

Review

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The microbiology of biomining: development and optimization of mineral-oxidizing microbial consortia

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Biomining, the use of micro-organisms to recover precious and base metals from mineral ores and concentrates, has developed into a successful and expanding area of biotechnology. While careful considerations are made in the design and engineering of biomining operations, microbiological aspects have been subjected to far less scrutiny and control. Biomining processes employ microbial consortia that are dominated by acidophilic, autotrophic iron- or sulfur-oxidizing prokaryotes. Mineral biooxidation takes place in highly aerated, continuous-flow, stirred-tank reactors or in irrigated dump or heap reactors, both of which provide an open, non-sterile environment. Continuous-flow, stirred tanks are characterized by homogeneous and constant growth conditions where the selection is for rapid growth, and consequently tank consortia tend to be dominated by two or three species of micro-organisms. In contrast, heap reactors provide highly heterogeneous growth environments that change with the age of the heap, and these tend to be colonized by a much greater variety of micro-organisms. Heap micro-organisms grow as biofilms that are not subject to washout and the major challenge is to provide sufficient biodiversity for optimum performance throughout the life of a heap. This review discusses theoretical and pragmatic aspects of assembling microbial consortia to process different mineral ores and concentrates, and the challenges for using constructed consortia in non-sterile industrial-scale operations.

Background

The use of micro-organisms to facilitate the extraction and recovery of precious and base metals from primary ores and concentrates, referred to generically as 'biomining', has developed into a successful and expanding area of biotechnology (Rawlings & Johnson, 2007). In contrast to most other industries that use microbial processes (such as fermentation technologies and drug production), the selection, control and monitoring of the microbial cultures in biomining has often been either minimal or non-existent. The question arises, therefore, as to whether the microbial populations in commercial operations are the most suitable and efficient consortia (and the most effective strains and species) that could be used for processing different minerals. Even if superior microbial consortia were to be identified in laboratory investigations, there is the challenge of how populations of mineral-oxidizers could be modified and controlled, particularly within heap leaching operations. Moreover, there is considerable debate regarding how an optimal microbial consortium for the biooxidation of a particular mineral under a given set of operation conditions (e.g. temperature or pH) might be developed. This debate includes whether there is much advantage in importing an active consortium from elsewhere compared with selecting

a consortium from indigenous micro-organisms found in the locality of a deposit.

In this article we discuss many of the theoretical considerations concerning the development of microbial cultures for continuous-flow stirred-tank reactors and describe how these are significantly different in the case of heap reactors.

Engineered configurations for biomining

Engineering options for biomining have evolved from relatively inexpensive, partly controlled, irrigated dump or heap reactors to sophisticated, highly controlled and expensive stirred-tank reactors. Dump or heap reactors range from randomly packed, low-efficiency dumps to carefully designed heaps that are stacked, aerated, irrigated and sometimes thermally insulated for higher levels of mineral leaching efficiency. Dump and heap reactors are typically used for leaching low-grade, run-of-mine rock that would otherwise be discarded (used widely for copper ores), or for low-value mineral ores that do not allow for the use of expensive reactors. Stirred-tank reactors consist of a series of aerated continuous-flow tanks that are used mostly in a pretreatment process for the recovery of high-value metals,

such as gold, from mineral concentrates. These reactors are more expensive to construct and operate than heap reactors but allow for the precise control of parameters such as temperature, pH and aeration, all of which have a major impact on the microbial populations and metal recovery efficiency. Details of these different engineering configurations and variants on them, such as the coating of inert rock particles with high-value sulfide concentrates in heap reactors, can be found in various reviews (e.g. Rawlings *et al.*, 2003; Harvey & Bath, 2007). Mineral heaps and stirred tanks provide very different environments and challenges for mineral-leaching micro-organisms, and different 'optimal' populations might be expected to emerge with similar target minerals depending on the reactor used.

Biomining processes provide a highly specialized growth environment

Irrespective of whether tank or heap processes are used, the micro-organisms that catalyse biomining processes are required to grow in an essentially inorganic, aerobic, low-pH environment. The most important micro-organisms are therefore autotrophic and, although the exact nature of the energy sources may vary from mineral to mineral, they grow by oxidizing reduced forms of sulfur or ferrous iron (or both). The pH within tanks and heaps may also vary, but is highly acidic and typically within the range 1.5 to 2.0. The characteristics of biomining micro-organisms have been reviewed in detail elsewhere (e.g. Rawlings, 2005; Hallberg & Johnson, 2001) but the rather extreme conditions in stirred tanks and heaps mean that the number of micro-organisms that are likely to play a major role in biomining processes is limited.

Characteristics of mineral degradation in stirred-tank reactors

The environment in a mineral-biooxidation continuous-flow stirred-tank reactor is highly homogeneous as it is operated at a set pH and temperature and with controlled aeration. However, conditions (such as concentrations of soluble metals and metalloids) will vary in a continuous-flow series of tanks as mineral oxidation becomes increasingly extensive, and this can have a significant impact on diversity and numbers of indigenous microbial species (e.g. Okibe *et al.*, 2003). The homogeneity within an individual tank results in a limited ecological niche that tends often to be dominated by two to four species, although smaller numbers of other micro-organisms may be present (Table 1). For example, Mikkelsen *et al.* (2006) found that the microbial populations in thermophilic (78 °C) stirred tanks leaching chalcopyrite were entirely archaeal (as would be predicted from the known thermotolerance of acidophilic prokaryotes) and comprised relatively few species of the order *Sulfolobales* (Table 1).

Stirred-tank reactors possess three major advantages that are also shared by sewage treatment but by very few other industrial processes. These advantages are linked and together result in the selection of highly efficient microbial consortia. Firstly, the process operates in continuous-flow mode. The advantage derived from continuous-flow operation is that it results in the continual selection for those micro-organisms that are able to grow most efficiently in the tanks. The most efficient growers will be subject to less cell washout and therefore dominate the microbial population. Secondly, the objective of the process is to degrade the substrate (mineral) as rapidly as possible. The mineral

Table 1. Acidophilic prokaryotes identified in stirred-tank mineral bioleaching and biooxidation operations

