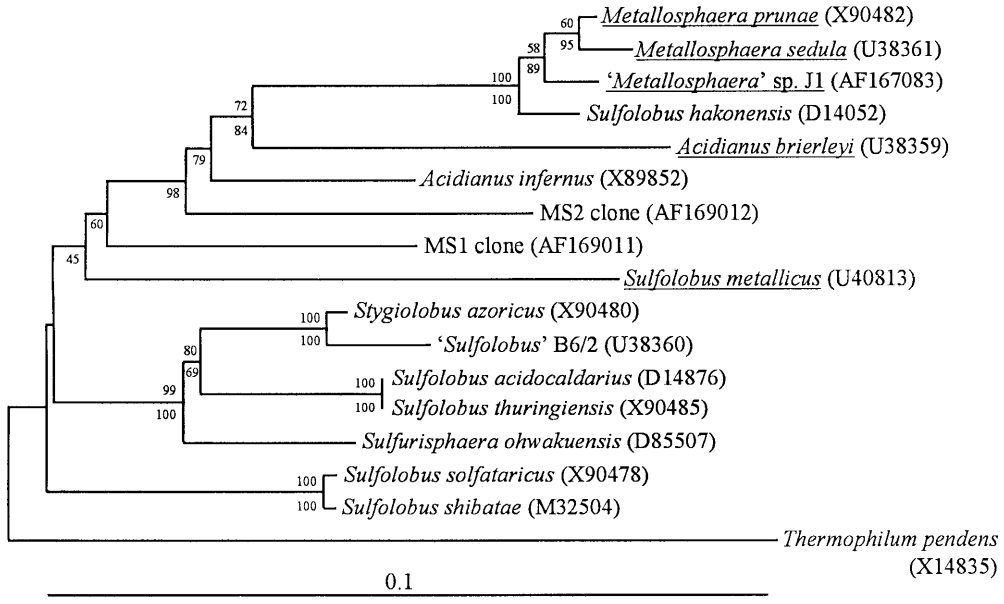

Fig. 3. An unrooted phylogenetic distance tree showing the relationship between 16*S* rRNA gene sequences of thermophilic Archaea (GenBank sequence accession numbers in *brackets*). Strains known to oxidize ferrous iron and pyrite are *underlined*. MS1 and MS2 are sequences of rRNA genes that were cloned from Montserrat hot spring sample DNA. "*Metallosphaera*" sp. J1 was isolated from a Javan enrichment culture (see text) but has not been fully characterized. Bootstrap values at nodes are from 100 estimations using maximum parsimony (*above the lines*) and distance methods (*below the lines*). The *scale bar* represents 0.1 substitutions per nucleotide position. Analysis used the PILEUP programme of GCG (Genetics Computer Group, University of Wisconsin) and DNADIST, DNAPARS, and FITCH programmes of PHYLIP version 3.57 (Felsenstein 1995)

placement of these novel sequences and those of *Acidianus infernus* and *Sulfolobus metallicus* is currently uncertain, with low bootstrap values (Fig. 3).

The capacity for iron and mineral sulfide oxidation among these thermoacidophiles might be restricted to a major phylogenetic subdivision of the order (Fig. 3) but is nevertheless found in several distinct evolutionary branches, which reflects the varied potential of strains for efficient mineral sulfide oxidation under industrial conditions. The more widespread distribution of the capacity for sulfur oxidation among the thermoacidophiles increases the possibility that mineral processing at high temperatures might involve mixed cultures of iron- and sulfur-oxidizing species from different genera, in a similar fashion to the bacterial combinations seen at lower temperature and noted earlier (for example, *T. caldus* with *L. ferrooxidans*). In addition to the capacities of iron and sulfur oxidation either in a single strain or in a mixed culture, application of the extreme thermophiles will require strains with tolerance of high concentrations of metals in solution and of agitation in the presence of high concentrations of mineral solids.

Mineral oxidation at high temperature in continuous culture

The enrichment cultures from the United States, Iceland, Java, and Montserrat noted earlier have all been used to oxidize mineral sulfides at high temperature and, as might be expected from the indications of strain diversity in the cultures, there were differences in mineral oxidation rates and efficiencies (unpublished data). A demonstration of the capacity of one of the enrichment cultures to maintain extraction of copper from chalcopyrite in laboratory continuous reactors is presented (Fig. 4). The steady-state pH was 1.3. The inoculum in this case was from another continuous reactor that was fed continuously with chalcopyrite for more than 30 days with a 3-day residence time. Details of the metal extraction performance are not considered here, but the attainment of roughly stable concentrations of metals in solution indicated continuous growth and mineral dissolution and a noteworthy tolerance of high metal ion concentrations. The concentration of copper in solution in such experiments varies with the mineral feed concentration and the copper content of the mineral concentrate. The 10% w/v mineral feed would ideally be more concentrated for a commercial process, but the limited tolerance of the thermoacidophilic Archaea of high solid concentrations is an area of concern (Lindström et al. 1993; Norris 1997; Nemati and Harrison 1999). Some *Sulfolobus*-like thermoacidophiles are inhibited by a few grams of copper per liter (Huber et al. 1989; Miller et al. 1992), but the tolerance shown in Fig. 4 of more than 20 g copper/l has been exceeded in other experiments in which the tolerance has approached that of *S. metallicus* at more than 40 g copper/l (data not shown).