Mineral concentrate	T (°C)	Prokaryotes identified	Reference
Zinc/lead pyrite	35–40	<i>Leptospirillum ferrooxidans</i> *, <i>Acidithiobacillus thiooxidans</i> †, <i>Acidiphilium cryptum</i> ‡, <i>Acidithiobacillus ferrooxidans</i> ‡	Goebel & Stackebrandt (1994)
Pyrite/arsenopyrite (gold Biox® culture)	40	<i>L. ferrooxidans</i> *, <i>At. thiooxidans</i> †, <i>At. ferrooxidans</i>	Dew <i>et al.</i> (1997)
Cobaltiferous pyrite	35	<i>L. ferrooxidans</i> , <i>At. thiooxidans</i> , <i>Sulfobacillus thermosulfidooxidans</i>	Battaglia-Brunet <i>et al.</i> (2002)
Polymetallic (copper, zinc and iron sulfides)	45	<i>Leptospirillum ferriphilum</i> , <i>Acidithiobacillus caldus</i> , <i>Sulfobacillus</i> sp., <i>Ferroplasma acidophilum</i>	Okibe <i>et al.</i> (2003)
Pyrite, arsenical pyrite and chalcopyrite	45	<i>At. caldus</i> , <i>Sb. thermosulfidooxidans</i> , ' <i>Sulfobacillus montserratensis</i> '	Dopson & Lindström (2004)
Chalcopyrite	78	(<i>Sulfolobus shibitae</i> §), (<i>Sulfurisphaera ohwakuensis</i> §), <i>Stygiolobus azoricus</i> §, <i>Metallosphaera</i> sp.§, <i>Acidianus infernus</i> §	Mikkelsen <i>et al.</i> (2006)

**L. ferrooxidans* was almost certainly *L. ferriphilum*, as identification methods at the time did not permit the two species to be distinguished from each other.

†*At. thiooxidans* was almost certainly *At. caldus* for the same reason as footnote *.

‡These two species were found in batch tanks but not in continuous-flow tanks.

§Nearest affiliated cultivated archaea to recovered clones.

||Clones probably represent new species within the order *Sulfolobales*.

provides the energy source and some nutrients for the micro-organisms, and those organisms that are most efficient at degrading the mineral will tend to dominate the process. This means that there will be continual selection for micro-organisms that either catalyse mineral breakdown the fastest or create the conditions in which mineral breakdown occurs most rapidly. Thirdly, process sterility is not required. As the object of the process is the degradation of the mineral, it does not matter which specific organisms carry out this decomposition, and those organisms that do this most efficiently are typically the most desirable. Since the process is non-sterile there is continual selection for micro-organisms that may enter the tanks (e.g. in the concentrate feed) that are more efficient than the resident organisms. This selection includes the selection for genes present in the horizontal gene pool (e.g. genes for metal resistance) that might improve the efficiency of the resident micro-organisms (Tuffin *et al.*, 2005, 2006).

Adaptation of stirred-tank micro-organisms for efficient growth

It is well established that: (i) micro-organisms that are isolated after a period of growth in continuous-flow biooxidation tanks are very much more efficient at mineral biooxidation than the consortium that was originally placed in the tanks, and (ii) a gradual improvement in microbial consortium performance may be experienced over a period of several years before a steady state is reached after which further improvements are slow or imperceptible (Rawlings & Silver, 1995). Unfortunately, microbial consortia before and after selection have not been studied to determine to what extent the improvement in microbial performance has been due to the recruitment of new micro-organisms or the selection of more efficient cells from within the original consortium that was inoculated into the tanks. Irrespective of whether the micro-organisms originated from the inoculum or from later arrivals, it is highly likely that a substantial amount of the improvement in the efficiency of a microbial culture would have occurred during growth in the tanks. The time required for micro-organisms to adapt to efficient growth in continuous-flow tanks may have been overlooked. The theoretical argument for this is as follows.

Iron- and sulfur-oxidizing organisms that are the principal contributors to mineral decomposition are widely distributed in many natural environments. However, these environments would seldom provide the steady-state 'near-optimal' conditions of a biooxidation tank. Rather, 'wild' micro-organisms are likely to experience substantial variations in pH, day–night temperatures, the availability of energy sources, nutrients and water, and a number of other possible variables. The fittest 'wild' organisms would be expected to be those that have become adapted to survival in highly variable, 'feast and famine' conditions. Consequently, the gene regulation systems of natural isolates would be expected to have become adapted for survival under frequently adverse conditions. These micro-organisms would not have been 'tuned' for efficient growth in the

near-optimal conditions provided by stirred-tank bioreactors. This means that potentially there is considerable scope for the selection of mutants with altered gene regulation and expression that permits rapid mineral breakdown under the conditions provided by the bioreactor. This adaptation may take an extended period of time that is difficult to predict, partly due to the random nature of a sequence of mutations that improve growth efficiency. Empirical experience obtained during the adaptation of the microbial consortium to continuous growth in the gold-bearing arsenopyrite concentrate biooxidation tanks at the Fairview mine (Barberton, South Africa) in the late 1980s suggests that the time taken for consortia to reach a state in which further improvement is imperceptibly slow is protracted. The total residence time in the series of tanks was reduced from 7 to 3.5 days over a period of approximately 3 years, before being again lengthened to 4 days to provide a margin of safety against cell washout (Rawlings & Silver, 1995). The nature of the adaptations that permitted more efficient growth and a reduction in residence time is not known. This adaptation is likely to be at two levels: the enrichment of certain types of micro-organisms capable of efficient growth in the tanks followed by mutations that further enhance this efficiency. What the nature of any DNA-level adaptations may be is not yet clear and this is likely to be a fruitful, albeit challenging area for future research.

Development of biomining consortia for stirred-tank processes: 'top down' and 'bottom up' approaches

Two starting points for the development of microbial consortia for the processing of new minerals have been proposed (Fig. 1). In the first case (the 'top down' approach) a mixture of micro-organisms is used to inoculate the test material (in laboratory- or pilot-scale operations) and the assumption is made that a limited number of these acidophiles will emerge as a stable and effective bioleaching consortium. Micro-organisms to be used in this 'see-who-wins' approach can be derived either from natural environments or from biooxidation plants used to treat a related (or different) mineral that are capable

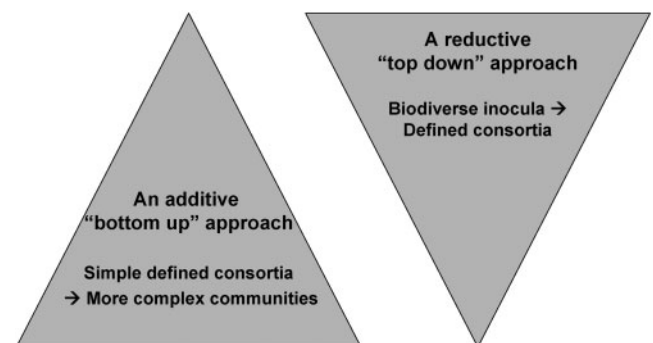


Fig. 1. Alternative experimental approaches for devising and evaluating microbial consortia for mineral bioleaching.

of growth at a broadly similar temperature and pH range to the process under development. These can be supplemented by known strains of micro-organisms from similar environments that have desirable characteristics, such as resistance to a particular metal or the ability to grow heterotrophically or mixotrophically on waste carbon metabolites (Table 2). Preliminary screening of these mixed populations on a metal ore or concentrate can be carried out in shake flasks or laboratory-scale bioreactors. To more closely simulate the industrial operation it is then necessary to assess the microbial consortia that establish on different minerals in an aerated tank (or a series of tanks) that is operated in continuous-flow mode. In this 'see-who-wins' approach the aim is to have sufficient microbial biodiversity (both physiological and phylogenetic) available in the starting material from which to adapt and select an efficient microbial consortium for use in a new mineral processing plant. An additional advantage of the 'top down' approach is that the large biodiversity afforded by the inoculum could make the bioleaching system more robust, in that it should be better able to adapt to and recover from sudden operational changes (such as interruption in pH and temperature control on stirred tanks).

The philosophy behind the 'bottom up' approach is radically different. The rationale here is to construct a 'logically designed' consortium to leach a particular ore or concentrate, on the basis of the operational parameters in the stirred tanks. Temperature, in particular, and pH will have major determinative roles, and other factors, such as potential metal and anion (e.g. chloride) toxicities, will also have a bearing on the consortium design. The bioleaching population will necessarily need to include at least one iron-oxidizer (to generate ferric iron, the main oxidant of sulfide minerals in acidic liquors) and one sulfur-oxidizer (to generate the required acidity). Given their known distribution in operating stirred tanks, *Leptospirillum ferriphilum* and *Acidithiobacillus caldus* are often selected for these roles for processes that operate in the range 35–45 °C although, as data in Table 3 indicate, these may be less important when leaching some mineral concentrates. Analyses of commercial bioleaching populations have shown, invariably, that mixotrophic or heterotrophic acidophiles are present, although usually in smaller numbers than the autotrophic iron/sulfur-oxidizers (e.g. Okibe *et al.*, 2003). These 'secondary' prokaryotes appear to have an important role in bioleaching communities in that they metabolize organic

Table 2. Microbial inocula used in a 'top down' approach to evaluate mesophilic and moderately thermophilic mineral leaching consortia

Species/strain	Physiological traits	Reference
(i) Mesophilic (30 °C) consortium		
<i>Acidithiobacillus ferrooxidans</i> ^T	Autotrophic Fe ²⁺ /S-oxidizer, Fe ³⁺ -reducer	Temple & Colmer (1951)
<i>At. ferrooxidans</i> -like (strain NO37)	Autotrophic Fe ²⁺ /S-oxidizer, Fe ³⁺ -reducer	Johnson <i>et al.</i> (2001a)
<i>Leptospirillum ferrooxidans</i> (strain CF12)	Autotrophic Fe ²⁺ -oxidizer	Coram & Rawlings (2002)
β-Proteobacterium isolate PSTR	(Autotrophic Fe ²⁺ -oxidizer)	Hallberg <i>et al.</i> (2006)
' <i>Ferrimicrobium acidiphilum</i> ' (proposed type strain)	Heterotrophic Fe ²⁺ -oxidizer, Fe ³⁺ -reducer	Johnson <i>et al.</i> (2001b)
Gram-positive iron-oxidizing isolate SLC66	Heterotrophic Fe ²⁺ -oxidizer	Johnson <i>et al.</i> (2001b)
' <i>Sulfobacillus montserratensis</i> ' (strain L15)	Mixotrophic Fe ²⁺ /S-oxidizer, Fe ³⁺ -reducer	Yahya & Johnson (2002)
<i>Thiomonas intermedia</i> (strain WJ68)	Mixotrophic Fe ²⁺ /S-oxidizer	Battaglia-Brunet <i>et al.</i> (2006)
<i>Acidithiobacillus thiooxidans</i> ^T	Autotrophic S-oxidizer	Waksman & Joffe (1921)
<i>Acidiphilium cryptum</i> -like (strain SJH)	Heterotrophic Fe ³⁺ -reducer	Hallberg & Johnson (2001)
' <i>Acidocella aromatica</i> ' (strain PFBC)	Heterotrophic Fe ³⁺ -reducer	Hallberg <i>et al.</i> (1999)
(ii) Moderately thermophilic (45 °C) consortium		
<i>Leptospirillum ferriphilum</i> (strain MT6)	Autotrophic Fe ²⁺ -oxidizer	Okibe <i>et al.</i> (2003)
<i>Acidimicrobium ferrooxidans</i> (strain TH3)	Mixotrophic Fe ²⁺ -oxidizer, Fe ³⁺ -reducer	Clark & Norris (1996)
<i>Ferropasma acidiphilum</i> (strain MT17)	Heterotrophic Fe ²⁺ -oxidizer, Fe ³⁺ -reducer	Okibe <i>et al.</i> (2003)
<i>Actinobacterium</i> isolate Y005	Heterotrophic Fe ²⁺ -oxidizer, Fe ³⁺ -reducer	Johnson <i>et al.</i> (2003)
<i>Sulfobacillus thermosulfidooxidans</i> ^T	Mixotrophic Fe ²⁺ /S-oxidizer, Fe ³⁺ -reducer	Tourova <i>et al.</i> (1994)
<i>Sulfobacillus acidophilus</i> (strain YTF1)	Mixotrophic Fe ²⁺ /S-oxidizer, Fe ³⁺ -reducer	Norris <i>et al.</i> (1996)
<i>Sulfobacillus</i> isolate BRGM2	(Mixotrophic) Fe ²⁺ /S-oxidizer	D. B. Johnson and others, unpublished
Firmicute isolate G1	(Mixotrophic Fe ²⁺ -oxidizer)	D. B. Johnson and others, unpublished
<i>Acidithiobacillus caldus</i> ^T	(Mixotrophic) S-oxidizer	Hallberg & Lindström (1994)
<i>Acidicaldus organivorans</i> ^{T*}	Heterotrophic S-oxidizer, Fe ³⁺ -reducer	Johnson <i>et al.</i> (2006)
<i>Alicyclobacillus</i> isolate Y004	Heterotrophic Fe ³⁺ -reducer	Johnson <i>et al.</i> (2003)

*Original proposed name '*Acidicaldus organivorans*' (in Johnson *et al.*, 2006).