The efficiency of copper extraction from chalcopyrite by thermoacidophiles is influenced by the characteristics of individual mineral concentrates, but typical results with laboratory-scale reactors (see Fig. 4) operated in series have indicated between 40% and 60% copper extraction in

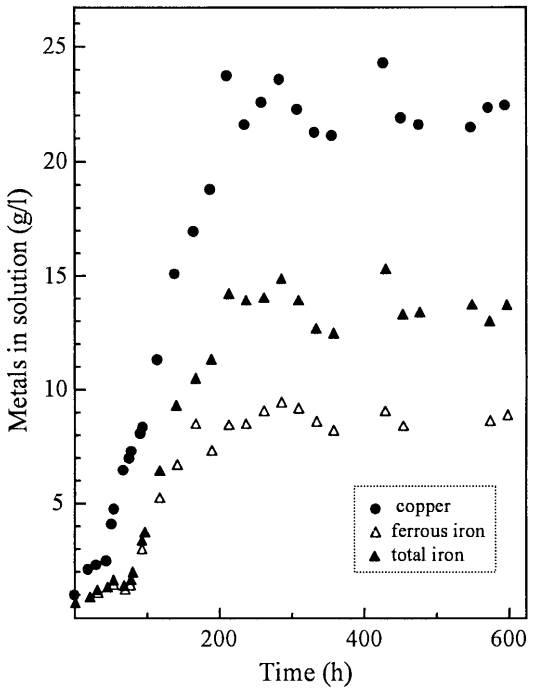

Fig. 4. Metals in solution during continuous oxidation of a chalcopyrite concentrate (27% w/w copper) by a mixed culture of autotrophic thermoacidophiles at 78°C in a stirred tank reactor. The culture was established as a batch culture with phased mineral additions until the medium flow and a 10% w/v mineral feed were started after 116 h, giving a residence time thereafter of 3 days

primary reactors and more than 90% extraction in secondary reactors with overall residence times of about 6 days (data not shown). The use of similar cultures in pilot-scale trials has confirmed their capacity to perform successfully under industrial conditions and increased the likelihood of the development of mineral processing by thermoacidophilic Archaea at industrial scale (Dew et al. 1999). Specific 16*S* rRNA probes have been used successfully to indicate the predominant species in a mixed culture in a high-temperature, mineral-processing reactor in the laboratory (N. Burton, D. Clark, and P. Norris, unpublished data), and it is anticipated that their use at larger scale will facilitate process understanding and development, at least from the microbiological perspective.

Acknowledgments Original work included in this article was funded by the Biotechnology and Biological Sciences Research Council and the Natural Environment Research Council. Samples from Java were kindly provided via Dr. R.A.D. Williams.

References

Brierley JA, Brierley CL (1986) Microbial mining using thermophilic microorganisms. In: Brock TD (ed) Thermophiles: general, molecular and applied microbiology. Wiley, New York, pp. 279–305

Briggs AP, Millard M (1997) Cobalt recovery using bacterial leaching at the Kasese project, Uganda. In: International biohydrometallurgy symposium/biomine 97. Australian Mineral Foundation, Glenside, pp. M2.4.1–M2.4.12

Clark DA, Norris PR (1996) *Acidimicrobium ferrooxidans* gen. nov., sp. nov.: mixed-culture ferrous iron oxidation with *Sulfobacillus* species. Microbiology 142:785–790

Dew DW, Lawson EN, Broadhurst JL (1997) The BIOX® process for biooxidation of gold-bearing ores or concentrates. In: Rawlings DE (ed) Biomining. Springer, Berlin, pp. 46–80

Dew DW, van Buren C, McKewan K, Bowker C (1999) Bioleaching of base metal sulphide concentrates: a comparison of mesophile and thermophile bacterial cultures. In: Amils R, Ballester A (eds) Biohydrometallurgy and the environment toward the mining of the 21st century, part A. Elsevier, Amsterdam, pp. 229–246

d'Hugues P, Cezac P, Battaglia F, Morin D (1999) Bioleaching of a cobaltiferous pyrite at 20% solids: a continuous laboratory scale study. In: Amils R, Ballester A (eds) Biohydrometallurgy and the environment toward the mining of the 21st century, part A. Elsevier, Amsterdam, pp. 167–176

Felsenstein J (1995) PHYLIP (Phylogeny Inference Package), version 3.57c. Department of Genetics, University of Washington, Seattle

Fuchs T, Huber H, Burggraf S, Stetter KO (1996) 16S rDNA-based phylogeny of the archaeal order of *Sulfolobales* and reclassification of *Desulfurolobus ambivalens* as *Acidianus ambivalens* comb. nov. Syst Appl Microbiol 19:56–60

Golovacheva RS, Karavaiko GI (1979) *Sulfobacillus* – a new genus of spore-forming thermophilic bacteria. Microbiology (Mikrobiologiya) 48:658–665

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

Hallman R, Friedrich A, Koops H-P, Pommerening-Röser A, Rohde K, Zenneck C, Sand W (1992) Physiological characteristics of *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans* and physicochemical factors influence microbial metal leaching. Geomicrobiol J 10:193–206

Helle U, Onken U (1988) Continuous microbial leaching of a pyritic concentrate by *Leptospirillum*-like bacteria. Appl Microbiol Biotechnol 28:553–558

Huber G, Spinnler C, Gambacorta A, Stetter KO (1989) *Metallosphaera sedula* gen. and sp. nov. represents a new genus of aerobic, metal-mobilizing, thermoacidophilic archaebacteria. Syst Appl Microbiol 12:38–47

Lawson EN (1997) The composition of mixed populations of leaching bacteria active in gold and nickel recovery from sulphide ores. In: International biohydrometallurgy symposium/biomine 97. Australian Mineral Foundation, Glenside, pp. QP4.1–QP4.10

Lindström EB, Wold S, Kettaneh-Wold N, Saaf S (1993) Optimization of pyrite bioleaching using *Sulfolobus acidocaldarius*. Appl Microbiol Biotechnol 38:702–707

Miller KW, Risanico SS, Risatti JB (1992) Differential tolerance of *Sulfolobus* strains to transition metals. FEMS Microbiol Lett 93:69–74

Miller PC (1997) The design and operating practice of bacterial oxidation plant using moderate thermophiles (the Bactech Process). In: Rawlings DE (ed) Biomining. Springer, Berlin, pp. 81–102

Nemati M, Harrison STL (1999) Effects of solid particles on thermophilic bioleaching of mineral sulphides. In: Amils R, Ballester A (eds) Biohydrometallurgy and the environment toward the mining of the 21st century, part A. Elsevier, Amsterdam, pp. 473–482

Norris PR (1983) Iron and mineral oxidation studies with *Leptospirillum*-like bacteria. In: Rossi G, Torma AE (eds) Recent progress in biohydrometallurgy. Associazione Mineraria Sarda, Iglesias, pp. 83–96

Norris PR (1990) Acidophilic bacteria and their activity in mineral sulfide oxidation. In: Ehrlich HL, Brierley CL (eds) Microbial mineral recovery. McGraw-Hill, New York, pp. 3–27

Norris PR (1997) Thermophiles and bioleaching. In: Rawlings DE (ed) Biomining. Springer, Berlin, pp. 248–258

Norris PR, Kelly DP (1982) The use of mixed microbial cultures in metal recovery. In: Bull AT, Slater JH (eds) Microbial interactions and communities. Academic Press, London, pp. 443–474

Norris PR, Owen JP (1992) Strain selection for high temperature oxidation of mineral sulfides in reactors. In: Ladisch MR, Bose A (eds) Harnessing biotechnology for the 21st century. American Chemical Society, Washington, DC, pp. 445–448

Norris PR, Owen JP (1993) Mineral sulphide oxidation by enrichment cultures of novel thermoacidophilic bacteria. FEMS Microbiol Rev 11:51–56

Norris PR, Marsh RM, Lindström EB (1986) Growth of mesophilic and thermophilic acidophilic bacteria on sulfur and tetrathionate. Biotechnol Appl Biochem 8:318–329

Norris PR, Clark DA, Owen JP, Waterhouse S (1996) Characteristics of *Sulfobacillus acidophilus* sp. nov. and other moderately thermophilic mineral sulphide-oxidizing bacteria. Microbiology 142:775–783

Rawlings DE (1997) (ed) Biomining. Springer, Berlin

Rawlings DE, Tributsch H, Hansford GS (1999a) Reasons why "*Leptospirillum*"-like species rather than *Thiobacillus ferrooxidans* are the dominant iron-oxidizing bacteria in many commercial processes for the biooxidation of pyrite and related ores. Microbiology 145:5–13

Rawlings DE, Coram NJ, Gardner MN, Deane SM (1999b) *Thiobacillus caldus* and *Leptospirillum ferrooxidans* are widely distributed in continuous flow biooxidation tanks used to treat a variety of metal containing ores and concentrates. In: Amils R, Ballester A (eds) Biohydrometallurgy and the environment toward the mining of the 21st century, part A. Elsevier, Amsterdam, pp. 777–786

Schrenk MO, Edwards KJ, Goodman RM, Hamers RJ, Banfield JF (1998) Distribution of *Thiobacillus ferrooxidans* and *Leptospirillum ferrooxidans*: implications for generation of acid mine drainage. Science 279:1519–1522

Walton KC, Johnson DB (1992) Microbiological and chemical characteristics of an acidic stream draining a disused copper mine. Environ Pollut 76:169–175