Table 3. Bioleaching consortia established on different ores and concentrates, and derived from a moderately thermophilic 'top down' inoculum (Table 2ii) in cultures incubated at 45 °C

Ore/concentrate	Numerically dominant prokaryotes	Others detected*
Polymetallic (Ni/Cu/Mn) ore	<i>Acidimicrobium ferrooxidans</i> , <i>Acidithiobacillus caldus</i>	<i>Sulfobacillus acidophilus</i> , <i>Acidicaldus organivorans</i>
Chalcocite black shale concentrate	<i>Sulfobacillus thermosulfidooxidans</i> , <i>At. caldus</i>	
Chalcopyrite/silver concentrate	<i>Sb. thermosulfidooxidans</i>	
Cobaltiferous pyrite concentrate	<i>Leptospirillum ferriphilum</i> , <i>At. caldus</i> , <i>Am. ferrooxidans</i>	<i>Acid. organivorans</i>
Sphalerite concentrate	<i>At. caldus</i> , <i>Am. ferrooxidans</i>	

*Present at <1% of the total population.

materials (lysates, exudates, etc.) that might otherwise inhibit some of the more sensitive primary producers, from which these materials derive (Johnson & Roberto, 1997; Okibe & Johnson, 2004). One or more mixotrophic or heterotrophic acidophiles is therefore usually included in a 'logically designed' consortium and, since many of these prokaryotes (e.g. *Sulfobacillus*, *Acidimicrobium* and *Ferroplasma*) also catalyse the oxidation of iron and/or sulfur, they also contribute to mineral dissolution. Where a stirred-tank system is operating, a more appropriate starting point for the 'bottom up' approach is to determine the composition of the indigenous microflora (using a biomolecular approach) and to isolate the different micro-organisms identified on 'overlaid' solid media (Johnson *et al.*, 2005). Isolates may then be tested, both as pure cultures and in all of the possible consortium permutations, to determine relative rates of mineral oxidation, acid production, etc., and the contribution of each type of organism to the efficiency of the process. This approach is usually facilitated by the observation that small numbers of different species (typically two to four) are present in significant numbers in stirred-tank leachates (e.g. Okibe *et al.*, 2003; d'Hugues *et al.*, 2003; Mikkelsen *et al.*, 2006). An advantage of this approach is that micro-organisms that are present and which do not enhance (directly or indirectly) mineral oxidation, or possibly add to operational costs (e.g. by production of excess acid), can be identified. Once the optimum combination of 'indigenous' micro-organisms has been determined in the laboratory, others are introduced (usually one species at a time) to determine what effect these have on net mineral oxidation, as well as to find out whether they are able to establish themselves within the consortium or are eliminated, e.g. due to competition with other micro-organisms. The overall objective is to arrive at an optimum bioleaching consortium for a particular ore or concentrate. This exercise can be very protracted, although the number of candidate bacteria and archaea can be minimized by reference to the prevailing conditions (temperature, pH, etc.) in the stirred tank, and to known interactions between different acidophiles.

In both the 'top down' and 'bottom up' approaches, those micro-organisms capable of growing most efficiently on the mineral (by direct oxidation or by utilizing intermediates of

mineral oxidation) under the conditions provided will dominate the population, while other prokaryotes (for example, those which use organic compounds arising from the primary producers) may persist as minor members of the community (Table 3). It does not matter which micro-organisms eventually dominate the culture, as the objective is the efficient breakdown of the mineral rather than which micro-organisms should be used. In both approaches, the assumption is that a sufficient variety of micro-organisms is present in the initial inoculum such that after adaptation, those in the final consortium are as capable of efficient mineral decomposition as any other adapted consortium is likely to be under similar conditions.

Issues to be considered when using the 'top down' or 'bottom up' approaches

Adaptation for efficient growth versus adaptation to a mineral. Irrespective of whether the 'top down' or 'bottom up' approach is adopted, the micro-organisms to be used may be obtained from different sources. One can either take natural 'wild' micro-organisms that have been exposed to, and therefore possibly already adapted, or at least partially adapted to, the mineral, and then adapt them to efficient growth in a continuous-flow tank. Alternatively one could take micro-organisms already adapted for efficient growth from an existing continuous-flow tank and adapt them to a new mineral. The empirical observations (described above) suggest that adaptation to efficient growth may take years rather than weeks or months and that the advantage of this adaptation may be substantial. There is probably not a 'one-size-fits-all' solution as to where to source organisms for a new consortium. For example, microbial consortia dominated by *L. ferriphilum* and *At. caldus* used for gold-bearing arsenopyrite concentrates have become highly resistant to arsenic. This was found to be due to the acquisition of transposons containing genes that confer high-level arsenic resistance that are present in addition to the low-level arsenic resistance genes found in most other isolates of the same species (Tuffin *et al.*, 2005, 2006). How long it takes for such transposons to be acquired from the horizontal gene pool would be expected to vary, depending on the access that the adapting biomining consortium has

to the horizontal gene pool. If the metal resistance is readily acquired, it may be better to begin with micro-organisms capable of efficient growth and adapt them to metal resistance. If metal resistance is rare (e.g. silver resistance), it might be better to begin with an already metal-resistant culture and adapt to efficient growth even though this may be a slow process. If a new mineral concentrate to be processed is similar to a mineral for which a growth-efficient consortium is already available, it is likely to be advantageous to use this consortium and adapt it to the new mineral.

How significant an advantage a previously selected ability to grow efficiently is in the selection of micro-organisms for consortium development needs to be verified experimentally. This would include testing how easy it is for unadapted 'wild' micro-organisms to become established in a continuous-flow biooxidation tank or how much pre-adaptation is required for them to become competitive.

Is there only one ideal combination of micro-organisms? It is important to know whether, for a given process and mineral, there is only one ideal combination of micro-organisms or whether combinations of different microbial isolates (or species) are likely to be as efficient as each other once they are equally adapted to the mineral. Put differently, how likely is it that a particular consortium of micro-organisms can be completely replaced either by different strains of the same species or by an overlapping or even different combination of species without affecting the metal-leaching performance of the consortium? Evidence that it is possible to change the microbial consortium without affecting the biooxidation rate comes from a comparison of arsenopyrite biooxidation plants at the Fairview mine (South Africa) and Tamboraque (Peru). When the first five Biox plants were built by Gencor in different parts of the world, they were inoculated with consortia that had originally been adapted to growth on arsenopyrite in the stirred tanks at the Fairview mine. Under typical operating conditions the Fairview consortium is dominated by the iron-oxidizer *L. ferriphilum* and the sulfur-oxidizer *At. caldus*. However, when the biooxidation plant was commissioned at Tamboraque it was decided to use a microbial consortium adapted from acid drainage in the Coricancha Mine situated 4000 m above sea-level in Peru. After several years of operation this culture was examined and the dominant iron-oxidizer was found to be *Leptospirillum ferrooxidans* rather than *L. ferriphilum* (unpublished observations). A direct comparison between the mineral-oxidation rates of the different consortia is not exact because the arsenopyrite concentrates are not identical, but there is nothing to suggest that one consortium is noticeably less efficient than the other. This suggests that bioleaching consortia containing different micro-organisms can be adapted to be as efficient or almost as efficient as each other.

Advantages of constructing bioleaching microbial consortia. Selection of the members of a bioleaching consortium for a given mineral and process is likely to be

a labour-intensive process, but it has the advantage that only the most advantageous organisms need be included. Once the most effective consortium has been determined in the laboratory, the next challenge is to evaluate if the efficiency of that consortium will improve with selection in a continuous-flow process, and whether or not individual species will be replaced by 'wild' strains when non-sterile mineral feed is used. This will depend, in part, on the extent of adaptation of the laboratory consortium to the process before it is subjected to competition from other micro-organisms. The more highly adapted the consortium the less likely it is that unadapted 'wild' cells will displace members of the new consortium. Since stirred tanks are not sterile, it may not be possible to keep unwanted micro-organisms from contaminating the consortium inoculum.

The 'logical design' approach is likely to be particularly advantageous when mineral biooxidation conditions that are outside of typical operating conditions are required. For example, if it is required to operate a stirred-tank process at as low a pH as possible (to avoid problems of passivation with jarosites, etc.; Stott *et al.*, 2000), there might be limitation to the pH tolerance of the indigenous microbial consortium. In the past several years, novel iron- and sulfur-oxidizing micro-organisms have been discovered that may be described as 'hyper-acidophilic', in that they are active at pH < 1. Examples are *Ferroplasma* spp. (Golyshina *et al.*, 2000) and '*Sulfobacillus montserratensis*' (Yahya & Johnson, 2002). Consortia containing these more extreme acidophiles have the potential to accelerate mineral dissolution at lower pH than has been used so far at commercial scale (Yahya & Johnson, 2002). A second example is the development of consortia for stirred-tank processes that operate at temperatures of 75 °C or higher (Rawlings *et al.*, 2003). These high-temperature organisms are not as widely distributed in the environment as mesophiles and moderate thermophiles, and a logically designed consortium may be more advantageous than the 'see-who-wins' approach. High-temperature consortia are less likely to be contaminated by unwanted 'wild' micro-organisms from the environment.

Characteristics and challenges of heap reactors

The engineering design of heaps used to leach ores (and concentrates, in the case of the Geocoat process; Harvey & Bath, 2007) continues to be refined. Heaps are constructed to pre-determined dimensions using graded ores, irrigated from above with acidic liquors and aerated from below (to provide carbon dioxide required by autotrophic mineral-oxidizing micro-organisms, as well as the oxygen to promote iron- and sulfur-oxidation). However, even the most carefully engineered heap reactors are inevitably heterogeneous (both spatially and temporally), in terms of irrigation efficiency, temperature, pH, the presence of anaerobic pockets, redox potential, dissolved solutes, available nutrients, etc. This lack of homogeneity results in a large number of microenvironments compared with the relatively homogeneous environment provided by a stirred

tank. The variability in microenvironment would be expected to support a much greater diversity of mineral-oxidizing and other micro-organisms that colonize different zones and microsites within them. For example, temperatures will be determined by climatic conditions (particularly in the outer layers of a heap), exothermic chemical reactions and heat transfer (conduction, convection and radiation at the heap surface). The oxidation of sulfidic minerals is an exothermic reaction, although heat generation varies between minerals, and is related to their reactivities. Pyrrhotite (FeS) is a more reactive mineral than pyrite (FeS₂) and consequently significant heat is often generated in a pyrrhotite-rich heap, shortly after construction and commissioning. Mineral-oxidizing and other acidophilic prokaryotes often have widely different temperature optima and ranges, and may be conveniently grouped into mesophiles (20–40 °C; predominantly bacteria), moderate thermophiles (40–60 °C; bacteria and archaea) and (extreme) thermophiles (60–80 °C; predominantly archaea). In a heap reactor that experiences fluctuations in temperature, these different groups would be predicted to become more or less dominant, as temperatures increase or decline, assuming that they are present in the first place. Some prokaryotes, notably *Sulfobacillus* spp. and other *Firmicutes*, are better adapted to survive adverse conditions, such as excessively high or low temperatures, or water stress (zones and microsites within heaps may experience periodic drying, in contrast to stirred tanks) due to their ability to survive as endospores.

It may therefore be predicted that, unlike stirred tanks that are dominated by a small number of indigenous prokaryotes, heap reactors contain a much greater biodiversity, and that the dominant species will vary spatially and during different stages of the life of a heap. There have been relatively few studies on the microbiology of heap bioreactors, and some of these have analysed the liquid phases [pregnant leach solutions (PLS), raffinate, etc.] rather than the ore itself. Most studies have been on chalcocite (Cu₂S) heaps, as this copper mineral is particularly amenable to bioleaching. Microbiological data

from analysis of heap populations show that a considerable diversity of acidophiles may be present in these reactors (Table 4).

Consortium development for heap reactors

Selection for rapid cell division is not as important. There are likely to be some important differences between micro-organisms that are competitive in a heap reactor compared to those in a stirred-tank reactor. One of these is that the ability to divide rapidly so as not to be eliminated by being washed out is not likely to be important in heap reactors. This is because many bioleaching prokaryotes grow on the surface of the mineral phase in the form of a biofilm (e.g. Sand *et al.*, 1995; Sand & Gehrke, 2006) or burrow into the mineral (e.g. Rodriguez-Leiva & Tributsch, 1988; Edwards *et al.*, 2001). Micro-organisms that are attached to a mineral will not be subject to washout. Since there is less need to adapt cells to rapid cell division, micro-organisms isolated from natural environments should become established in a heap provided that they encounter a suitable niche in which they can grow competitively.

Ensuring sufficient biodiversity. As discussed above, there is likely to be a requirement for a large amount of microbial diversity within a heap reactor at different stages in its life cycle. The challenge is therefore to ensure that there is enough biodiversity within a heap to ensure its optimal performance. Should one rely on the observation that iron- and sulfur-oxidizing micro-organisms are naturally ubiquitous or should one inoculate a heap with organisms that are not likely to be present? At least part of the answer to this is a knowledge of how widely distributed different iron- and sulfur-oxidizing micro-organisms are in the environment. In general, acidophiles that grow from ambient to approximately 40–45 °C appear to be widely distributed in naturally acidic environments. Wherever a mineral containing iron and sulfur is exposed to air and water it is likely that iron- and sulfur-oxidizing organisms will establish on the mineral within a very

Table 4. Acidophilic prokaryotes identified in heap reactors

Heap type and location	Prokaryotes identified	Reference
Chalcopyrite overburden (Australia)	<i>Acidithiobacillus ferrooxidans</i> , <i>Acidithiobacillus thiooxidans</i> , <i>Acidiphilium cryptum</i>	Goebel & Stackebrandt (1994)
Copper sulfide/oxide heap (SW USA)	<i>Acidithiobacillus</i> spp., <i>Leptospirillum ferrooxidans</i> , <i>Acidiphilium</i> spp., 'Ferrimicrobium acidiphilum'	Bruhn <i>et al.</i> (1999)
Copper sulfide/oxide heap (SW USA)	<i>Sulfobacillus</i> spp. and other <i>Firmicutes</i> , 'Ferrimicrobium acidiphilum', <i>Acidisphaera</i> sp., <i>At. thiooxidans</i> , <i>At. ferrooxidans</i>	C.G. Bryan & D.B. Johnson (unpublished)
Chalcocite heap (Australia)	<i>Leptospirillum ferriphilum</i> , <i>Acidithiobacillus caldus</i> , 'Ferroplasma cupricumulans'*	Hawkes <i>et al.</i> (2006)
Run-of-mine copper heap (Chile)	<i>At. ferrooxidans</i> , <i>L. ferriphilum</i> , <i>Ferroplasma acidiphilum</i> , novel firmicutes, novel crenarchaeotes	Demergasso <i>et al.</i> (2005)

*Original proposed name (in Hawkes *et al.*, 2006) 'Ferroplasma cypraxacervatum'.

short period of time. Micro-organisms growing at 45–55 °C ('moderate thermophiles') are also widely distributed in warm to hot acidic environments. For example several *Sulfobacillus* and *At. caldus* strains have been isolated from coal spoils in the UK (Marsh & Norris, 1983) and from geothermal areas such as Yellowstone National Park, USA (Johnson *et al.*, 2003). Similarly, moderate thermophiles are often readily isolated from heap reactors (Hawkes *et al.*, 2006; C. G. Bryan & D. B. Johnson, unpublished data).

Temperature biodiversity might be greater than expected because mineral oxidation is an exothermic process and the tendency of many bioleaching micro-organisms to grow at temperatures above ambient is therefore not too surprising. However, more extremely thermophilic (60–80 °C) iron- and sulfur-oxidizers are less likely to be as ubiquitous as mesophiles and moderate thermophiles. Where extreme thermophiles are required (such as for bioleaching of chalcopyrite concentrates at high temperatures), it has been necessary to go to high-temperature iron- and sulfur-containing acid environments to isolate suitable organisms from which to adapt suitable cultures (Norris *et al.*, 2000).

Whether to inoculate, and with which micro-organisms. It is likely that there will be considerable time saving in inoculating a new heap with a microbial consortium rather than waiting for micro-organisms to grow naturally. Heap inoculation has been patented by the Newmont Mining Corporation (Denver, CO, USA). The best procedure for ensuring uniform distribution within a heap is a complex subject that will not be discussed here. Which micro-organisms to include in an inoculum is another challenge. Some operators appear to rely on natural development of micro-organisms within a heap (e.g. Nifty Copper and other operations; Plumb *et al.*, 2006) and once these have developed, cycling the drainage liquors through a new heap can serve as an inoculum. Others, such as Newmont Mining, prepare inocula containing mesophilic to moderately thermophilic bacteria (*Acidithiobacillus ferrooxidans*, *Leptospirillum* spp., *Sulfobacillus* spp.) and, because some parts of their heaps reach 80 °C, also inoculate with extremely thermophilic archaea (*Acidianus* spp., *Sulfolobus* spp. and *Metallosphaera* spp.; Logan *et al.*, 2007). This requires several inoculum preparation vessels. Although it might be possible to rely on the natural development of mesophilic and possibly moderately thermophilic micro-organisms, inoculation with extreme thermophiles is likely to be a necessity when such organisms are required. A new heap is likely to take some time before regions with temperatures as high as 80 °C develop, and how long extreme thermophiles will persist in cool regions of a heap, and the best time to inoculate with them, remains unclear.

Two-stage mineral bioprocessing: alternative approaches for metal recovery from sulfidic ores

An alternative approach for bioprocessing metal ores that has been demonstrated at both laboratory and pilot scale

involves separating ferric iron oxidation of minerals (a chemical process) and the regeneration of ferric iron (a biological process). One advantage of this 'indirect' approach is that it allows conditions for both processes to be optimized. For example, oxidation of sulfide minerals, such as chalcopyrite, may be more effective at temperatures (> 80 °C) that exceed those at which known iron-oxidizing thermoacidophilic archaea are able to grow. Ferric iron liquors, generated biologically, are heated and reacted with the target minerals (ores or concentrates), causing dissolution and release of soluble metals. The ferrous iron-rich liquor that drains the mineral phase is cooled and reoxidized in a bioreactor. The efficiency of the bioreactor in regenerating ferric iron is of major importance in these systems. Kinnunen & Puhakka (2004) developed a reactor that regenerated ferric iron at the rate of 8.2 g l⁻¹ h⁻¹, at a hydraulic retention time of 0.6 h. The bioreactor operated at 37 °C and was shown to be dominated by the chemolithotrophic bacterium *L. ferriphilum*, and *Ferroplasma*-like archaea.

Another approach being explored is to operate individual stirred tanks within a series at different temperatures (van Aswegen *et al.*, 2007). The perceived advantage of this approach relates to the biooxidation of refractory gold ores where formation of reduced inorganic sulfur compounds (RISCs) in tanks operated at conventional (~40 °C) temperatures causes problems in downstream processing of the gold-containing residues (due to the formation of thiocyanate, cyanide being used to solubilize and extract gold from oxidized concentrates). It has been found that RISCs can be completely eliminated in tanks operated at 65–80 °C, thereby significantly lowering consumption of cyanide.

Conclusions

Broadly similar types of micro-organisms are likely to be present in stirred-tank and heap reactors. However, a difference often not appreciated is that, although microbial consortia isolated from tanks that have been operating for a number of years may be of the same species as those found in the environment, they have been selected for their ability to grow efficiently. Furthermore, empirical observations suggest that adaptation to rapid growth might take years rather than weeks. When developing new inocula there is likely to be a distinct advantage to using cultures that have been pre-adapted to efficient growth rather than natural isolates. The use of unadapted micro-organisms might be necessary if a characteristic is required that no pre-adapted micro-organisms possess, such as resistance to a metal for which resistance is not commonly found. The majority of heap reactor micro-organisms attach themselves to minerals and are not easily washed out. Therefore they need not have been adapted to efficient growth.

The top down 'see-who-wins' approach is easy to apply to stirred-tank consortium development, as all that is required is sufficient biodiversity for the selection of a competitive consortium. It is also likely that more than one combination

of micro-organisms, once fully adapted, is likely to be equally efficient at biooxidizing a particular mineral. Although a lot of preparative work is required, the logically designed 'bottom up' approach has the advantage that only beneficial micro-organisms need be included. There is a danger that since mineral biooxidation processes are not sterile, it may be difficult to prevent nuisance micro-organisms (such as those producing excess acid) from invading the consortium. The logical design approach probably has its biggest advantage where atypical conditions are required for which ubiquitous organisms are not available, such as stirred-tank operation at exceptionally high acidity (pH <1) or high temperatures (~80 °C). Under such circumstances contamination by nuisance micro-organisms is less of a danger.

Environmental conditions in a continuous-flow stirred tank are uniform spatially and constant over time. This allows for limited microbial diversity and, once established, the composition of the consortium may remain relatively constant. In contrast, heap reactors are not homogeneous and provide a large number of very different environments that support a large diversity of micro-organisms. Furthermore, the environments change as the heap ages and the most reactive minerals are removed. From a consortium development point of view, the main challenge is to provide sufficient microbial diversity throughout the life of the heap to ensure optimum heap performance. To do that effectively, more studies on the microbial composition at different stages in the life of heap reactors containing minerals of different compositions are required.

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References

- Battaglia-Brunet, F., Clarens, M., d'Hugues, P., Godon, J. J., Foucher, S. & Morin, D. (2002). Monitoring of a pyrite-oxidising bacterial population using DNA single strand conformation polymorphism and microscopic techniques. *Appl Microbiol Biotechnol* **60**, 206–211.
- Battaglia-Brunet, F., Joulain, C., Garrido, F., Dictor, M.-C., Morin, D., Coupland, K., Johnson, D. B., Hallberg, K. B. & Baranger, P. (2006). Oxidation of arsenite by *Thiomonas* strains and characterization of *Thiomonas arsenivorans* sp. nov. *Antonie van Leeuwenhoek* **89**, 99–108.
- Bruhn, D. F., Thompson, D. N. & Naoh, K. S. (1999). Microbial ecology assessment of a mixed copper oxide/sulfide dump leach operation. In *Biohydrometallurgy and the Environment. Toward the Mining of the 21st Century, Process Metallurgy 9A*, pp. 799–808. Edited by R. Amils & A. Ballester. Amsterdam: Elsevier.
- Clark, D. A. & Norris, P. R. (1996). *Acidimicrobium ferrooxidans* gen. nov., sp. nov. mixed culture ferrous iron oxidation with *Sulfobacillus* species. *Microbiology* **141**, 785–790.
- 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. *Appl Environ Microbiol* **68**, 838–845.
- Demergasso, C. S., Galeguillos, P. A., Escudero, L. V., Zepeda, 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.
- Dew, D. W., Lawson, E. N. & Broadhurst, J. L. (1997). The BIOX[®] process for biooxidation of gold-bearing ores or concentrates. In *Biomining: Theory, Microbes and Industrial Processes*, pp. 45–80. Edited by D. E. Rawlings. Georgetown, TX: Springer/Landes Bioscience.
- d'Hugues, P., Battaglia-Brunet, F., Clarens, M. & Morin, D. (2003). Microbial diversity of various metal-sulphides bioleaching cultures grown under different operating conditions using 16S-rDNA analysis. In *Biohydrometallurgy; a Sustainable Technology in Evolution*, pp. 1323–1334. Edited by M. Tsezos, A. Hatzikioseyan & E. Remoudaki. Zografou, Greece: National Technical University of Athens.
- Dopson, M. & Lindström, E. B. (2004). Analysis of community composition during moderately thermophilic bioleaching of pyrite, arsenical pyrite, and chalcopyrite. *Microb Ecol* **48**, 19–28.
- Edwards, K. J., Hu, B., Hamers, R. J. & Banfield, J. F. (2001). A new look at microbiological leaching patterns on sulfide minerals. *FEMS Microbiol Ecol* **34**, 197–206.
- Goebel, B. M. & Stackebrandt, E. (1994). Cultural and phylogenetic analysis of mixed microbial populations found in natural and commercial bioleaching environments. *Appl Environ Microbiol* **60**, 1614–1621.
- Golyshina, O. V., Pivovarova, T. A., Karavaiko, G. I., Kondrat'eva, T. F., Moore, E. R. B., Abraham, W. R., Lunsdorf, H., Timmis, K. N., Yakimov, M. M. & Golyshin, P. N. (2000). *Ferroplasma acidiphilum* gen. nov., sp. nov., an acidophilic, autotrophic, ferrous-iron-oxidizing, cell-wall-lacking, mesophilic member of the *Ferroplasmaceae* fam. nov., comprising a distinct lineage of the *Archaea*. *Int J Syst Evol Microbiol* **50**, 997–1006.
- Hallberg, K. B. & Johnson, D. B. (2001). Biodiversity of acidophilic prokaryotes. *Adv Appl Microbiol* **49**, 37–84.
- Hallberg, K. B. & Lindström, E. B. (1994). Characterization of *Thiobacillus caldus* sp. nov., a moderately thermophilic acidophile. *Microbiology* **140**, 3451–3456.
- Hallberg, K. B., Johnson, D. B. & Williams, P. A. (1999). A novel metabolic phenotype among acidophilic bacteria: aromatic degradation and the potential use of these microorganisms for the treatment of wastewater containing organic and inorganic pollutants. In *Biohydrometallurgy and the Environment. Toward the Mining of the 21st Century, Process Metallurgy 9A*, pp. 719–728. Edited by R. Amils & A. Ballester. Amsterdam: Elsevier.
- Hallberg, K. B., Coupland, K., Kimura, S. & Johnson, D. B. (2006). Macroscopic "acid streamer" growths in acidic, metal-rich mine waters in north Wales consist of novel and remarkably simple bacterial communities. *Appl Environ Microbiol* **72**, 2022–2030.
- Harvey, T. J. & Bath, M. (2007). The GeoBiotics GEOCOAT technology – progress and challenges. In *Biomining*, pp. 113–138. Edited by D. E. Rawlings & D. B. Johnson. Heidelberg: Springer.
- Hawkes, R. B., Franzmann, P. D. & Plumb, J. J. (2006). Moderate thermophiles including "*Ferroplasma cypraxacervatum*" sp. nov., dominate an industrial scale chalcocite heap bioleaching operation. *Hydrometallurgy* **83**, 229–236.
- Johnson, D. B. & Roberto, F. F. (1997). Heterotrophic acidophiles and their roles in the bioleaching of sulfide minerals. In *Biomining: Theory, Microbes and Industrial Processes*, pp. 259–280. Edited by D. E. Rawlings. Georgetown, TX: Springer/Landes Bioscience.

- Johnson, D. B., Rolfe, S., Hallberg, K. B. & Iversen, E. (2001a). Isolation and phylogenetic characterisation of acidophilic microorganisms indigenous to acidic drainage waters at an abandoned Norwegian copper mine. *Environ Microbiol* **3**, 630–637.
- Johnson, D. B., Bacelar-Nicolau, P., Okibe, N., Yahya, A. & Hallberg, K. B. (2001b). Role of pure and mixed cultures of Gram-positive eubacteria in mineral leaching. In *Biohydrometallurgy: Fundamentals, Technology and Sustainable Development, Process Metallurgy 11A*, pp. 461–470. Edited by V. S. T. Ciminelli & O. Garcia, Jr. Amsterdam: Elsevier.
- Johnson, D. B., Okibe, N. & Roberto, F. F. (2003). Novel thermo-acidophiles isolated from geothermal sites in Yellowstone National Park: physiological and phylogenetic characteristics. *Arch Microbiol* **180**, 60–68.
- 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). *J Microbiol Methods* **60**, 299–313.
- Johnson, D. B., Stallwood, B., Kimura, S. & Hallberg, K. B. (2006). Characteristics of *Acidicaldus organovorvus*, gen. nov., sp. nov.; a novel thermo-acidophilic heterotrophic proteobacterium. *Arch Microbiol* **185**, 212–221.
- Kinnunen, H.-M. & Puhakka, J. A. (2004). High-rate ferric sulfate generation by a *Leptospirillum ferriphilum*-dominated biofilm and the role of jarosite in biomass retainment in a fluidized-bed reactor. *Biotechnol Bioeng* **85**, 697–705.
- Logan, T. C., Seal, T. & Brierley, J. A. (2007). Whole-ore heap biooxidation of sulfidic gold-bearing ores. In *Biomining*, pp. 113–138. Edited by D. E. Rawlings & D. B. Johnson. Heidelberg: Springer.
- Marsh, R. M. & Norris, P. R. (1983). The isolation of some thermophilic, autotrophic, iron- and sulphur-oxidizing bacteria. *FEMS Microbiol Lett* **17**, 311–315.
- Mikkelsen, D., Kappler, U., McEwan, A. G. & Sly, L. I. (2006). Archaeal diversity in two thermophilic chalcopyrite bioleaching reactors. *Environ Microbiol* **8**, 2050–2055.
- Norris, P. R., Clark, D. A., Owen, J. P. & Waterhouse, S. (1996). Characteristics of *Sulfobacillus acidophilus* sp. nov. and other moderately thermophilic mineral-sulphide-oxidizing bacteria. *Microbiology* **141**, 775–783.
- Norris, P. R., Burton, N. P. & Foulis, N. A. M. (2000). Acidophiles in bioreactor mineral processing. *Extremophiles* **4**, 71–76.
- Okibe, N. & Johnson, D. B. (2004). Biooxidation of pyrite by defined mixed cultures of moderately thermophilic acidophiles in pH-controlled bioreactors: the significance of microbial interactions. *Biotechnol Bioeng* **87**, 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. *Appl Environ Microbiol* **69**, 1936–1943.
- Plumb, J. J., Hawkes, R. B. & Franzman, P. D. (2006). The microbiology of moderately thermophilic and transiently thermophilic ore heaps. In *Biomining*, pp. 217–235. Edited by D. E. Rawlings & D. B. Johnson. Berlin: Springer.
- Rawlings, D. E. (2005). Characteristics and adaptability of iron- and sulfur-oxidizing microorganisms used for the recovery of metals from minerals and their concentrates. *Microb Cell Fact* **4**, 13. <http://www.microbialcellfactories.com/content/4/1/13>
- Rawlings, D. E. & Johnson, D. B. (editors) (2007). *Biomining*. Heidelberg: Springer.
- Rawlings, D. E. & Silver, S. (1995). Mining with microbes. *Bio/Technology* **13**, 773–779.
- Rawlings, D. E., Dew, D. & du Plessis, C. (2003). Biomineralization of metal-containing ores and concentrates. *Trends Biotechnol* **21**, 38–44.
- Rodriguez-Leiva, M. & Tributsch, H. (1988). Morphology of bacterial leaching patterns by *Thiobacillus ferrooxidans* on pyrite. *Arch Microbiol* **149**, 401–405.
- Sand, W. & Gehrke, T. (2006). Extracellular polymeric substances mediate bioleaching/biocorrosion via interfacial processes involving iron(III) ions and acidophilic bacteria. *Res Microbiol* **157**, 49–56.
- Sand, W., Gehrke, T., Hallmann, R. & Schippers, A. (1995). Sulfur chemistry, biofilm, and the (in)direct attack mechanism – critical evaluation of bacterial leaching. *Appl Microbiol Biotechnol* **43**, 961–966.
- Stott, M. B., Watling, H. R., Franzmann, P. D. & Sutton, D. C. (2000). The role of iron-hydroxy precipitates in the passivation of chalcopyrite during bioleaching. *Miner Eng* **13**, 1117–1127.
- Temple, K. L. & Colmer, A. R. (1951). The autotrophic oxidation of iron by a new bacterium: *Thiobacillus ferrooxidans*. *J Bacteriol* **62**, 605–611.
- Tourova, T. P., Poltoraus, A. B., Lebedeva, I. A., Tsaplina, I. A., Bogdanova, T. I. & Karavaiko, G. I. (1994). 16S ribosomal RNA (rDNA) sequence analysis and phylogenetic position of *Sulfobacillus thermosulfidooxidans*. *Syst Appl Microbiol* **17**, 509–512.
- Tuffin, I. M., de Groot, P., Deane, S. M. & Rawlings, D. E. (2005). An unusual Tn21-like transposon containing an *ars* operon is present in highly arsenic-resistant strains of the biomining bacterium *Acidithiobacillus caldus*. *Microbiology* **151**, 3027–3039.
- Tuffin, I. M., Hector, S. B., Deane, S. M. & Rawlings, D. E. (2006). The resistance determinants of a highly arsenic resistant strain of *Leptospirillum ferriphilum* isolated from a commercial biooxidation tank. *Appl Environ Microbiol* **72**, 2247–2253.
- van Aswegen, P. C., van Niekerk, J. & Olivier, W. (2007). The BIOX[®] process for the treatment of refractory gold concentrates. In *Biomining*, pp. 1–34. Edited by D. E. Rawlings & D. B. Johnson. Heidelberg: Springer.
- Waksman, S. A. & Joffe, J. S. (1921). Acid production by a new sulfur-oxidizing bacterium. *Science* **53**, 216.
- Yahya, A. & Johnson, D. B. (2002). Bioleaching of pyrite at low pH and low redox potentials by novel mesophilic Gram-positive bacteria. *Hydrometallurgy* **63**, 181–188.